ISSN 0030-9885

Coden: PSIRAA 48 (2) 75-148 (2005)

Pakistan Journal of Scientific and Industrial Research

Vol. 48, No. 2, March-April, 2005

Published Bimonthly by
Scientific Information Centre
Pakistan Council of Scientific and Industrial Research
Karachi, Pakistan

Pakistan Journal of Scientific and Industrial Research

This Journal is indexed/abstracted in Biological Abstracts and Biological Abstracts Reports, Chemical Abstracts, Geo Abstracts, CAB International, BioSciences Information Service, Zoological Record, BIOSIS, NISC, NSDP, Current Contents, CCAB, Rapra Polymer Database, Reviews and Meetings and their CD-ROM counterparts, etc.

Subscription rates (including handling and Air Mail postage): ***Local:*** Rs. 2000 per volume, single issue Rs. 350; ***Foreign:*** US$ 400 per volume, single issue US$ 70.

Electronic format of this journal is available with: Bell & Howell Information and Learning, 300 North Zeeb Road, P.O. 1346, Ann Arbor, Michigan 48106, U.S.A; Fax. No.313-677-0108; http://www.umi.com

Photocopies of back issues can be obtained through submission of complete reference to the Executive Editor against the payment of Rs. 25 per page per copy (Registered Mail) and Rs. 115 per copy (Courier Service), within Pakistan; US$ 10 per page per copy (Registered Mail) and US$25 per page per copy (Courier Service), for all other countries.

Printed by: Saad Publications, Karachi, Pakistan.

Published by: Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Editorial Address:

Executive Editor,
Pakistan Journal of Scientific and Industrial Research, PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Tel: 92-21-4651739-40, 4651741-43

Fax: 92-21-4651738

E-mail: pcsirsys@super.net.pk;

pcsir-sic@cyber.net.pk

Pakistan Journal of Scientific and Industrial Research

Vol. 48, No. 2 **Contents** **March - April 2005**

Physical Sciences

Short Communications

Biological Sciences

Short Communications

Technology

Review

Pak. J. Sci. Ind. Res. 2005 **48**(2) 75-80

The Impact Parameters of the Broadening and Shift of Spectral Lines

M. S. Helmi and G. D. Roston*
Department of Physics, Faculty of Science, Alexandria University, Alexandria, Egypt

(received February 25, 2003; revised January 19, 2005; accepted January 25, 2005)

Abstract. The classical theory of collisional broadening and shift coefficients (β, δ) of an isolated spectral line was used to obtain simple analytical formulae for calculating both β and δ. These formulae were obtained on the assumption that the short range interaction was effective only in the broadening, while the long range was effective in the shift of the spectral line. These coefficients, β and δ depended on the limiting phase shifts responsible for broadening η_{0b} and shift $\eta_{0\delta}$. It was found that the values of η_{0b} and $\eta_{0\delta}$ were not equal to each other, as was proposed by Weisskopf $\eta_{0b} = \eta_{0\delta} = 1$, but were instead $\eta_{ob} = \pm \pi / 5$ and $\eta_{o\delta} = \pm \pi / 2$. The correct signs of these phases were obtained and defined. When these phases were applied with their correct signs in the approximate formulae, the broadening β_c and shift δ_c coefficients for some interactions of Tl, Hg, Cd, Zn, Ar and Ne with inert gases and self-interactions were in agreement with the corresponding values obtained numerically by other authors. The limit at which the shift changed its sign was also obtained. New impact parameters which were not known up to now have been discussed and obtained.

Keywords: spectral line, broadening coefficient β, shift coefficient δ, impact parameters, spectral line shift, spectral line broadening

Introduction

It has long been appreciated that studying the collision broadening and shift of spectral lines contain information concerning the interatomic potentials between the radiating and perturbing atoms. The theoretical treatment of this process is of great interest for the region of low densities at which the interactions of two particles are predominant, where the impact approximation takes place, and the half-width and shift are proportional to the density of the perturbing gas. In this case, the broadening and shift coefficients β and δ, respectively, are specified for such interactions. Quantum mechanical formulation of the impact approximation to the theory of collision broadening and shift of spectral lines gives results which differ little from those of the classical theory. It seems worthwhile, therefore, to use the classical theory of collision broadening and shift developed for any interaction potential. To interpret the experimental data, the theoretical values of broadening and shift parameters in the impact limit of line broadening theory have been calculated for van der Waals and Lennard-Jones potentials by many authors (Dygdala *et al.*, 1989; Dygdala, 1988; Bielski *et al.*, 1985; Czychaj and Sienkiewicz, 1984). The results of these calculations were obtained by the numerical solution of the Lindholm and Foley impact theory of broadening developed by Hindmarsh *et al.* (1967) and Helmi (1994), who concluded that the impact parameters for the broadening and shift coefficients of spectral lines must be different, contrary to the proposed value $\rho_{0\beta} = \rho_{0\delta} = \rho_0$ due to the Weisskopf phase shift $\eta_0 = 1$ (Helmi, 1994), which has no basis. Helmi and Roston (2000) obtained simple analytical formulae for calculating β and δ in case of Lennard-Jones potential. These formulae were based on the assumption that the phase shifts for the broadening η_{ob} and shift $\eta_{o\delta}$ were different. These values are given by $\eta_{ob} = \pm \pi / 5$ and $\eta_{o\delta} = \pm \pi / 2$. Comparing the calculated values of β and δ with that calculated by the numerical method (Hindmarsh *et al.*, 1967), it was found that there was a good agreement between the two values for some interactions, when η_{ob} and $\eta_{o\delta}$ were positive and when other interactions were negative.

The aim of this work was to obtain the correct sign of the broadening and shift phases η_{ob} and $\eta_{o\delta}$ and how to apply these to calculate the values of the new impact parameters, ρ_{ob} and $\rho_{o\delta}$, responsible for the broadening and shift of spectral line, and also to obtain the critical value of the impact parameter, ρ_δ, which separates between the red and blue shifts of the spectral line.

Theoretical Background

According to the adiabatic phase shift theory, the broadening β and shift δ coefficients are given by:

$$\beta = 4\pi \hat{v} \int_0^\infty \rho\,[1 - \cos \eta\,(\hat{v}, \rho)]\, d\rho \qquad (1a)$$

$$\delta = 2\pi \hat{v} \int_0^\infty \rho\, \sin \eta\,(\hat{v}, \rho)\, d\rho \qquad (1b)$$

where:

$\eta(\hat{v}, \rho)$ = the total phase shift caused by a single collision occurring at the impact parameter ρ and relative velocity $\hat{v}$.

*Author for correspondence; E-mail: gamal_daniel@yahoo.com

If the perturber follows a straight line trajectory, $\eta(\hat{\upsilon}, \rho)$ can be written as (Findeisen *et al.*, 1987):

$$\eta(\hat{\upsilon}, \rho) = \frac{2}{\hbar \hat{\upsilon}} \int_0^\infty \frac{R\Delta V(R)}{[R^2 - \rho^2]^{1/2}} \, dR \tag{2}$$

where:

R = the interatomic separation

$\Delta V(R)$ = the difference between the adiabatic potentials describing the interaction between the perturber and emitting atom in its upper and lower states

For the simplest case of monatomic inverse-power potentials $\Delta V(R) = \hbar C_n R^{-n}$, the phase shift $(\hat{\upsilon}, \rho)$ takes the form:

$$\eta(\hat{\upsilon}, \rho) = \frac{\alpha_n C_n}{\hat{\upsilon} \rho^{n-1}} \tag{3}$$

where:

$$\alpha_n = \sqrt{\pi} \, \frac{\Gamma(n-1)/2}{\Gamma(n/2)} \tag{4}$$

The approximate formulae for β and δ. If we assume that the effective part for broadening β in (1a) comes from the near distances $(0 - \rho_{0b})$ and the effective part for the shift δ in (1b) comes from the far distances $(\rho_{0\delta} \to \infty)$, then (1a) and (1b) will take the form:

$$\beta = 4\pi \hat{\nu} \int_0^{\rho_{ob}} \rho \, [1 - \cos \eta(\hat{\upsilon}, \rho)] \, d\rho \tag{5a}$$

$$\delta = 2\pi \hat{\nu} \int_{\rho_{o\delta}}^\infty \rho \, \sin \eta(\hat{\upsilon}, \rho) \, d\rho \tag{5b}$$

It may be seen from (3) that $\eta(\hat{\upsilon}, \rho)$ will be large in the short range $(0 - \rho_{0b})$ and cos $\eta(\hat{\upsilon}, \rho)$ is quickly oscillating, so that $\int \cos \eta(\hat{\upsilon}, \rho) = 0$, while $\eta(\hat{\upsilon}, \rho)$ will be very small in the long range $(\rho_{0\delta} \to \infty)$, so that $\sin \eta(\hat{\upsilon}, \rho) \approx \eta(\hat{\upsilon}, \rho)$. From this was found that (Helmi and Roston, 2000):

$$\beta = 2\pi \hat{\nu} \int_0^{\rho_{ob}} \rho \, d\rho = 2\pi \rho_{0b}^2 \tag{6a}$$

$$\delta = 2\pi \hat{\nu} \int_{\rho_{0\delta}}^\infty \rho \, \eta(\hat{\upsilon}, \rho) \, d\rho \tag{6b}$$

where:

ρ_{0b} and $\rho_{0\delta}$ = respectively, the broadening and shift impact parameters

Applications to potentials. ***Different types of inverse-power potentials.*** The approximate formulae (6) are used to express β and δ in terms of the limiting values of impact parameter ρ, and hence, via (3), the limiting values of η. Introducing the van der Waal's difference potential $\Delta V(R) = hC_n R^{-n}$ in (2), the phase parameters due to the broadening η_{0b} and shift $\eta_{0\delta}$ are given as (Helmi and Roston, 2000):

$$\eta_{0b} = [2 \int_0^\infty x \, [1 - \cos x^{1-n}] \, dx]^{\frac{1-n}{2}} \tag{7a}$$

$$\eta_{0\delta} = [(n-3) \int_0^\infty x \, \sin x^{1-n} \, dx]^{\frac{1-n}{3-n}} \tag{7b}$$

The average values of η_{0b} and $\eta_{0\delta}$ for diffrent values of n [n = 3, 4 and 6] were calculated by Helmi and Roston (2000), which gives $\eta_{ob} = \pm \pi / 5$ and $\eta_{o\delta} = \pm \pi / 2$.

Lennard-Jones potential. The Lennard-Jones difference potential between the excited and the ground states of the quasimolecule consisting of radiating and perturbing atoms is given by:

$$\Delta V(R) = \hbar \, \Delta C_{12} R^{-12} - \hbar \, \Delta C_6 R^{-6} \tag{8}$$

where:

R = the distance between the colliding atoms

ΔC_6, ΔC_{12} = constants, depending on the states of these atoms

The pressure broadening β and shift δ coefficients in the case of L-J potential are given as (Hindmarsh *et al.*, 1967):

$$\beta = 8\pi \left(\frac{3\pi}{8}\right)^{\frac{2}{5}} \hat{\upsilon}^{\frac{3}{5}} (\Delta C_6)^{\frac{2}{5}} B(\alpha) \tag{9a}$$

$$\delta = 2\pi \left(\frac{3\pi}{8}\right)^{\frac{2}{5}} \hat{\upsilon}^{\frac{3}{5}} (\Delta C_6)^{\frac{2}{5}} S(\alpha) \tag{9b}$$

where:

the broadening and shift functions $B(\alpha)$ and $S(\alpha)$ are defined by the following integrals:

$$B(\alpha) = \int_0^\infty x \sin^2 \frac{1}{2} (\alpha x^{-11} - x^{-5}) \, dx \tag{10a}$$

$$S(\alpha) = \int_0^\infty x \sin (\alpha x^{-11} - x^{-5}) \, dx \tag{10b}$$

where:

$$\alpha = 0.536 \, \hat{\upsilon}^{\frac{6}{5}} (\Delta C_6)^{-\frac{11}{5}} \Delta C_{12}$$

The functions $B(\alpha)$ and $S(\alpha)$ were obtained numerically for some chosen values of α. The phase shift $\eta(\hat{\upsilon}, \rho)$ in case of L-J potential is given by introducing (8) into (2) as:

$$\eta(\hat{\upsilon}, \rho) = \left(\frac{\alpha_{12} \Delta C_{12}}{\hat{\upsilon}}\right) \rho^{-11} - \left(\frac{\alpha_6 \Delta C_6}{\hat{\upsilon}}\right) \rho^{-5} \tag{11}$$

Introducing the obtained average values $\eta_{0b} = \pm 0.63$ and $\eta_{0\delta} = \pm 1.57$ into (11), the broadening and shift impact parameters ρ_{0b} and $\rho_{0\delta}$ in the case of L-J potential are obtained as:

$$\left(\frac{\alpha_{12} \Delta C_{12}}{\hat{\upsilon}}\right) \rho_{0b}^{-11} - \left(\frac{\alpha_6 \Delta C_6}{\hat{\upsilon}}\right) \rho_{0b}^{-5} = \pm \frac{\pi}{5} = \pm 0.63 \tag{12a}$$

$$\left(\frac{\alpha_{12}\Delta C_{12}}{\hat{\upsilon}}\right)\rho_{0\delta}^{-11} - \left(\frac{\alpha_6 \Delta C_6}{\hat{\upsilon}}\right)\rho_{0\delta}^{-5} = \pm\frac{\pi}{2} = \pm 1.57 \quad (12b)$$

The positive sign in (12a, b) denotes that the repulsion forces are more than the attraction forces, while the negative sign denotes that the attraction forces are more than the repulsive forces at the collision time.

To obtain the broadening β and shift δ coefficients in case of L-J potential, we introduce (11) into (6a, b), which gives:

$$\beta = \hat{\upsilon}\,\rho_{0b}^2 \quad (13a)$$

$$\delta = \hat{\upsilon}\left(\frac{\alpha_{12}\Delta C_{12}}{9\hat{\upsilon}}\right)\rho_{0\delta}^{-9} - \left(\frac{\alpha_6 \Delta C_6}{3\hat{\upsilon}}\right)\rho_{0\delta}^{-3} \quad (13b)$$

Knowing ρ_{0b} and $\rho_{0\delta}$ from (12a, b), β and δ can be obtained directly from the approximated formulae (13a, b).

Applying the Lennard-Jones (L-J) potential with the same obtained average values of η_{0b} and $\eta_{0\delta}$, and comparing the results obtained by the approximated formulae (6a, b) for β and δ with the results obtained for these coefficients by numerical calculations of (Hindmarsh *et al.*, 1967), Helmi and Roston (2000) found that there was an agreement between the two results for some interactions when η_{0b} and $\eta_{0\delta}$ were positive, and other interactions were negative, but Helmi and Roston (2000) did not clarify the reason of the different signs, and as to when these signs could be applied.

To obtain the necessary sign used in (12a, b), the following was proceeded:

Let ρ_0, ρ_E, ρ_{0b} and $\rho_{0\delta}$ be the impact parameters corresponding, respectively, to the phase shifts $\eta = 0$, $\eta = \eta_E$ (the phase shift well-depth), $\eta_{0b} = \pm 0.63$ and $\eta_{0\delta} = \pm 1.57$. When $\eta(\hat{\upsilon}, \rho)$ given by (11) was plotted against ρ for different interactions, the plotted curves had the form shown in Fig. 1-3. The parameters ρ_o, ρ_E and η_E are given from (11) as:

$$\rho_0 = \left[\frac{21\,\Delta C_{12}}{32\,\Delta C_6}\right]^{1/6}, \quad \rho_E = 1.14\,\rho_0$$

$$\eta_E = \left(\frac{\alpha_{12}\,\Delta C_{12}}{\bar{V}}\right)\rho_E^{-11} - \left(\frac{\alpha_6\,\Delta C_6}{\bar{V}}\right)\rho_E^{-5} \quad (14)$$

It may also be seen from (13b) that the impact parameter ρ_δ, which separates between the negative and positive signs of the shift coefficients δ, is given by:

$$\rho_\delta = \left[\frac{7\,\Delta C_{12}}{32\,\Delta C_6}\right]^{1/6} = 1.2\,\rho_0 \quad (15)$$

So that if $\rho_{0\delta} < \rho_\delta$ in (12b), then δ has a positive sign and the spectral line will be shifted to the blue wing, but if $\rho_{0\delta} > \rho_\delta$, then δ has a negative sign and the spectral line will be shifted to the red wing.

Results and Discussion

To obtain the appropriate sign of $\eta_{0b} = \pm 0.63$ and $\eta_{0\delta} = \pm 1.57$, we proceed as follows:

knowing, ΔC_6, ΔC_{12} and $\hat{\upsilon}$, η_E can be obtained using (14);

(1) if $\eta_E > -0.63$

then:

ρ_{0b} and $\rho_{0\delta}$ can be obtained using the positive sign for η_{0b} and $\eta_{0\delta}$ according to (12a, b), in this case $\rho_{0\delta} < \rho_{0b} < \rho_E$ (Fig. 1),

(2) if $\eta_E \leq -1.57$

then:

ρ_{0b} and $\rho_{0\delta}$ will be taken with the negative sign for η_{0b} and $\eta_{0\delta}$, in this case $\rho_{0b} > \rho_{0\delta} > \rho_E$ (Fig. 2),

(3) if $\eta_E < -0.63$, but > -1.57

then:

ρ_{0b} will be taken with the negative sign for η_{0b}, so that, $\rho_{0b} > \rho_E$, however, $\rho_{0\delta}$ will be taken with the positive sign for $\eta_{0\delta}$, so that $\rho_{0\delta} < \rho_E$ (Fig. 3).

The calculated coefficients β_c and δ_c for different interactions are illustrated in Table 1, with the corresponding Hindmarsh values β_H and δ_H, for the interactions of Ar, Ne, Tl, Hg, Cd and Zn atoms with the inert gases Xe, Kr, Ar, Ne and He.

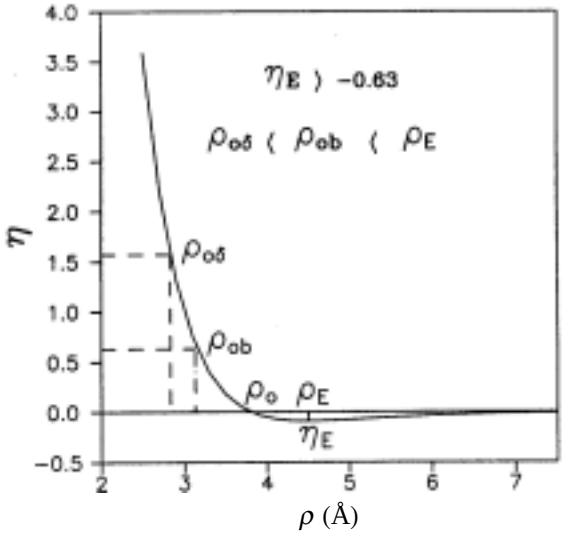


Fig. 1. The phase shift η as a function of the impact parameter ρ, when $\eta_E > -0.63$.

Table 1. The calculated values of pressure broadening β_c and shift δ_c coefficients with the corresponding Hindmarsh values β_H and δ_H in units 10^{-20} /cm/atom /cm^3 for Ar, Ne, Tl, Hg, Cd and Zn perturbed by inert gases*

Perturber	Parameters		Phase	Impact parameters				Broadening		Shift potential	
	ΔC_6	ΔC_{12}	η_E	ρ_δ	ρ_E	ρ_{ob}	$\rho_{o\delta}$	β_H	β_C	δ_H	δ_C
Ar line 703 nm, T = 330 K											
Ar[1]	531.8	83750	-0.060	18.0	24.7	18.8	17.6	7.040	6.930	0.200	0.200
Ne[1]	130.0	55870	-0.005	21.3	29.2	18.4	17.0	8.250	8.170	0.990	1.000
He[1]	70.50	48190	-0.001	23.0	31.7	17.3	16.0	13.89	13.75	1.890	1.780
Ne line 540 nm, T = 330 K											
Ne[4]	5.400	1.2400	-0.100	6.09	8.34	6.52	6.15	1.153	1.170	-0.028	-0.028
He[4]	2.900	0.8800	-0.025	6.37	8.73	6.22	5.79	1.870	1.870	0.150	0.150
Tl line 377.68 nm, T = 860 K											
Xe[2,3]	145.2	30.910	-5.290	5.99	8.21	14.1	11.7	3.270	3.160	-1.100	-1.170
Kr[2,3]]	49.40	20.610	-1.900	6.08	9.49	12.4	10.0	2.840	2.840	-1.400	-1.100
Ar[2,3]	58.70	15.250	-1.170	6.20	8.49	7.72	7.12	1.900	1.949	-1.180	-1.200
Ne[2,3]	14.30	6.5800	-0.130	6.82	9.34	7.42	7.00	1.640	1.800	-0.080	-0.090
Hg line 253.6 nm, T = 860 K											
Hg[5]	11.700	0.0572	-10.98	3.19	4.38	8.76	7.29	1.079	1.085	-0.396	-0.394
Xe[6]	0.2970	0.0010	-0.270	3.01	4.28	3.52	3.36	0.207	0.202	-0.037	-0.036
Kr[7]	0.7920	0.0080	-0.300	3.61	4.94	4.08	3.93	0.304	0.314	-0.068	-0.067
Ar[8]	0.0630	0.4970	-0.003	5.09	6.97	4.20	3.87	0.438	0.443	0.055	0.059
Ne[8]	0.7450	0.0640	-0.027	5.16	7.05	5.07	4.72	0.850	0.847	0.064	0.066
Cd line 326.1 nm, T = 860 K											
Cd[9]	7.2200	0.0451	-4.080	3.33	4.56	7.45	6.15	1.140	1.070	-0.365	-0.396
Xe[10]	1.3900	0.0110	-0.670	3.46	4.75	4.50	3.90	0.359	0.370	-0.154	-0.156
Kr[11]	0.2190	0.0127	-0.018	4.85	6.61	4.58	4.26	0.428	0.437	0.039	0.041
Ar[10]	0.2197	0.0306	-0.458	3.82	5.21	4.37	4.22	0.549	0.510	-0.180	-0.175
Ne[10]	0.3740	0.0388	-0.760	3.63	4.96	4.63	4.10	0.775	0.753	-0.355	-0.370
He[10]	0.3170	0.0106	-0.010	4.43	6.03	4.04	3.75	1.190	1.200	0.123	0.127
Zn line 307.6 nm, T = 860 K											
Zn[12]	1.7875	0.0199	-0.475	3.67	5.02	4.22	4.07	0.491	0.441	-0.163	-0.161
Xe[13]	10.532	0.1926	-2.558	3.98	5.46	9.36	7.71	1.220	1.188	-0.665	-0.705
Kr[14]	2.6630	0.0028	-5.369	2.47	3.39	5.91	4.95	0.829	0.817	-0.268	-0.291
Ar[14]	0.1650	0.0072	-0.012	4.61	6.31	4.25	3.95	0.514	0.515	0.051	0.053
Ne[15]	0.1337	0.0030	-0.014	4.12	5.63	3.83	3.56	0.529	0.531	0.052	0.054

* the values of ρ are in Å units; ΔC_6 in units 10^{-32} cm^6 rad s^{-1}, and ΔC_{12} in units 10^{-74} cm^{12} rad s^{-1}, which are taken from the references given below:

[1] Bielski *et al.* (1985), [2] Dygdala (1988), [3] Dygdala *et al.* (1989), [4] Bielski *et al*. (1980), [5] Czuchaj *et al.* (1997), [6] Okunishi *et al.* (1990), [7] Kurosawa *et al.* (1998), [8] Petzold and Behmenburg (1978), [9] Helmi *et al.* (1996) , [10] Czuchaj and Stoll (1999), [11] Czajkowski *et al.* (1991), [12] Czajkowski and Kopersk (1999), [13] Wallace *et al.* (1991), [14] Wallace *et al.* (1992), [15] Koperski and Czajkowski (2000)

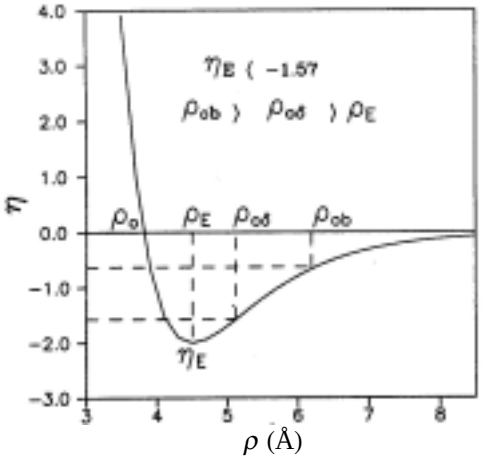


Fig. 2. The phase shift η as a function of the impact parameter ρ, when $\eta_E \leq -1.57$.

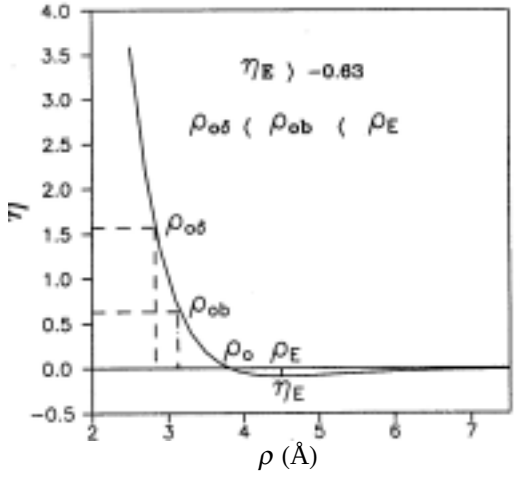


Fig. 3. The phase shift η as a function of the impact parameter ρ, when $-1.57 < \eta_E < -0.63$.

Conclusions

The following conclusions are based on the fact that the calculated coefficients β_C and δ_C by simple analytical formulae obtained by the present authors, when the Lennard-Jones potential was applied, are in good agreement as shown in Table 1, with the corresponding coefficients obtained numerically by other authors. This has led to the following considerations:

(1) new impact parameters ρ_o, ρ_E, ρ_{ob}, $\rho_{o\delta}$ and ρ_δ, which are firstly defined and obtained,

(2) the impact parameters ρ_{ob}, and $\rho_{o\delta}$ responsible, receptively, for the broadening and shift of spectral lines are different, and depend strongly on the values of ΔC_6 and ΔC_{12}, the values and signs of the phase shifts η_{ob} and $\eta_{o\delta}$ due to the broadening and shift,

(3) the phases η_{ob} and $\eta_{o\delta}$ at which the broadening and shift, respectively, start to take place are also different in values, which are the same for all interactions, $\eta_{ob} = \pm 0.63$ and $\eta_{o\delta} = \pm 1.57$,

(4) the sign of phases η_{ob} or $\eta_{o\delta}$ depended on the value of the phase η_E at the equilibrium position of phases, which value is given by (14),

(5) the impact parameter ρ_δ which separates between the positive and negative signs of the shift of spectral line was obtained and given by a simple formula (15), so that if $\rho_{o\delta} < \rho_\delta$, then the line was shifted to the blue wing (δ = positive value), but if $\rho_{o\delta} > \rho_\delta$, then it was shifted to the red wing (δ = negative value),

(6) as the calculated coefficients β_c and δ_c, using the approximated formulae, are in good agreement with those obtained before by numerical calculations, then all assumptions leading to the approximated formulae are valid and the obtained formulae, furthermore, can be easily used with other complicated potentials.

References

Bielski, A., Bobkowski, R., Dokurno, W., Szudy, J. 1980. Low pressure shift of the $2p^5 3p$-$2p^5 4d$ spectral lines of neon perturbed by neon and helium. *Acta Phys. Polonika* **A59:** 107-114.

Bielski, A., Wawrzynski, J., Wolnikowski, J. 1985. Low pressure broadening and shift of the $3P^5 4P$-$3P^5 6s$ argon spectral lines perturbed by halium, neon and argon. *Acta Phys. Polonica* **A67:** 621-629.

Czajkowski, M., Bobkowski, R., Krause, L. 1991. Spectroscopy of $AO^+ \leftarrow XO^+$ and $B1 \leftarrow XO^+$ transitions in cadmium - krypton (Cd Kr). *Phys. Rev.* **A 44:** 5730-5736.

Czajkowski, M., Kopersk, J. J. 1999. The Cd_2 and Zn_2 van der Waals dimers revisited. Correction for some molecular potential parameters. *Spectrochimica Acta* **A 55:** 2221-2229.

Czuchaj, E., Rebentrost, F., Stoll, H., Preuss, H. 1997. Calculation of the ground- and excited-state potential energy curves for the Hg_2 molecule in a pseudopotential approach. *Chem. Phys.* **214:** 277-289.

Czuchaj, E., Sienkiewicz, J. 1984. Pseudopotential calculation of the adiabatic potentials and oscillator strengths of cadmium-rare gas pairs. *J. Phys.* **B17:** 2251-2267.

Czuchaj, E., Stoll, H. 1999. Calculation of ground and excited-state potential energy curves for the Cd-rare gas complexes. *Chem. Phys.* **248:** 1-16.

Dygdala, R. S. 1988. Broadening and shift of the resonance line of thallium due to collisions with krypton and xenon atoms. *J. Phys. B:At. Mol. Opt. Phys.* **21:** 2039-2047.

Dygdala, R. S., Bobkowski, R., Lisicki, E. 1989. Broadening

and shift of the resonance line of thallium due to collisions with neon and argon atoms. *J. Phys. B:At. Mol. Opt. Phys.* **22:** 1563-1572.

Findeisen, M., Grycuk, T., Bielski, A., Szudy, J. 1987. On the role of Maxwellian overaging in the interpretation of the pressure broadening and shift of spectral lines. *J. Phys. B:At. Mol. Opt. Phys.* **20:** 5997-6003.

Helmi, M. S. 1994. On the validity of Weisskopf phase shift in the pressure broadening and shift of spectral lines. *Pak. J. Sci. Ind. Res.* **37:** 499-502.

Helmi, M. S., Grycuk, T., Roston, G. D. 1996. Interatomic potentials and the van der Waals' coeffiicient for the intercombination Cd 326.6 nm absorption line broadened by cadmium pressure. *Spectrochimica Acta* **B 51:** 633-641.

Helmi, M. S., Roston, G. D. 2000. Determination of the phase shifts leading to the broadening and shift of spectral lines. *Physica Scripta* **62:** 36-40.

Hindmarsh, W. R., Petford, A. D., Smith, G. 1967. Interpretation of collision broadening and shift of spectral lines. *Proc. Roy. Soc.* **A297:** 296-304.

Koperski, J., Czajkowski, M. 2000. Spectroscopic characterization of the ZnNe van der Waals molecule in the $XO^+(4^1S_o)$ and D1 (4^1P_1) energy states. *Phys. Rev. A: At. Mol. Opt. Phys.* **62:** 012505/1-012505/9.

Kurosawa, T., Ohmori, K., Chiba, H., Okunishi, M., Ueda, K., Sato, Y. , Devdariani, A. Z., Nikitin, E. E. 1998. Collision induced absorption in mercury-rare gas collisions. *J. Chem. Phys.* **108:** 8101-8109.

Okunishi, M., Nakazawa, H., Yamanouchi, K.,Tsuchiya, S. 1990. Interatomic potentials of mercury-krypton and mercury-xenon (HgKr and HgXe) van der Waals complexes in the A^3O^+ and B ^{3}1 states. Revisited. *J. Chem. Phys.* **93:** 7526-7527.

Petzold, H. C., Behmenburg, W. 1978. Interaction potentials of mercury- argon from temperature dependent absorption spectra. *Z. Naturforsch.* **33A:** 1461-1468.

Wallace, I., Kaup, J. G., Breckenridge, W. H. 1991. Excitation of Zn ($4s4P'P_1$) within the zinc - xenon van der Waals complex: effect of *p*-orbital alignment on the production of zinc ($4s4p^3P_j$). *J. Phys. Chem.* **95:** 8060-8065.

Wallace, I., Ryter, J., Breckenridge, W. H. 1992. Spectroscopic characterization of the $X^1\Sigma^+_o$ and C $^1\Pi_1$ states of the zinc-krypton (Zn-Kr) molecule. *J. Chem. Phys.* **96:** 136-143.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 81-85

Isomerization of 1,5-Hexadiene Catalyzed by Bis-(Cyclopentadienyl) Lanthanide Schiff Base/NaH Systems; Ln = Sm, Dy, Y, Er

Muhammad Yousaf*[ac], Yanlong Qian[a] and Muhammad Khalid Saeed[b]

[a]Laboratory of Organometallic Chemistry, East China University of Science and Technology, Meilong Road 130[#], 200237, Shanghai, Peoples Republic of China

[b]Biotechnology and Food Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan

[c]Present address: Government Ghazali College, Jhang, Pakistan

(received December 8, 2003; revised January 18, 2005; accepted January 27, 2005)

Abstract. Catalytic isomerization of 1,5-hexadiene by Cp_2Ln Schiff base/NaH (Schiff base = $C_{14}H_{14}NO_2$, Ln = Sm, Dy, Y, and Er) systems was studied. The isomerization resulted in a mixture of 1,4-hexadiene, 2,4-hexadiene, 1,3-hexadiene, methylene-cyclopentane, and methylcyclopentene. 1,4-Hexadiene and methylenecyclopentane were the intermediate products, while 2,4-hexadiene and methylcyclopentene were the end-products. The effects of the nature of catalyst, temperature, amount of the catalyst, time and solvent, on the isomerization rate and product composition were also studied. The ratio of the linear to the cyclic product in the reaction depended on the amount of catalyst used.

Keywords: isomerization, 1,5-hexadiene, 2,4-hexadiene, lanthanocene complexes, Schiff base/NaH systems, methylcyclopentene

Introduction

The recent growth in organolanthanide chemistry has primarily focused on complexes stabilized by tridentate Schiff base ligand system. Further interest in exploring the metal ion complexes with Schiff base ligands has continually increased, since it has been recognized that many of such complexes may serve as biologically important, naturally occuring ionophores. On the other hand, metal hydride complexes are fundamental components in a wide range of stoichiometric and catalytic organometallic reactions (Teller and Bau, 1981). However, the hydrides of the lanthanide metals have been previously described only for interstitial metallic compounds. The simple binary hydrides, LnH_x, have been known for many years (Mueller *et al.*, 1968). The literature survey verifies that hydride complexes of lanthanide metals are important factors for the rapid advancement of the developing organolanthanide chemistry. The investigations concerning lanthanide complexes with Schiff bases have been devoted to their synthesis, structural studies, and biological application of metal enzymes or protein bondings. A large number of Schiff bases and their complexes have been studied for their interesting and important properties, such as, their ability to reversibly bind oxygen (Jones *et al.*, 1979) catalytic activity in hydrogenation of olefins (Henrici-Olie and Olive, 1984), transfer of amino group (Dugas and Penny, 1981), photochromic properties (Margerrum and Miller, 1971), complexing ability towards some toxic metals (Sawodny and Reiderer, 1977), catalytic synthesis of polymethylmethacrylate (Yousaf *et al.*, 2000 a; b), and so on. Untill now, such type of complexes have not been attempted for isomerization of olefins. Earlier works indicate that the catalytic isomerization of 1,5-hexadiene by Cp_2TiCl_2/i-C_3H_7MgBr system results in a mixture of 1,4-hexadiene, 1,3-hexadiene, 2,4-hexadiene, methylenecyclopentane, and methylcyclopentene (Akita *et al.*, 1983; Lehmkuhi and Qian, 1983). Later on, it was verified that isomerization of 1,5-hexadiene with cyclopen-tadienyl titanium complexes resulted into a mixture of simi-lar isomeric products as reported above (Qian *et al.*, 2000). Thus, there was an interest in the synthesis of monomeric lanthanocene complexes with tridentate Schiff base [N-I-(ortho-methoxyphenyl)] as a ligand, which is the electronic equivalent to cycloentadienyl ligand, and then catalytic isomerization of 1,5 hexadiene.

*Author for correspondence

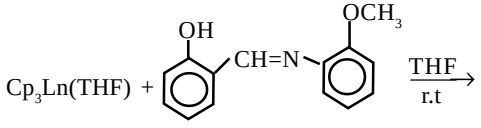
Materials and Methods

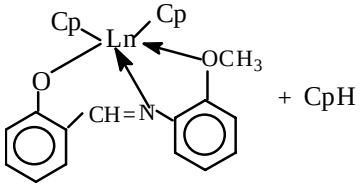
All the operations were carried out under prepurified argon by use of schlenk techniques. All the solvents were refluxed and distilled over blue sodium benzophenone under argon immediately before use. All the complexes were newely synthesized by using the reported procedures (Yousaf *et al.*, 2000c; d; Liu and Ding, 1998) and were characterized by MS, EA, and IR. Sodium hydride was washed with THF and dried under vacuum. 1,5-hexadiene was dried by treating with CaH_2 and distilled under argon. A general procedure was as follows (refer to entry 1 in Table 1): a 25 ml schlenk tube, equipped with a teflon stopcock, was charged under argon with 0.052 g (0.10 mmole) of $(C_5H_5)_2Sm(OC_{14}H_{13}NO)$ and 0.12 g of NaH,

then 3 ml THF was poured along with stirring, cooled to -78°C, and 0.164 g (0.24 ml) of 1, 5-hexadiene was introduced by a syringe. The reaction mixture was allowed to warm to room temperature, and then was continued at 60°C. After the given time, the reaction was quenched with 1 ml of methanol. The reaction mixture was then distilled out under reduced pressure and the distillate was collected in schlenk tube at -78°C. The solution of the products thus obtained was injected into the GC, and products were identified by comparing with authentic compounds.

Results and Discussion

The Cp_2Ln Schiff base complexes were synthesized as follows:





Ln = Sm, Dy, Y, Er

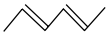
The results of isomerization of 1,5-hexadiene catalyzed by different Cp_2LnSb catalysts are shown in Table 1.

It was observed that neither these novel lanthanocene complexes, nor NaH alone, did show any encouraging activity. When the complexes were, however, embedded with NaH (1:50), then they exhibited reasonable efficiency.

As is explained by the observations listed in Table 1, the catalytic efficiency of $(C_5H_5)_2Sm(OC_{14}H_{13}NO)$ was more than any other attempted complexes. Further, the synthetic yield of this complex was much more than others. So, this complex was used as a representative of all the samples tested to study the effect of catalyst, tempareture, catalyst/substrate ratio, time, and solvent on the isomerization of 1,5-hexadiene. At 60 °C, the $(C_5H_5)_2Sm(OC_{14}H_{13}NO)$ favours the conversion of 1,5-hexadiene into linear products, especially 1,4-hexadiene (80.6%), whereas $(C_5H_5)_2Er(OC_{14}H_{13}NO)$ conveys 1,5-hexadiene into cyclic ones, preferably methylenecyclopentane (25.2%). There was no significant difference between the convertion efficiency of $(C_5H_5)_2Er(OC_{14}H_{13}NO)$ and $(C_5H_5)_2ErCl(OC_{14}H_{13}NO)$.THF. This suggests that both complexes may offer the same kind of hydride system. Generally, the catalytic efficiency of $(C_5H_5)_2Sm(OC_{14}H_{13}NO)$ was better (28.7%) than any other complex used, which may be because of the ionic radius of samarium being larger than any other metal used. It means that greater the ionic radius, the greater will be the coordination sphere of the metal. Hence, easier the active species, such as CpLnH will be produced, and also easier the monomeric unit will make attachment with the metal during the isomerization process. Moreover, it should be kept in mind that the role of Schiff base ligand in such type of the complexes is to facilitate the generation of active species, like CpLnH, because Schiff base, being polar, can react with NaH easily to produce such active species. Further, there was found no catalytic activity with $ErCl_3$/NaH system.

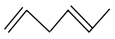
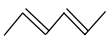
The effect of temperature, as shown in Table 2, indicates that at high temperature, 1,4-hexadiene was the prominent product, whereas at the lower temperature, methylenecyclopentane was the major one. Conclusively at 60 °C, as is clear from Fig. 1, the catalytic efficiency was appreciable.

Table 1. Effect of catalysts on the isomerization of 1,5-hexadiene*

Catalyst	Conversion (%)	Selectivity (%)				Linear/cyclic
			 cc,ct,tt			
$(C_5H_5)_2Sm(OC_{14}H_{13}NO)$	28.7	80.6	2.4	12.4	4.6	83.0/17.0
$(C_5H_5)_2Dy(OC_{14}H_{13}NO)$	25.8	81.3	2.7	11.7	4.2	84.0/16.0
$(C_5H_5)_2Y(OC_{14}H_{13}NO)$	25.7	75.0	7.1	13.1	4.8	82.1/17.9
$(C_5H_5)_2Er(OC_{14}H_{13}NO)$	20.6	55.8	9.1	25.2	9.9	64.9/35.1
$(C_5H_5)_2ErCl(OC_{14}H_{13}NO)$. THF	20.0	62.1	7.0	23.3	7.6	69.1/30.9
$ErCl_3$	1.1	100	trace	trace	trace	

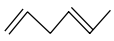
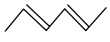
*reaction conditions: catalyst/hexadiene = 1:20; catalyst/NaH = 1:50; time 24 h; temperature = 60 °C ; solvent: THF

Table 2. Effect of temperature on the isomerization of 1,5-hexadiene*

Temperature (°C)	Conversion (%)	Selectivity (%)				Linear/cyclic
			 cc,ct,tt			
30	7.9	28.8	5.2	49.0	17.0	36.7/63.3
45	21.3	75.3	3.5	15.6	5.6	78.8/21.2
60	28.7	80.6	2.4	12.4	4.6	30.0/17.0

*reaction conditions: catalyst/hexadiene = 1:20; catalyst/NaH = 1:50; solvent = THF; time = 24 h

Table 3. Effect of mole ratio on the isomerization of 1,5-hexadiene*

Temperature (°C)	Conversion (%)	Selectivity (%)				Linear/cyclic
			 cc,ct,tt			
1:10	31.3	84.0	3.0	9.5	3.5	87.0/13.0
1:20	28.7	80.6	2.4	12.4	4.6	83.0/17.0
1:40	14.4	46.1	10.9	32.4	10.5	51.0/43.0

*reaction conditions: catalyst/NaH 1:50; time = 24 h; temperature = 60 °C; solvent = THF

In the case of catalyst/substrate, as shown in Table 3, for 1:10 to 1:20, there was no significant difference in the conversion of 1,5-hexadiene into either linear (87.0% and 83%) products, or the cyclic ones (13% and 17%). However, at 1:40, a remarkable difference was observed. The linear products were smaller but cyclic were higher than the above ones. This indicates that the more the catalyst, the more linear the products, which is in accordance with the results reported earlier (Liu *et al.*, 1998). The results listed in Table 3 were further verified by Fig. 2.

The results listed in Table 4 show that untill 10 h, the catalytic efficiency of the catalyst was very rapid, being 25.6%. Then, during the further period of 10 h, it was comparatively slower, 27.4%, and after this there was no significant increase, being only 1.3% in the next 10 h. It means that after 10 h the isomerization equilibrium had been reached. It was possible perhaps to initiate further isomerization of the remaining 75% material on the removal of the product. Conclusively, isomerization of 1,5-hexadiene can be completed during 20 h by using such type of a catalytic system. The whole discussion was verified through Fig. 3, which shows that the conversion of isomerization flattens after 10 h.

The results as presented in Table 5 prove justifiably that THF was the best solvent for this study, showing (7.9%) maximum conversion, whereas toluene had shown the least activity

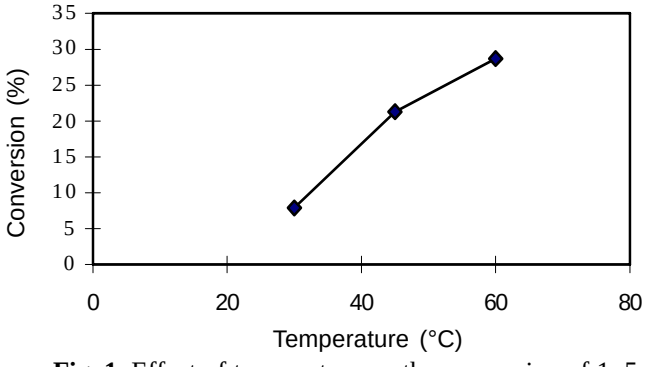


Fig. 1. Effect of temperature on the conversion of 1, 5-hexadiene.

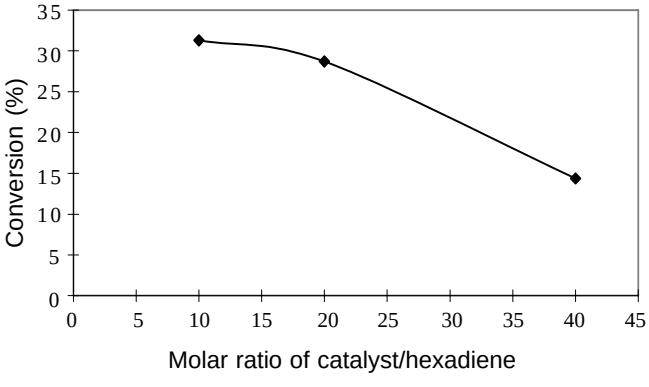


Fig. 2. Effect of catalyst/hexadiene molar ratio on the isomerization of 1, 5-hexadiene.

(0.5%). From these results it was concluded that polarity of the solvent played a vital role for the isomerization of 1,5-hexadine. In other words, solubility of the catalyst was more in THF than any other solvents used (Et_2O and toluene). Moreover, in the case of THF, 1,3-hexadiene and methylene-cyclopantane were the main components.

The proposed mechanism is presented in the reaction scheme as shown in Fig. 4 (Qian *et al.*, 2000; Akita *et al.*, 1983; Lehmkuhi and Qian, 1983).

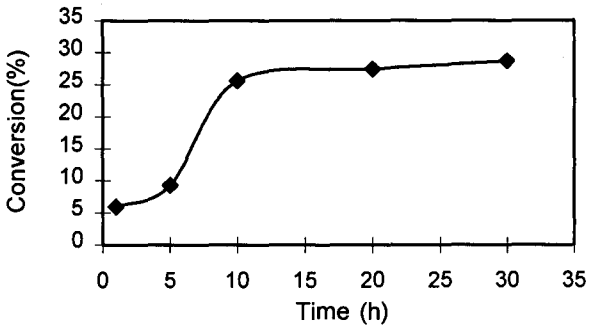


Fig. 3. Effect of time on the isomerization of 1, 5-hexadiene.

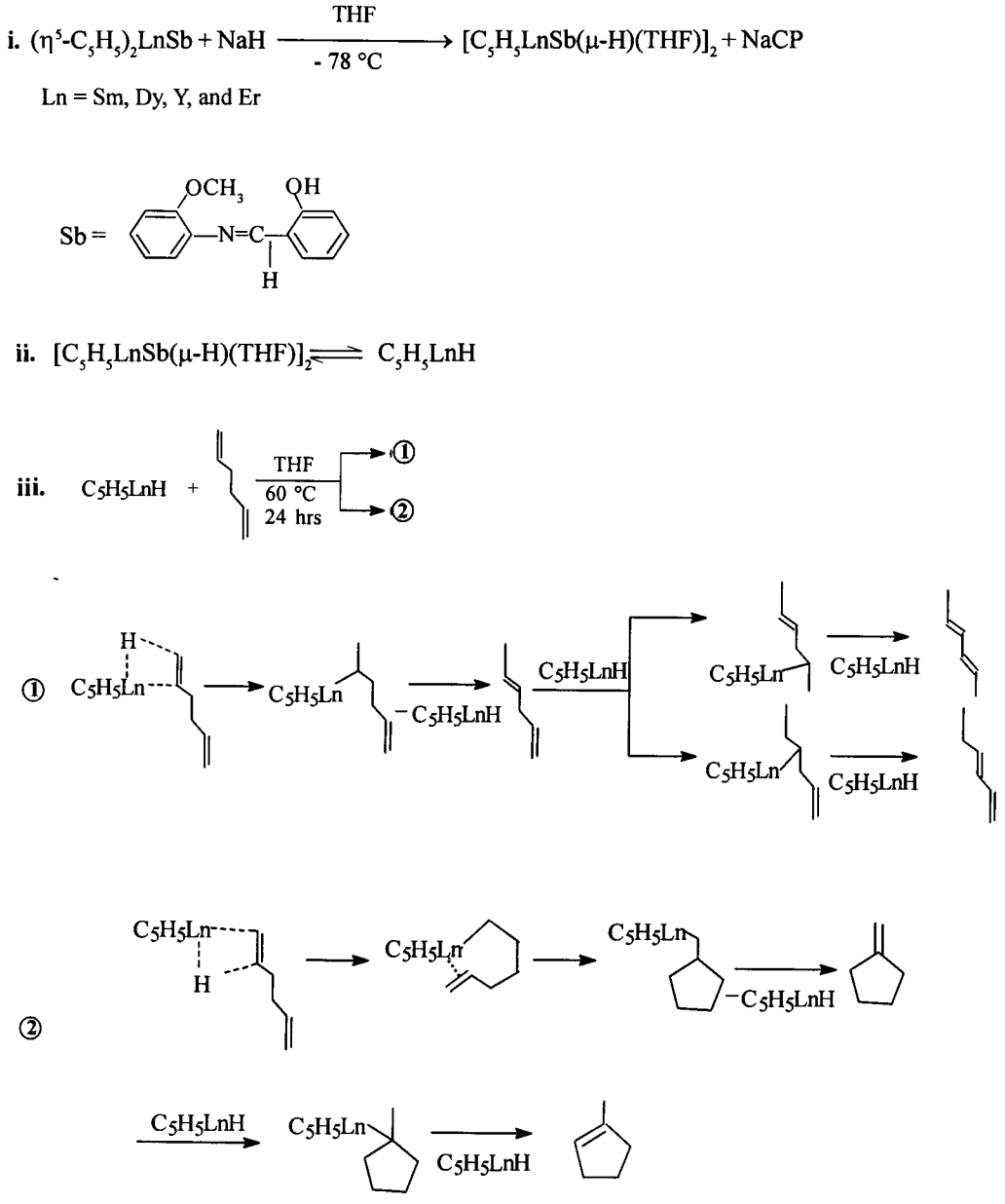
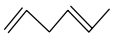
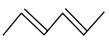


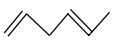
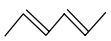
Fig. 4. Proposed mechanism of the isomerization of 1,5-hexadiene.

Table 4. Effect of time on the isomerization of 1,5-hexadiene*

Temperature (°C)	Conversion (%)	Selectivity (%)				Linear/cyclic
			 cc,ct,tt			
1	5.9	8.2	18.4	53.7	19.7	26.6/73.4
5	9.3	5.5	33.2	41.9	19.4	38.7/61.3
10	25.6	81.7	3.2	11.2	9.0	84.8/15.2
20	27.4	82.4	2.3	11.1	4.2	84.7/15.3
30	28.7	80.6	2.4	12.4	4.6	83.0/17.0

*reaction conditions: catalyst/hexadiene = 1:20; catalyst/NaH = 1:50; temperature = 60 °C ; solution = THF

Table 5. Effect of solvent on the isomerization of 1,5- hexadiene*

Solvent	Conversion (%)	Selectivity (%)				Linear/cyclic
			 cc,ct,tt			
THF	7.9	28.8	5.2	49.0	17	34/ 66
Et_2O	3.5	100	trace	trace	trace	
Toluene	0.5	100	nil	nil	nil	

*reaction conditions: catalyst/hexadiene = 1: 20; catalyst/NaH = 1:50; time = 24 h; temperature = 60 °C

References

Akita, M., Yasuda, H., Nagasun, K., Nakamura, A. 1983. Bis-(cyclopentadienyl) titanium chloride complexes for the isomerization of 1,5-hexadiene. *Bull. Chem. Soc. Japan* **10:** 554-562.

Dugas, H., Penny, C. 1981. *Bioorganic Chemistry*, pp. 435-475, 5th edition, New York Springer Press, New York, USA.

Henrici-Olie, G., Olive, S. 1984. *The Chemistry of the Catalyzed Hydrogenation of Carbon Monooxide*, pp. 152-158, 3rd edition, Berlin Springer Press, Berlin, Germany.

Jones, R.D., Summerville, D.A., Basalo, F. 1979. Schiff base complexes for the reversibly binding of oxygen. *Chem. Rev.* **79:** 133-136.

Lehmkuhi, H., Qian, Y. 1983. Isomerization of 1,5-hexadiene catalysed by CP_2 Ti Cl_2/(i-C_3H_7 Mg Br) systems. In: *Chemistry*, pp. 247-251, Berlin Springer Press, Berlin, Germany.

Liu, Q., Ding, M. 1998. Synthesis and crystalographic study of novel organolanthanocene complex. *J. Organomet. Chem.* **553:** 179-182.

Liu, Q., Ding, M., Lin, Y., Xin, Y. 1998. Effect of catalyst molar ratio on the isomerization of 1,5-hexadiene. *Polyhedron* **17:** 555-557.

Margerrum, J.D., Miller, L.J. 1971. *Photochromism*, pp. 569-585, 3rd edition. Wiley Interscience Press, John Wiley and Sons, USA.

Mueller, W.M., Blackledge, T.P., Libowtz, G.G. 1968. *Metal Hydrides*, New York Academic Press, pp. 546-565, 4th edition, New York, USA.

Qian, Y., Saleque, M.A. Huang, J. 2000. Effect of cyclopenthadienyl titanium complexes for the isomerization of 1,5-hexadiene. *J. Catalysis* **21:** 1-5.

Sawodny, W.J., Reiderer, M. 1977. Complexing ability towards certain toxic metals. *Angew. Chem. Int.* **16:** 59-63.

Teller, R.G., Bau, R. 1981. *Structural Bonding*, pp. 44-60, 1st edition, Berlin Springer Press, Berlin, Germany.

Yousaf, M., Huang, J., Feng, F.Z., Qian, Y. 2000a. Polymerization of MMA catalyzed by different mixed ligand lanthanoene/A1 $(i\text{-}Bu)_3$ systems. *Chinese J. Chem.* **18:** 759-763.

Yousaf, M., Huang, J., Feng, F.Z., Qian, Y., Quan, S.J., Zhida, P. 2000b. Catalytic synthesis of polymethyl methacrylate by mixed ligand Schiff base lanthanocene complexes. *Chinese J. Catalysis* **21:** 293-294.

Yousaf, M., Liu, Q., Huang, J., Qian, Y., Sunchen, A. 2000c. Synthesis and X-ray crystal structure of organolanthanocene aryloxide complexes with tridentate Schiff base ligand. *Inorg. Chem. Commun.* **3:** 105-106.

Yousaf, M., Liu, Q., Huang, J., Qian, Y. 2000d. Synthesis, characterization and catalytic applications of tridentate Schiff base derivatives of bis and mono cyclopentadienyl lanthanocene complexes. *Chinese J. Chem.* **18:** 740-744.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 86-89

Beneficiation Studies on the Low-Grade Chromite of Muslim Bagh, Balochistan, Pakistan

S. Atiq*, Irfan Hafeez, K. Khan and M.A. Malik
PCSIR Laboratories, Quetta, Pakistan

(received October 5, 2002; revised December 16, 2004; accepted January 1, 2005)

Abstract. Low-grade chromite of Muslim Bagh, Balochistan, Pakisktan was beneficiated to produce chromite concentrate by cationic flotation using disodium *n*-octadecyl sulfosuccinamate as collector. Effect of various parameters such as grind size, pulp density, pH, and conditioning time on the overall grade and recovery of chromite was also investigated. Particle liberation studies during this investigation revealed that grinding of the ore upto -80 # liberated over 89% of chromite. However, presence of excessive amounts of fines inhibited the flotation. The ore, initially containing 38% Cr_2O_3, was upgraded to concentrates assaying Cr_2O_3 upto 60% with an overall Cr_2O_3 recovery of 82%.

Keywords: beneficiation, cationic flotation, disodium *n*-octadecyl sulfosuccinamate, chromite ore, Pakistan chromite, low-grade chromite

Introduction

Chromite is the only commercial source of chromium, which is used in metallurgical, refractory and chemical industries. The most extensive deposits of chromite in Pakistan exist around Muslim Bagh in the Zhob Valley (Ahmed, 1975). These deposits may be placed in five different groups according to their locations, namely, Khanozai, Jungtogarh, Saplitogarh, Nasai, and Fort Sandeman (Ahmed, 1969; Bilgrami, 1964). The host rocks are restricted to serpentine and dunite, although chromite grains are also found in harzburgite, contact carbonate rocks, and residual cherts (Bilgrami, 1968). Chromite deposits occur in the form of stringers, bands, pods, nodules, and as disseminations in the host rocks. These deposits are quite variable in size and grade. No concrete data on the quantity of chromite reserves is available. However, total reserves in the Zhob-Muslim Bagh area are estimated to be of the order of millions of tonnes. Chromite of Muslim Bagh is being mined since 1903, and until now over 1.5 million tonnes of the ore has been mined (Kazmi and Abbas, 2001). Selective mining of exportable higher grades of chromite has resulted in such deposits being close to exhaustion. The present scenario requires utilization of the indigenous low-grade chromite for the production of value-added chromium-based chemicals to replace their imports (R & D-PCSIR, 1988).

The gravity concentration of low-grade chromite ore is uneconomical and technologically complex (Fillip and Junaka, 1956), whereas froth flotation technique is considered to be an adequate method for upgrading low-grade chromite ore. Using this technique, minerals can be separated based on their surface properties. The valuable minerals are made aerophilic and the gangue minerals aerophobic by adjusting the pulp properties with the help of suitable flotation reagents (Wills, 1977). The present investigation deals with the beneficiation of low-grade chromite ore from deposits, which at present have little or no commercial value.

*Authors for correspondence

Materials and Methods

Sample preparation. Ore sample (about 10 tonnes) procured from Muslim Bagh, Balochistan, Pakistan was crushed by using a jaw crusher and a roll crusher. The representative samples were then prepared by coning-quartering and riffling the roll product. Five samples were drawn from the representative lot and analysed for their chemical composition using gravimetric and spectrophotometric techniques. The average chemical analysis of the ore is presented in Table 1. The size analysis of the ore, after intermediate crushing, was carried out using an Octagon 200 sieving apparatus, which is given in Fig. 1.

Table 1. Average chemical analysis of the Muslim Bagh, Balochistan, Pakistan low-grade chromite ore

Constituents	Percentage
Cr_2O_3	38.06
Fe_2O_3	13.80
SiO_2	20.02
Al_2O_3	10.50
MgO	14.40
LOI	3.20

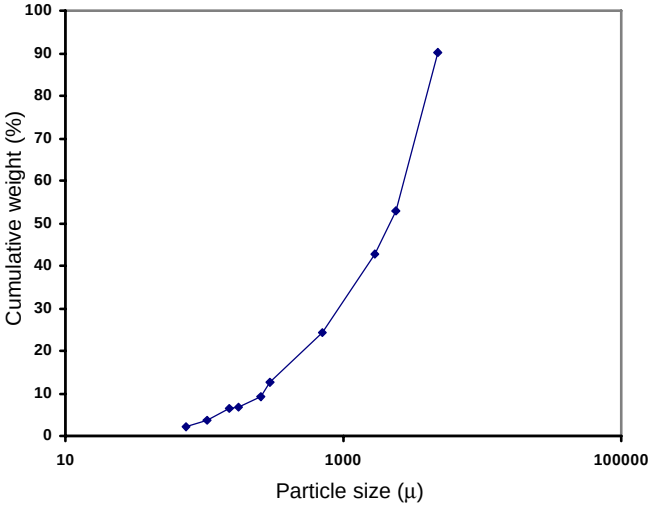


Fig. 1. Cumulative weight (%) as a function of particle size (μ), after intermediate crushing of chromite ore.

Particle liberation studies. The fractions obtained from roll product were analysed for liberation of chromite grains. The optical microscopic examination revealed that the ore required grinding upto -80 # for adequate liberation of the chromite grains. Data showing liberation of chromite at various size fractions is presented in Table 2. The percentage chromite liberation was calculated using the following relationship (Hafeez *et al.*, 1989; Sullian and Workentine, 1964):

$$\text{Chromite liberation (\%)} = \frac{\text{Free chromite (\%) x 100}}{\text{Free chromite + Locked chromite (\%)}}$$

Table 2. Liberation of the chromite ore at different particle size fractions

Particle size (mesh #)	Free chromite grains (%)	Compound grains (%)	Free gangue (%)	Chromite liberation (%)
-8+10	15.38	58.91	25.70	20.68
-10+25	30.4	34.16	35.43	47.00
-25+50	39.2	22.38	38.41	63.65
-50+60	49.0	10.23	40.71	82.72
-60+80	50.1	9.0	40.8	84.71
-80	52.2	6.4	41.3	89.08

Experimental reagents. In the present investigation, disodium octadecyl sulfosuccinamate (American Cyanamide Company) was used as the cationic collector for chromite flotation. The other reagents included hydrofluoric acid (Merck), which acted as an activator. In order to stabilize the froth, small quantity of polypropylene glycol was used. In addition, tannic acid and sulfuric acid were used to act as depressant and pH regulator, respectively.

Beneficiation studies. Flotation tests were carried out in a Denver flotation machine (Model D-12) at a speed of 1000 rpm for the recovery of chromite. The ore for flotation feed was prepared by grinding the roll product in laboratory ball mill for 5 min to obtain a product of 100% passing mesh size -80 #. Due to the brittle nature of the ore, crushing and grinding produced considerable quantity of chromite fines. The sieve analysis of the material so prepared along with Cr_2O_3 contents of different size fractions is given in Table 3.

Table 3. Sieve analysis of the chromite ore ground in ball mill for 5 min

Mesh size #	Weight (%)	Cr_2O_3 (%)
-80+100	7.34	41.3
-100+150	31.00	45.4
-150+200	14.23	43.3
-200+325	17.46	40.0
-325+400	12.97	31.5
-400	17.00	20.7

Results and Discussion

Presence of slime in the flotation feed is known to adversely affect the flotation process. The detrimental effect of slimes was thought to be two-folds: firstly, slimes consumed reagents because of high specific surface, and secondly, the factor of interference of slime particles with the air-mineral contact (Iwasaki, 1983). The ore was, therefore, thoroughly deslimed prior to flotation.

The flotation of chromite, using a cationic collector, can be improved by the addition of adequate amount of a suitable activator (Abido, 1971). It was observed during the present investigations that the chromite ore floated in an acidic environment, after activation by fluoride ions, in the form of HF. The pH required to activate the chromite ore was in the vicinity of 3.0.

An extensive investigation on chromite of Heroshah (NWFP, Pakistan) has been done previously (Hafeez *et al.*, 1999). These authors were able to upgrade the chromite ore containing 31-32% Cr_2O_3 to 43-44 % Cr_2O_3 with 90% recovery using oleic acid as collector. Fatty acids, such as oleic acid, have been successfully used in the anionic flotation by a host of researchers (Hussain and Hafeez, 1989; Hafeez *et al.*, 1988). However, in the present studies, ore was beneficiated by using a cationic collector, namely, disodium *n*-octadecyl sulfo-succinamate. This collector is an amine derivative and relatively more expensive. However, the fact that disodium *n*-octadecyl sulfosuccinamate was required in much lower quantity yiel-

ding better grades with higher recoveries, made the whole process very competitive.

The particle size was optimized at -80 # after carrying out a series of tests. Size, coarser than -80 #, had adverse effects on the overall recovery and grade, whereas feed size finer than -80 # generated excessive quantities of slime which inhibited the flotation process. Pulp density is another vital parameter in any flotation process. Present investigations revealed that the ore under investigation can be effectively floated at the pulp density of 35%, with the conditioning time ranging from 12-15 min.

Tannic acid (300 g/tonne) was used to effectively depress the gangue minerals in the ore. Any increase in the quantity of depressant resulted in lower yield and grade of the concentrate. The optimum quantity of the collector (disodium-*n*-octadecyl sulfosuccinamate) was determined to be 400 g/tonne, along with 300 g/tonne HF, which acted as an activator.

The flotation parameters, as optimized during present research, are summarized in Table 4 and the beneficiation process flowsheet is presented in Fig. 2.

The flotation process yielded concentrate assaying 60.1% Cr_2O_3, corresponding to chromite recovery of 82.65%. The metallurgical balance of the flotation process is presented in Table 5.

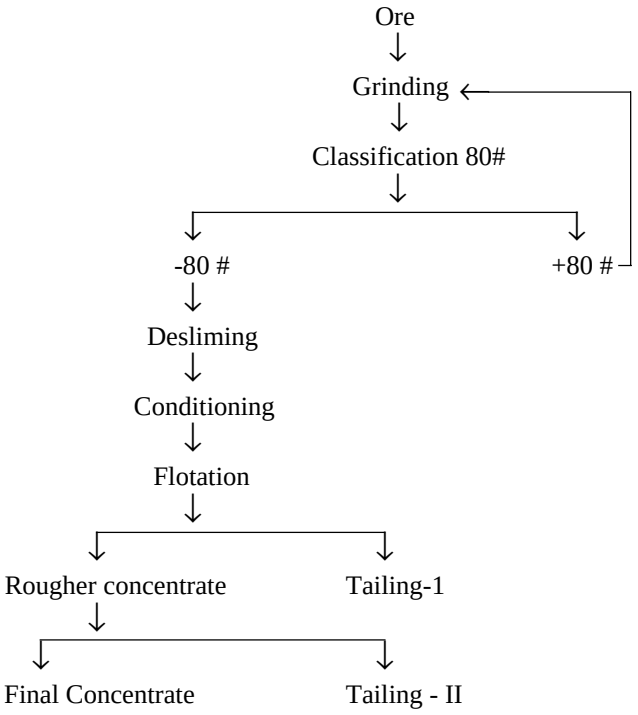


Fig. 2. Flow-sheet for the processing of Muslim Bagh, Balochistan, Pakistan low-grade chromite ore.

Table 4. Optimum parameters for flotation of chromite ore

Parameters	Conditions	
	Rougher	Cleaner
Grind size	-80 + 200 #	-80 + 200 #
Pulp density	35% solids	20% solids
pH	~3	~3
Activator	200 g/tonne HF	100 g/tonne HF
Conditioning time	15 min	10 min
Fuel oil	120 g/tonne	50 g/tonne
Depressant	200 g/tonne tannic acid	100 g /tonne tannic acid
Frother	150 g/tonne polypropylene glycol	50 g/tonne polypropylene glycol
Collector	300 g/tonne disodium *n*-octadecyl sulfosuccinamate	100 g/tonne disodium *n*-octadecyl sulfosuccinamate

Table 5. Metallurgical balance of feed size -80 # of chromite ore

Product	Weight (%)	Grade (%)	Recovery (%)
Cleaner concentrate	52.35	60.0	82.65
Cleaner tail	4.3	17.8	2.01
Rougher tail	17.64	6.06	2.81
Slimes	25.7	18.5	12.51
Calculated heads	99.99	38.0	99.98

Conclusion

The results of these investigations indicated that it is beneficial to upgrade the Muslim Bagh low-grade chromite ore by the flotation technique to produce chromite concentrate assaying 60.1% Cr_2O_3 with a recovery of 82.65% using disodium *n*-octadecyl sulfosuccinamate as the collector. However, upgradation through floatation technique required desliming of the feed without which no significant grade or recovery was obtained. Moreover, when the feed containd finer particles, the loss of material to the slimes increased, which ultimately decreased the recovery of the concentrate. It is concluded that the concentrate so produced can be gainfully utilized in the metallurgical, chemical and refractory making industries.

References

Abido, A.M. 1971. Fluoride activation in the flotation of chromite. *J. Appl. Chem. Biotechnol*. **21:** 328-334.

Ahmed, Z. 1975. *Geology of Mineral Deposits of Balochistan*, vol. **36**, p. 25, Records of the Geological Survey of

Pakistan, Geological Survey of Pakis-tan, Quetta, Pakistan.

Ahmed, Z. 1969. *Directory of Mineral Deposits of Pakistan*, vol. **15**, p. 11, Records of the Geological Survey of Pakistan, Geological Survey of Pakistan, Quetta, Pakistan.

Bilgrami, S.A. 1968. Geology and mineralogy of the Zhob Valley chromite deposits, Pakistan. *Amer. Mineral* **54:** 134-148.

Bilgrami, S.A. 1964. The regional distribution of chemically different chromites from Zhob Valley. *Geol. Bull. Punjab* **4:** 1-16.

Fillip, S., Junaka, N. 1956. A Method of Concentrating Chromite Containing Ore, U.S. Patent No.3, 473, 656.

Hafeez, A., Ahmed, S., Hussain, K. 1989. Gravity concentration of Muslim Bagh (Balochistan) chromite. *Pak. J. Sci. Ind. Res*. **32:** 490-494.

Hafeez, A., Ahmed, S., Hussain, K. 1988. Beneficiation of Landi Raud (Malakand, NWFP) chromite ore. *Pak. J. Sci. Ind. Res*. **31:** 593-598.

Hafeez, A., Anwar, M.S., Ahmad, S., Ahmad, I., Ahmad, J. 1999. Flotation study of a chromite ore. *J. Pak. Inst. Chem. Engrs.* **28:** 47-53.

Hussain, K., Hafeez, A. 1989. Recovery of chromite fines by froth flotation. *Pak. J. Sci. Ind. Res*. **32:** 495-499.

Iwasaki, I. 1983. Iron ore flotation, theory and practice. *Mining Engineering* **35:** 622-631.

Kazmi, A.H., Abbas, G.S. 2001. *Metallogeny and Mineral Deposits of Pakistan*, pp. 112-119, Orient Petroleum Incorporation, Islamabad, Pakistan.

R&D-PCSIR. 1988. *Research and Development Work by PCSIR on Indigenous Ores: Chromite of Muslim Bagh (Balochistan) and Malakand (NWFP)*, vol. **III**, Pakistan Council of Scientific and Industrial Research Laboratories, Lahore, Pakistan.

Sullian, G.V., Workentine, G.F. 1964. Beneficiating low-grade chromite from the still water complex. *Montana, U.S. Bureau of Mines*, R.I. 6448.

Wills, B.A. 1977. *Mineral Processing Technology*, p. 486, 6th edition, Butterworth and Heineman, Oxford, UK.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 90-95

Studies on the Adsorption of Copper (II) by Activated Charcoal and its Application in the Treatment of Textile Industry Effluents

Hajira Tahir[a], M. Nasiruddin Khan*[a] and Anila Sarwar[b]
[a]Department of Chemistry, University of Karachi, Karachi-75270, Pakistan
[b]Fuel Research Centre, Pakistan Council of Scientific & Industrial Research, Karachi, Pakistan

(received February 2, 2003; revised March 15, 2005; accepted March 17, 2005)

Abstract. The adsorption of divalent copper ions on activated charcoal was studied as a function of pH, amount of charcoal and the concentration of copper ions to optimize the operational conditions for the removal of copper ions using activated charcoal. The cross-interferences with other ions or matrix components of the textile industries effluents were also investigated. The applicability of Freundlich and Dubinin-Radushkevich equations for the said system was tested. Thermodynamic parameters, such as free energy change (ΔG), enthalpy change (ΔH), and entropy change (ΔS) during the adsorption were computed. The treatment of textile industries wastes, containing higher concentrations of copper ions, has been evaluated using activated charcoal based on adsorption technique. The overall performance of the treatment system indicated that activated charcoal can be utilized as a potential decontaminate for the removal of copper ions from textile effluents before discharge into the hydrosphere.

Keywords: adsorption isotherms, activated charcoal, textile industries waste, copper ions removal, industrial effluents, wastewater treatment

Introduction

Copper is an essential mineral, which is a component of several important enzymes in the body and is thus essential for good health. It is found in all the body tissues. Copper deficiency leads to a variety of abnormalities, including anaemia, skeletal defects, degeneration of the nervous system, reproductive failure, pronounced cardiovascular lesions, and elevated blood cholesterol. However, high copper intake can be toxic and may cause headaches, hypoglycemia, increased heart rate, nausea, damage to the kidney and liver, and hair loss in women. It may also cause psychological problems, such as brain dysfunction (Nolan, 1983), and depression and schizophrenia (Pfeiffer and Iliev, 1972).

Trace metals are natural elements in the aquatic ecosystems. However, deposits of anthropogenic origin have caused a progressive increase in their concentration, thus creating environmental problems in coastal zones, lakes and rivers. In most cases, the source is traceable to untreated industrial and sewage deposits. The concentration of these elements above tolerable levels is a disturbance factor for the survival of species and stability of the ecosystem. Hussain *et al.* (2000) have reported high level of trace metal pollution in sediments and liquid wastes from textile industries of Pakistan. It is, therefore, particularly necessary to treat textile effluents prior to their discharge into the receiving waterbodies (Manju *et al.,* 1998; Choi and Cho, 1996; Sapari, 1996). The most common methods used for the removal of trace metals from industrial wastewaters include, chemical precipitation, sedimentation, flotation, coagulation, and ion exchange. Adsorption, however, has an edge over other methods, mainly due to its simplicity and the sludge-free clean operation. Several adsorbents have been used for the treatment of effluents rich in Cu (II) at the solid-solution interface (Sameer and Banat, 2002; Samra, 2002; Ajmal *et al.,* 1998; Giles *et al.,* 1960). During recent years, carbon as an adsorbent has become the more accepted medium for the physicochemical treatment of wastewaters (Malik and Stelko, 2002; Sameer and Duvnjak, 1999). Activated charcoal has found many applications in several industries because of its large surface area, high adsorption capacity, microporous structure, selective adsorption, radiation stability, and high purity in removing the toxic and health hazardous particles and ions from gases and solutions. Activated charcoal is evaluated in the present study for the removal of copper from aqueous solutions under optimized conditions. The adsorbent was successfully applied for the removal of Cu (II) from the textile industry wastewaters.

*Author for correspondence; E-mail: khann210@yahoo.com

Materials and Methods

Instrumentation. All analyses for Cu determination were done on atomic absorption spectrometer (Perkin-Elmer 3100, flame atomic absorption spectrometer), equipped with a hollow cathode lamp. An Orion 710 pH meter attached with a combined electrode was used for adjusting the pH of the solutions. Shaker-incubator (1000 - Heidolph) was used for sha-

king, and Eppendorf 5417 microcentrifuge was used to centrifuge the solution.

Activated charcoal and reagents. Activated charcoal (Merck) with an average particle size 0.02-0.08 mm and the diameter of micropores in the range of 30-90 Å was used as the adsorbent. The stock solution of Cu (II) was prepared (0.01 mol/l) by dissolving $CuCl_2.2H_2O$ (BDH, Analar) in distilled water. All other reagents used were of AR grade.

General procedure. Copper sample solutions were transferred to 250 ml Erlenmeyer flasks, adjusted to pH 2.0 by using appropriate buffer system. 0.05 g of activated charcoal was added and the mixture was agitated in shaker incubator for 60 min and subsequently centrifuged at 4000 rpm for 10 min. The concentrations of metal ions remaining in the solution were assayed by standard atomic absorption spectrometry procedures and corrected for the adsorption loss of copper ions on the walls of flasks by running a blank experiment (without adding activated charcoal). The distribution coefficients (K_D in ml/g) were computed as follows:

$$K_D = \frac{C_1}{C_2} \times \frac{\text{volume of solution (ml)}}{\text{mass of solute (g)}} \quad (1)$$

where:

C_1 = the amount of adsorbed copper ions per gram of the activated charcoal
C_2 = the concentration of copper ions per ml of the aqueous solution at equilibrium

The percentage removal was calculated using the following expression:

$$\text{Cu removal}\,(\%) = \frac{100\,K_D}{K_D + \frac{V}{m}} \quad (2)$$

where:

K_D = distribution coefficient
V = volume of solution
m = amount of activated charcoal used

Results and Discussion

Effect of the amount of activated charcoal. The dependence of copper ion adsorption on the amount of activated charcoal was investigated by varying the amount of activated charcoal from 0.01 to 0.50 g. The effect of these variations is presented in Fig. 1. The observations obtained indicate that the values of distribution coefficient (K_D) and percentage removal increased with the increase in the quantity of activated charcoal and attained maximum values at 0.05 g, which then started decreasing with further increase in the amount of activated charcoal. This behaviour reflects the decrease in the effective surface area resulting in the conglomeration of the adsorbent particles, especially at the higher quantities. Therefore, for all further studies 0.05 g of activated charcoal was used as the optimum quantity.

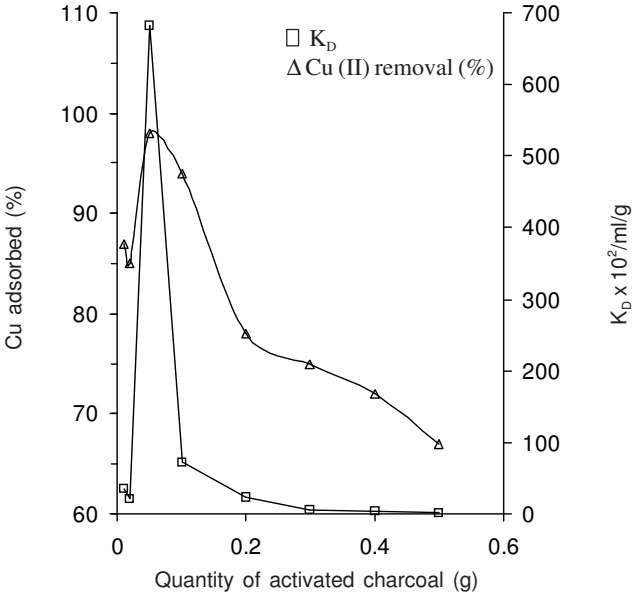


Fig. 1. Effect of the quantity of activated charcoal on the adsorption of Cu (II).

Effect of pH. The influence of pH on copper ion adsorption by activated charcoal was studied by keeping the other parameters constant. The dependence of copper (II) adsorption by activated charcoal, as a function of pH varied from 1 to 6, was evident from the variation of distribution coefficients (K_D) and percentage adsorption values, which were the maximum at pH 2.0 (Fig. 2). Therefore, for all subsequent studies pH 2.0 was used.

Effect of concentration of copper (II) ions. The effect of copper ion concentration on its adsorption by activated charcoal was studied in the metal concentration range of 5 to 50 mg/l. The variation in the distribution coefficients (K_D) and percentage adsorption at different metal concentrations at temperatures 303 through 318 K are shown in Fig. 3(a) and 3(b). The comparison of distribution coefficients (K_D) and percentage adsorption values for the activated charcoal range from 3.52 to 19.74 x 10^2 ml/g and from 58.2 to 90.8%, respectively, at concentrations ranging from 5 to 50 mg/l, indicate the quantitative recovery of copper ions at relatively higher temperatures.

Adsorption isotherms. The adsorption isotherms measured at temperatures ranging from 303 to 318 K are shown in Fig. 4.

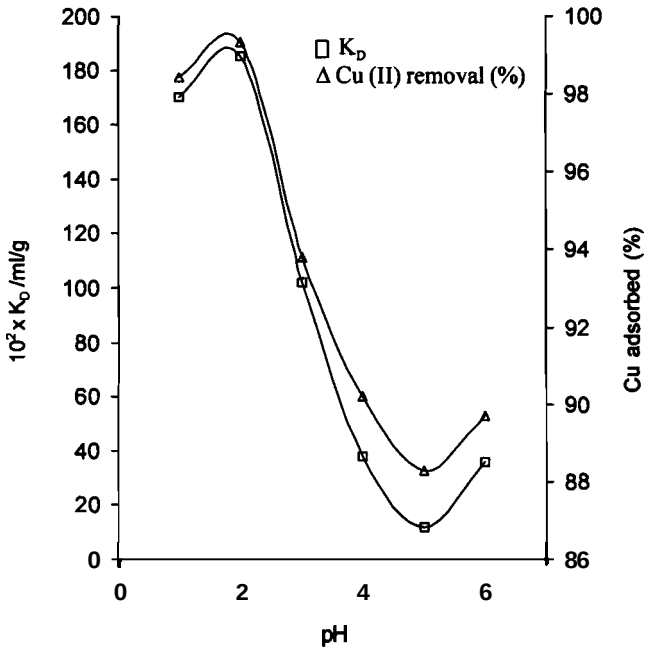


Fig. 2. Effect **of pH** on the adsorption of Cu **(II)** by activated charcoal.

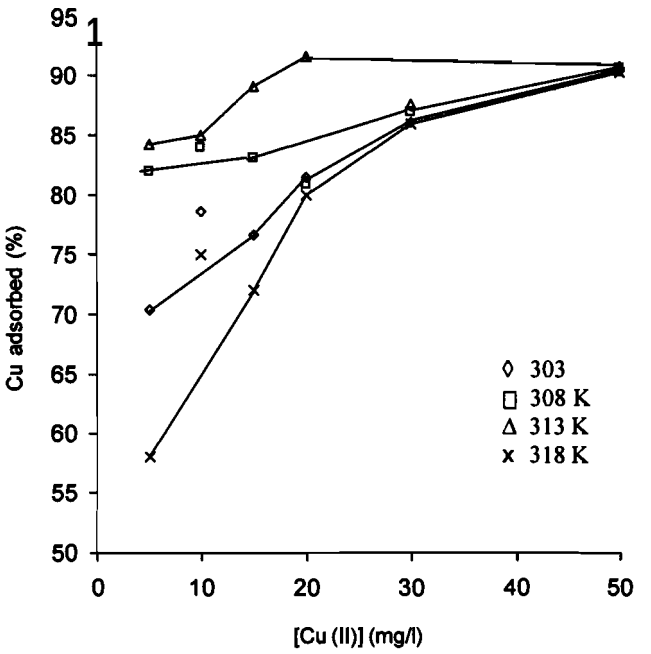


Fig. 3(b). Effect of [Cu (11)] on its percentage adsorption by activated charcoal at different temperatures.

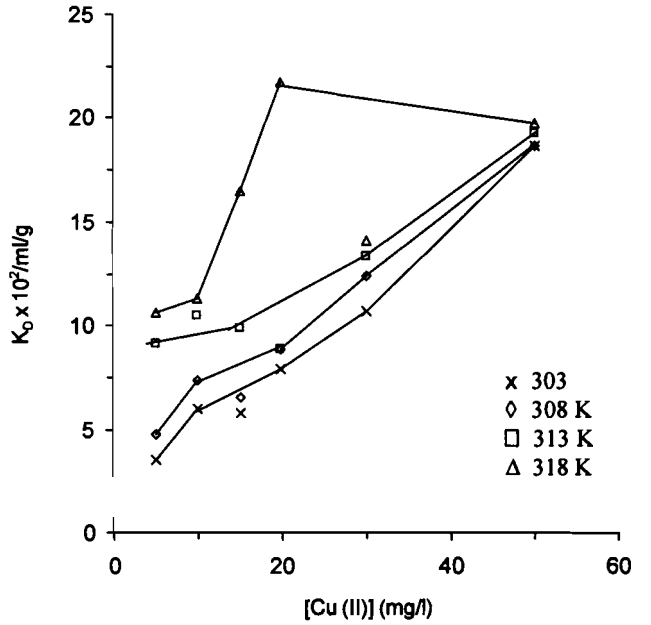


Fig. **3(a).** Effect of [Cu (IT)] on the K_D values of adsorption by activated charcoal at different temperatures.

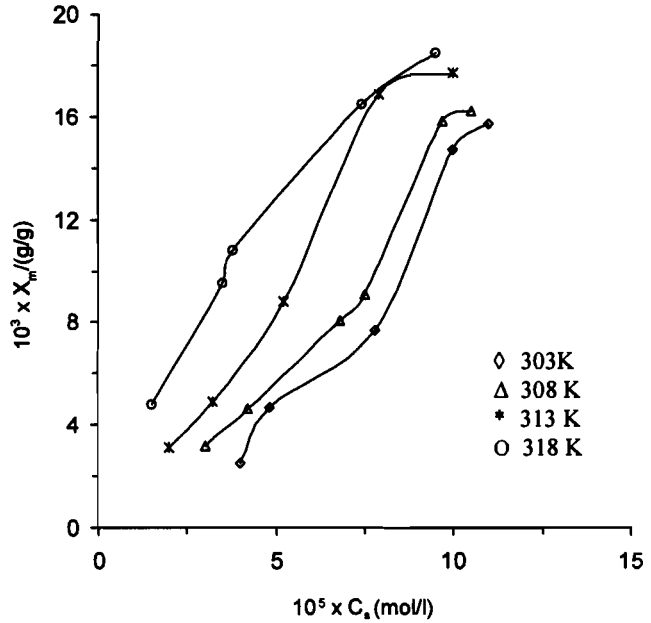


Fig. 4. Adsorption **isotherms** of charcoal for the adsorption of copper ions at different temperatures.

The data have been plotted in terms of the amount of Cu ions adsorbed (x) per gram activated charcoal (g) versus the equilibrium concentration (C,) expressed in **mol/l.** The **x/m** expres-sed in **g/g** was calculated from the formula:

$$x/m = (C_o - C_e)\,V/m \qquad (3)$$

where:

x/m = amount of Cu **(II)** adsorbed per g activated charcoal

C_o = initial concentration of copper ions in the system

C_e = equilibrium concentration

V = volume of solution

m = amount of activated charcoal used

All the isotherms were of L_3-type according to the classification of Giles *et al.* (1960). The initial portion of isotherms provides information about the availability of the active sites for an adsorbate, while the plateau signifies the monolayer formation. The increase in adsorption with temperature indicates that adsorption of copper ions is an endothermic process. The data were also put to the Freundlich, Langmuir and Dubinin-Radushkevich (D-R) isotherms equations. The Freundlich isotherms have been expressed in the form of:

$$x/m = A\,C_s^{1/n} \quad (4)$$

where:

A and 1/n = empirical constants
x/m and C_s have their usual meanings as detailed for equa-tion (3)

Fig. 5 depicts the Freundlich plots obtained at the various temperatures studied for the present system. Values of A and n computed from the respective slopes and intercepts of these plots are listed in Table 1. The values of n relate to the nature and strength of the adsorptive forces involved. The higher values of 1/n signify that strong adsorption forces were operating on activated charcoal (Ajmal *et al.,* 1998). However, the values of log A decreased with increasing temperature, which shows that the adsorption was more favourable at higher temperatures.

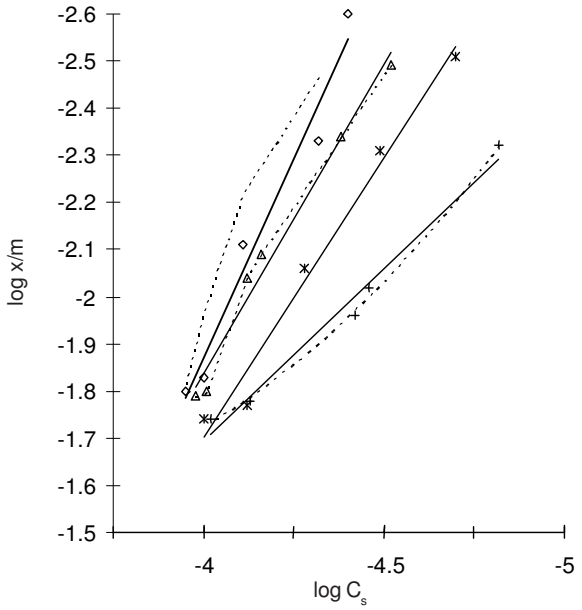


Fig. 5. Freundlich plots for the adsorption of Cu (II) by activated charcoal at different temperatures.

The Langmuir adsorption isotherms is the best known model and most frequently utilized to determine the adsorption parameters. The Langmuir expression can be represented as:

Table 1. Freundlich and Dubinin-Radushkevich (D-R) isotherms parameters of adsorption of Cu (II) ions by activated charcoal

Temperature (K)	Freundlich parameters		D-R parameters		
	n	A	$x_m \times 10^4$ /mol/g	-K x 10^3 /mol^2 kJ^2	Es/kJ/mol
303	0.584	145.5	17.1	7.87	7.97
308	0.770	28.5	7.90	6.50	8.77
313	0.852	19.91	1.48	3.57	11.83
318	1.365	10.09	1.36	2.64	13.76

K= absolute temperature on the Kelvin scale; 0 °C= 273K, 100 °C= 373K

$$\frac{1}{X} = \frac{1}{x_m K}\,\frac{1}{C_s} + \frac{1}{x_m} \quad (5)$$

where:

$X = \frac{x}{m}$ having the same meaning as described for equation (3)
K = Langmuir adsorption constant
x_m = the limited amount of the adsorbate that can be taken up per mass of the adsorbent
C_s = the same meanings as in the Freundlich isotherms as described for equation (3)

Thus, a plot of 1/X against $1/C_s$ is linear with a gradient of $1/x_m$, K and intercept of $1/x_m$ (Fig. 6).The dotted and solid lines represent linear and non-linear regression fits for both the isotherms. Their correlation coefficient values are summarized in Table 2, which indicates that the data fit the Langmuir isotherms better than the Freundlich isotherms, both in the case of linear and non-linear regressions.

The Freundlich type adsorption isotherms is an indication of surface heterogeneity of the adsorbent, whereas Langmuir type isotherms hint towards surface homogeneity of the adsorbent. This leads to the conclusion that the surface of activated charcoal is made up of small homogeneous adsorption patches, which are very much similar to each other in respect of adsorption phenomenon. The good correlation coefficient values for Langmuir isotherms also explain a strictly localized monolayer adsorption phenomenon.

The data were put to the Dubinin-Radushkevich (D-R) equation in the linearized form as:

$$\ln \frac{x}{m} = \ln x_m - K'\varepsilon^2 \quad (6)$$

where:

$$\varepsilon = RT \ln (1 + 1/C_s) \quad (7)$$

x_m = monolayer capacity of adsorbent
K' = constant related to adsorption energy

ε = adsorption potential
R = gas constant
T = absolute temperature
x/m and C, = usual meanings as described for equation (3)

The D-R plots of ln (x/m) versus ε^2 obtained at various temperatures are shown in Fig. 7. Values of x_m and K′ were calculated fiom the intercept and slope of these plots, and the values of mean free energy of sorption (E,) were calculated fiom K' according to the following eqation:

$$E_s = (-2K')^{-1/2} \quad (8)$$

All these values are listed in Table 1, which show that the value of E_s increases with temperature, indicating an increase in copper ion sorption with increasing temperature.

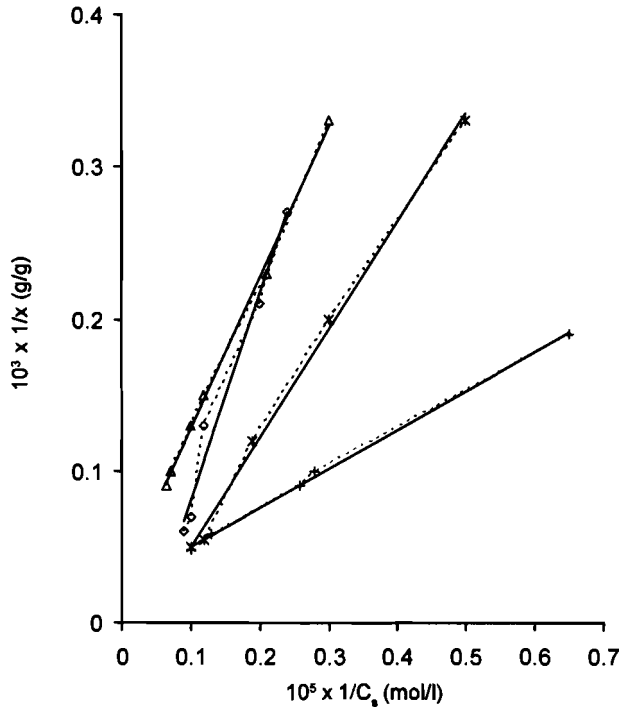


Fig. 6. Langmuir isotherms plots for the adsorption of Cu (II) ions by activated charcoal at different temperatures.

Table 2. Correlation coefficients for linear and non-linear regression fits of different adsorption isotherms

Temperature (K)	Correlation coefficients			
	Linear regression		Non-linear regression	
	Freundlich isotherms	Langmuir isotherms	Freundlich isotherms	Langmuir isotherms
303	0.9715	0.9893	0.9868	0.9953
308	0.9777	0.9974	0.9953	0.9988
313	0.9827	0.9964	0.9827	0.9969
318	0.9882	0.9981	0.9974	0.9983

The thermodynamic quantities, such as free energy change (AG), enthalpy change (AH), and entropy change (AS) for copper adsorption were calculated using the following equations:

$$\Delta G = -RT \ln K_D \quad (9)$$

$$\Delta G = \Delta H - T\Delta S \quad (10)$$

The values of thermodynamic parameters are given in Table 3. The positive values of AH and AS, and the decrease in AG values with increasing temperature, show that the adsorption of copper ions on activated charcoal was favoured at higher temperatures. This may be due to desorption of the copper ions at higher temperatures leading to an endothermic adsorption process, which may be chemical in nature.

Study of interferences. A study of the interferences of Co, Cd, Zn, Ni, Cr (VI), Mn, Ag, Mg and Pb on the Cu ion adsorption by activated charcoal was also done. Solutions of 50 mg/l Cu (II) were treated with 0.05 g of the adsorbent in the presence of increasing concentrations of interfering ions fiom

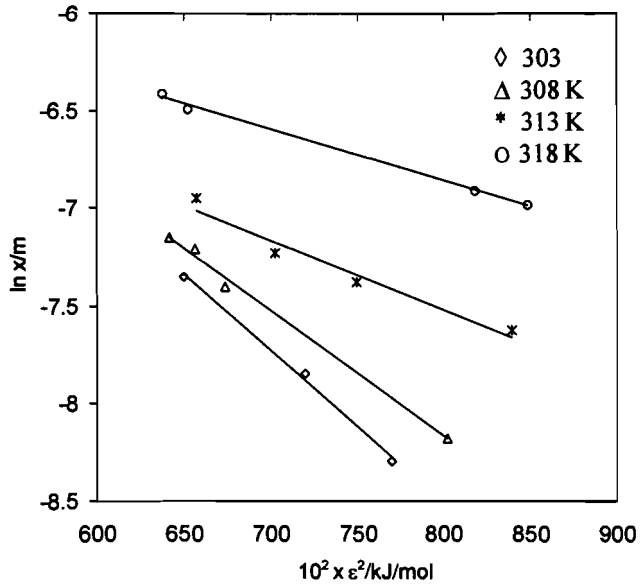


Fig. 7. D-R isotherms for the adsorption of copper ions by activated charcoal at different temperatures.

Table 3. Thermodynamic parameters of activated charcoal for the adsorption of Cu (II) ions at different temperatures

Concentration/mg/l	ΔH/kJ/mol	ΔS/kJ/K/mol	ΔG/kJ/mol			
			303 K	308 K	313 K	318 K
5.000	52.49	0.184	-14.77	-15.77	-17.45	-18.43
15.00	45.14	0.163	-16.02	-16.60	-17.94	-19.58
20.00	37.17	0.139	-16.81	-16.99	-17.66	-20.31
30.00	12.00	0.060	-17.58	-18.24	-18.73	-19.17
50.00	2.47	0.032	-18.97	-19.29	-19.68	-20.06

0 to 200 mg/l and the results obtained were evaluated in terms of Cu removal from the solution. No significant influence of these ions was noted under optimized conditions indicating selective Cu (II) adsorption.

Adsorption of copper ions from textile waste. A 25 ml textile effluent sample TXS_3 containing the maximum copper concentration of 339 mg/kg was diluted seven times and treated against 0.05 g activated charcoal at 318 K under optimized conditions. A removal percentage of total copper in the order of 80±5% was obtained. This comparativly low percentage removal, 90.8% as mentioned in Fig. 3(b), was probably due to the influence of the textile waste matrix components on the adsorption of copper by activated charcoal.

Conclusion

Activated charcoal was noted to be a good adsorbent for the adsorption of Cu (II) from aqueous solutions, the process being influenced by pH, temperature, mass of adsorbent and copper concentration. The equilibrium data fit well in the Langmuir, Freundlich and D-R models of adsorption isotherms. pH 2.0 and 0.05 g of activated charcoal were most appropriate for the quantitative removal of copper (II) in the presence of other ions or other matrices of textile industry effluents.

References

Ajmal, M., Khan, A.H., Ahmed, S., Ahmed, A. 1998. Role of sawdust in the removal of copper (II) from industrial wastes. *Water Res.* **32:** 3085-3091.

Choi, Y.S., Cho, J.H. 1996. Color removal from dye waste water using vermiculite. *Environ. Technol.* **17:** 1169-1180.

Giles, C.H., MacEwan, T.H., Nakhwa, S.N., Smith, D. 1960. Adsorption. Part-X. A system of classification of solution adsorption isotherm and its use in diagnosis of adsorption mechanism and in measurement of specific surface areas of solids. *J. Chem. Soc.* 3973-3993.

Hussain, S.T., Hajira, T., Saleem, M., Afzal, M. 2000. Trace metal pollution assessment of sediments and liquid waters from textile industries of Pakistan. *J. Trace Microprobe Techniques* **18:** 99-106.

Malik, D.J., Strelko, V., Streat, M. 2002. Characterization of novel modified active carbons and marine algal biomass for the selective adsorption of lead. *Water Res.* **36:** 1527-1538.

Manju, G.N., Raji, C., Anirudhan, T.S. 1998. Evaluation of coconut husk carbon for the removal of arsenic from water. *Water Res.* **32:** 3062-3070.

Nolan, K.R. 1983. Copper toxicity syndrome. *J. Orthomolecular Psychiatry* **12:** 270-282.

Pfeiffer, C., Iliev, V. 1972. A study of zinc deficiency and copper excess in the schizophrenias. *Intern. Rev. Neurobiol.* Supp. **1:** 141-185.

Sameer, A.A., Banat, F. 2002. Adsorption of copper ions to tyre rubber. *Adsorption Sci. Technol.* **18:** 685-700.

Sameer, A.A., Duvnjak, Z. 1999. Sorption of heavy metals from synthetic metal solutions and industrial waste water using plant materials. *Water Qual. Res. J. Can.* **34:** 481-503.

Samra, S.E. 2002. Removal of Ni^{2+} and Cu^{2+} ions from aqueous solution of lignite based carbons. *Adsorption Sci. Technol.* **18:** 761-775.

Sapari, N. 1996. Treatment of textile wastewater by overland flow. *Desalination* **106:** 179-182.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 96-102

Biomagnification of Some Heavy and Essential Metals in Sediments, Fishes and Crayfish from Ondo State Coastal Region, Nigeria

S. S. Asaolu* and O. Olaofe
Department of Chemistry, University of Ado-Ekiti, Ado-Ekiti, Ekiti State, Nigeria

(received September 29, 2003; revised March 19, 2005; accepted March 21, 2005)

Abstract. The biomagnification levels of some essential (Fe, Zn, Cu) and toxic metals (Pb, Ni, Cd, Cr, Co, Mn) were determined in sediments, three kinds of fish (*Oreochromis niloticus*, *Synodonthis* sp., and *Clarias gariepinus*), and crayfish from the Ondo State coastal region. The metal biomagnification in the fish and crayfish was several times greater than in water, while that in the sediments was several thousand-folds greater than in both the organisms and water. Among the metals examined in water, Fe was the most abundant with average values of 146.7 and 74.3 mg/l, respectively, for wet and dry seasons, while Co was the least with average values of 2.4 and 1.6 mg/l. In the sediments, concentrations of Pb, Ni, Fe, Cr, Co and Mn in the wet season were relatively higher than those obtained for the dry season. Fe with an average of 50.9 mg/kg in *C. gariepinus* was the most abundant metal in the fish samples, while Cu with an average value of 0.3 mg/kg in *O. niloticus* was the least. The metal biomagnification for most of the metals for both seasons was found to vary widely from one location to the other. This was confirmed by the coefficient of variation that ranged from 31% to 144% and 29% to 130% in the wet and dry seasons, respectively. The present study has shown that fish, crayfish and sediments can be used to monitor the pollution level of metals in the Nigerian coastal water.

Keywords: metal biomagnification, heavy metals, sediments, crayfish, metal pollution, metal accumulation in fish

Introduction

The occurrence of metals in excess of natural loads is a problem of serious concern (Adeyeye, 2000). This is attributed to the rapid population increase, industrial development, urbanization, and agricultural practices (Calamari and Naeve, 1994). Some of these metals are extremely dangerous to human health, such as Cd accumulation is associated with hypertension, osteomalacia and itai-itai disease (Ipinmoroti *et al.,* 1997; Oloyede *et al.,* 1990). Lead poisoning has been associated with permanent brain damage, behavioural disorders and impaired hearing (Ipinmoroti *et al.,* 1997; Mirian *et al.,* 1994). Toxic and essential metals enter the aquatic environment through natural and artificial processes that involve weathering of rocks and soil, dissolution of aerosol particles in the atmosphere, oil spillage, sewage effluents, auto-emissions, dredging activities, and industrial effluents (Asaolu *et al.,* 1997; Ipinmoroti and Oshodi, 1993). With increased diversification in industrialization and extensive use of metal based fertilizers, such as phosphate and ammonia fertilizers in Nigeria, the concentration of metal pollutants in the freshwater reservoirs is expected to rise through natural run-offs (Finerty *et al.,* 1990). High percentage of acid leachable metals, such as Fe, Mn, Zn, Pb, Cu and Ni, have been reported for some lakes in and around Ibadan, Nigeria (Ajayi and Mombeshora, 1989). After entering the water, metals may precipitate, get adsorbed on solid surface, remain soluble or suspended in water, or are taken-up by fauna and flora, eventually accumulating in marine organisms that are consumed by human beings (Asaolu *et al.,* 1997; Mohammed *et al.,* 1982; Gutheric *et al.,* 1979). The presence of metal pollutants in fresh and marine waters has been found to disturb the delicate balance of the aquatic ecosystem, including concentration of some metals in the body tissues of fish (Asaolu *et al.,* 1997; Munshi and Singh, 1989; Kakulu and Osibanjo, 1986).

In the natural aquatic ecosystems, metals occur normally in nanogram to microgram levels. However, some of these metals occuring at low concentrations in surface waters are found in high concentrations in the corresponding sediments and fishes in the aquatic environments (Asaolu *et al.,* 1997; Calamari and Naeve, 1994; Kakulu and Osibanjo, 1986). It is, therefore, necessary to understand the biomagnification levels of some of these metals in the sediments and biota in the aquatic ecosystems. This work was designed to examine the biomagnification levels of some metals in the sediments, fishes and crayfish from the coastal regions of Ondo State, Nigeria with a view to creating baseline documentation and environmental awareness.

Materials and Methods

Surface water samples were collected from fourteen different locations between Igbokoda and Jirinwo where the sea incursion takes place (Fig. 1). The water samples were collected in 2.5 litre polythene bottles, previously washed and leached with

*Author for correspondence; E-mail: ssasaolu@yahoo.com

10% NHO_3 for 48 h. The water samples were chemically preserved by the addition of 5 ml conc HNO_3 per litre, kept in refrigerator prior to analysis.

Sediment samples were collected by divers from each location from where the surface water samples were collected. The sediment samples were collected in polythene bags that were previously soaked in 10% HNO_3 for 48 h, followed by rinsing with distilled water and then allowed to drain to dryness.

Three different types of fish (*Oreochromis niloticus, Synodontis* sp., and *Clarias gariepinus*) and crayfish were bought randomly from fishermen fishing along the coastal area of study. The samples were carefully washed with distilled deionised water to remove any adhering contaminants and then dried in filter paper folds. The samples were then wrapped in aluminium foils and kept in the deep freezer at -18 °C prior to analysis.

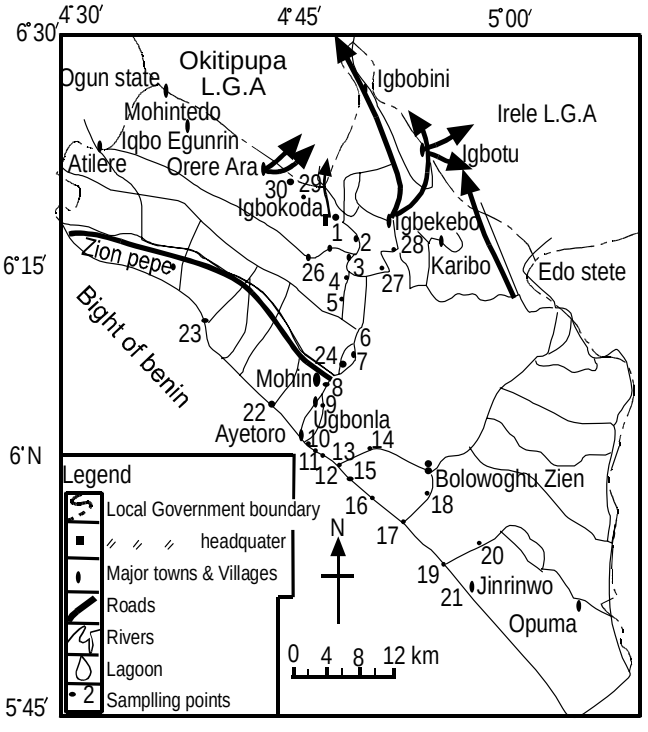


Fig. 1. Map of Ilaje/Ese Odo Local Government of Ondo State showing water and sediment sampling points.

Sample treatment and metal determination. Metals were extracted from the water samples, using a mixture of ammonium pyrolidinedithiocarbamate (APDC) and sodium diethyldithiocarbamate (NaDDC) solution as reported by Lo *et al.* (1982) and Ipinmoroti *et al.* (1997). The method has been reported to extract nearly all the metals from solution at pH 4.0-4.5, with an extraction efficiency of about 95%, 94%, 100%, 88%, 96% and 92% for Cd, Co, Fe, Ni, Pb and Zn, respectively. Metal concentrations in the extract were determined by atomic absorption spectrophotometer (Varian Model Spectral AA-10).

The sediment samples were air dried, ground and sieved through a nylon sieve of 200 mesh size. 0.5 g of the sieved sample was digested in a teflon beaker with a mixture of HNO_3, HCl_4 and HF in the ratio 7:2:8 ml, respectively (Asaolu *et al.*, 1997). The resultant solutions from the digest were filtered, in each case, into a 100 ml volumetric flask and made up to the mark with distilled deionised water. The metals were determined in the resultant solutions by atomic absorption spectrophotometer.

About 4 g of the homogenized samples of the fishes and crayfish were digested with a mixture of conc HNO_3 and 72% HCl_4 in the ratio 100:3 in an air tight nelgene bottle in a temperature controlled waterbath at about 85 °C for 3 h (Asaolu *et al.*, 1997). Resultant solutions from the digest were filtered into 100 ml volumetric flasks and then made up to the mark with 0.5% HNO_3. The metals from the solutions were determined by atomic absorption spectrophotometer.

Results and Discussion

The map of the coastal region showing the sampling locations is given in Fig. 1. Tables 1 and 2 present the mean metal levels (mg/l) of some toxic and essential metals in water samples for both the wet and dry seasons, respectively. Except for Cu, Zn and Cd, metal concentrations in the water during the wet season were relatively higher than the dry season. This may be attributed to the natural run-offs during the raining period from various sources, including the mineralised areas which eventually end up in the aquatic system (Ipinmoroti *et al.*, 1997). Iron was the most abundant metal during the wet and dry seasons with average values of 146.7 and 74.3 mg/l, respectively, while Co was the least with average values of 2.4 and 1.6 mg/l for the wet and dry seasons.

Metal concentrations in water from this area (Tables 1 and 2) have been discussed exhaustively (Asaolu, 1998; Ipinmoroti *et al.*, 1997). Tables 3 and 4 present mean metal levels (mg/kg) in sediments of the coastal region for both the wet and dry seasons. Concentrations of Pb, Ni, Fe, Cr, Co and Mn in the wet season were relatively higher than those obtained in the dry season. This is similar to the trends observed for the surface water in the present study. This is probably due to the high flushing rate during the rains in the wet season (Asaolu *et al.*, 1997).

The higher concentration of Fe, as compared to other metals, in the sediments from the locations of sampling has been

Table 1. Metal levels (mg/l x 10^{-3}) in the Ondo coastal waters during the wet season

Location of sampling/ statistical analysis	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Igbokoda	2.5	9.2	197.5	6.9	7.4	7.5	3.1	2.1	13.4
Kajola	1.3	9.9	135.8	5.6	10.1	3.3	2.1	2.4	12.6
Ibila	2.0	10.35	121.1	5.2	7.8	4.1	2.4	0.7	10.6
Elegboro	4.7	15.5	141.3	6.1	8.9	5.5	14.4	1.0	14.1
Legha	5.0	7.8	145.4	4.4	7.7	2.7	1.4	3.7	10.0
Mahin	4.7	14.4	122.6	5.8	8.0	5.1	14.4	2.3	13.4
Ugbonla	3.4	9.2	161.8	5.8	7.4	3.8	4.5	0.6	12.3
Ayetoro	3.8	11.1	159.3	5.8	6.5	3.6	4.9	5.3	12.3
Alagbo	4.4	20.3	161.3	4.4	6.3	2.4	0.7	1.4	8.4
Asumaga	4.0	19.8	153.0	4.3	5.4	5.3	14.0	3.8	12.8
Ilepete	0.8	12.1	188.2	4.8	6.0	2.8	0.8	4.1	8.6
Obenle	0.4	6.7	69.5	2.3	3.9	1.7	0.4	0.3	8.2
Ojumole	0.9	7.8	141.5	2.5	6.2	2.2	1.5	1.1	11.9
Jirinwo	1.5	18.4	156.4	6.3	8.1	3.9	2.3	3.5	12.6
Mean values	2.8	12.3	146.7	5.0	7.1	3.9	4.8	2.4	11.5
sd	1.7	4.6	30.9	1.3	1.5	1.6	5.3	1.5	2.0
cv	0.59	0.37	0.21	0.27	0.22	0.40	1.10	0.6	0.17

sd: standard deviation; cv: coefficient of variation

Table 2. Metal levels (mg/l x 10^{-3}) in the Ondo coastal waters during the dry season

Location of sampling/ statistical analysis	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Igbokoda	2.2	6.8	23.2	6.4	11.1	4.1	1.2	2.0	9.3
Kajola	1.1	5.5	45.7	7.0	9.7	4.0	1.6	1.1	10.4
Ibila	1.6	6.6	31.9	6.5	10.9	5.2	1.6	0.9	11.5
Elegboro	4.1	5.4	44.4	7.5	11.8	4.9	10.2	5.0	12.7
Legha	1.2	4.4	29.0	5.7	9.5	3.1	0.7	0.5	9.0
Mahin	4.2	8.0	26.6	8.3	11.3	4.4	6.9	1.0	12.1
Ugbonla	2.9	7.4	31.0	7.5	10.4	3.7	6.9	1.0	12.1
Ayetoro	3.3	7.8	51.6	6.7	10.3	3.9	3.5	2.0	12.3
Alagbo	7.0	3.4	38.3	7.5	10.4	2.7	0.4	0.8	11.6
Asumaga	3.5	7.7	52.9	7.8	9.6	6.3	10.0	5.0	11.9
Ilepete	0.6	6.1	29.6	5.4	7.6	2.7	0.2	0.4	8.4
Obenle	0.4	3.1	76.8	3.5	5.3	2.4	0.4	0.6	8.0
Ojumole	0.6	4.4	316.5	7.4	8.5	3.1	0.6	1.1	11.0
Jirinwo	1.4	5.7	242.4	7.0	5.7	3.6	1.5	2.0	12.1
Mean values	2.4	5.9	74.3	6.7	9.7	3.9	3.3	1.6	10.9
sd	1.9	1.6	89.2	1.2	1.7	1.1	3.6	1.6	1.6
cv	0.77	0.27	1.2	0.18	0.17	0.27	1.1	0.97	0.15

sd: standard deviation; cv: coefficient of variation

attributed to the high iron concentrations in the Nigerian soils (Asaolu *et al.,* 1997; Okoye, 1991). Table 5 presents the mean metal concentrations (mg/kg) for the three different types of fishes and crayfish from the coastal region. Among the fish samples, *Synodontis* sp., tended to accumulate more metals, such as Pb, Ni, Cu, Zn, Cr and Co, than *C. gariepinus* and *O. niloticus*. Iron with an average of 50.9 mg/kg in *C. gariepinus* was the most abundant metal in the fish samples, while Cu with an average value of 0.3 mg/kg in *O. niloticus* was the least. Similar observations have been reported earlier (Okoye, 1991). Concentrations of Pb, Fe, Cu and Zn in crayfish were higher than in the fishes. Present study suggests that higher concentrations of such metals in the crayfish were due to the bottom feeding habit, since the concentrations of these metals were higher in the sediments. It is quite possible that the crayfish ingest more metals during feeding on benthos and hence concentrated the metals more than the fishes (Asaolu, 1998).

The high concentration of the metals in fish and crayfish samples, as compared with the water samples, could be an advantage particularly in respect of some of the essential minerals. For example, Fe has been found to play a vital role in the formation of haemoglobin, Co as a component of vitamin B (cyanocobalamin) is essential for the prevention of anaemia, and Cu and Zn have been found to play important role in enzymatic activities (Cater and Fernando, 1979). However, high concentrations of the toxic metals in this respect would be dangerous as some of these metals, such as Cd and Pb have been reported to be extremely toxic even at very low concentrations (Adeyeye, 2000; Ipinmoroti *et al.,* 1997).

Table 3. Metal levels (mg/kg) in sediments collected from the Ondo coastal waters during the wet season

Location of sampling/ statistical analysis	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Igbokoda	7.4	16.8	214.1	7.5	7.0	10.4	16.9	5.7	62.5
Kajola	9.5	18.1	120.7	7.1	8.2	9.6	18.0	6.1	57.9
Ibila	6.8	16.7	198.9	6.7	7.7	8.5	15.3	9.2	49.0
Elegboro	9.5	22.5	233.9	6.3	7.8	9.9	19.3	6.4	55.0
Legha	10.1	25.8	515.0	8.1	9.5	10.0	20.9	6.5	203.5
Mahin	7.0	18.6	216.7	5.5	9.3	8.9	16.1	8.3	61.1
Ugbonla	7.4	20.8	214.3	7.6	8.3	9.2	17.5	9.8	47.8
Ayetoro	9.8	22.6	328.4	8.3	8.4	9.8	19.1	6.6	97.2
Alagbo	8.7	20.8	196.2	7.4	8.3	9.0	18.4	6.0	72.5
Asumaga	9.1	22.6	235.5	8.8	8.5	9.3	18.8	6.5	177.8
Ilepete	4.3	17.5	193.2	4.8	6.3	8.0	11.9	9.6	58.1
Obenle	8.5	20.1	240.0	7.8	8.7	9.3	17.6	13.0	78.5
Ojumole	9.8	26.1	348.9	7.9	9.4	10.2	19.4	12.7	149.3
Jirinwo	5.8	22.2	194.2	5.7	7.0	0.1	15.1	12.1	65.3
Mean values	8.1	20.8	246.4	7.1	8.2	9.3	17.5	8.5	88.3
sd	1.7	3.0	95.6	1.2	0.94	0.75	2.3	2.6	50.8
cv	0.21	0.15	0.39	0.16	0.11	0.08	0.13	0.31	0.58

sd: standard deviation; cv: coefficient of variation

Table 4. Metal levels (mg/kg) in sediments collected from the Ondo coastal waters during the dry season

Location of sampling/ statistical analysis	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Igbokoda	7.0	10.9	134.7	7.7	7.9	8.5	12.9	1.4	56.2
Kajola	8.9	15.5	124.6	10.0	9.3	10.5	15.4	1.6	51.8
Ibila	6.6	8.3	100.5	7.6	7.2	8.3	11.2	1.0	45.0
Elegboro	8.9	12.2	155.2	9.4	10.0	10.1	15.2	1.7	52.0
Legha	9.7	18.9	338.2	10.7	11.7	12.0	17.2	2.3	190.0
Mahin	6.6	9.4	122.2	8.4	7.5	8.5	11.6	1.2	55.6
Ugbonla	6.6	10.0	112.5	7.3	8.9	8.6	12.9	1.3	41.6
Ayetoro	9.0	16.1	256.4	9.4	10.2	10.5	15.5	1.8	91.5
Alagbo	8.0	10.3	199.2	8.5	9.9	9.0	14.3	1.5	72.1
Asumaga	9.3	18.3	172.7	10.0	11.2	9.3	13.8	1.9	170.0
Ilepete	5.6	8.6	85.1	7.0	7.0	8.3	10.7	1.4	52.1
Obenle	8.5	11.7	155.9	9.5	9.3	9.8	16.9	1.5	71.1
Ojumole	9.8	16.9	312.6	10.6	10.6	11.7	18.8	2.0	146.0
Jirinwo	6.5	10.2	124.2	7.9	7.9	8.6	13.1	1.6	58.3
Mean values	7.8	12.0	167.5	8.9	9.2	9.6	14.3	1.6	82.4
sd	1.3	4.8	78.4	1.2	1.5	1.2	2.4	0.34	49.2
cv	0.18	0.40	0.47	0.14	0.17	0.13	0.17	0.31	0.60

sd: standard deviation; cv: coefficient of variation

Table 5. Metal levels (mg/kg) in the three fish species and crayfish

Fish species/ crayfish samples	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Oreochromis niloticus	1.2	4.8	13.8	0.3	1.5	2.2	2.7	1.6	41.3
Synodontis sp.	1.5	5.8	42.6	0.6	1.9	14.0	6.1	3.8	30.0
Clarias gariepinus	1.4	5.3	50.9	0.4	1.7	21.3	3.6	3.6	22.5
Crayfish	4.5	1.9	78.4	12.8	19.1	0.8	nd	2.9	nd

nd: not detected

Tables 6 and 7 present the level of metal biomagnification in sediments for wet and dry seasons, respectively, while Table 8 presents the level of metal biomagnification in fishes and crayfish. From Tables 6 and 7 it may be observed that the biomagnification of Ni, Cu, Zn, Cd, Co and Mn in the wet season was higher than that obtained in the dry season. Except for Co and Ni, the concentrations of these metals in the sediments in the wet season were lower than those of the dry season. The direct relationship between the concentration and biomagnification of Co and Ni in the wet season suggests that the coastal environment might be naturally rich in Co and Ni, and these could be so leached into the aquatic system during rains. The levels of metal biomagnification determined in the sediments reveal that the metals were present in greater amount (several thousand-folds) than in the corresponding surrounding water (Tables 1-7). Similar observations have been made by Ipinmoroti and Oshodi (1993) in their study of some metals present in fish, water and sediment samples from a pond. The present study suggests that the pollution level of all the metals can be better monitored in the sediments of an aquatic environment. Also, the presence of some metals that may seem to be absent or present at relatively low concentrations, for example Pb and Co, in the surface water can be greater and detectable in the sediment samples. The biomagnification of most of the metals for both seasons varied widely from one location to the other.

This is indicated by the coefficient of variation that ranged from 31% to 144% and 29% to 130% in the wet and dry seasons, respectively (Tables 6 and 7). This indicates that some of the locations were heavily loaded with some of the metals as compared with their values in the corresponding surface water. Such locations, as Igbokoda, Legha, Alagbo, Ilepete, Obenla and Ojumole can be used to monitor the accumulation of metals like Pb, Cr, Co, Mn and Cd (Tables 1-7).

Table 6. The levels of metal biomagnification in sediment samples collected during the wet season

Location of sampling/ statistical analysis	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Igbokoda	2960	1826	1054	1087	946	1387	5452	2714	4664
Kajola	7308	1828	889	1268	812	2909	8571	2542	4595
Ibila	3400	1606	1642	1288	987	2073	6375	13142	4623
Elegboro	2021	1452	1655	1033	876	1800	1340	6400	3900
Legha	2020	3308	3542	1841	1234	3703	1492	81667	20350
Mahin	1489	1292	1768	948	1163	1961	1118	3609	4560
Ugbonla	2176	2261	1324	1310	1122	2421	3889	16333	3886
Ayetoro	2579	2036	2062	1431	1292	1722	3898	1245	7902
Alagbo	1977	10246	1216	1681	1317	3750	26286	4286	8631
Asumaga	2275	1141	1539	2047	1574	1755	1343	1712	13891
Ilepete	5375	1446	1027	1000	1050	2857	23875	2341	6756
Obenle	21250	3000	3453	3391	2231	5471	4400	43333	9573
Ojumole	10888	3346	3466	3160	3116	4636	1293	6045	12521
Jirinwo	3867	1207	1242	905	864	2077	6565	3457	5159
Mean values	4970	2571	1850	1599	1213	2823	7167	7773	7929
sd	5354	2332	941	758	375	1184	7121	11159	4802
cv	1.08	0.91	0.51	0.49	0.31	0.42	0.99	1.44	0.61

sd: standard deviation; cv: coefficient of variation

Table 7. The levels of metal biomagnification in sediment samples collected during the dry season

Location of sampling/ statistical analysis	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Igbokoda	3182	1588	5806	1203	712	2073	10750	700	6043
Kajola	8091	2818	2726	1429	959	2625	9625	1455	4981
Ibila	4125	1258	3150	1169	667	1596	7000	1111	3913
Elegboro	2170	2259	3495	1253	847	2061	1490	340	4094
Legha	8083	4295	11662	1877	1232	3871	24571	4600	21111
Mahin	1571	1175	4594	1012	664	1932	1681	12000	4595
Ugbonla	2276	1351	3629	973	856	2324	1869	1300	3438
Ayetoro	2727	264	4969	1403	990	2692	4429	900	7439
Alagbo	1143	3029	3896	1133	952	3444	35750	1875	16207
Asumaga	2657	2377	3265	1282	1167	1476	1380	380	14285
Ilepete	9333	1410	2875	1296	921	3037	53500	3500	6190
Obenle	21250	3774	2030	2714	1755	8083	42250	2500	8887
Ojumole	16333	3841	988	1432	1282	3774	31333	1818	13272
Jirinwo	4642	1789	512	1129	814	2389	8667	800	4858
Mean values	6256	2231	3828	1379	987	2670	16735	2377	7808
sd	5982	1184	2668	444	294	852	17436	3021	5065
cv	0.96	0.53	0.70	0.322	0.29	0.32	1.04	1.30	0.65

sd: standard deviation; cv: coefficient of variation

Table 8. Biomagnification factor of metals in fish

Fish type	Pb	Ni	Fe	Cu	Zn	Cd	Cr	Co	Mn
Oreochromis niloticus	429	390	94	60	211	564	563	667	3591
Synodontis sp.	536	471	290	120	268	3590	1271	1583	2609
Clarias gariepinus	500	431	347	80	239	5462	750	1500	1975
Crayfish	2885	209	710	2188	2262	231	nd	1500	nd

nd: not detected

The biomagnifications of the metals in fishes and crayfish samples were lower than those obtained for the sediment samples (Tables 6-8). However, metal concentrations in the fish and crayfish samples were greater than their corresponding values in water. Similar trends are usual in the aquatic ecosystems (Adeyeye, 2000; Ipinmoroti and Oshodi, 1993). The higher value of the biomagnification of metals in the fishes, and particularly in the crayfish, indicates that the organisms have greater tendency to concentrate the metals in their body tissues. These organisms, therefore, can be considered as pollution indicators of metals in the aquatic environments. The levels of biomagnification of the metals in fishes and crayfish indicate some degree of consistency for which *Synodontis* sp. was noted to concentrate nearly all the metals. It was further observed that *O. niloticus* can be useful for Mn monitoring, *C. gariepinus* for Cd and few other metals, and crayfish for Pb, Cu, Zn and possibly Co.

References

Adeyeye, E.I. 2000. Determination of major elements in fish, associated water and soil sediments from some fresh water ponds. *Ghana J. Chem.* **43:** 367-373.

Ajayi, S.O., Mombeshora, C. 1989. Sedimentary trace metals in lakes in Ibadan, Nigeria. *Sci. Total Environ.* **87-88:** 77-84.

Asaolu, S.S. 1998. Chemical Pollution Study of Ondo State Coastal Region. *Ph. D. Thesis*, Federal University of Technology, Akure, Nigeria.

Asaolu, S.S., Ipinmoroti, K.O., Olaofe, O., Adeeyinwo, C.O. 1997. Interrelationship of heavy metal concentration in water, sediments and fish samples from Ondo State Coastal Area, Nigeria. *Afri. J. Sci.* **1:** 55-61.

Calamari, D., Naeve, H. 1994. *Trace Metals Concentration in Fish and Sediment Samples,* pp. 1-118, CIFA Technical Paper 25, FAI, Rome, Italy.

Cater, D.E., Fernando, Q. 1979. Metals in the environment. Chemical toxicology. *J. Chem. Educ.* **56:** 490-495.

Finerty, M.W., Madden, J.D., Feagley, S.E., Grodner, R.M. 1990. Enzymatic activities of some trace metals. *Arch. Environ. Contam. Toxicol.* **44:** 461-467.

Gutheric, R.K., Davis, E.M., Cherry, D.S., Murray, H.E. 1979. Accumulation of heavy metals in marine organisms. *Bull. Environ. Contam. Toxicol.* **21:** 53.

Ipinmoroti, K.O., Asaolu, S.S., Adeeyinwo, C.E., Olaofe, O. 1997. Distribution of some heavy metals in coastal waters of Ondo State, Nigeria. *J. Techno-Science* **1:** 46-51.

Ipinmoroti, K.O., Oshodi, A.A. 1993. Determination of trace metals in fish, associated waters and soil sediments from fish ponds. *Discov. Innov.* **2:** 135-138.

Kakulu, S.E., Osibanjo, O. 1986. Trace metal analysis. *Environ. Pollut. Ser.* **8:** 315-322.

Lo, J.M., Yu, J.C., Hutchison, F.I., Wal, C.M. 1982. Solvent extraction of dithiocarbamate complexes and back extraction with mercury (II) for determination of trace metals in seawater by atomic absorption spectrometry. *Anal. Chem.* **54:** 2536-2539.

Mirian, E., Baron, M.D., Russell, M., Boyle, M.A. 1994. Are pediatricians ready for the new guidelines on lead poisoning. *Pediatrics* **93:** 178-182.

Mohammed, S., Tahir, H.Z., Amir-ul-Hoda, Atique, A. 1982. Heavy metal concentration in shrimps, crab and sediments. *Bull. Environ. Contam. Toxicol.* **29:** 313-319.

Munshi, J.S.A., Singh, N.K. 1989. Assessment of some pollutants in fresh and marine waters. *Environ. Ecol.* **7:** 790-792.

Okoye, B.C. 1991. Heavy metals in the environment. *Inter. Environ. Studies* **37:** 285-292.

Oloyede, O.B., Adediran, G.O., Oyinloye, J.A. 1990. Some heavy metal composition of some Nigerian soup condiments. *Niger. J. Nut. Sci.* **11:** 13-20.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 103-107

Indoor NO_2 Sampling in a Large University Campus in Benin City, Southern Nigeria, Using Palmes Diffusion Tubes

E. E. Ukpebor*, Y. T. Sadiku, and S. I. Ahonkhai
Chemistry Department, University Of Benin, Benin City, Nigeria

(received January 28, 2004; revised October 28, 2004; accepted November 6, 2004)

Abstract. Monitoring of NO_2 in different indoor environments (without cooking and with cooking using different fuels) was done. Palmes diffusion tubes were used for the monitoring. The sampling duration was two weeks. The highest NO_2 concentration of 38.61 ppb (73.74 μg/m^3) was monitored in the room where the cooking was done with a gas burner. This was followed by the room with firewood cooking, where the concentration was 36.75 ppb (70.19 μg/m^3) and the least concentration of 24.05 ppb (46.80 μg/m^3) was noted in the room, where kerosene stove was used for cooking. It is of significance to observe that the WHO annual average guideline value of 40 μg/m^3 was exceeded in all the rooms where cooking was done. Levels obtained in this study, therefore, suggest a need for precautionary mitigation. However, the outdoor concentration of NO_2 was almost the same as that obtained indoors in the rooms without cooking. This suggests high penetration indoors of outdoor NO_2. A background level of 3.40 ppb (6.49 μg/m^3) was established for the environment in Ugbowo, Benin City, Nigeria.

Keywords: indoor No_2, outdoor No_2, cooking fuels, nitrogen dioxide, air pollution, Palmes diffusion tubes

Introduction

Although overwhelming evidence exists on the anthropogenic source of air pollutants in Nigeria, yet only scanty information and data are available on this subject. Informations on the tropospheric levels of the solid and liquid droplets have been provided (Baumbach *et al.,* 1995; Adejumo *et al.,* 1994; Ogunsola *et al.,* 1993). Ambient concentrations of some gaseous pollutants have also been reported recently (Ukpebor and Ahonkhai, 2000). Most people spend more time indoors than outdoors, thus making indoor spaces important microenvironments when addressing risks from air pollution. A recent report indicates that a person is perhaps 1000 times more likely to inhale a chemical molecule if it is emitted indoors than outdoors (Nazaroff and Singer, 2004). Furthermore, estimates from the World Health Organization indicate that indoor air pollution from the use of solid fuels, accounts for 1.6 million deaths globally per year (WHO, 2002).Though it has been estimated that about 1.9 million people die annually as a result of exposure to high concentrations of suspended particulate matter and other pollutants in the indoor air environment in the developing countries (WHO, 2000), information and resources to control the indoor air quality are often lacking (Ferrari *et al.,* 1995). The only reported indoor measurement till date in Nigeria is the 1999 study on the indoor levels of NO_2 in an operating room using a nitrous oxide as an anaesthetic (Ukpebor and Imarengiaye, 2002). Prohibitive cost of air sampling equipment, erratic electric power supply, lack of trained personnel and unreliability of some of the available passive samplers are some of the reasons advanced for the lack of data on indoor air quality status. NO_2 has been primarily selected for air monitoring because of the recent confirmation of the reliability and sensitivity of Palmes diffusion tubes for NO_2 (Ukpebor *et al.,* 2004; Hansen *et al.,* 2001; Hangartner, 1999). Secondly, NO_2 is an important indicator of air pollution because the concentration of NO_2 is well correlated with the concentration of carbon monoxide, particulates, polycyclic aromatic hydrocarbons (Lewis *et al.,* 1995), and soot (Bower *et al.,* 1991).

The indoor NO_2 is generated from a series of sources, such as tobacco smoke or from cooking with biomass fuelled stoves. While cooking, unvented gas and kerosene heaters may increase the indoor NO_2 levels (Bardana, 2001). Elevated levels of NO_2 have been measured routinely in kitchens during conventional gas cooking (Spengler and Cohen, 1985). NO_2 may cause irritation of nose and eyes and may induce lower respiratory system symptoms through penetration of the conducting airways (Alberts, 1994). In healthy subjects, any exposure to NO_2 at levels found indoors may cause airway inflammation, affect blood cells, and augment susceptibility of airway epithelial cells to injury from respiratory viruses (Frampton *et al.,* 2002). High indoor levels of NO_2 have been associated with an increased prevalence of respiratory symptoms in healthy children (Garret *et al.,* 1998) and adults (Simoni *et al.,* 2002), or asthmatic subjects (Ng *et al.,* 2001).

*Author for correspondence; E-mail: ukpebehi@uniben.edu

The work reported here is a pilot study designed to assess the NO_2 levels in different indoor environments within the Ugbowo Campus, University of Benin, Nigeria. It is hoped that this report will stimulate further indoor air pollution studies. Furthermore, it is anticipated that the data generated would help in policy development and prioritization of management actions.

Materials and Methods

Study areas. Ugbowo Campus, University of Benin, Nigeria, is populated with about 40,000 inhabitants, approx 30,000 of which are students and the rest include staff and their families. About 70% of the student population resides on the campus. Different indoor environments were carefully selected on the campus in this pilot study, which included a room with a kerosene stove used exclusively for cooking, a room with a gas burner, a kitchen with firewood as the means of cooking, and a room with no cooking to serve as the control. Two outdoor measuring sites were also selected, a remote site about three km from the residential area with no motorized traffic density and another site with very low traffic density. The former remote site was to act as a control and also to provide the background NO_2 concentration in the environment. The latter outdoor site was to facilitate the calculation of outdoor/indoor NO_2 ratio.

NO_2 monitoring. NO_2 measurement was carried out by using Palmes diffusion tubes. The diffusion tubes used in this study consisted of a small acrylic tubing, 8.20 cm long with a cross-sectional area of 0.82 cm^2, having two stainless steel mesh as support for adsorbing material at its one end. Triethanolamine was used as the adsorbent for NO_2. The sensitivity and utmost accuracy of this particular tube length and the selectivity and specificity of triethanolamine for NO_2 have been documented (Ukpebor *et al.*, 2004; Gold, 1977). In preparing the tubes, uniformity was maximized, for example, the same drying time for each of the steel grids, and the use of a freshly prepared triethanolamine/acetone mixture. The required steel grids were cleaned with acetone and dried properly. A mixture of two parts of acetone and one part of triethanolamine was prepared and stirred thoroughly. The grids were dipped in the mixture and dried for 20 min. Dipping in solution prior to assembly was found to give significantly more precise NO_2 concentrations than by the pipetting method (Hamilton and Heal, 2004). After drying, two of the steel grids were placed at one end of the tube and the tube was capped. The prepared tubes were stored in a refrigerator and finally exposed at the different monitoring sites. Two tubes from each of the prepared sets were retained in storage as 'blanks' for later analysis along with the exposed tubes. A two-week sampling period was observed to allow a reasonable quantity of nitrogen dioxide to be adsorbed.

After exposing the passive samplers, the amount of NO_2 adsorbed was determined colourimetrically as nitrite with Saltzmann reagent (Palmes *et al.*,1976). A visible spectrophotometer (Spectronic, 21D) at zero extinction, previously calibrated with known concentrations of nitrite (NO_2^-), was used to determine the absorbance of both blanks and the air samples at 540 nm, using the reagent as referred. Absorbance readings from the unexposed 'blank' diffusion tubes were averaged for each preparation set and the value subtracted from the readings for the exposed tubes. The atmospheric concentration of NO_2 obtained during the measuring period was calculated as described by Palmes *et al.* (1976), using Fick's first law, the dimensions of the tube, and the diffusion coefficient of NO_2 in the air with the following equation:

$$C = \frac{22.4 \times 10^3 \times \frac{T}{273} \times F \times Z \times E}{t \times D \times A}$$

where:
C = NO_2 conc (ppb)
T = temperature (K)
F = calibration factor
Z = length of the diffusion tube (cm)
E = extinction minus extinction of the blank
t = exposure time (sec)
D = diffusion coefficient of the compound in air (cm^2/sec)
A = cross–sectional area of the tube (cm^2)

note: 1 ppb NO_2 = 1.91 μg NO_2/m^3

Results and Discussion

The time-weighted average concentrations of NO_2 obtained from the different indoors and the two outdoor environments during June and July are presented in Table 1. NO_2 levels measured from three of the four indoor environments seem high. However, these values can best be assessed by a comparison with the approved National and International Standards. The Federal Environmental Protection Agency (FEPA, 1991) allows daily average limit for NO_2, which is presently 75-113 μg/m^3. The US National Ambient Air Quality Standard (USEPA, 1990) for NO_2 is presently 100 μg/m^3 annual average, while the World Health Organization annual average guideline for exposure to NO_2 is 40 μg/m^3, and the 1 h guideline is of 200 μg/m^3 (WHO, 2000). It is significant to indicate that the outdoor air had a different pollutant composition than that found in the indoor air. Not all of these compositions have been taken into account in developing the air quality guide-

Table 1. Measured indoors and outdoors NO_2 concentrations during the two months of study

Sampling site	NO_2 conc ppb (μg/m^3)		
	Analysis during June	Analysis during July	Mean values
Room with kerosene stove	25.98 (49.62)	23.02 (43.97)	25.05 (46.80)
Room with gas burner	40.83 (77.98)	36.38 (69.49)	38.61 (73.74)
Room with firewood	37.86 (72.31)	35.64 (68.07)	36.75 (70.19)
Room with no cooking	11.88 (22.69)	8.91 (17.02)	10.40 (19.86)
Vice-chancellor's lodge	11.92 (22.77)	8.17 (17.60)	10.05 (19.19)
Control site	3. 82 (7.30)	2.97 (5.67)	3. 40 (6.49)

lines given above and, therefore, may not be applicable under all circumstances. Despite this limitation, the data obtained would be discussed in line with the set standards.

The mean background NO_2 concentration obtained in this study was 3.40 ppb (6.49 μg/m^3). This level was obtained in the control site, a very remote location devoid of any of the known anthropogenic source group of NO_2. This, therefore, indicates a natural unpoluted source and hence the expected minimum in the monitoring environment. The highest NO_2 range of 36.38 ppb (69.49 μg/m^3) to 40.83 ppb (77.98 μg/m^3) measured in this study was recorded in the indoor site where exclusively gas burner was used for cooking. The mean NO_2 concentration in this room was found to be a factor of 11 times higher than the background concentration and a factor of 4 time higher than what was measured at the outdoor control environment selected for the study (near the Vice Chancellor's lodge). The second highest mean NO_2 concentration of 36.75 ppb (70.19 μg/m^3) was obtained in the room where firewood was used as the means of cooking. This room gave a calculated outdoors/indoors NO_2 ratio of 0.27, and a factor of 10 times higher than the background NO_2 level (Table 2). The least concentration range of 23.02-24.05 ppb NO_2 was measured in the room with kerosene stove. The room with no cooking recorded a mean NO_2 concentration of 19.86 μg/m^3. This is a factor of 3 times higher than the background NO_2 concentration. The calculated outdoors/indoors NO_2 ratio for this room was found to be 0.97 (Table 2). This reveals no significant difference between the outdoor control site concentration and the concentration measured in this room. The outdoors site selected near the Vice-chancellor's lodge gave NO_2 level of 10.05 ppb (19.19 μg/m^3). This value is a factor of 3 times higher than the background concentration. The difference of (13.37 μg/m^3) between the background level (6.49 μg/m^3) and the measured NO_2 concentration (19.86 μg/m^3) in the room with no cooking may be due to penetration indoors of the outdoors NO_2. Indoor concentrations of air pollutants are influenced by outdoors levels, indoor sources, the rate of exchange between indoor and outdoor air, and the characteristics and furnishings of the buildings (WHO, 2000). Indoor values of 20% to 80% of the outdoor concentrations have been reported (Yocum, 1982).

Table 2. Calculated outdoors/indoors NO_2 ratio

Sampling site	Outdoors/indoors ratio
Room with kerosene stove	0.42
Room with gas burner	0.26
Room with firewood	0.27
Room with no cooking	0.97

The differences in the NO_2 levels obtained in the different indoor environments could be a function of the mode of combustion, the type of fuel used for cooking and the quantity of fuel combusted. However, since the samplers gave only average values for the exposure period, no attempt was made to record the pattern or extent of fuel usage. In the developed countries, indoor levels of NO_2, for example, are affected by gas heaters and cooking ranges (used in 20-80% of houses in some countries). In five European countries, the average NO_2 concentrations (over 2-7 days) for dwellings with gas equipment were in the range of 20-40 μg/m^3 in living rooms and 40-70 μg/m^3 in kitchens, and 10-20 μg/m^3 in dwellings without gas equipment (WHO, 2000). In addition, short-term measurements reveal NO_2 concentrations that may be five-folds higher than those averaged over several days. Peak values of up to 3800 μg/m^3 for 1 min have been measured in the Netherlands in kitchens with unvented gas cooking ranges (Seifert, 1993; ECA, 1989).

Relatively few studies have been conducted to determine the health effects of indoor exposures to air pollutants in the developing countries. Enough data have become available in recent years, however, to obtain some preliminary information on the type and very approximate magnitude of effects (Chen *et al.,* 1990). NO_2 levels of about 940 μg/m^3 (0.5 ppm) increase

susceptibility to bacterial and viral infections. Epidemiological studies evaluating the effects of NO_2 exposures in homes with gas cooking appliances have been conducted. In general, epidemiological studies of adults and infants (less than 2-year olds) show no significant effect of the use of gas cooking appliances on respiratory illnesses, nor do the few available studies of infants and adults show any association between pulmonary function changes and gas stove use. However, children 5-12 years old are estimated to have a 20% increased risk for respiratory symptoms and disease for each increase of 28 $\mu g/m^3$ NO_2 (2-week average), where the weekly average concentrations are in the range of 15-128 $\mu g/m^3$ or possibly higher (WHO, 2000).

Conclusions

With growing public concerns about the indoor air quality, action has been taken in many developed countries to characterize the levels of indoor air pollutants. This present pilot study is the first attempt at initiating a large-scale indoor air pollution characterization process in Nigeria. The following can be inferred from this study. The NO_2 levels in the entire indoor/outdoor environments monitored, complied with the threshold values set nationally. However, it is of great significance that the WHO annual average guideline value of 40 $\mu g/m^3$ was exceeded in all the rooms where cooking was done. Levels obtained in this study, therefore, suggest a need for precautionary mitigation. Cooking with a gas burner indoors generated the highest NO_2 concentration of 77.98 $\mu g/m^3$. The room where cooking was done with a kerosene stove gave the lowest NO_2 level of 49.62 $\mu g/m^3$. There was no significant difference between the outdoor NO_2 concentration and the concentration in the room where no cooking was done, suggesting high penetration indoors of the outdoor NO_2.The background NO_2 concentration for Ugbowo environment was found to be 3.40 ppb (6.49 $\mu g/m^3$).

References

Adejumo, J. A., Obioh, I. B., Ogunsola, O. J., Akeredolu, F. A., Olaniyi, H. B., Asubiojo, O. F., Oluwole, A. F., Akanle, O. A., Spyrou, N.M. 1994. The atmospheric deposition of major, minor and trace elements within and around three cement factories. *J. Radio. Analyt. Nuclear Chem*. **179:** 195-204.

Alberts, W.M. 1994. Indoor air pollution, NO, NO_2, CO, CO_2. *J. Allergy Clin. Immunol.* **94:** 289-295.

Bardana, E.J. 2001. Indoor pollution and its impact on respiratory health. *Ann. Allergy Asthma Immunol.* **87:** 33-40.

Baumbach, G., Vogt, U., Hein, K.R.G., Oluwole, A.F., Ogunsola, O. J., Olaniyi, H.B., Akeredolu, F. A. 1995. Air pollution in a large tropical city with a high traffic density: results of measurements in Lagos, Nigeria. *Sci. Total Environ.* **169:** 25-31.

Bower, J.S., Lampert, J.E., Stevenson. K.J., Atkins, D.H.F., Lewis, D.V. 1991. A diffusion tube survey of NO_2 levels in urban areas of UK. *Atmos. Environ.* **25B:** 255- 265.

Chen, B.H., Hong, C.J., Pandey, M.R., Smith, K.R. 1990. Indoor air pollution and its effect in developing countries. *WHO Statistics Quarterly* **43:** 127-138.

ECA. 1989. *Indoor Pollution by NO_2 in European Countries; European Concerted Action on Indoor Air Quality and its Impact on Man*, Report No. 3, Office for Official Publications of the European Communities, Luxembourg, Belgium.

FEPA. 1991. *Guidelines and Standards for Environmental Pollution Control in Nigeria*, Federal Environmental Pollution Contral in Nigeria, Lagos, Nigeria.

Ferrari, L., Chowdhury, B., Younes, M. 1995. Indoor air pollution in developing countries. What are the problems and how can they be resolved? In: *Indoor Air: An Integrated Approach,* L. Morawska, N. D. Bofinger, M. Maroni (eds.), pp. 27-28, Elsevier, Oxford, UK.

Frampton, M.W., Boscia, J., Roberts Jr., N.J., Azadniv, M., Torres, A., Cox, C., Morrow, P.E., Nochols, J., Chalupa, D., Frasier, L.M., Gibb, F.R., Speers, D.M., Tsai,Y., Utell, M.J. 2002. Nitrogen dioxide exposure; effects on airway and blood cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **282:** 155-165.

Garret, M.H., Hooper, M.A., Hooper, D.M., Abramson, M.J. 1998. Respiratory symptoms in children and indoor exposure to nitrogen dioxide and gas stoves. *Am. J. Respir. Crit. Care Med.* **158:** 891-895.

Gold, A. 1977. Stoicheiometry of nitrogen dioxide determination in triethanolamine trapping solution. *Anal. Chem.* **49:** 1448-1450.

Hamilton, R.P., Heal, M. R. 2004. Evaluation of method of preparation of passive diffusion tubes for measurement of ambient nitrogen dioxide. *J. Environ. Monit*. **6:** 12-17.

Hangartner, M. 1999. Passive sampling for the measurement of nitrogen dioxide emission. *Dragger Review* **83:** 6-8.

Hansen, T.S., Kruse, M., Lohse, C. 2001. Monitoring of NO_2 in Greenland. *J. Environ. Monit.* **3:** 139-145.

Lewis, A.C., Kupiszewska, D.,Bartle, K.D., Pilling, M.J. 1995. City center concentrations of polycyclic aromatic hydrocarbons using critical fluid extraction. *Atmos. Environ.* **29:** 1537-1542.

Nazaroff, W.W., Singer, B.C. 2004. Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. *J. Exposure Analysis Environ. Epidemiol.* **14:** S71-S77.

Ng, T.P., Seet, C.S., Tan, W.C., Foo, S.C. 2001. Nitrogen dioxide exposure from domestic gas cooking and airway response in asthmatic women. *Thorax* **56:** 596-601.

Ogunsola, O.J., Oluwole, A.F., Obioh, I.B., Asubiojo, O.I., Akeredolu, F.A., Akanle, O.A., Spyrou, N.M. 1993. Analysis of suspended air particulates along some motorways in Nigeria by PIXE and EDXRF. *Nucl. Instr. Methods Phys. Res.* **B79:** 404- 407.

Palmes, E.D., Gunnison, A.F., di Mattio, J., Tomczyk, C. 1976. Personal sampler for nitrogen dioxide. *Am. Ind. Hyg. Assoc. J.* **37:** 570-577.

Seifert, B. 1993. *Innenräume*: *Handbuch der Umweltmedizin*, Ecomed-Fachverlag, Landsberg.

Simoni, M., Carrozzi, L., Baldacci, S., Angino, A., Scognamiglio, A., di Pede, F., Sapign, T., Viegi, G. 2002. The Po River Delta (North Italy) epidemiological study; effect of pollutant exposure on acute respiratory symptoms and respiratory function in adults. *Arch. Environ. Health* **57:** 130-136.

Spengler, J.D., Cohen, M. 1985. Emissions from indoor combustion sources. In: *Indoor Air and Human Health,* R.B. Gammage, C.V. Kaye (eds.), Chelsea, ML.

Ukpebor, E.E., Ahonkhai, S.I., Heydtmann, H. 2004. NO_2 measurement with a passive sampler: assessment of the sensitivity of two types of Palmes diffusion tubes for NO_2. *Intern. J. Environ. Stud.* **61:** 67-71.

Ukpebor, E.E., Ahonkhai, S.I. 2000. Spatial and temporal variation of nitrogen dioxide concentration in Benin City, using passive samplers. *Nig. J. Appl. Sci.* **18:** 72-77.

Ukpebor, E.E., Imarengiaye, C.O. 2002. The use of simple diffusion tube samplers for the measurement of nitrogen dioxide in an operating room using nitrous oxide as an anaesthetic. *West Afr. J. Med.* **21:** 192-194.

USEPA. 1990. *User's Guide for the Urban Airshed Modes,* vol. **1:** *User's Manual for UAM (CB4), EPA-450/4-90 007a,* Office of Air Quality Planning and Standards, US Environmental Protection Agency, Research Triangle Park, NC, USA.

WHO. 2002. *Reducing Risks, Promoting Healthy Life*, pp. 69-70, Word Health Organization, Geneva, Switzerland.

WHO. 2000. *Guidelines for Air Quality,* World Health Organization, Geneva, Switzerland.

Yocum, E. 1982. Indoor-outdoor air quality relationship. *J. Air Pollut. Control Assoc.* **32:** 500.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 108-109

Short Communication

Quantitative Determination of Sinensetin in *Orthosiphon stamineus* Leaves by Thin-Layer Chromatography and Imaging Densitometry

M. Amzad Hossain[*ab], Zakri Zakaria[a] and Zhari Ismail[a]

[a] School of Pharmaceutical Sciences, University Sains Malaysia, 11800 Pulau Pinang, Malaysia
[b] Chemistry Division, Atomic Energy Centre, GPO Box No. 164, Ramna, Dhaka-1000, Bangladesh

(received August 27, 2003; revised November 25, 2004; accepted December 04, 2004)

Abstract. An analytical method for the determination of sinensetin in *Orthosiphon stamineus* leaves by a thin-layer chromatography-imaging densitometric method was developed. The procedure consisted of extraction of dry leaf powder with 50% methanol and high performance preparative thin layer chromatography (HPTLC). HPTLC was performed on silica gel plate, using chloroform-ethylacetate (60:40) as the developing solvent for sinensetin. The plate was scanned with a reflectance densitometer at 190 nm. The quantification was done by the external standard method.

Keywords: *Orthosiphon stamineus*, sinensetin quantification, sinensetin extraction, reflectance densitometry

Orthosiphon stamineus, family Lamiaceae, is commonly found in the rain forests of several tropical countries. The leaves of this plant are used as diuretic and to treat urinary lithiasis, diabetes, edema, jaundice, hypertension, biliary lithiasis (Hossain and Zhari, 2003), rheumatism (Beaux *et al.*, 1999), and eruptive fever, influenza and hepatitis (Awale *et al.*, 2002). Owing to its pharmaceutical utility, the plant is under systematic cultivation in Malaysia and consumed as a health drink tea to facilitate body detoxification. Extracts of *O. stamineus* are widely used in Malaysia for the treatment of diabetes and kidney stone diseases. Over 4000 chemically unique flavonoids have been identified in plant sources. These low-molecular-mass substances, found in all vascular plants, are phenylbenzopyrones with an assortment of basic structures (Harborne, 1993). On an average, the daily western diet contains approximately 1 g of mixed flavonoids in fruits, vegetables, nuts, seeds, stems, flowers, tea and wine (Kuhnau, 1976). This quantity provides pharmacologically significant amounts of flavonoids in the body fluids and tissues. The most important components of *O. stamineus* leaves are the polyphenols, the polymethoxylated flavonoids, such as sinensetin, eupatorin, and the caffeic acid derivatives, such as rosmarinic and caffeic acids (Olah *et al.*, 2003). The polyphenols from *O. stamineus* have been studied by different chromatographic and spectral methods (Pietta *et al.*, 1991; Wollenweber and Mann, 1985; Gracza and Ruff, 1984). So far, no report has appeared on the HPTLC of sinensetin, which is described as a rapid and simple method for its quantitative determination in the leaves of *O. stamineus.*

Preparative thin layer chromatographic plates (20x10 cm), precoated with l mm silica gel GF_{254} (Merck) were used. The silica gel GF_{254} suspended in water, well homogenized by electric stirring, was applied on the TLC glass plates with a Camag applicator. The tuff was previously sieved and the fraction with particle size < 40 mm was used for the preparation of 0.5 mm thickness wet layer. The water used was purified by the Nonpure-Unit (Barnstead, Boston, USA). The Camag analyser used in the studies was a reflectance spectrometre equipped with an IBM computer, monitoring range 190-700 nm. Data acquisition and processing were performed using the winCATS software programme.

The *O. stamineus* leaves, collected from Penang, Malaysia, were dried at room temperature, or in the oven below 40 °C. The dried leaves were pulverised and sifted through 500 µm mesh size sieve. Accurately weighed 10 g of the leaf powder was transferred to 70 ml 50% methanol (Merck). After ultrasonication for 1 h in an ultrasonic cleaning bath, the extract was filtered and evaporated to dryness (later referred to as extract powder) using rotary evaporator and vacuum pump. Solutions for the standard calibration curve were made in methanol at the concentrations of 0.01, 0.0125, 0.015, 0.0175, and 0.2 mg/ml, using pure crystalline sinensetin. The standard curve was used for the determination of sinensetin in the extract powder. The sinensetin solution of the 50% methanol extract was prepared by dissolving 5 mg extract powder/ml methanol and used for quantitative determination.

The standard sinensetin and the extract powder solutions were applied to the TLC plates (solution volume applied: 10 µl; spot diameter: 10 mm). The solution application was done at 15 mm from the bottom edge of the plate and the spots were 20.1 mm apart from each other. The TLC plates were developed at room temperature in an unsaturated glass twin-trough chamber. The solvent system consisted of chloroform (Merck):

*Author for correspondence; [b] present address;
E-mail: dramzadh@yahoo.com

ethylacetate (60:40), allowing the ascending migration of the mobile phase over a distance of 8.5 cm. The developed TLC plates were dried in a stream of warm air. The chromatograms were read under UV light and further scanned with spectrodensitometre.

The plates were not sprayed with any reagent and the spots were visualised under UV at 365 nm. The chromatograms showed that the spots of the *O. stamineus* extract had their colour and R_f values similar to those of the standard in sinensetin solutions (Fig.1). The calculated R_f values and densitometry for the standards and the components in the extract powder from the leaf extracts of *O. stamineus* further demonstrated the presence of sinensetin (Table 1).

The quantitative determination of sinensetin was done byTLC densitometry using the calibration curve method. The calibration curve already prepared with known concentrations of sinensetin as detailead above, was read using the winCATS software programme. The concentration of the extract powder was obtained using the formula:

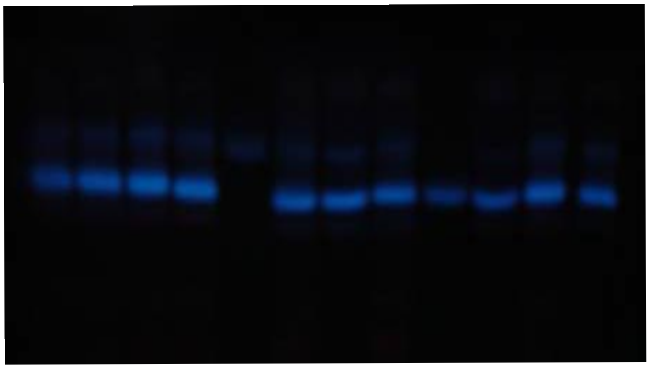


Fig. 1. Chromatograms of the sinensetin extract of *Orthosiphon stamineus* and the sinensetin standard at 365 nm without any reagent sprayed.

Table 1. The concentration of the sinensetin standard solutions and the leaf extract of *Orthosiphon stamineus* applied to TLC plates, their R_f values and the area under reflectance densitometre scanning at 190 nm

Samples /standard	Concentration	Rf values	Area
Standard 1	100 ppm (10 µl)	0.42	790
Standard 2	125 ppm (10 µl)	0.42	902
Standard 3	150 ppm (10 µl)	0.42	1234
Standard 4	175 ppm (10 µl)	0.42	1370
Standard 5	200 ppm (10 µl)	0.42	1532
50% Methanol extract	10 µl	0.42	3784*

* means of three values

$$\% C = V_e C_{et} / 10 m$$

where:

V_e = volume of the standard solution
C_{et} = concentration of the standard solution
10 = quantity of the extract sample in µl
m = weight of the plant material used for extraction

The amount of sinensetin in the extract powder, calculated from the standard curve by the densitometric method was determined to be 0.36%. The results obtained with this method were found to be in agreement with the confirmatory determinations done on HPLC. The observations on the qualitative and quantitative determinations obtained by the densitometric method were in conformity with chromatographic and spectral methods. The analytical procedure reported here thus provides a fast and reliable method for the determination of sinensetin having the potential of application to other pharmaceutical preparations for the qualitative and quantitative determination of drugs.

References

Awale, S., Tezuka, Y., Banskota, A.H., Shimoji, S., Taira, K., Kadota, S. 2002. Norstaminane and isopimarane-type diterpene of *Orthosiphon stamineus* from Okinawa. *Tetrahedron* **58:** 5503-5512.

Beaux, D., Fleurentin, J., Mortier, F. 1999. Effect of extracts of *Orthosiphon stamineus* Benth. *Phytother. Res.* **13:** 222-225.

Gracza, L., Ruff, P. 1984. Phytochemical and pharmacological studies on *Orthosiphon stamineus. Arch. Pharm.* **317:** 339-345.

Harborne, J.B. 1993. *The Flavonoids-Advances in Research*, pp. 790-810, Chapman and Hall, London, UK.

Hossain, M. A., Zahri, I. 2003. Hydroxy betulinic acid from the leaves of *Orthosiphon stamineus*. *Asian Coordinating Group Chem.* **16:** 69-73.

Kuhnau, J. 1976 Isolation of flavonoids from the leaves of tea. *World Rev. Nutr. Diet* **24:** 117-121.

Olah, N.K., Radu, L., Mogosan, C., Hanganu, D., Gocan, S. 2003. Phytochemical and pharmacological studies on *Orthosiphon stamineus* Benth (Lamiaceae) hydroalcoholic extracts. *J. Pharm. Biomed. Anal.* **33:** 117-123.

Pietta, P. G., Mauri, P. L., Rava, A., Sabbatini, G. 1991. Quantitative and qualitative determination of toxic compounds from Malaysian medicinal plant Java tea. *J. Chromatogr.* **549:** 367-382.

Wollenweber, E., Mann, K. 1985. Quantification of caffeic acid derivatives from *Orthosiphon stamineus. Planta Medica* **51:** 459-460.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 110-112

Short Communication

Effect of Storage on the Physicochemical Properties of Palm Oil

F. O. Abulude*, M. O. Ogunkoya and O. S. Eluyode
Department of General Studies, Federal College of Agriculture, Akure-340001, Ondo State, Nigeria

(received June 19, 2003; revised November 18, 2004; accepted January 24, 2005)

Abstract. The effect of 4-month storage on the physicochemical properties of palm oil stored in earthenware pots, plastic and tin containers is reported. Significant increase was noted in the iodine value (tin, from 48.22 to 57.95; plastic, from 48.22 to 56.68) free fatty acids (tin, from 0.65 to 0.93 %; plastic, from 0.65 to 0.93 %), and peroxide value (tin, from 5 to 8.07 mEq/kg; plastic, from 5 to 7.87 mEq/kg) of the oil during the period of storage. The increase was even more pronounced in the earthenware pot in iodine value (from 48.22 to 60.91), free fatty acids (from 0.65 to 0.95%), and peroxide value (from 5 to 8.40 mEq/kg). The sensory quality characteristics were adversely affected during storage in the earthenware pots after 4th month of storage. The results suggest that plastic was the best storage container for palm oil.

Keywords: oil storage, physicochemical properties, earthenware pot, plastic container, tin container

Palm (*Elaeis guineensis*) is a "wonder tree", being very useful to humans (Abulude and Lawal, 2002). Oil is among its most useful products, which finds application in cooking, manufacturing of paints, cloth, linoleum, printing ink, insecticides, pharmaceuticals, cosmetics, leather making, production of animal feeds, baking products, and confectioneries. The storing of large volumes of oil for long periods under diverse conditions is not an easy task, since many of its characteristics (colour, flavour and clarity), which are necessary to be maintained, deteriorate. In order to maintain stability of palm oil for a long period for consumption and industrial usage, without the loss of quality and quantity, good storage methods need to be developed. This study was, therefore, carried out to determine the effect of storage on the physicochemical properties of palm oil when stored in containers made of different materials.

The palm oil used in this investigation was procured from the Federal College of Agriculture, Akure, Ondo State Processing Unit. The analytical studies were carried out between May and August. The storage containers (earthenware pots, plastic and tin) were purchased from the local market in Akure. These were washed with detergent, rinsed in distilled water and sun-dried for 5 h. Two litres of oil was stored in each of the containers for 4 months prior to analyses. The initial analytical values, determined before the commencement of storage, were used as the reference values. Two determinations on the physicochemical properties were carried out at 1-month intervals. Iodine value, free fatty acids, peroxide value, and colour were determined by the methods described by Pearson (1976). The relative humidity (%) and temperature (˚C) were recorded daily. Dirt and sediments were removed using the filtration method. Sensory evaluations were done by a taste panel of a nine judges, who evaluated the sample in terms of colour, taste, and odour using a nine point hedonic scale (9 = super-good, downwards to 1 = super-bad). All determinations were done in duplicate. Means and standard deviations were calculated according to the methods of Steel and Torrie (1980). Duncan's multiple range test was used to determine the significant differences (Duncan, 1955).

Table 1 records the average ambient temperature (˚C) and relative humidity (%) during the storage period, by month. The temperature ranged between 26.5-29.0 ˚C, whereas humidity ranged between 90.0-95.4% during the storage period. The physicochemical properties of the oil stored in different containers are presented in Table 2. The free fatty acids (%) was low at the commencement as well as in the stored samples. It varied between 0.65 at commencement and 0.95 in August in the earthenware pots. It was, nevertheless, observed that the free fatty acids increased during storage. The reason for this increase may be attributed to the absorption of moisture by the earthenware pots from the surroundings. When this occurs there is a likelihood of microorganisms affecting the oil, which in turn may lead to spoilage. This is in agreement with the earlier reports for milk during storage (Rehman

Table1. Ambient temperature and humidity of palm oil during storage period

Month	Temperature (˚C)	Humidity (%)
Commencement	26.7	91.0
May	27.0	95.4
June	28.0	92.0
July	29.0	90.0
August	26.5	94.0

*Author for correspondence; E-mail: Wilolud@yahoo.com

et al., 2002; Pearson, 1976). The low free fatty acids values suggest that the sample was good edible oil and that the storage in containers was observed to be effective. With appreciable further increase, the oil may tend to spoil quickly. The peroxide value, which was also low (ranging between 5 to 8.40 mEq/kg), is a measure of the peroxide oxygen present, and is the value used in assessing the extent of oil spoilage.

Table 2. Physicochemical properties of palm oil stored in different kinds of containers

Parameter storage period	Earthenware pots	Plastic containers	Tin containers
Values at commencement			
Free fatty acid (%)	0.65	0.65	0.65
Peroxide value (mEq/kg)	5.00	5.00	5.00
Iodine value	48.22	48.22	48.22
Taste	801±0.2	8±03	8.0±0.3
Odour	81±0.2	8.1±0.2	8.1±0.3
Colour	red	red	red
Dirt/sediments	nil	nil	nil
Values in May (after one month storage)			
Free fatty acid(%)	0.72	0.67	0.71
Peroxide value (mEq/kg)	5.40	5.00	5.00
Iodine value	53.47	49.91	51.18
Taste	5.2±0.25	8.2±0.3	7.6±0.3
Odour	5.1±0.03	8.3±0.4	7.5±0.3
Colour	orange	red	red
Dirt/sediments	2.0±0.01	nil	nil
Values in June (after two month storage)			
Free fatty acid (%)	0.76	0.73	0.75
Peroxide value (mEq/kg)	6.40	6.20	6.20
Iodine value	56.26	50.76	54.15
Taste	5.4±0.30	8.0±0.2	7.5±0.2
Odour	5.3±0.25	8.1±0.3	7.5±0.3
Colour	orange	red	red
Dirt/sediments	4.0±0.01	nil	nil
Values in July (after three month storage)			
Free fatty acid (%)	0.92	0.85	0.89
Peroxide value (mEq/kg)	7.20	6.67	7.10
Iodine value	58.54	53.72	57.28
Taste	5.6±0.20	8.1±0.2	7.4±0.3
Colour	orange	red	red
Dirt/sediments	6.0±0.01	nil	nil
Values in August (after four month storage)			
Free fatty acid (%)	0.95	0.90	0.93
Peroxide value (mEq/kg)	8.40	7.87	8.07
Iodine value	60.91	56.68	57.95
Taste	5.7±0.20	8.4±0.2	7.2±0.3
Odour	5.6±0.30	8.2±0.2	7.0±0.2
Colour	orange	red	red
Dirt/sediments	6.0±0.01	nil	nil

These values were noted increase as the time of storage increased. The oil stored in earthenware pots had the highest peroxide value. However, the obtained values were within the acceptable value of 10 mEq/kg (Pearson, 1976). The low peroxide values indicated that the oil had a low susceptibility to oxidative rancidity and was suitable to be kept for sometime in different storage containers without appreciable deterioration.

During storage in the container with closed lids, increase in iodine value (from 48.22 to 60.91) was noted. To an extent, these changes were more pronounced in earthenware pots. The higher the iodine value, the greater is the liability of the oil to go rancid by oxidation. It then suggests that the oil stored in the earthenware pots may go rancid quickly if there was a further increase in the period of storage.

As shown in Table 3, the analytical observations at the commencement of storage for free fatty acids, peroxide value and iodine value were much different as compared with the results of samples stored in different containers. It was observed, however, that there were no significant differences ($p < 0.05$) in the parameters, when compared with the variations between the replicates throughout the period of storage in the three kinds of containers. After the storage periods of 1st month to 4th month, the taste, colour and odour of samples stored in the earthenware pots were significantly less ($p < 0.05$) than the sample stored in plastic and tin containers. The total amount of dirt and sediments increased during the storage in earthenware pots, whereas there was none in the other containers. The dirt and sediments were, therefore, on account of the structure breakdown of the earthenware pots. The variation in the physicochemical properties may be attributed to oxidation, and rancidity accelerated by exposure to heat, light and the contact with metals of the containers.

Table 3. Analysis of variance of free fatty acids, proxide value and iodine value of palm oil stored for 4 months

Parameter	F-Value	Test of significance ($p < 0.05$)
Free fatty acids	0.69	ns
Peroxide value	0.22	ns
Iodine value	0.13	ns

ns: no significant difference

From the results of this study, it can be inferred that deterioration in the physicochemical parameters in the containers was in the following order:

earthenware pots > tin containers > plastic containers

It can thus be concluded that plastic containers were the most suitable for the storage of palm oil over the period of four month storage.

References

Abulude, F.O., Lawal, L.O. 2002. Analysis of selected nutrients of wines obtained from felled palm tree. *Pak. J. Sci. Ind. Res.* **45:** 386-387.

Duncan, D.B. 1955. Multiple range and multiple F-test. *Biometrics* **11:** 1-42.

Pearson, D. 1976. *The Chemical Analysis of Foods*, pp. 324-335, Churchill Livingstone, Edinburgh, UK.

Steel, R.G.D., Torrie, J.H. 1980. *Principles and Procedures of Statistics*, p. 311, McGraw-Hill, New York, USA.

Rehman, Z., Salariya, A.M., Habib, F., Zafar, S.I. 2002. Physicochemical changes during storage of ultrahigh temperature processed whole and skimmed buffalo milk. *Pak. J. Sci. Ind. Res.* **45:** 394-399.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 113-117

Hybridization Studies in Normal and Cracked-Skull Diseased African Catfish, *Clarias gariepinus*

O. Fagbuaro*[a], J.I. Awopetu[b], M.A. Faluyi[c] and P.O. Aluko[d]

[a]Department of Zoology, University of Ado-Ekiti, PMB 5363, Ado-Ekiti, Nigeria

[b]Department of Zoology, Obafemi Awolowo University, Ile Ife, Nigeria

[c]Department of Plant Science and Forestry, University of Ado-Ekiti, PMB 5363, Ado-Ekiti, Nigeria

[d]National Institute for Freshwater Fisheries Research, PMB 6006, New Bussa, Nigeria

(received March 8, 2003; revised May 26, 2004; accepted January 25, 2005)

Abstract. Intraspecific hybridization studies were carried out in two strains of *Clarias gariepinus* with acute and mild cephalic-abnormality or cracked-skull disease and non-cephalic or normal species from Ibadan, Oyo State, Southwestern Nigeria, and the Maiduguri strain from Maiduguri, Borno State, Northern Nigeria. The highest percentage of hatchability (84.5%) was recorded in the Maiduguri strain of *C. gariepinus* and the least (67.5%) in the strain with mild cephalic-abnormality. Among the intraspecific hybrids, the highest percentage (76.7%) was recorded in the cross between the female of Maiduguri strain and the male of non-cephalic strain, while the least (72.2%) was recorded in the cross involving the female of Maiduguri strain and the male of strain with cephalic-abnormality. The maximum growth recorded was 4.321g in six weeks in the strain with mild cephalic-abnormality and minimum 3.758 g in the Maiduguri strain. Among the hybrids, maximum growth of 4.086 g was recorded in the cross between the female of Maiduguri strain and the male of non-cephalic, and minimum of 3.582 g in the cross between the female with cephalic-abnormality and the male of Maiduguri strain. There was no occurrence of cephalic-abnormality in the cross between the female of *C. gariepinus* with cephalic-abnormality and the male of Maiduguri strain.

Keywords: hybridization, cephalic-abnormality, hatchability, productivity, *Clarias gariepinus*

Introduction

Clariid catfish (*Clarias* spp., and *Heterobranchus* spp.) are highly valued edible fishes in Africa and constitute prominent commercial aquaculture species. These are widely cultivated in Africa, mainly under semi-intensive systems with an average production level of 40 metric ton/ha/yr (Hecht, 1996). *Clarias gariepinus* has been introduced for intensive culture in some European and Asian countries. Huisman and Richter (1987) reported that *Clarias gariepinus* belongs to an economically important group of fishes in the tropical and subtropical regions. According to Aluko and Popoola (2002), the rapid development of fish production, along with the introduction of super-intensive fish farming systems, have necessitated the concurrent improvement of productivity and adaptability of fish species. This has necessarily involved hybridization which simply means heterospecific insemination. The method is used to combine desirable characteristics of one species with those of another in order to produce offsprings of superior quality than the parent species. Aluko and Popoola (2002) reported that scientists and fish farmers have successfully used the methods of hybridization, selection and inbreeding for fish quality improvement. Intraspecific hybridization is the cross between strain stocks of the same species and has been applied to cause increased fish production, while interspecific hybridization is the cross between two different species. At low stocking densities, diseases are not a serious problem in polyculture or monoculture operations of African catfish. Although some fungal parasites and bacterial diseases may occur, cephalic-abnormality, also known as cracked-skull disease, is a catfish disease which has been reported from intensive pond rearing systems and hatcheries across Africa. The outbreak of the disease has been reported in the Asian catfish species *C. batrachus* and *C. macrocephalus* (Kabata, 1985), and *C. gariepinus* in Israel (Viveen *et al*., 1985), in Central Africa (Huisman and Richter, 1987) and in Nigeria (Awa and Alegbeleye, 1991).

The present study has been carried out to find a permanent solution to the problem of cephalic-abnormality in catfish culture.

Materials and Methods

Source of broodstock. Male and female brooders were obtained from different locations in Nigeria. These included

*Author for correspondence; E-mail: omofagbuaro@yahoo.com

Ibadan (Oyo State) and Maiduguri (Borno State). The affected strains, which were mainly from Ibadan and Maiduguri, were stocked separately in holding concrete tanks at the National Institute for Freshwater Fisheries Research (NIFFR), New Bussa, Niger State, Nigeria for almost two seasons for fattening and were fed on the NIFFR prepared fish feed containing 40% crude protein. Brooders were collected from the tanks by using drag-net and were separated into the two sexes.

Hormone injectcon. Synthetic ovaprim hormone was used for induced breeding of the fish at the dose of 0.5 ml/kg of fish for a latency period of 12 h.

Fertilization procedure. After the latency period of 12 h, milt was collected by sacrificing the male because of the testicular anatomy, which hampers stripping of milt in *Clarias*. Eggs were stripped from females by gently pressing the abdominal region. The collected milt was diluted by physiological saline solution (0.9% NaCl) at a ratio of 1:5 and was used in fertilizing the eggs by using a clean dry feather for mixing.

The parental and intraspecific crosses were carried out, in triplicate, as detailed below.

Parentals. The parental crosses were: female cephalic (cp) x male (cp) (acute); female cephalic (cp) (mild) x male cephalic (cp) (mild); female non-cephalic (ncp) x male non-cephalic (ncp); and female Maiduguri (mai) x male Maiduguri (mai).

Intraspecific hybrids. The intraspecific hybridization was done between: female cephalic (cp) x male non-cephalic (ncp); female cephalic (cp) x male Maiduguri(mai); female non-cephalic (ncp) x male cephalic (cp); female non-cephalic (ncp) x male Maiduguri (mai); female Maiduguri (mai) x male cephalic (cp); and female Maiduguri (mai) x male non-cephalic (ncp).

Incubation. Incubation was carried out in 30 well aerated aquarium tanks with dimensions 60 x 30 x 30 cm^3, each filled with fifteen litres of water. Egg collectors, made up of shreads of loose nylon sack called "kakabans", were placed in each aquarium for the eggs to attach. The temperature range was between 25 °C and 26 °C in the aquaria, with pH 7.1. Hatching occurred 22 h after fertilization, which lasted 6 h. Hatchlings or larvae were carefully counted. The hatching percentage was also determined for each aquarium. 100 post-hatchlings from each mating group were put in different aquaria and the length and weight measurements were done. Feeding was started on the fourth day after hatching, which was done twice a day with the zooplankton, *Moina micrura,* for a period of 10 days. The survival rate for each treatment was determined everyday by direct counting of the fry. Counting was done in small beakers with minimum stress to the fry.

After the indoor rearing, all the ten mating groups were duplicated. From each cross, 80 fry were stocked in 2 x 2 x 1 m^3 concrete tanks. The fry were fed on artificial fish diet with 40% crude protein for a period of six weeks (42 days). Weekly samplings were done for fry growth in length (cm) and weight (g).

Results and Discussion

The percentage hatchability in the ten mating groups involving intraspecific crosses of two strains of *Clarias gariepinus*, a strain with cephalic-abnormality or cracked-skull disease plus normal species of the same strain and another from a different stock was recorded (Table 1). The highest of percentage (84.5%) hatchability was recorded in the Maiduguri strain (female Maiduguri x male Maiduguri) among the parental crosses, and the least (67.5%) in the Ibadan strain with mild

Table 1. Mean percentage daily survival of four strains and six intraspecific hybrids of *Clarias gariepinus*

Mating groups (f x m)	Hatchability (%)	Mean length (mm)	Initial mean weight (g)	Final mean weight (g)	Mean weight gain (g)	Mean weight gain/day (g)
Parental strains						
cp (acute) x cp (acute)	82.5	4.6	0.02	1.25	1.230	0.123
cp (mild) x cp (mild)	67.5	4.4	0.01	0.95	0.940	0.094
ncp x ncp	69.2	3.6	0.15	0.90	0.885	0.089
mai x mai	84.5	4.4	0.01	1.05	1.040	0.104
Intraspecific hybrids						
cp x ncp	73.7	2.6	0.02	1.05	1.035	0.104
cp x mai	75.9	5.0	0.01	1.00	0.990	0.099
ncp x cp	75.5	4.6	0.02	1.05	1.030	0.103
ncp x mai	73.5	4.6	0.01	1.50	1.490	0.149
mai x cp	72.2	2.2	0.01	0.90	0.800	0.080
mai x ncp	76.7	4.5	0.01	0.95	0.940	0.094

f = female; m = male; cp = cephalic-abnormality strain; ncp = non-cephalic-abnormality strain; mai = Maiduguri strain

cephalic-abnormality [female cephalic (cp) (mild) x male cephalic (cp) (mild)].

The highest percentage of hatchability (75.9%) was recorded in the cross between the female cephalic-abnormality (cp) and male Maiduguri (mai) among the hybrids, and the least (72.2%) in the female Maiduguri (mai) and male cephalic-abnormality (cp). In this study, cases with low percentage hatchabilities may have arisen from cephalic-abnormality, poor water quality and probably over-ripening of eggs. The low hatchability of the parental strain with mild cephalic-abnormality may probably be due to other hereditary factors.

The mean length (mm) and weight (g) of hatchlings of the ten mating groups are also shown in Table 1. In the parental groups, the highest final mean weight and length of hatchlings 1.25 g and 4.6 mm, respectively, were recorded for *C. gariepinus* strain with acute cephalic-abnormality, while non-cephalic-abnormality strain had the least final mean weight and were even shorter 0.90 g and 3.6 mm, respectively. Among the intraspecific hybrids, hatchlings with shorter body (about 2.2 mm and 2.6 mm) were produced in the cross between female Maiduguri and male cephalic-abnormality, and female with cephalic-abnormality and male non-cephalic-abnormality, respectively. The hatchlings of the hybrids between female non-cephalic and male Maiduguri strain were heavier (1.50 g), while the hatchlings of the cross between the female cephalic-abnormality strain and the male Maiduguri strain were the longest. The hybrids of the cross between the female Maiduguri strain and the male cephalic-abnormality strain had the least weight (0.90 g), and were likewise shorter in length (2.2 mm).

The mean percentage of the daily survival of hatchlings of the four strains of *C. gariepinus* and their six intraspecific hybrids reared for ten days in indoor aquaria, are shown in Fig 1. Among the pure line strains, *C. gariepinus* with mild cephalic-abnormality had the highest survival rate (78.0%) and the strain with acute cephalic-abnormality had the least survival value (70.0%). Hybrids of the female *C. gariepinus* with cephalic-abnormality and the male without cephalic-abnormality had the highest survival value (89.0%), while the least (39.0%) was in the hybrids of the female *C. gariepinus* without cephalic-abnormality and the male Maiduguri strain at the end of ten days of rearing in aquaria. The reason for the possible low survival value may be due to the trait of cephalic-abnormality.

The average weight of the fry of four strains and six intraspecific hybrids of *C. gariepinus* reared in open concrete tanks for a period of six weeks are shown in Fig.2. Among the parental crosses, the *C. gariepinus* strain with cephalic-abnormality had the highest weight (7.94 g), while the least

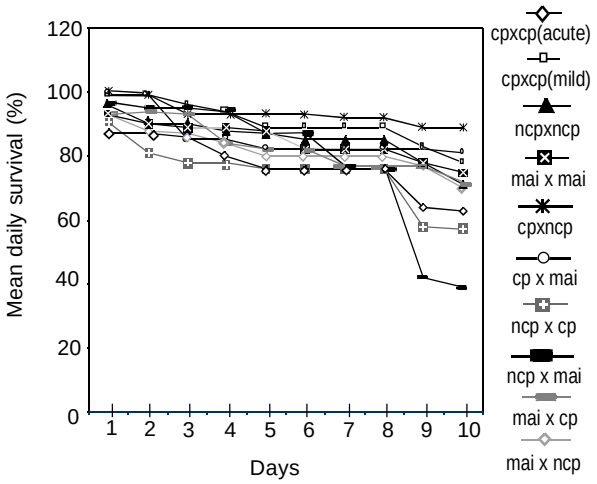


Fig. 1. Mean percentage daily survival of four strains and six intraspecific hybrids of *Clarias gariepinus* maintained in indoor aquaria.

(4.50 g) was noted in the Maiduguri strain of *C. gariepinus*. By the third week in concrete tanks, the Maiduguri strain was heavier than other mating groups (2.01 g), which however later depreciated in weight. It was observed that the disease is hereditary and that the *C. gariepinus* with cephalic-abnormality normally had high growth rate at the early stage of their life. It is possible that the abnormality hastened more digestion and assimilation of food nutrients than occurred in the other strains, suggesting that the possible cause of cephalic-abnormality of cracked-skull disease may not be due to the lack of vitamin C and other nutritional deficiencies as reported by Kabata (1985), Ashley *et al.* (1975) and de Graaf and Janssen (1996). Ashley *et al.* (1975), have further stated that the vitamin C deficiency results in poor wound healing, failure of granulation tissue to fibrose, and bizzarre development of gill, skeletal cartilages and spinal deformities. This further confirmed that cephalic-abnormality or cracked-skull disease is hereditary and could possibly be eradicated by intraspecific and interspecific hybridization of species of *Clarias*.

Awa and Alegbeleye (1991) and Huisman and Richter (1987) reported that the cause of the cracked-skull disease is phytoplankton blooms. This was impossible as there usually were traces of the cephalic-deformation few days after hatching even during the indoor rearing where there were no traces of blooms. Among the intraspecific hybrids, the female Maiduguri strain and the male non-cephalic strain hybrid showed the highest weight in the six weeks of maintenance, while the cross between the female non-cephalic strain and the male Maiduguri strain had the least weight after six weeks of maintenance outside the concrete tanks. The reason for this weight

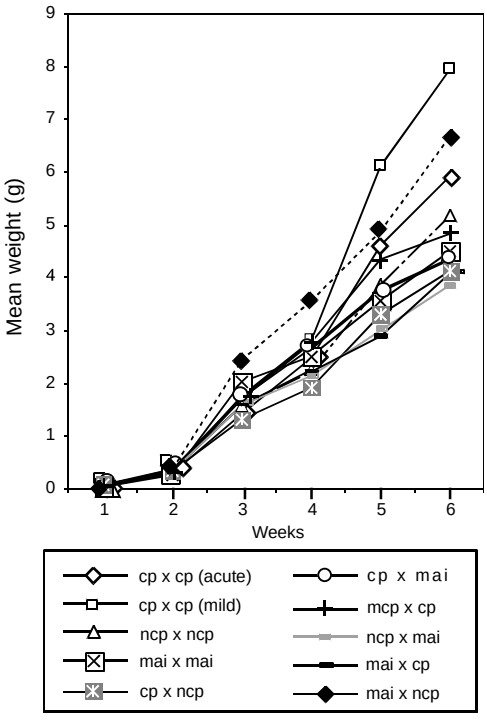


Fig. 2. Mean weight of fry of four strains and six intraspecific hybrids of *Clarias gariepinus* reared in concrete tanks.

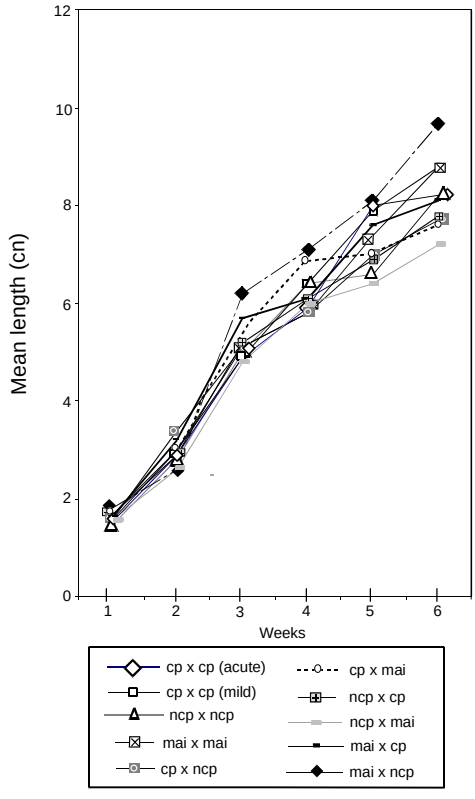


Fig. 3. Mean length of fry of four strains and six intraspecific hybrids of *Clarias gariepinus* reared in concrete tanks.

decrease may not be due to the rate of conversion of food, but may be due to other environmental factors such as stress during sampling and water quality.

The mean length of the fry of the ten genetic groups of *C. gariepinus* reared in concrete tanks for six weeks is shown in Fig.3. The highest mean length of fry (8.84 cm) was recorded for the strain with mild cephalic-abnormality of the parental group and there were no significant differences in the mean length of the parental group. Among the hybrids, the fry with short body (7.20 cm) was produced in the cross between the female non-cephalic strain and the male Maiduguri strain, while the longer ones (9.74 cm) were observed in the cross between the female Maiduguri strain and the male non-cephalic strain of *C. gariepinus.*

The percentage frequency of occurrence of the cephalic-abnormality among the fry of four strains and six intraspecific hybrids of *C. gariepinus* is shown in Fig. 4. There was a drastic reduction in the incidence of cephalic-abnormality in this study with the total elimination of cephalic problems in the cross between the strains with cephalic-abnormality and Maiduguri strain. This hybrid could probably solve the problem of cephalic-deformation, or mating of *C. gariepinus* with cephalic-abnormality with another species of *Clarias* (interspecific hybridization) that could be properly screened for

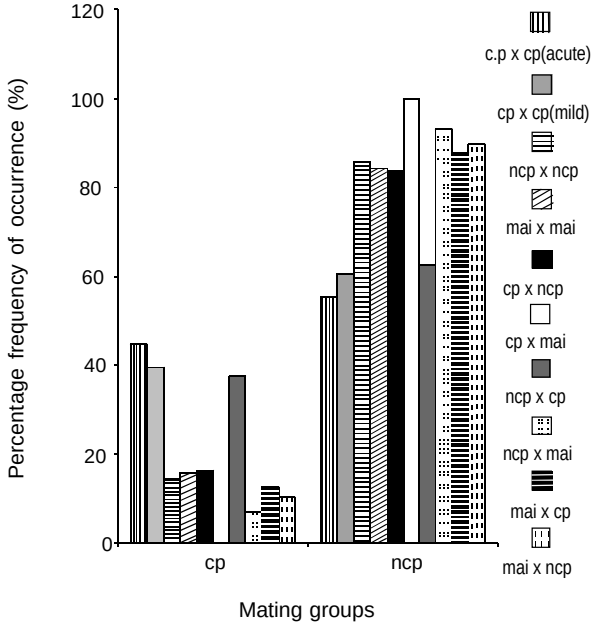


Fig. 4. Percentage frequency of occurrence of cephalic-abnormality among the fry of four strains and six intraspecific hybrids of *Clarias gariepinus* cp, cephalic; ncp, non-cephalic.

cephalic problems and could be properly paired with *C. gariepinus* in terms of chromosomes. The results obtained from this study generally showed that the traces of cephalic-abnormality are common to all strains of *C. gariepinus* regardless of the source or geographical distribution. It is, therefore, recommended that source and occurrence of cephalic abnormality (cracked-skull disease) should be the area of focus in other species of *Clarias* so as to help aquaculturists in finding total solution to this problem and possibly to overcome the problems of inbreeding in hatcheries.

Reference

Aluko, P. O., Popoola, E. O. 2002. Intraspecific hybridization studies in three wild strains of *Heterobranchus longifilis* (Valencienns, 1840). *J. Aquatic Sci.* **17:** 9-12.

Ashley, L.M., Halver, J.E., Smith, R.R. 1975. Ascorbic acid deficiency in rainbow trout and coho salmon and effects on wound healing. In: *The Pathology of Fishes*, W.E. Ribel, G.L. Migaki (eds.), pp. 769-786, The University of Wisconsin Press, Wisconsin, USA.

Awa, J.H., Alegbeleye, W.O. 1991. Occurrence and treatment of cracked-skill disease affecting *Clarias* spp. in homestead ponds in Nigeria. *J. Fish Disease* **14:** 467-473.

de Graaf, G., Janssen, H. 1996. *Artificial Reproduction and Pond rearing of the African Catfish Clarias gariepinus in Sub-Saharan Africa,* FAO Fisheries Technical Paper 362, pp. 1- 73, Food and Agriculture Organization, Rome, Italy.

Hecht, T. 1996. The culture of *Clarias gariepinus* in South Africa with comments on the subsistence aquaculture in Africa. In: *World Fisheries Congress,* pp. 121-135, Oxford and IBM Publishing, New Delhi, India.

Huisman, E.A., Richter, C.J.J. 1987. Reproduction, growth, health control and aquacultural potentials of the African catfish, *Clarias gariepinus* (Burchell, 1822). *Aquaculture* **63:** 1-14.

Kabata, Z. 1985. *Parasites and Disease of Fish Cultured in the Tropics,* pp. 1-150, Taylor and Francis, London, UK.

Viveen, W.J.R., Richter, C.J.J., Van Oordt, P.G.W.J., Janssen, J. A. L., Huisman, E. A. 1985. *Practical Manual for the Culture of African Catfish Clarias gariepinus,* pp. 1-128, Directorate General International Cooperation of Ministry of Foreign Affairs, The Hague, the Netherlands.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 118-121

A Comparative Study of the Food and Feeding Habits of *Chrysichthys nigrodigitatus* and *Brycinus nurse* in a Tropical River

C. G. Oronsaye* and F. A. Nakpodia
Zoology Department, University of Benin, Benin City, Nigeria

(received December 15, 2003; revised November 10, 2004; accepted January 24, 2005)

Abstract. A comparative study of food and feeding habits of two fish species, *Chrysichthys nigrodigitatus* (Lacepede) and *Brycinus nurse* (Ruppel) was carried out in the Ethiope, a tropical river. Fish species were procured from fishermen, using cast-nets, fish traps, hooks and lines for fish catch. Specimens were chilled with ice-blocks in a heat-insulated cooler and transported to the laboratory at the University of Benin for analysis. One hundred fish specimens were examined and their stomach contents analysed. Two methods were applied for the analysis, namely, the frequency of occurrence method and the volumetric method. The result of the analysis showed that *C. nigrodigitatus* was an omnivorous detritivore, while *B. nurse* was a herbivore. Thus, they occupied different ecological niches and hence were found in abundance in the same water body.

Keywords: food/feeding habits, fish species, tropical river, *Chrysichthys nigrodigitatus*, *Brycinus nurse*, Ethiope river

Introduction

Studies on the food and feeding habits of fish species is a subject of continuing research, since it constitutes the basis for the development of a successful fisheries management programme on fish capture and culture. The published work from Africa shows that intensive investigations on the fish species started when Boulenger (1916) produced a catalogue of the freshwater fishes of Africa. Later, Robert (1975) reported the geographical distribution of African freshwater fishes and Welcomme (1979) reviewed food and feeding habits of fish species in the African flood plain rivers. Durr and Gonzalez (2002) studied the feeding habits of *Beryx splendens* and *B. decadactylus* (Berycidae) off the Canary Islands. The effects of metazoan parasites on the feeding behaviour of some fish species in the North Sea were investigated by Klimpel *et al.* (2003). A comparative study on the feeding habits of co-occurring sprat (*Sprattus sprattus*) and cod (*Gadus morhua*) larvae in the Baltic Sea was carried out by Voss *et al.* (2003). The sediment grain size, with regards to the feeding, and assemblage structure of ground fish in the Northeastern continental shelf were studied by Methratta and Link (2004). In respect of Nigeria, Reed *et al.* (1967) had produced a comprehensive record of fish and fisheries of Northern Nigeria, while Fagade and Olaniyan (1973) studied the food and feeding relationships of fishes of the Lagos Lagoon in Western Nigeria. Other investigators who worked on the fishes of Nigeria include, Olatunde (1979) in the upper Ogun River, Adebisi (1981) in Lagos and Lekki Lagoons, Tetsola (1988) in the Niger Delta area and the Warri River, Ikomi and Sikoki (2001) at the River Jamieson, and Oboh *et al.* (2003) also at the Jamieson River.

*Author for correspondence

The Ethiope River is very important for commercial fisheries and the production of palm-wine from the numerous raffia palms (*Raphia hookerii*) growing luxuriantly along its bank. The only published work reported on the river is on the distribution of the fish species by Odum (1995). Thus, this study aims to continue research on the fishes of this river providing a more detailed comparative investigation into the food and feeding habits of the two fish species, *C. nigrodigitatus* and *B. nurse*, which were most abundant in the river. The two fish species selected for this study are also of very high commercial and economic value.

Materials and Methods

Study area. The Ethiope River is located between latitude 5° 57'-5° 45/N and longitude 5° 00'-6° 05/E. It has its source at Umuaja Hills (Fig. 1). From its origin it moves westwards to Sapele where it empties into the Benin River, covering a distance of 88 km. The river traverses an area having the tropical rain forest climate. The rainy season lasts from April to November, while the dry season lasts from December to March.

Procedure of sampling. The sampling zone for this study extended from Umeghe through Jerhe to the Ethiope-Jamieson Rivers Confluence at Sapele. Fish specimens were collected with the help of fishermen hired from Umeghe, using cast-net, fish traps, hooks and lines. The study was carried out from June to October 1999. A total of 100 fish specimens, collected through the fishermen, were chilled with ice-blocks in a heat-insulated cooler and transported to the laboratory at the University of Benin, Nigeria for analysis. Fish identification was done, using the published works and identification

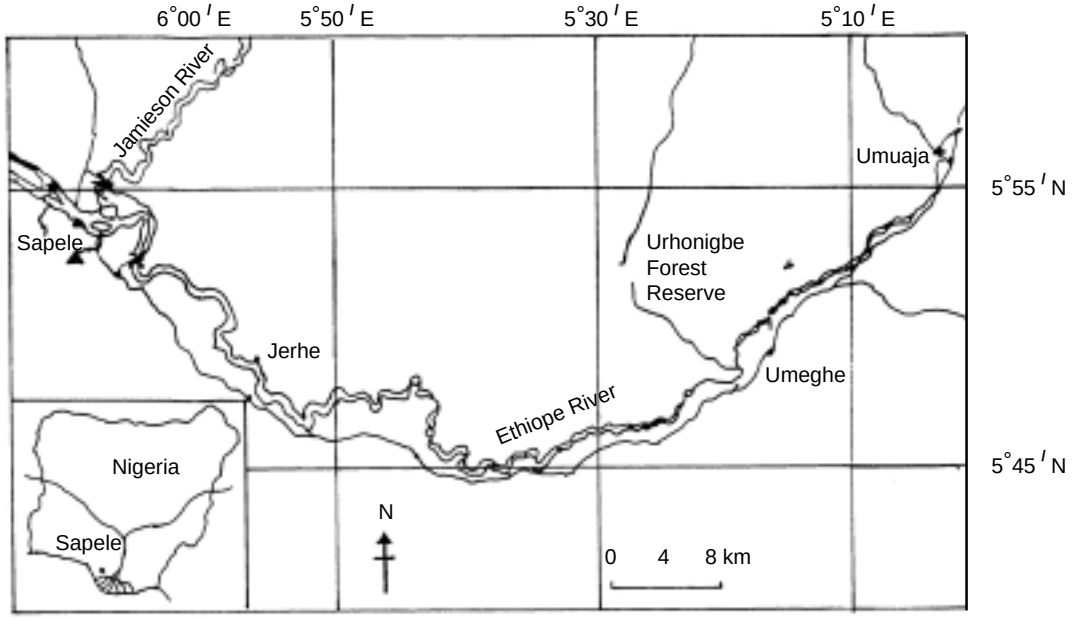


Fig. 1. Map showing the course of Ethiope River and the sampling zones.

guides of Odum (1995), Robert (1975), Holden and Reed (1972), Reed *et al.* (1967) and Boulenger (1916). Each fish specimen was weighed on a top loading balance (Mettler E200) after mopping off excess water with a filter paper. Standard and total lengths of each fish were measured to an accuracy of 1 mm (Lagler, 1964). Standard length was measured from the tip of the snout to the end of the caudal peduncle, while total length was measured from the tip of the snout to the end of the caudal fin. The stomach, from the oesophagus to the pylorus, of each fish was removed and preserved in 5% formalin by dissecting the fish, mid-ventrally from the throat to the anal pore. In order to establish the diet of the fish, each stomach was then slit open and its contents emptied into a petri-dish for analysis. Two methods were employed in the analysis, namely, the frequency of occurrence method and volumetric method.

Frequency of occurrence method. The number of stomachs of each fish species, having different types of food items was recorded. These observations were expressed as the percentage of the total number of stomachs of the species examined and the proportion of the fish population of that species that fed on a particular food item (Hynes, 1950). The percentage frequency of occurrence was then tabulated against each type of food item. This method has also been used by Oboh *et al.* (2003) and Ugwumba and Mbu-Oben *et al.* (1990).

Volumetric method. The volume of the stomach and its food contents for each fish species was determined by the application of Archimedes principle (displacement of water) using a measuring cylinder. The volume of the stomach contents of each fish was then expressed as percentage (%) of the volume of the stomach. Only those stomach samples were used for volume determination that had food contents.

Results and Discussion

The stomach content analysis, using the frequency of occurrence method, is given in Table 1. *Chrysichthys nigrodigitatus* was observed to be clearly a detritivore, having 68% frequency of detritus materials, whereas *Brycinus nurse* had only 24% of this category of food. On the other hand, *B. nurse* was observed to be a herbivore with 70% frequency of plant materials. The breakdown of different kinds of food items eaten by the two fish species is also shown in Table 1. It was most significantly noted that chironomid larvae and threads were absent in *B. nurse*, while fish scales were absent in *C. nigrodigitatus*. The frequency of 28% filamentons algae in the stomachs of *B. nurse*, which was only 2% in the stomachs of *C. nigrodigitatus*, clearly indicates further the herbivorous feeding habit of *B. nurse*. However, the appreciably high percentage frequencies of plant materials, insect parts and fish remains in the stomachs of *C. nigrodigitatus* suggest that this fish species is an omnivorous detritovore.

The stomach contents as analysed by the volumetric method are shown in Table 2. In the case of *C. nigrodigitatus,* 21 fish samples had 87-96%, followed by 1 sample with 75-77%, 6 samples with 50-55%, and 10 samples with 25-27% volume of stomachs filled with food samples. These values respec-

Table 1. Stomach contents analysis of *Chrysichthys nigrodigitatus* and *Brycinus nurse* using frequency of occurrence method

Food items	*C. nigrodigitatus*	*B. nurse*
	Frequency occurrence (%)	Frequency occurrence (%)
Detritus materials	68	24
Filamentous algae	2	28
Plant materials	36	70
Insect parts	34	22
Fish remains	26	20
Chironomid larvae	12	0
Sand grains	42	14
Threads	2	0
Seeds	8	22
Fish scales	0	20
Shrimps	6	8
Unidentified materials	18	16

Table 2. Stomach contents analysis of *Chrysichthys nigrodigitatus* and *Brycinus nurse* as done by the volumetric method*

Fish stomach category by volume	*C. nigrodigitatus*		*B. nurse*	
	Volume of stomach content (as % of the total)	Number of stomachs	Volume of stomach content (as % of the total)	Number of stomachs
¼-Full stomach	25 - 27	10	25 - 27	5
½-Full stomach	50 - 55	6	50 - 55	9
¾-Full stomach	75 - 77	1	75 - 78	3
Full stomach	87 - 96	21	88 - 97	26

*81 stomachs out of 100 were found with food contents, while 19 were found empty

tively corresponded to: full stomach, ¾-full stomach, ½-full stomach, and ¼-full stomach in the ratio of 21:1:6:10 fish samples. The trend for *B. nurse* on the basis of the stomach volume-fil was 26:3:9:5 stomach samples, respectively, for full, ¾-full, ½-full, and ¼-full stomach of this fish species.

The food and feeding habits of *B. nurse* and *C. nigrodigitatus* in the Ethiope River as reported in the present study is in agreement with earlier reports for these fish species found in some other water bodies. For instance, Ikusemiju and Olaniyan (1977) noted that *C. nigrodigitatus* fed on gastropods, ostracods, detritus and plant materials in the Lekki Lagoon. Brown (1985) further reported that this fish species fed mainly on detritus, insects and plant materials in Ikpoba River. In the case of *B. nurse*, Reed *et al.* (1967) recorded that the fish species feed on insects, snails and plant materials in Northern Nigeria. Ikomi and Sikoki (2001) also observed that the presence of tiny unicuspid teeth in the mouth of the fish suggest that this fish species feeds on plants, leaf buds and seeds of water lilies, and is thus a herbivorous feeder.

Conclusion

The comparative study on the food and feeding habit of *C. nigrodigitatus* and *B. nurse* from the Ethiope River has revealed that the former is an omnivorous detritivore, whereas the latter is a herbivore. These two fish species thus occupy different ecological niches, which explains their presence in abundance within the same water-body. Also, *C. nigrodigitatus* is known to be a bottom dweller, while *B. nurse* is a mid-water swimmer, which confirms the observation that they occupy different ecological niches within the same river.

Acknowledgement

The corresponding author expresses gratitude to Dr. S.C. Okoli, Associate Professor of Education, University of Benin, Nigeria, for encouragement in the education and research interests of the author (C.G. Oronsaye).

References

Adebisi, A.A. 1981. Analysis of stomach contents of Piscivorous fishes of upper Ogun rivers in Nigeria. *Hydrobiologia* **79:** 167-177.

Boulenger, G.A. 1916. *Catologue of the Freshwater Fishes of Africa in the British Museum*, vol. **4,** pp. 1-392, Natural History Museum, London, UK.

Brown, A.C. 1985. The Food and Feeding Relationships of Non-Cichlid Fish in Ikpoba River Downstream of Dam, *M.Sc Thesis*, Department of Zoology, University of Benin, Nigeria.

Durr, J., Gonzalez, J.A. 2002. Feeding habits of *Beryx splendens* and *Beryx decadactylus* (Berycidae) off the Canary Islands. *Fish. Res.* **54:** 363-374.

Fagade, S.O., Olaniyan, C.I.O. 1973. The food and feeding interrelationship of the fishes in the Lagos Lagoon. *J. Fish Biol.* **5:** 205-225.

Holden, M., Reed, W. 1972. *West African Freshwater Fishes*, pp. 1-68, 1st edition, Longman, London, UK.

Hynes, H.B.N. 1950. Food of freshwater sticklebacks *(Gasterosteus aculeeatus* and *Pygosteus pungitius)* with a review of methods used in the studies of the food of fishes. *J. Animal Ecol.* **19:** 36-58.

Ikomi, R.G., Sikoki, F.D. 2001. Studies on the distribution, abundance, growth pattern and dietary habits of *Brycinus nurse* Ruppel 1832 (Osteichthyes: Characidae) in the River Jamieson, Nigeria. *Acta Ichthyl. Piscat.* **31:** 27-44.

Ikusemiju, K., Olaniyan, C.I.O. 1977. The food and feeding

habits of the catfishes, *Chrysichthys walkari* (Gunther), *Chrysichthys nigrodigitatus* (Lacepede) and *Chrysichthys filamentous* (Boulenger) in the Lekki Lagoon, Nigeria. *J. Fish Biol.* **10:** 105-112.

Klimpel, S., Seehagen, A., Palm, H.W. 2003. Metazoan parasites and feeding behaviour of four small-sized fish species from the Central-North Sea. *Parasitol. Res.* **91:** 290-297.

Lagler, K.F. 1964. *Freshwater Fishery Biology,* pp. 1-545, 2nd edition, John Wiley and Sons, New York, USA.

Methratta, E., Link, J. 2004. Sediment grain size and ground fish assemblage structure in the Northeastern continental shelf ecosystem. *NEFSC Science Symposium,* February, 2004, Atlantic City, NJ, USA.

Oboh, I.P., Edema, C.O., Tongo, O.L. 2003. The food and feeding habits of fishes of the Jamieson River, Nigeria. *Pak. J. Sci. Ind. Res.* **46:** 255-260.

Odum, O. 1995. Fish distribution in Ethiope River, Southern Nigeria. *J. Trop. Freshwater Biol.* **4:** 55-64.

Olatunde, A. A. 1979. The food and feeding habits of *Physailia pellucida* and *Schilbe mystus* with notes on the diet of *Siluranodon auritus* and *S. uranoscopus,* Family: Schilbeidae (Osteichthyes: Siluriformes) from Lake Kainji, Nigeria. *Freshwater Biol.* **9:** 183-190.

Reed, W., Burchard, J., Hopson, A. J., Jannes, J., Yaro, I. 1967. *Fish and Fisheries of Northern Nigeria,* pp. 1-266, 1st edition, Ministry of Agriculture, Northern Nigeria, Zaria, Nigeria.

Robert, T.R. 1975. Geograpahical distribution of African freshwater fishes. *Zool. J. Lim. Soc.* **57:** 247-319.

Tetsola, F.A. 1988. Distribution and Feeding Relationship of the Fishes of Warri River, Nigeria, *Ph.D Thesis*, Department of Zoology, University of Benin, Nigeria.

Ugwumba, A., Mbu-Oben, A. 1990. Food, feeding ecology of the mormyrids of Lekki Lagoon, Nigeria. *Nig. J. Nat. Sci.* **5:** 38-46.

Voss, R., Koster, F. W., Dickmann, M. 2003. Comparing the feeding habits of co-occuring sprat (*Sprattus sprattus*) and cod (*Gadus morhua*) larvae in the Bornholm Basin, Baltic Sea. *Fish. Res.* **63:** 97-111.

Welcomme, R.I. 1979. *Fisheries Ecology of Flood Plain Rivers,* pp.1-317, 1st edition, Longman, London, UK.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 122-125

Biological Evaluation of Extracts and Triterpenoids of *Euphorbia hirta*

M. Abu-Sayeed*[a], M. Abbas Ali[b], P.K. Bhattacharjee[b], Anwarul Islam[b], G.R.M. Astaq[b], Mohal Khan[c] and Sharmina Yeasmin[c]

[a]Department of Applied Chemistry and Chemical Technology, Rajshahi University, Rajshahi-6205, Bangladesh
[b]Department of Chemistry, Rajshahi University of Engineering and Technology, Rajshahi-6204, Bangladesh
[c]BCSIR Laboratories, Rajshahi-6206, Bangladesh

(received November 17, 2003; revised January 10, 2005; accepted January 25, 2005)

Abstract. Antibacterial and antifungal activities of crude extracts and two triterpenoids, taraxerone (EH-1) and 11α, 12α-oxidotaraxerol (EH-2) isolated from the plant *Euphorbia hirta* were tested against fourteen pathogenic bacteria and six fungi. Crude extracts and pure compounds exhibited significant activity against most of the bacteria tested. On the other hand, all the crude extracts and pure compounds were active, but not significant enough, against most of the tested fungi. The minimum inhibitory concentrations (MICs) of the isolated compounds were also determined against the tested organisms (10^7 cells/ml) and the effective values were found to be between 64-128 μg/ml. In the brine shrimp lethality bioassay, the compounds were screened for their probable cytotoxic activity, and the LC_{50} values of EH-1 and EH-2 were found to be 17.78 and 10 μg/ml, respectively.

Keywords: *Euphorbia hirta*, triterpenoids, brine shrimp lethality bioassay, antibacterial activity, antifungal activity, taraxerone, 11α,12 α-oxidotaraxerol

Introduction

Many microorganisms can cause several diseases and now, in this world of modern science, human beings are capable of facing any challenge against these diseases. In spite of the tremendous advancement of medical science and technology, nevertheless, diseases are the leading health problem, particularly in the under-privileged populations in the remote rural areas in the developing countries. In Bangladesh, a very poor country with poor hygiene, diarrhoea, cholera, typhoid, malaria, diptheria, etc., are major causes of morbidity and mortality.

Euphorbia hirta, Family Euphorbiaceae, is commonly grown in almost all the districts of Bangladesh, hotter parts of India, and other tropical and subtropical countries, where the plant is used in traditional medicine for several illnesses since the time immemorial. Juice of the plant is given in the treatment of aphthae, dysentery and colic diseases, and is used by women to increase the flow of milk. A decoction is used to treat asthma and chronic bronchial affections. The plant is chiefly prescribed in the treatment of cough, gonorrhoea and bowel complaints. On the Gold Coast, it is ground and mixed with water and used as an enema (Kirtikar and Basu, 1996).

Due to the fact that this plant is very useful, as found by previous reports and the fact that little information is available on its biological activity, there is a need to find out more about the potential of this plant as an antimicrobial agent. The present study is, therefore, designed to assess the potency of the plant extracts and triterpenoids isolated therefrom on selected microorganisms and also to determine the cytotoxic effect of these compounds.

*Author for correspondence E-mail: radwiya44@yahoo.com

Materials and Methods

Source of the plant and microbiological cultures. The plant *Euphorbia hirta,* was collected from the Rajshahi University Campus, Bangladesh. Organisms used in the present studies were obtained from the Department of Pharmacy, Rajshahi University, pure cultures of which were previously procured from the Institute of Food and Nutrition, University of Dhaka, and also from ICDDR, Bangladesh. All solvents used during this study were redistilled and purified. Other chemicals, including the culture media used, were of analytical grade unless otherwise specified.

Plant material preparation, extraction procedure, and compound identification. The whole plant of *E. hirta* was cut into small pieces, dried in an oven at 40 °C to a constant weight, pulverized into fine powder in a grinding machine, and stored in an airtight container. One kg of the powdered plant material was exhaustively extracted with rectified spirit at room temperature. Solvent of the extract was evaporated under reduced pressure and the extract was fractionated with petroleum ether

(40-60 °C), chloroform and methanol. Compounds EH-1 and EH-2 (Fig .1) were isolated from the petroleum ether extract by column chromatography (Beckett and Stenlake, 1986), followed by TLC and preparative TLC (Egon, 1969). The compounds were identified as taraxerone (EH-1) and 11α, 12α-oxidotaraxerol (EH-2) on the basis of spectral data coupled with physical and chemical evidences (Bhattacharjee, 2002),

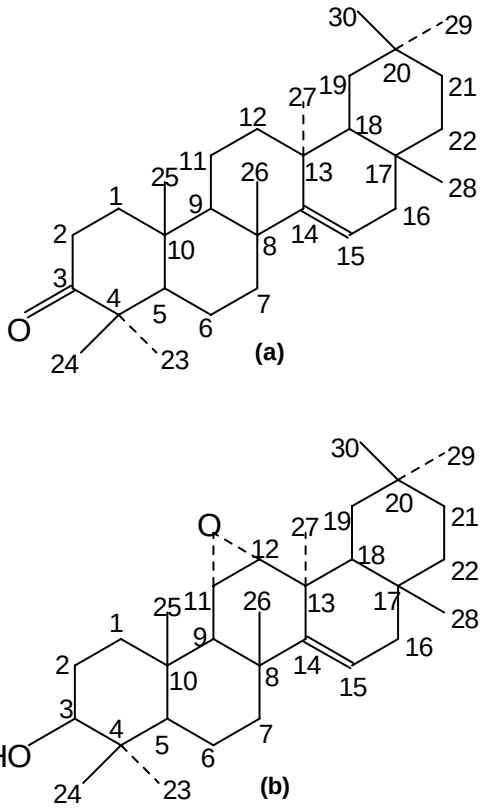


Fig. 1. Compounds isolated from the petroleum ether extract of *Euphorbia hirta,* identified as (a) taraxerone (EH-1) and (b) 11α, 12 α-oxidotaraxerol (EH-2).

and also by comparison with the previously reported values (Kuo *et al.,* 1996; Tanaka and Matsunaga, 1988).

Antimicrobial screening. *In vitro* antibacterial and antifungal screenings were performed with the crude petroleum ether, chloroform and methanol extracts, as well as the isolated pure compounds (EH-1 and EH-2) against 14 pathogenic bacteria, 5 of which were gram-positive and 9 were gram-negative, and 6 pathogenic fungi by the standard disc diffusion method (Barry, 1980; Bauer *et al.,* 1966). Nutrient agar medium was used for determining the antibacterial activity, whereas potato dextrose agar medium (PDA) was selected for antifungal screening. Standard antibiotic discs of kanamycin (30 µg/disc) and fluconal (50 µg/disc) were used for the comparison of antibacterial and antifungal efficiency tests, respectively.

The crude extracts were dissolved in sufficient amounts of the respective solvents so that each 15 µl solution contained 400 µg of the test material. On the other hand, compounds EH-1 and EH-2 were dissolved separately in sufficient volume of chloroform to get a concentration of 200 µg/10 µl. The antimicrobial activities were determined by measuring the diameter of the inhibitory zones in mm using a transparent scale. The diameters of the zones of inhibition produced by the tested samples were then compared with the diameters of the zones of inhibition produced by the standard antibiotic discs used.

Minimum inhibitory concentration (MIC). The MIC values of compound EH-1 were determined against the gram-positive *Sarcina lutea* and the gram-negative *Shigella dysenteriae* (10^7 cells/ml), while the MIC values of compound EH-2 were evaluated against the gram-positive *Streptococcus aureus* and the gram-negative *Shigella dysenteriae* (10^7 cells/ml) by the serial dilution technique (Reiner, 1982). Nutrient agar and nutrient broth were used as bacteriological media.

Brine shrimp lethality bioassay. Cytotoxic effects of compounds EH-1 and EH-2 were evaluated by determining LC_{50}, using the brine shrimp lethality test (Mayer *et al.,* 1982; Persoone, 1980). The test sample was dissolved in DMSO and specific volumes were transferred to the different vials containing 10 living shrimp nauplii to which seawater was added to make the volume upto 5 ml in each vial. The final concentrations of the test sample in the vials was 6, 12, 24, 48 and 96 µg/ml. Three replicates were done for each concentration. A control was also run similarly, by taking 10 living shrimps nauplii in 5 ml seawater, but without any test sample isolated from the plant. The same assay procedure was performed with standard ampicillin trihydrate for comparison of the efficiency of the test samples.

After incubation for 24 h, the vials were observed and the number of shrimp nauplii deaths in each vial was counted, using a magnifying glass. From these data, the mean percentage of mortality of the nauplii was calculated for each concentration of the test sample used.

Results and Discussion

As shown in Table 1, all the extracts (petroleum ether, chloroform, and methanol) displayed mild to moderate activity against most of the tested bacteria. The results were compared with those of kanamycin as the standard antibiotic. Of the three extracts, chloroform extract did not show any activity against gram-negatives, *Pseudomonas aeruginosa* and

Escherichia coli, whereas methanol extract was inactive only against the gram negative, *Pseudomonas aeruginosa*. On the other hand, the purified compounds, EH-1 and EH-2, isolated from *E. hirta,* were found to be active against all the tested bacteria. The compound EH-1 exhibited strong activity against the gram-positive *Sarcina lutea* (16 mm) and gram-negative *Shigella dysenteriae* (17 mm), whereas the compound EH-2 showed strong activity against the gram-positive *Streptococcus aureus* (18 mm) and the gram-negative *Shigella dysenteriae* (16 mm). Results depicted in Table 2 demonstrate that all the extracts and the pure compounds, EH-1 and EH-2, were active but not significantly enough, against most of the fungi tested. Furthermore, they did not have any activity against *Aspergillus niger* and *Trichoderma viride*.

During the preliminary screening work, it was found that the compound EH-1 was strongly active against the gram-positive *Sarcina lutea* and the gram-negative *Shigella dysenteriae,* whereas compound EH-2 displayed strong activity against the gram-positive *Streptococcus aureus* and the gram-negative *Shigella dysenteriae*. An attempt was, therefore, made to determine the minimum inhibitory concentrations (MICs) of these compounds against the above mentioned organisms. As shown in Table 3, MIC values of the compound EH-1 were found to be 64 µg/ml and 128 µg/ml against *Sarcina lutea* and *Shigella dysenteriae,* respectively, and those of EH-2 were the same, i.e., 128 µg/ml against *Streptococcus aureus* and *Shigella dysenteriae*. It is expected that further work on the plant may yield clinical success for controlling diseases caused by pathogenic bacteria.

Compounds EH-1 and EH-2 exhibited positive results on brine shrimp lethality bioassay, indicating toxic biological activity. The mortality rate of brine shrimp nauplii was found to increase with the increase in concentration of the compounds and a plot of log of concentration against percentage of mortality gave almost linear correlation (Fig. 2). From the graph, the LC_{50} (concentration at which 50 % mortality of the nauplii occured), as estimated by extrapolation, was found to be 17.78 µg/ml for EH-1, and 10.00 µg/ml for EH-2 (Table 4).

Table 1. Antibacterial activities of different extracts and the purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta*

	Diameter of zone of inhibition (mm)					
Test organisms	A	B	C	D	E	F
Gram-positive bacteria						
Bacillus subtilis	10	12	14	10	11	29
B. cereus	12	9	13	13	10	28
B. megaterium	10	12	11	9	14	30
Sarcina lutea	14	16	13	16	12	25
Streptococcus aureus	13	10	14	10	18	23
Gram-negative bacteria						
Escherichia coli	9	0	10	13	10	20
Shigella dysenteriae	12	10	14	17	16	22
S. sonnei	10	11	12	12	11	25
S. shiga	11	10	13	11	10	22
S. boydii	13	9	11	14	13	24
S. flexneriae	10	11	13	12	11	22
Pseudomonas aeruginosa	10	0	0	10	12	30
Salmonella typhi	10	10	12	12	14	24
Klebsiella sp.	13	10	12	14	13	23

A= petroleum ether extract (400 µg/disc); B = chloroform extract (400 µg/disc); C = methanol extract (400 µg/disc); D = EH-1 (200 µg/disc); E = EH-2 (200 µg/disc); F = kanamycin (30 µg/disc)

Table 2. Antifungal activities of different extracts and the purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta*

	Diameter of zone of inhibition (mm)					
Test organisms	A	B	C	D	E	F
Aspergillus flavus	10	8	12	12	10	17
Aspergillus niger	0	0	0	0	0	0
Penecillum sp.	7	8	10	8	10	11
Trichoderma viride	0	0	0	0	0	10
Candida albicans	10	8	12	13	10	16
Botryodiplodia theobromae	7	9	10	10	9	14

A = petroleum ether extract (400 µg/disc); B = chloroform extract (400 µg/disc); C = methanol extract (400 µg/disc); D = EH-1 (200 µg/disc); E = EH-2 (200 µg/disc); F = fluconal (50 µg/disc)

Table 3. Minimum inhibitory concentration (MIC) values of purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta* against the tested bacterial organisms

Test organisms	Tested sample	Concentrations of the tested sample (µg/ml)									
		512	256	128	64	32	16	8	4	2	1
Sarcina lutea	EH-1	-	-	-	-	+	+	+	+	+	+
Shigella dysenteriae		-	-	-	+	+	+	+	+	+	+
Strept.aureus	EH-2	-	-	-	+	+	+	+	+	+	+
Shigella dysenteriae		-	-	-	+	+	+	+	+	+	+

- = no growth; + = growth

Table 4. Cytotoxicity of purified compounds, EH-1 and EH-2, isolated from *Euphorbia hirta,* using brine shrimp lethality bioassay

Test samples	Conc (µg/ml)	Log conc (Log C)	Number of shrimps died* vial 1	vial 2	vial 3	Average number of deaths	Mortality (%)	LC_{50} (µg/ml)
Ampicillin trihydrate	6	0.7781	4	5	4	4.333	43.33	
	12	1.0791	6	6	7	6.333	63.33	
	24	1.3802	7	8	8	7.666	76.66	
	48	1.6812	9	9	10	9.333	93.33	
	96	1.9822	10	10	9	9.666	96.66	7.49
EH-1	6	0.7781	3	3	3	3.000	30.00	
	12	1.0791	4	5	4	4.333	43.33	
	24	1.3802	6	6	5	5.666	56.66	
	48	1.6812	7	7	6	6.666	66.66	
	96	1.9822	8	8	8	8.000	80.00	17.78
EH-2	6	0.7781	4	4	4	4.000	40.00	
	12	1.0791	5	5	6	5.333	53.33	
	24	1.3802	6	7	7	7.000	70.00	
	48	1.6812	8	8	9	8.333	83.33	
	96	1.9822	9	10	10	9.666	96.66	10.00

*10 shrimp nauplii were taken in each vial; control comprised of no test sample added, in which no deaths occurred

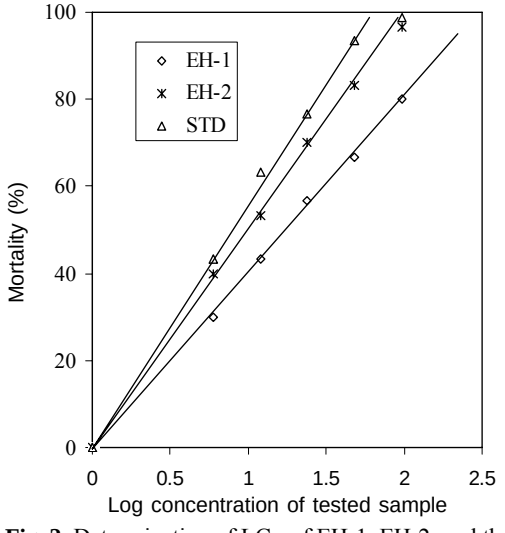


Fig. 2. Determination of LC_{50} of EH-1, EH-2, and the standard ampicillin trihydrate (STD) against brine shrimp nauplii.

References

Barry, A. L. 1980. *Procedures for Testing Antimicrobial Agents in Agar Media: Antibiotics in Laboratory Medicine*, pp. 1-23, Williams and Wilkins Co., Baltimore, USA.

Bauer, A.W., Kirby, W.W.M., Sherris, J.C., Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clinic. Pathol*. **45:** 493-496.

Beckett, A.H., Stenlake, J.B. 1986. *Chromatography: Practical Pharmaceutical Chemistry*, vol **2**, pp. 75-76, 3rd edition, New Delhi, India.

Bhattacharjee, P.K. 2002. Physicochemical and Biological Studies on *Euphorbia hirta* Linn. and *Trema orientalis* (L.) BL. *M.Sc. Thesis,* Department of Applied Chemistry and Chemical Technology, Rajshahi University, Bangladesh.

Egon, S. 1969. *Thin Layer Chromatography: A Laboratory Handbook*, p. 112, 2nd edition, Springer Verlag, New York, USA.

Kirtikar, K.R., Basu, B.D. 1996. *Indian Medicinal Plants*, vol. **3**, pp. 2197-2199, International Book Distributors, Dehradun, India.

Kuo, Y.H., Way, S.T., Wu, C.H. 1996. A new triterpene and a new lignin from *Saussurea japonica*. *J. Nat. Prod.* **59:** 622-624.

Mayer, B.N., Ferringni, N.R., Putnam, J.E, Jacobsen, J.B., Nichols, D.E., McLaughlin, J.L .1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* **45:** 31-34.

Persoone, G. 1980. *Proceeding of the International Symposium on Brine Shrimp Artemia salina*, vol **3**, pp. 1-3, Universal Press, Belgium.

Reiner, R. 1982. *Detection of Antibiotics Activity: Antibiotics, An Introduction,* pp. 21-25, Roche Scientific Service, Switzerland.

Tanaka, R., Matsunaga, S. 1988. Triterpene constituents from *Euphorbia supina*. *Phytochemistry* **27:** 3579-3584.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 126-127

Short Communication

Blood Clotting Effect of Leaf Extracts of *Bryophyllum pinnatum*

J. M. Okuo* and I. M. Ejimadu
Chemistry Department, Faculty of Science, University of Benin, Benin City, Nigeria

(received February 20, 2003; revised December 9, 2004; accepted December 13, 2004)

Abstract. Clotting time of blood samples drawn from eight patients was determined using three variants of plant extracts (crude, aqueous and chloroform) of the leaf of *Bryophyllum pinnatum*. These extracts clotted the test blood samples faster than the untreated blood samples, which ranged between 0.34-3.27 min. Blood samples pretreated with heparin (anticoagulant) did not clot, to any extent, when treated with the *B. pinnatum* extracts. All the test blood samples showed different clotting times. The clotting efficiency of the different extracts was crude > aqueous > chloroform > control. The clotting time for different test blood samples, not treated with any variant of the plant extracts (control samples), ranged between 10.363±0.012 and 14.483±0.008 (min). Normal blood clotting time is between 6-10 min, which may indicate deficiencies in one or more clotting factors in the untreated test blood samples drawn from the study subjects.

Keywords: blood clotting, *Bryophyllum pinnatum*, anticoagulant

Blood is a very vital medium of life in the humans. Plasma is the fluid portion of the blood, which constitutes 55% of its volume (Guyton, 1986). Plasma clots on standing, but remains in liquid form if an anticoagulant is added. It is important that the fluid nature of blood is maintained in normal living systems. In certain situations, blood is lost or spilled from open injuries or internal haemorrhage (Guyton, 1996). Bleeding is arrested when clots are formed in the blood (hemostasis). Excessive bleeding can be caused by deficiency of any one of the many blood-clotting factors. However, three major types of bleeding tendencies, namely, vitamin K deficiency, thrombocytopeonia (platelet deficiency), and haemophilia have been studied (Harker, 1987). Excessive bleeding time is desirable to be reduced. The aim of this study was to find out natural remedies based on a plant material for controlling excessive bleeding. This study is expected to be further useful for finding natural agents that may be applied to solve the bleeding problems of haemophiliacs. These natural agents will replace the very expensive clotting factor VIII (antihaemophilic factor A, antihaemophilic globulin), or factor IX (plasma thromboplastin component, antihaemophilic factor B), and become widely available, if discovered (Charin, 1984). Anti-coagulants prevent clot formation, for example, heparin. These act by preventing the activation of factor XI (plasma thromboplastin) and by inhibiting the action of thrombin (Cleavon, 1993), histamine, oxalates and coumarin (dicoumarol derivatives, which inhibit the action of vitamin K). Coagulants, on the other hand, are chemical principles that activate the formation of thrombin and encourage clot formation.

*Author for correspondence; E-mail: okuojm@yahoo.com

Chemical agents present in some plant extracts are known to have coagulant properties. The leaf sap of *Bryophyllum pinnatum* is used in Ivory Coast to stop bleeding of cuts and in Mexico for the treatment of failure of menstruation. The leaf mash compounded with palm oil or butter is applied to treat wounds, burns, abscesses, ulcers, sores, swellings and pains. The plant is an ingredient of a prescription used in Congo to hasten the expulsion of the after-birth fluids. Among the numerous medicinal attributes, the plant is reported to have antidiarrhoea, antiulcer, antiinflamatory, antidiabetic, antipyretic, antibacterial, antifungal, and spasmogenic effects. It is used in the Philippines as analgesic to treat headache and rheumatism, in the Ivory Coast for earache and ophthalmic emergencies, in Gabon for itching, in Congo against allergic inflammation, fungal and eczematous infections, and in Nigeria as a diuretic. On account of the wide spectrum of medicinal uses, including those related with blood problems, the leaf extract of *B. pinnatum* was investigated for its blood clotting ability.

Prepration of the extracts. Fresh leaves of *B. pinnatum* were collected (September) from around the Pharmacognosy Department, University of Benin and from the adjacent Ekosodin Village. About 3.5 kg of the leaves were chopped and immersed in distilled water (6 litres) and boiled (1 h). The supernatant was decanted, cooled and collected as the aqueous extract, which was concentrated in a rotatory evaporator and stored in amber coloured bottles as the crude extract. To a part of this crude extract was added 100 ml distilled water and twice its volume of chloroform in a separatory funnel. Both the chloroform and aqueous extracts were separated and further concentrated. Three different extracts were thus obtained

Table 1. Coagulation time (min) of human blood samples treated with crude, aqueous and chloroform extracts of *Bryophyllum pinnatum* leaves

Blood sample code	Crude	Aqueous extract	Chloroform extract	No extract**
A	11.21±0.01	11.34±0.01	14.41±0.18	14.48±0.01
B	10.47±0.01	11.02±0.02	12.18±0.03	12.23±0.02
C	10.06±0.02	10.33±0.02	11.25±0.83	11.11±0.01
D	10.02±0.02	10.07±0.06	10.39±0.01	10.36±0.01
E	10.50±0.02	11.02±0.01	11.14±0.02	11.19±0.01
F	12.10±0.01	12.20±0.01	12.55±0.03	13.20±0.01
G	11.58±0.01	12.11±0.01	12.54±0.01	13.03±0.02
H (control-1)*	no coagulation	no coagulation	no coagulation	no coagulation

*blood samples pretreated with the anticoagulant, heparin (control-1); **blood samples not treated with *B. pinnatum* extracts (control-2).

by this process, namely, the crude extract (obtained on boiling the leaves in distilled water), the chloroform extract, and the aqueous extract. These three extracts were used in the blood clotting tests.

Blood clotting tests. Eight blood samples were tested for clotting; seven with the three variants of the *B. pinnatum* extracts and one with the anticoagulant heparin as the control-1. To 0.2 ml each of the three extracts were separately added 0.5 ml of the test blood samples. Control-2 comprised of blood samples to which neither heparin (as anticoagulant), nor *B. pinnatum* extracts (as coagulants), were added. Runs were done in triplicate. Observations for blood clotting were recorded every 30 sec and the time taken for clotting was recorded in min (Table 1).

The seven blood samples showed different clotting time. The crude extract induced clotting at relatively faster rate than the aqueous or the chloroform extracts. The order of clotting was observed to be: crude > aqueous > chloroform > control-2 (untreated test blood). The rate of clotting was fastest in the presence of crude extract, which in comparison with control-2 was faster between 0.34-3.27 min. No clotting in control-1 containing heparin was observed. It is significant to note that the normal clotting time of blood samples to which no *B. pinnatum* extracts were added (control-2) was quite high (10.36-14.48 min) than the usually expected 6-10 min, which may indicate a deficiency of one or more clotting factors in the population from which the blood samples were taken. Further studies on the chemical constituents in the leaves of *B. pinnatum* responsible for blood clotting are indicated.

Acknowledgement

University of Benin is acknowledged for funding this project, and University of Benin Teaching Hospital is acknowledged for supplying the blood samples. We are grateful to our colleagues in the Chemistry Department, particularly Dr. P. O. Okolo, from whom we received informations relevant to this study.

References

Chavin, S.I. 1984. Factor III: structure and function in blood clotting. *Am. J. Hematol.* **16:** 279-297.

Cleavon, G. 1993. *The Design of Synthetic Inhibitors to Thrombin,* pp. 81-143, Plenum Publishing Corp., New York, USA.

Guyton, A.C. 1996. *Medical Physiology,* pp. 469-472, 9th edition, W.B. Saunders and Co., New York, USA.

Guyton, A.C. 1986. *Medical Physiology,* pp. 146-148, 8th edition, W.B. Saunders and Co., New York, USA.

Harker, I.A. 1987. Acquired disorders of platelet function. *Am. N. Y. Acad. Sci.* **509:** 18.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 128-130

Short Communication

Development of Stabilized Vegetable Amylases for Enzymatic Desizing of Woven Fabric with Starch Containing Sizes

S. Ishrat Ali* and A. Farhan Khan
Department of Applied Chemistry and Chemical Technology, University of Karachi-75270, Pakistan

(received August 24, 2002; revised February 28, 2005; accepted March 4, 2005)

Abstract. Investigations have been carried out on the development of stabilized vegetable amylases for enzymatic desizing of woven fabric. Vegetable amylases from barley and germinated mango seeds were extracted and stabilized for industrial use. The desizing of woven cotton fabric was carried out with these amylases. Their desizing performance was also compared with commercially available enzymes. As a result of this study, highly active and stabilized amylases were obtained from barley and germinated mango seeds. The method used for the enzyme recovery was also noted to give good yield from both the sources of plant origin.

Keywords: barley amylase, germinated mango seed amylase, desizing enzyme, enzyme stabilization, woven fabric

The first crude use of enzymes in textile processing was done in 1857 when starch-sized cloth was soaked with liquor containing barley. Later, in 1900, this process was slightly improved by using malt extract. The process of enzymatic desizng, using animal and bacterial amylases, was introduced in many textile factories in 1912 (Cavaco and Gubitz, 2003). Weaving is one of the oldest arts known. Woven fabric consists of sets of yarns, interlaced at right angle in some established sequence or pattern. The yarns that run parallel to the selvage or the longer diameters of a bolt of fabric are called warp yarns, those that run crosswise of the fabric are called weft yarns. Starch-containing sizes are applied to the warp yarn of woven fabrics to assist in the weaving process, which however must be removed prior to dyeing and printing processes. The removal of the starch size from the cotton yarn is called the desizing process (Shenai, 1991). Amylase enzyme that specifically acts on starch is considered to be the favourable option for the solubilization of starch into glucose and maltose (Bergmeyer, 1974). Enzymatic desizing is now regarded as the most safe and economical method. In order to reduce the cost of production of the desizing enzymes, an attempt has been made to extract amylases from the indigenous resources available in Pakistan. For this purpose, germinated barley and mango seeds were used for the extraction of amylase. The study on this aspect is reported here.

Extraction of barley amylase. Barely is one of the earliest known grains. It is grown in tropical regions of Pakistan. Barley is used in soups, in animal feed, in the production of malt for beer and commercial alcohol (Brooks, 1962). Selected seeds of barley were steeped in water for 2-7 days. Steeping for three days at 20-25 °C was found to be the optimum period. The steeped seeds were dried for 3 days at room temperature. After drying, the barley seeds were spread over cotton sheet and turned periodically to maintain uniformity of moisture, temperature and proper aeration. Germination started, after 4 days, which continued upto 10-15 days in dark conditions at 20-25 °C. By checking the amylase activity everyday it was found that the activity was low during first 7 days, while the optimum activity was reached after 11 days, and decline was observed after 15 days. Amylase activity was less when germination was allowed to proceed during daylight. The loss of moisture during the germination process, was replenished by sprinkling water. The germination process was terminated after 11 days by freezing the germinated seeds at -10 °C for 24 h. For better extraction of amylases the germinated seeds were crushed and soaked in equal amount of water (1:1) for 10-15 days. Best results were obtained after 10-11 days of soaking. For preservation, 0.1-0.2% sodium benzoate was used. After 10-11 days, extract was removed by filtration. From 1 kg of germinated barley seeds, about 470 g malt extract was obtained. It was a light brown liquid extract.

Extraction of amylase from germinated mango seeds. Selected seeds of mango were collected and washed. After washing, the seeds were buried in soil for germination. After 15 days, the seed stone broke and shoots appeared. When shoots grew up, the germinated seeds were separated from the hard stone, washed and frozen for 24 hours at -10 °C. These germinated seeds were ground and soaked in equal amount of water (1:1) in dark conditions for 7 days at 20-25 °C. For preser-

*Author for correspondence; E-mail: syd_ishratali@yahoo.com

vation, 0.1-0.2% sodium benzoate was used. After 7 days the water extract was separated by filtration through a fine cloth. The filtrate was passed through a bed of activated carbon to remove the brownish colour. From 1 kg of germinated mango seeds about 300 g enzyme extract was obtained.

The exhaust enzymatic desizing methods of AATCC (103-1989). The exhaust method is a simple method usually used for enzymatic desizing. Malt amylase usually requires 3 h to desize the fabric. By appropriate changes in washings, the enzyme concentration, temperature, wetting agents and electrolytes, the desizing time can be reduced. Certain electrolytes were added to the amylase extracts or the desizing liquor to improve desizing efficiency so as to shorten the desizing duration. Sodium chloride or calcium chloride were used for this purpose. When sodium or calcium chloride was used in the desizing liquor, the thermal resistance as well as the solubility of amylases increased, as these salts combine with the carboxyl groups, allowing the amylases to form a complex with starch and eventually to break it down (Peters, 1975).

Before starting the desizing process it was necessary to prewash the fabric for 8-10 min at 90-95 °C in 2 g/l detergent. This resulted in the swelling of starch and facilitated the subsequent amylase action. After pre-washing, the fabric was squeezed as much as possible. The desizing cycles were repeated with different types of enzyme preparations for the sake of comparison.

Rotary dyeing machine tube was filled with the desizing liquor and the machine started. When the temperature of 40 °C was achieved, the machine was stopped and the prewashed and squeezed fabric was impregnated into the desizing solution. This warm desizing solution provided the necessary conditions for amylase to quickly penetrate into the fabric. The machine was started again and temperature was raised to 60-62 °C. When temperature reached 62 °C, the breakdown of starch started. After the time required (2 h for barley and 3 h for germinated mango seed amylases), the machine was stopped and the fabric was washed. This washing process is very important for removing the degraded starch from the fabric. It was best obtained by a subsequent washing with a detergent or soda ash 2 g/l at 95-100 °C for 15 min. Cold washing coagulates the liquefied starch on the fabric, which is very difficult to remove otherwise. After detergent washing, the fabric was rinsed with warm water at 60 °C for 10 min followed by a cold rinse.

The pad batch enzymatic desizing method. The pad batch method is also simple and is well used in the textile industry. Before starting the desizing process, fabric was prewashed for 8-10 min at 90-95 °C in 2 g/l detergent or soda ash. It swelled the starch and facilitated the subsequent amylase action. The fabric was squeezed firmly before impregnation. The desizing liquor was heated up to 60-62 °C. This heating facilitated the penetration of the amylases into the fabric. The desizing liquor was poured in the padder machine. The padding cycles were repeated with different types of enzyme preparations.

The padder machine was started and the squeezed fabric was padded for 100% pick-up. After padding, the fabric was wrapped in polyethylene bags and kept revolving throughout the batching time for getting even distribution of the enzyme liquor. After 12 h, the polyethylene bags were unwrapped and the fabric was washed at 95-100 °C for 15 min with 2 g/l soda ash or a suitable detergent. After the detergent washing, the fabric was rinsed with warm water at 60 °C for 10 min followed by a cold rinse.

Detection of amylase activity. The activity of amylase enzymes was measured in terms of time required to breakdown the size starches. This digestion was checked by colour development, using iodine solution as the indicator (1 g iodine crystals and 15 g potassium iodide were dissolved in 1 litre of water). This solution was applied on the processed cloth. If the colour of the cloth changed to brown it showed the complete removal of starch and if it turned blue or violet then this showed that the size starch was still present on the fabric (Booth, 1968).

The enzyme extracts, obtained during the present studies, took 2 h (barley extract) and 3 h (germinated mango seed extract) in the exhaust process, and 12 h with both these extracts in the pad batch process. The two commercial extracts, namely, Bactasol MTN (Clarriant) and Nervanase 3x (ICI) completed the fabric desizing within the same time as was taken by the barley extract in the two types of processes. These findings are in agreement with the observations on fabric desizing reported by Troja (1970), Frantisek (1964) and Hans (1938; 1936). It was also noted that whereas the germinated mango seed extract took slightly longer period for fabric desizing, the extract concentration required was about twice more (30-35 g/l) in comparison with the barley extract and the two commercial preparations (10-15 g/l). The observations reported in the present study, therefore, indicate the possibility of commercial application of crude enzyme extracts made from cheap or waste plant materials for the desizing of fabrics in the textile industry.

References

Booth, J.E. 1968. *Principles of Textile Testing*, pp. 180-190, Newnes Butterworths, London, UK.

Bergmeyer, H.U. 1974. *Methods of Enzymatic Analysis*, pp. 65-85, Academic Press, New York, USA.

Brooks, A. 1962. *The Picture Book of Grains*, pp. 34-36, John Day Company, New York, USA.

Cavaco, P.A., Gubitz, G.M. 2003. *Textile Processing with Enzymes*, pp. 1-3, Woodhead Publishing Limited, Cambridge, UK.

Frantisek, S. 1964. Application and evaluation of enzymic desizing agents. *Textil-Praxis* **19:** 378- 384.

Hans, J.H. 1938. The use of enzymic textile assistants for sizing and desizing. *Deut. Wollen-Gewerbe* **70:** 1591-1592.

Hans, J.H. 1936. The role of the enzymes in the textile industry. *Monatschr. Textil-Ind.* **51:** 157-159.

Peters, R.H. 1975. *Textile Chemistry*, vol. **3**, pp. 40-55, Elsevier Scientific Publishing Company, New York, USA.

Shenai, V.A. 1991. *Technology of Bleaching and Mercerizing*, vol. **3**, pp. 20-35, Sevek Publications, Bombay, India.

Troja, P. 1970. Amylase rich malt extract for use in the textile industry. *Bull. Univ. Shteteror Tiranes. Ser. Shkencat Natyr.* **24:** 161 –173.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 131-134

Effect of Part Replacement of Mercaptobenzothiazole with Locust Bean Cake on the Thermal and Electrical Conductivities of Natural Rubber Vulcanizates

B.F. Adeosun*[a], A.E. Oyeleke[a] and E.G. Olumayede[b]

[a] Science Technology Department, Federal Polytechnic, PMB 5351, Ado-Ekiti, Nigeria

[b] Lems Department, Ondo State Polytechnic, PMB 1019, Owo, Nigeria

(received April 18, 2003; revised September 7, 2004; accepted January 24, 2005)

Abstract. The effect of part replacement of mercaptobenzothiazole (MBTS) with locust bean cake (LBC) on the thermodynamic parameters, thermal and electrical conductivities of natural rubber (NR) composite, was examined. Generally, all the thermodynamic parameters, the thermal conductivity and the electrical conductivity of the NR vulcanizates were altered on the inclusion of the MBTS/LBC mix in the formulations of the composites. The degree of alteration of these properties increased with increasing LBC contents in the MBTS/LBC mix. It appears that upto 50/50, MBTS/LBC mix, the lower the entropy change of the molecules of the composite, the higher was the crosslink density of the composite, and the better was the ability of the composite to conduct heat and electricity. It is, therefore, advantageous to replace MBTS with LBC upto 50% in the formulations of NR composites for improved thermal and electrical insulation.

Keyword: mercaptobenzothiazole, locust bean cake, natural rubber, thermodynamic parameters, rubber vulcanizates

Introduction

Locust bean, which is common in Nigeria particularly in the Northern and South-Western parts, has been studied extensively (Adewumi, 1997). It has been found to contain 39-40% oil, 31-40% protein, and 11.7-15.4% carbohydrates (Oladele *et al*., 1985). The oil has been reported to be suitable for the manufacture of soap (Owoyale *et al*.,1986). It has also been established that the leaves of the locust tree are rich in nitrogen, which are used as livestock feed and as a manure. The fermented products of locust bean are used as food condiments all over Nigeria (Adewumi, 1997). Recently, Olaofe *et al.* (1998) have reported on the chemical composition and functional properties of the locust bean.

The properties of natural rubber composites accelerated with mercaptobenzothiazole (MBTS) have been studied (Adeosun *et al.*, 1997; Adu and Adeosun, 1997; Elliot, 1987; Bristow, 1986). The effect of addition of locust bean cake on the properties of natural rubber composite accelerated with MBTS have also been studied. These studies have shown that at relatively low locust bean cake concentration of ≤ 0.3 in the locust bean cake/MBTS admixture, the tensile strength and modulus of natural rubber composite improved over the conventional MBTS composite. Also, the degradation resistance of the raw rubber, before compounding for vulcanization, and the reversion resistance of the resultant natural rubber compound were noted to improve on the addition of locust bean cake.

These observations on the positive effects of locust bean cake on the properties of natural rubber have aroused interest to examine the thermodynamics of elasticity, and the electrical and thermal conductivities of natural rubber composite accelerated with locust been cake in the admixture with the conventional MBTS accelerator.

Materials and Methods

Preparatory and analytical procedures. Filler preparation, latex compounding, dry rubber composite compounding formulation (Table 1), and curing procedures were followed as reported already (Adeosun and Olaofe, 2005; Adeosun *et al.*, 1997). The determination of thermodynamic parameters of elasticity was done as described by Adeosun *et al.* (1999). The measurement of electrical conductivity was done as reported earlier (Oyeleke, 2000). Thermal conductivity measurements were done by Lee's disc apparatus. The sample thickness was determined by micrometer screw gauge. After the determination of the steady state temperature of the lower plate it was heated directly to nearly 100 ^{0}C. The sample was then placed on the plate and the temperature monitored at intervals of thirty seconds. Data obtained were used to plot the cooling curve. The gradient of the curve at the steady state temperature of the lower plate was evaluated and used in the Fourier's equation.

Author for correspondence; E-mail: ojolo48@yahoo.com

Table 1. Compounding formulation of the natural rubber composites examined

Constituents	Sample code*						
	P_1	P_2	P_3	P_4	P_5	P_6	P_7
Natural rubber	100	100	100	100	100	100	100
Zinc oxide	5	5	5	5	5	5	5
Steanic acid	3	3	3	3	3	3	3
Sulphur	1	0.8	0.6	0.5	0.4	0.2	-
MBT [a]	-	0.2	0.4	0.5	0.6	0.8	1
LBC [b]							

*constituents as ratio by parts in different compounding formulations; [a] mercaptobenzothiazole (MBT); [b] locust bean cake (LBC)

Data treatment. (a) The thermodynamic equation of the state for elastic materials was used in the form shown in equation (1) below (Das and Behera, 1983):

$$f = \left(\frac{dH}{dL}\right)_{T.P} + T\left(\frac{df}{dT}\right)_{L.P} \quad (1)$$

When the elastic material under tension f was extended by an amount dL, the plot of tension f versus absolute temperature T showed linearity (Fig. 1) with slope $\left(\frac{df}{dT}\right)_{T.P}$, and intercept $\left(\frac{dH}{dL}\right)_{T.P}$ $\left(\frac{dS}{dL}\right)_{T.P}$ was evaluated using equation (2) (Das and Behera, 1983; Wall, 1942).

$$\left(\frac{dH}{dL}\right)_{T.P} = -\left(\frac{dS}{dL}\right)_{L.P} \quad (2)$$

and $\left(\frac{dG}{dL}\right)_{T.P}$ was evaluated using equation (3):

$$\left(\frac{dG}{dL}\right)_{T.P} = \left(\frac{dH}{dL}\right)_{T.P} - T\left(\frac{dS}{dL}\right)_{T.P} \quad (3)$$

values of $\left(\frac{df}{dT}\right)_{L.P}$, $\left(\frac{dS}{dL}\right)_{T.P}$, $\left(\frac{dG}{dL}\right)_{T.P}$ and $\left(\frac{dH}{dL}\right)_{T.P}$ are shown in Table 2.

(b) Thermal conductivity was evaluated using equation (4) (Bird *et al.*, 1960):

$$K = \frac{Q}{A(T_1 - T_2)_L} \ (Wm^{-1}K^{-1}) \quad (4)$$

where:

K = thermal conductivity

Q = heat flow

A = area of conducting material

L = thickness of conducting material

ΔT = change in temperature between surface within which the conducting material was enclosed

Results and Discussion

Values of $\left(\frac{df}{dT}\right)_{L.P}$, $\left(\frac{dH}{dL}\right)_{T.P}$, $\left(\frac{ds}{dL}\right)_{T.P}$ and $\left(\frac{dG}{dL}\right)_{T.P}$ of the composites examined, as evaluated by the least square method, are given in Table 2. The linear plots of tension f versus absolute temperature, using equation (1) for the composite are shown in Fig 1. The crosslink densities from the previous work on the components (Adeosun *et al.*, 1999) are also shown in Table 2.

For highly elastic rubber-like materials, $\left(\frac{df}{dT}\right)_{L.P}$ had a large positive value and the term $T\left(\frac{df}{dT}\right)_{L.P}$ predominated over the $\left(\frac{dH}{dL}\right)_{T.P}$ (equation 1). The $\left(\frac{df}{dT}\right)_{L.P}$ was, however, small and negative for materials such as steel (Das and Behera, 1983). It may be observed from Table 2 that the rubber-like nature of the natural rubber composite deteriorated steadily, reaching a minimum at 50/50 MBTS/LBC, and then improved as the concentration of LBC increased. At relatively low LBC concentration (0.2 to 0.5 parts per 100 rubber), elasticity decreased with increasing LBC concentrations, but at relatively high LBC concentrations (0.6 to 1.0 parts per 100 rubber), elasticity improved with increasing LBC concentration.

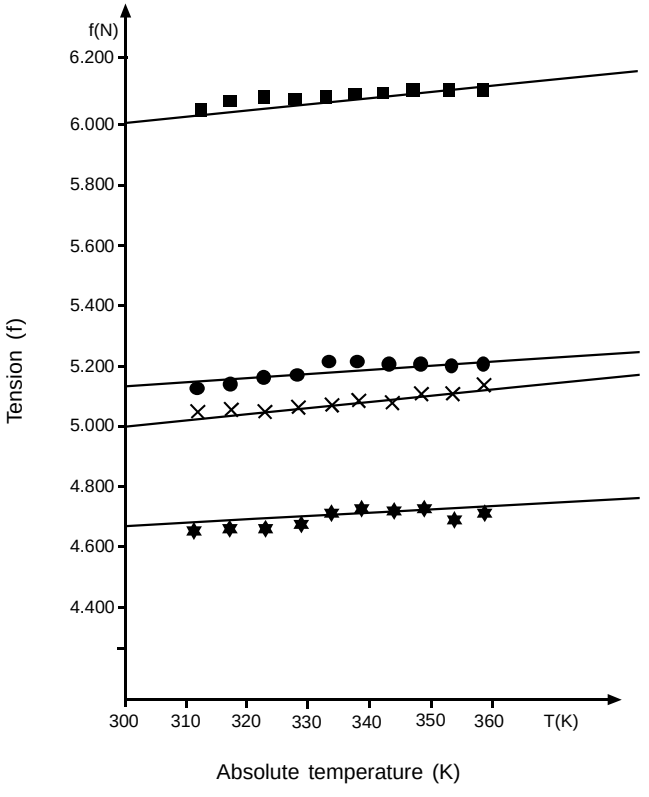


Fig. 1. Graph of tension f as a function of absolute temperature.

Table 2. Thermodynamic parameters of elasticity and thermal conductivities of the composites examined

Composite sample*	Locust bean cake : MBTS (pphr)	Elasticity $\frac{df}{dT} \times 10^4$ (J/mol)	Free energy $\frac{dG}{dL} \times 10^4$ (J/mol)	Enthalpy $\frac{dH}{dL} \times 10^4$ (J/mol)	Entropy $\frac{dS}{dL} \times 10$ (J/mol)	Crosslink density
P1	0:1.0	2.88	5.10	5.12	-2.88	5.76
P2	0.2:0.8	2.24	5.02	4.35	-2.24	5.25
P3	0.4:0.6	1.72	5.14	4.63	-1.72	5.32
P4	0.5:0.5	1.68	5.17	4.67	-1.68	3.65
P5	0.6:0.4	1.74	4.65	4.13	-1.74	5.45
P6	0.8:0.2	1.84	4.60	4.05	-1.84	5.65
P7	1.0:0	2.36	4.84	4.13	-2.36	3.55

*refer Table 1 for constituents of the composite samples; pphr: parts per hundred rubber; MBTS: mercaptobenzothiazole

Considering the entropy factor, $\left(\frac{ds}{dL}\right)_{T,P}$ results show negative values for all the composites. This is not surprising as entropy decreased (tends to a more orderly state) as the composite was extended. This happens due to the uncoiling of the long chain polymer molecules in the direction of stretching when the length was extended (Das and Behera, 1983). The change in entropy of elasticity becomes more positive as LBC concentration increased, reaching a maximum and then decreased progressively. The molecules of the natural rubber composites containing only the conventional MBTS accelerator seem more orderly than the composites containing MBTS/LBC mix. This orderliness decreased to a minimum at 50/50 composition of MBTS/LBC and then the molecules became progressively more orderly with increasing LBC concentrations. A cursory look at the change in free energy of elasticity revealed that values at relatively low LBC concentrations are observed to be more positive than at high LBC concentrations. A closer look revealed that the change in free energy increased with increasing LBC contents in the MBTS/LBC admixture, reaching a maximum at 50/50 mix and dropped thereafter. This connotes that as the LBC contents increased, the composite became progressively less spontaneous to elasticity–until the composites became more spontaneous after reaching 50/50 composite. The change in enthalpy of elasticity generally follows the same trend as the change in free energy of elasticity. The heat accompanying the composites under stress seems to increase as the spontaneity of elasticity decreased.

The variation of thermal conductivity of the composites as a function of LBC concentration is presented in Table 3. It may be observed that the thermal conductivity of the conventional composite drastically decreased from 6.07 W/m/K to 3.37 W/m/K on the introduction to the mix containing 0.2 parts per hundred rubber LBC. Also, the thermal conductivity decreased progressively, reaching a minimum and then increased as LBC concentration was further increased (but still inferior to the conventional composites in respect of conductivity). This trend connotes that the ability of the NR composites to insulate heat increased on the introduction of the MBTS/LBC mix, reaching a maximum and deteriorated thereafter. The composites containing the mix are, therefore, better heat insulators than the conventional composites with the 50/50 mix exhibiting the best ability to insulate heat.

Table 3. Thermal and electrical conductivities of the composites examined

Composite sample*	LBC: MBTS ratio (pphr)	Thermal conductivity (10) (W/m/k)	Electrical conductivity (10^4) (Ω/m)
P1	0:1.0	6.07	4.97
P2	0.2:0.8	3.37	3.39
P3	0.4:0.6	2.92	4.00
P4	0.5:0.5	2.27	3.75
P5	0.6:0.4	3.47	3.75
P6	0.8:0.2	2.73	4.12
P7	1.0:0	2.68	4.09

*refer Table 1 for constituents of the composite samples; pphr: parts per hundred rubber; LBC: locust bean cake; MBTS: mercaptobenzothiazole

The data on electrical conductivity are also presented in Table 3. It may be observed that the composite containing only the conventional MBTS accelerator had the highest ability to conduct electricity. On the introduction of the MBTS/LBC mix, electrical conductivity dropped and decreased progressively as the concentration of LBC in the mix increased, reaching a minimum and thereafter increased

with further increase in LBC contents in the MBTS/LBC mix. This trend observed for electrical conductivity agrees with the thermal conductivity trend, though they showed minima at slightly different MBTS/LBC ratio. When the crosslink densities of the composition were compared to their thermal and electrical conductivities it appeared that the higher the crosslink density, the higher was the ability of the composite to conduct heat and electricity.

Conclusion

Generally, all the thermodynamic parameters, the thermal conductivity and the electrical conductivity of the natural rubber composites deteriorated on the inclusion of the MBTS/LBC mix in the formulation of the composites. The degree of deterioration increased with increased LBC concentration in the MBTS/LBC mix. It appears that up to 50/50, MBTS/LBC mix, the less orderly is the molecule of the composite, the less is the crosslink density of the composite and the less is the ability of the composite to conduct heat and electricity. Hence, the higher is the possibility of utilizing the composite as thermal and electrical insulators. It is, therefore, advantageous to include MBTS/LBC in the formulation of natural rubber composites for improved thermal and electrical insulation.

References

Adeosun, B.F., Olaofe, O. 2005. The effect of local materials (fillers) on the crosslink density, hardness, resilience and hysteresis of natural rubber. *Pak. J. Sci. Ind. Res.* **48:** 63-67.

Adeosun, B.F., Adu, O.E., Ojo, M.M. 1997. Effect of cure temperature on the mechanical properties of natural rubber vulcanizate. *Consultation J.* **2:** 14 18.

Adeosun, B.F., Adu, E.O., Oyewusi, P.A., Ogunmade. 1999. Thermodynamics of stretching of filled NR vulcanizate. *J. Appl. Sci*. **3:** 690-697.

Adewumi, B.A. 1997. Development in the technology of locust bean processing. *J. Tech. Sci*. **1:** 9-14.

Adu, O.E., Adeosun, B.F. 1997. Quality parameters of some natural rubber clones indigenous to Nigeria. *J. Tech. Sci.* **1:** 31-34.

Bird, R.B., Stewart, W.E., Lightfoot, E.N. 1960. *Transport Phenomenon,* pp. 244-245, John Wiley and Sons Inc., New York, USA.

Bristow, G.M. 1986. Reversion resistance of accelerated sulphur systems. *Nat. Rubber Technol*. **17:** 7-17.

Das, R.C., Behera, B. 1983. *Experimental Physical Chemistry,* pp. 171-174, McGraw Hill, New Delhi, India.

Elliot, D.J. 1987. Properties of black reinforced blends of NR and butadiene rubber. *Nat. Rubber Technol.* **17:** 1-6.

Oladele, F.A., Fawole, M.O., Bhat, R.B. 1985. Leaf anatomy of *Parkia clappertoniare. J. Tech. Sci.* **1:** 9-14.

Olaofe, O., Akintayo, E.T. 1998. Mineral compositions and functional properties of African locust bean. In: *Proc. 20th Annual Conference of the Nigeria Institution of Food Science and Technology,* Shareton Hotel and Towers, Ikeja, Nigeria.

Owoyale, J.A., Shok, M.A., Olagbenro, T. 1986. Some chemical constituents of the fruits of *Parkia clappertoniare* as protein industrial raw material. In: *Proc. 2nd Annual Conference of Biotechnology Society of Nigeria,* Unilorin, Nigeria.

Oyeleke, A.E. 2000. The Effect of Locust Bean Cake on the Thermodynamic Parameters, Thermal and Electrical Conductivities of Natural Rubber Composites. *HND Thesis*, Federal Polytechnic, Ado-Ekiti, Nigeria.

Wall, F.T. 1942. Statistical thermodynamics of rubber. *J. Chem. Phys.* **10:** 132-134.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 135-142

Factors Affecting the Geometric and Tensile Properties of Stretch-Knitted Cotton Fabrics

S. Muhammad Nawaz*, Assad Farooq, Babar Shahbaz and Ghulam Murtza
Department of Fibre Technology, University of Agriculture, Faisalabad, Pakistan

(received June 11, 2003; revised February 15, 2005; accepted February 22, 2005)

Abstract. The present paper attempts to investigate the effect of elastane concentration and knitting processing variables, such as tightness factor and take-down tension upon spirality and density of rib and single jersey fabrics. Five different concentrations of elastane (0-7%), three different take-down tensions (2, 3 and 5 kg) and four levels of tightness factors (12, 13.5, 15, and 16.5) were selected. Fabric density has a direct relationship with the concentration of elastane and tightness factors. Weight of both types of knitted fabrics increased with the increase in the tightness factor and the percentage of elastane. It was also found that with the increase in take-down tension and reduction in the tightness factor, the spirality increased.

Keywords: knitted fabrics, spirality, elastane, tightness factor, fabric strength

Introduction

There has been an extraordinary development in the scope and applications of knitted fabrics during the recent years. The principal reason behind this growth is to be found in the structure of the knitted fabric itself. The most important development has been in the increasing applications of lycra and other elastane yarns. Elastane spandex yarns are used to provide fitness and comfort in garments, made mainly in fabrics produced by circular knitting. The form, amount and arrangement of the spandex yarn used in the fabrics depends upon the type and construction of the garments, the fabric weight and the amount of stretch (Corbman, 1983). The major knitting parameters that influence strength, dimension, stretch, and weight of such fabrics are yarn count, tightness of construction, tension on the spandex, and frequency of the spandex on the machine (Abou-iiana, 1998; Buehler and Haid, 1986). Also, fabric's extensibility affects its bursting strength and increases inversely with the tightness factor (Ertugrul and Nuray, 2000).

There are difficulties to be encountered in knitting such fabrics that provide garments with the desired combination of properties. The problem of spirality (spirality is the deviation of the courses and the wales line angle from 90°) greatly affects the knitted fabrics when they are made into garments. The spirality increases with the decrease in tightness factor, while greater take-down tension results in greater angle of spirality (Tariq, 1998; Banerjee and Alaiban, 1988a). Higgins *et al.* (2003) have carried out studies on the length and width shrinkages, skewness, and spirality of three weft-knitted cotton structures during tumble drying. Significant length and width shrinkages occurred in all the three structures. The tumbling action in a tumble drier has a significant influence on the dimensional stability and distortion of weft-knitted cotton fabrics. Jeon *et al.* (2003) have investigated the mechanical properties of warp-knitted fabrics that had differences in knitting structure, knitting density and yarn composition. Fabric weight showed the tendency of gradually decreasing as the number of abrasion cycles increased. It was due to the fact that pill was removed after the abrasion cycles. Tensile strength to rupture decreased with increasing the number of abrasions. The arrangement of yarn input to the machine principally affected this property.

This study attempts to examine the effect of the elastane concentration, tightness factor, the take-down tension on fabric density and spirality of the single jersey and 1/1 rib knitted fabrics.

Material and Methods

Source and characteristics of the yarn. The carded 20^S hosiery yarn (yarn count: 20 single) required for this study was obtained from the running stocks of Masood Textile Mills, Faisalabad, Pakistan. To assess the inherent potential of the raw stocks being utilized for knit fabric construction, the yarn was evaluated for the spinning parameters because physical characteristics of the yarn have a direct bearing on the knitting process. Lea-strength of the yarn was 122.2 lbs, count lea-strength of the product was 2426; thick and thin, places and neps were recorded as 51, 14 and 133 per kilometer, respectively. The hairiness value was 7.88, while 3.7 twists per inch were recorded in the sample yarn.

*Authors for correspondence; E-mail: fibretechnology@yahoo.com

Knitting process. The machines of Pailung brand of 30-inch dia were used for the construction of 1x1 rib and single jersey fabrics. Keeping all other knitting parameters standard, the variables selected for the study of their effects on both types of the fabric (rib and single jersey) are given in Table 1.

After making all possible combinations of the variables, samples were knitted and placed on a flat surface for 24 h at 65±2% relative humidity and 27±2 ˚C temperature for the purpose of conditioning. The following fabric characteristics were evaluated.

Fabric spirality. Spirality is the deviation of the courses and the wales line angle from 90˚. More the angle deviates, more will be the angle of spirality. It was measured in the tube form of fabric according to the procedure suggested by ASTM Committee (1997a).

Fabric density. It is the weight of one square metre of fabric expressed in grams. The weight of the fabric did not vary by more than ±5% according to the standard given by ASTM Committee (1997b).

Fabric strength. Fabric strength is the force expressed in pounds per square inch (PSI), which is used to burst the fabric and was determined according to the ASTM Committee (1997c).

Table 1. Knitting variables used to study their effects on rib and single jersey fabrics

Concentration of elastane (C)	Tightness factor (F)	Take-down tension (T)	Fibre type (S)
C0 = 0%	F1 = 12	T1 = 2kg	S_1 = rib fabric
C1 = 1%	F2 = 13.5	T2 = 3kg	S_2 = single jersey
C2 = 3%	F3 = 15	T3 = 5kg	
C3 = 5%	F4 = 16.5		
C4 = 7%			

Analysis of data. Completely randomized design was applied in the analysis of variance for testing differences among the various quality characteristics studied in these investigations (Steel and Torrie, 1984). Significance was checked at 1 and 5% confidence levels. The new Duncan's multiple range test was also applied for individual comparison of mean values among the various quality characters. M-stat microcomputer package was employed for statistical manipulation of the results (Freed, 1992).

Results and Discussion

Fabric density. The statistical analysis and individual comparison of fabric density are shown in Table 2a. The statistical analysis indicated that the effect of fabric type, concentration of elastane, tightness factor, take-down tension and the interactions SxC, CxF, CxT were highly significant, the interaction FxT was only significant, whereas all the remaining interactions were non-significant.

Table 2a. Analysis of variance for fabric density

SOV	DF	SS	MS	F-value	Probability
S	1	28999.12	28999.12	1374.74	0.00**
C	4	96454.14	24113.53	1143.13	0.00**
F	3	144333.16	48111.05	2280.76	0.00**
T	2	13848.59	6924.30	328.25	0.00**
SxC	4	458.68	114.67	5.43	0.00**
SxF	3	19.40	6.47	0.31	ns
CxF	12	1625.61	135.47	6.42	0.00**
SxT	2	100.61	50.31	2.38	0.09 ns
CxT	8	464.34	58.04	2.75	0.006**
FxT	6	284.00	47.33	2.24	0.04*
SxCxF	12	141.83	11.82	0.56	ns
SxCxT	8	126.92	15.86	0.75	ns
SxFxT	6	4.89	0.83	0.038	ns
CxFxT	24	19.89	0.83	0.039	ns
SxCxFxT	24	7.24	0.30	0.01	ns
Error	240	5062.62	21.09		
Total	359	291951.07			

S: fibre type; C: conc of elastane; F: tightness factor; T: take-down tension; **: highly significant; *: significant; ns: non-significant

Duncan's multiple range test for individual comparison of mean values of different elastane percentage values recorded maximum fabric density at 7% elastane feed (C_4), followed by C_3 (5%), C_2 (3%), C_1 (1%) and control (C_0) with their respective values as 170.62, 158.13, 146.13, 134.77 and 124.49 g/m^2 (Table 2b). All these values differ significantly from each other. The present results depicted a direct relationship between the concentration of the elastane with fabric density, indicating that more the elastane concentration, the greater the density of the fabric. This was so because of the fact that addition of elastane drew both the courses and wales closer, consequently the weight per unit area of the fabric also increased. Some earlier researchers (Abou-iiana, 1998; Corbman, 1983) reported the major knitting parameters that influenced power, stretch, and yield (ounce per square yard) of the fabric as yarn count and tension on the spandex and frequency of the spandex on the machine.

The individual comparison of mean values, regarding the tightness factor, indicated that the maximum value for fabric density was recorded at F_4, followed by F_3, F_2, and F_1, with their respective mean values as 174.53, 154.86, 137.08 and 120.82 g/m^2 (Table 2b). All these values differ significantly from

each other. The present observations depicted a direct relationship between the tightness factor and the fabric density, indicating that an increase in tightness factor resulted in an increase in the weight per unit area of the knitted fabric. It has been reported that if the size of the yarn remained constant, the increase of loop size produced a decrease in weight per unit area (Raz, 1993; Brackenbury, 1992).

Table 2b. Comparison of individual mean values for fabric density (g/m^2)

Concentration of lycra (C)	Tightness factor (F)	Take-down tension (T)	Fabric construction (S)
124.49 e	120.82 d	155.32 a	155.80
134.77 d	137.08 c	144.48 b	137.85
146.13 c	154.86 b	140.67 c	
158.13 b	174.53 a		
170.62 a			

values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)

The values of fabric density given in Table 2b, varied from 155.32, 144.48, 140.67 g/m^2 for different levels of take-down tension varying from 2 kg (T_1), 3 kg (T_2), and 5 kg (T_3), respectively. These results indicate that fabric density of the knitted fabric was inversely proportional to the take-down tension. When the take-down tension was increased, the count for courses per inch retrograded and ultimately the fabric weight per unit area decreased (Mumtaz, 2001; Gill, 2000).

The fabric density under rib (S_1) and plain fabrics (S_2) was recorded as 155.80 and 137.85 g/m^2, respectively. These results show that the rib fabric was heavier than the single knit fabric. It was due to the fact that rib fabric had loops on both the sides, whereas single knit fabric had loops only on one side. Similarly, rib structures are bulkier and heavier than plain knit structures made of similar yarn thickness on machines of similar gauge (Raz, 1993).

Table 2c represents the combined effect of the fabric type and elastane share (SxC) upon fabric weight. Under rib fabric, maximum weight, 179.11 g/m^2 was observed in the combination S_1xC_4, whereas minimum value of the fabric density 135.38 g/m^2 was achieved for combination S_1xC_0. Similarly, for single jersey fabric, the maximum fabric density (162.14 g/m^2) was noted under combination S_2xC_4 and minimum fabric density (113.57 g/m^2) was determined for combination S_2xC_0. However, the overall best combination was S_1xC_4 with 179.11 g/m^2 fabric density, whereas the combination S_2xC_0 represented the minimum fabric density (19.29 g/m^2).

Table 2c. Interactions (SxC) of fabric type (S) and concentration (C) of elastane for fabric density (g/m^2)

	C_0	C_1	C_2	C_3	C_4
S_1	135.38 g	143.73 f	155.24 d	165.56 b	179.11 a
S_2	113.57 i	125.80 h	137.04 g	150.71 e	162.14 c

S_1 : rib fabric; S_2 : single jersey

The interaction study of the elastane share and tightness factor (CxF) are shown in Table 2d. It depicts that in the control, the maximum fabric density (148.73 g/m^2) was achieved in combination C_0xF_4, which the minimum value of fabric density (101.92 g/m^2) was observed in combination C_0xF_1. Similarly, for 1% elastane (C_1), the maximum fabric density of 159.80 g/m^2 was noted in combination C_1xF_4, and the minimum weight (111.59 g/m^2) was noted in combination C_1xF_1. Under 3% elastane, the maximum fabric density (173.64 g/m^2) was achieved in the combination of C_2xF_4, whereas the minimum weight per unit area (120.20 g/m^2) was noted under combination C_2xF_1. Likewise, under 5% elastane, the maximum fabric density (187.87 g/m^2) was noted for combination C_3xF_4, whereas the minimum fabric density (130.21 g/m^2) was observed for combination C_3xF_3. For 7% elastane, the maximum fabric density of 202.63 g/m^2 was noted for the combination of C_4xF_4, and the minimum fabric weight (140 g/m^2) was determined for combination C_4xF_1. However, the overall best combination was C_4xF_4, whereas combination C_0xF_1 represented the minimum fabric density (101.92 g/m^2).

Table 2d. Interactions (CxF) of concentration (C) of elastane and tightness factor (F) for fabric density (g/m^2)

	F_1	F_2	F_3	F_4
C_0	101.92 p	115.70 n	131.53 k	148.73 h
C_1	111.50 o	125.80 l	141.97 i	159.80 f
C_2	120.20 m	136.46 j	154.25 b	173.64 d
C_3	130.21 k	147.66 h	166.78 e	187.64 b
C_4	140.27 i	159.80 f	179.80 c	202.63 a

Table 2e represents the interaction study of the elastane share and take-down tension (CxT). The interaction study reveals the best combination was C_4xT_1, with 181.01 g/m^2 fabric density, whereas combination C_0xT_3 represented the minimum fabric density (119.74 g/m^2). Table 2f represents the interaction study of the tightness factor F and take-down tension (FxT). It depicts that under take-down tension T_1 (2 kg) the maximum fabric density (184.76 g/m^2) was determined for combination F_4xT_1, whereas minimum fabric density 127.77 g/m^2 was found for combination F_1xT_1. Under take-down tension T_2 (3 kg), the maximum fabric density (171.67 g/m^2) was

noted for combination F_4xT_2, whereas minimum (118.94 g/m^2) was observed for combination F_1xT_2. Similarly, under T_3 (5 kg), the maximum weight per unit area (167.18 g/m^2) was found for combination F_4xT_3 and the minimum (115.76 g/m^2) was found for combination F_1xT_3. However, the best overall combination was F_4xT_1 of fabric weight (184.76 g/m^2), whereas the combination F_1xT_3 represented the minimum fabric weight per unit area (115.76 g/m^2).

Table 2e. Interactions (CxT) of concentration (C) of elastane and take-down tension (T) for fabric density (g/m^2)

	T_1	T_2	T_3
C_0	131.62 g	122.01 h	119.74 h
C_1	142.06 f	132.31 g	129.93 g
C_2	154.14 d	143.46 f	140.80 f
C_3	167.71 b	156.15 d	150.54 e
C_4	181.01 a	168.49 b	162.37 c

Table 2f. Interactions (FxT) of tightness factor (F) and take-down tension (T) for fabric density (g/m^2)

	T_1	T_2	T_3
F_1	127.77 j	118.94 k	115.76 l
F_2	144.94 g	134.92 h	131.40 I
F_3	163.80 d	152.42 e	148.37 f
F_4	184.76 a	171.67 b	167.18 c

Spirality. The statistical analysis and individual comparison of spirality are shown in the Table 3a, which indicates that the effect of fabric type (S), tightness factor (F), take-down tension (T) and the interactions SxC, SxF, SxCxT were highly significant. However, only the interaction SxT was significant, whereas concentration of elastane (C) and remaining interactions exerted non-significant effects.

The comparison of mean values for spirality showed that for different percentages of elastane C_0, C_1, C_2, C_3 and C_4, the spirality was 4.66, 4.49, 4.48, 4.52 and 4.50 degree (Table 3b). These results show that the spirality was independent of elastane concentration in the fabric. Spirality is the deviation of the courses and the wales line angle from 90^0. It greatly affects the knitted fabrics when they are made into garments (Banerjee and Alaiban, 1988b).

Duncan's multiple range test (Table 3b), for individual comparison of means for different levels of tightness factor recorded minimum spirality at F_4, followed by F_3, F_2, and F_1, with their respective values as 4.11, 4.41, 4.63 and 4.97 degree. The results of present study show an inverse relationship between the tightness factor and spirality, indicating that more the tightness factor, the lower the spirality. The

Table 3a. Analysis of variance for spirality

SOV	DF	SS	MS	F-value	Probability
S	1	3454.02	3435.02	14519.24	0.00**
C	4	1.61	0.40	1.69	0.15 ns
F	3	35.34	11.78	49.52	0.00**
T	2	14.97	7.48	31.46	0.00**
SxC	4	17.53	4.38	18.42	0.00**
SxF	3	4.72	1.57	6.61	0.00**
CxF	12	0.80	0.07	0.28	ns
SxT	2	1.74	0.87	3.65	0.03*
CxT	8	3.46	0.43	1.81	0.07 ns
FxT	6	0.50	0.08	0.35	ns
SxCxF	12	0.74	0.06	0.26	ns
SxCxT	8	6.42	0.80	3.37	0.00**
SxFxT	6	0.16	0.02	0.11	ns
CxFxT	24	0.70	0.03	0.12	ns
SxCxFxT	24	0.70	0.03	0.13	ns
Error	240	57.094	0.24		
Total	359	3600.55			

S: fibre type; C: conc of elastane; F: tightness factor; T: take-down tension; **: highly significant; *: significant; ns: non-significant

Table 3b. Comparison of individual mean values for spirality (degree of spirality)

Concentration of elastane (C)	Tightness factor (F)	Take down tension (T)	Fabric construction (S)
4.66	4.97 a	4.28 c	1.43
4.49	4.63 b	4.55 b	7.63
4.48	4.41 c	4.77 a	
4.52	4.11 d		
4.50			

values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)

alteration of spirality for equal changes of stitch length varied from one machine to another machine. When the stitch length was increased the tightness factor decreased and hence spirality increased (Banerjee and Alaiban, 1988a & b). The properties of raw materials and tightness of construction significantly affect fabric dimension.

The individual mean values for the data pertaining to spirality given in Table 3b, varied from 4.28, 4.55, and 4.77 degree for different levels of take-down tension, varying from 2, 3, and 5 kg, respectively. These results depicted a directly proportional relationship between the take-down tension and spirality, indicating that more the take-down tension the higher the spirality. The take-down tension is responsible for most of the lengthwise distortions of the fabric (Saleem, 2003; Black, 1974).

A comparison of individual means for spirality under rib (S_1) and single jersey (S_2) fabrics in the present study revealed

1.43 and 7.63 degree spirality, correspondingly. These results show that rib fabrics have less spiral degree than that of plain knit fabrics. In fact, 1x1 rib is balanced by alternate wales of face loops in each side. The plain-knitted fabrics made from single cotton yarn are most prone to spirality, the degree being related to the number of twists/unit length in the yarn. Spirality does not occur in 1x1 rib and interlock fabrics; the loops formed in opposite direction cancelling out the distortions. The lighter fabrics were more deformed in manufacturing than the heavier fabrics (Raz, 1993).

The interaction of the fabric type and the elastane concentration (SxC) is given in Table 3c, from which it is evident that for S_1, higher concentration of elastane reduced the spirality of the rib fabric. On the other hand, for the fabric type S_2, higher concentration of elastane increased the spirality of plain knit fabric.

Table 3d represents the interaction study of the fabric type and tightness factor (SxF). It depicts that under rib fabrics (S_1), the maximum 1.74° spirality was achieved for the combination S_1xF_1, and the minimum value of spirality (1.17°) was observed for combination S_1xF_4. Similarly, for single jersey fabric, the maximum spirality of 8.21° was noted under combination S_2xF_2, whereas minimum spirality of 7.06° was noted for combination S_2xF_4. However, the overall best combination was S_1xF_4 of spirality (1.17°), whereas combination S_2xF_1 represented the maximum spirality (8.21°).

Table 3c. Interactions (SxC) of fabric type (S) and concentration (C) of elastane for spirality (degree of spirality)

	C_0	C_1	C_2	C_3	C_4
S_1	1.86 c	1.53 d	1.43 de	1.27 ef	1.08 f
S_2	7.46 b	7.45 b	7.53 b	7.78 a	7.94 a

S_1: rib fabric; S_2: single jersey

Table 3d. Interactions (SxF) of fabric type (S) and tightness factor (F) for spirality (degree of spirality)

	F_1	F_2	F_3	F_4
S_1	1.74 e	1.46 f	1.38 f	1.17 g
S_2	8.21 a	7.81 b	7.45 c	7.06 d

S_1: rib fabric; S_2: single jersey

Table 3e shows the interaction of fabric type and the take-down tension (SxT) for spirality. It is evident from these observations that by increasing the take-down tension the fabric spirality increased gradually. Maximum spirality was recorded for S_2xT_3, while the combination S_1xT_1 produced the fabric with minimum spirality. Higgins *et al.* (2003) reported that the tumbling action in a tumble drier has the greatest influence on the dimensional stability and distortion of weft-knitted cotton fabrics. Spirality increased with decrease in the tightness factor and greater take-down tension resulted in greater angle of spirality (Tariq, 1998; Banerjee and Alaiban, 1988a; b).

Table 3e. Interactions (SxT) of fabric type (S) and take-down tension (T) for spirality (degree of spirality)

	T_1	T_2	T_3
S_1	1.27 e	1.44 de	1.60 d
S_2	7.28 c	7.66 b	7.95 a

S_1: rib fabric; S_2: single jersey

Table 3f represents the interaction study of the fabric type, the elastane share, and take-down tension (SxCxT). It depicts that under rib fabrics, minimum spirality (1.08°) was achieved under the combination, $S_1xC_4xT_1$, at maximum concentration of elastane and minimum level of take-down tension, whereas maximum value of spirality (2.14°) was observed for plain knit fabric. Minimum spirality (6.68°) was noted under combination $S_2xC_0xT_1$, whereas the maximum (8.5°) was obtained at combination $S_2xC_4xT_3$. However, the overall best combination was $S_1xC_4xT_1$ with spirality (1.08°), whereas the combination $S_2xC_4xT_3$ represented the maximum spirality (8.5°).

Fabric strength. The statistical analysis and individual comparison of the fabric strength are shown in the Table 4a, which indicates that the effect of fabric type (S), the concentration of elastane (C), the tightness factor (F), and the interactions SxC, SxT were highly significant, whereas the take-down tension (T), along with all the remaining interactions were non-significant.

Duncan's multiple range test for individual comparison of mean values for different elastane percentages recorded

Table 3f. Interactions (SxCxT) of fabric type (S), concentration of elastane (C) and take-down tension (T) for spirality (degree of spirality)

	S_1					S_2				
	C_0	C_1	C_2	C_3	C_4	C_0	C_1	C_2	C_3	C_4
T_1	1.56 ghi	1.27 ij	1.27 ij	1.17 ij	1.08 j	6.84 e	7.44 d	7.48 d	7.35 d	7.24 d
T_2	1.89 fg	1.50 ghij	1.42 hij	1.31 ij	1.08 j	7.55 d	7.44 d	7.55 d	7.74 cd	8.01 bc
T_3	2.14 f	1.83 fgh	1.61 ghi	1.34 ij	1.08 j	8.00 bc	7.46 d	7.55 d	8.23 ab	8.5 a

S_1: rib fabric; S_2: single jersey

Table 4a. Analysis of variance for fabric strength

SOV	DF	SS	M S	F-value	Prob
S	1	12822.96	12822.96	520.83	0.00**
C	4	21044.05	5261.01	213.68	0.00**
F	3	614.56	204.85	8.32	0.00**
T	2	147.62	73.81	2.99	0.053 ns
SxC	4	1193.36	298.38	12.12	0.00**
SxF	3	19.83	6.61	0.27	ns
CxF	12	157.25	13.10	0.53	ns
SxT	2	317.77	158.88	6.45	0.00**
CxT	8	199.00	24.87	1.01	0.43 ns
FxT	6	9.91	1.65	0.07	ns
SxCxF	12	85.73	7.14	0.29	ns
SxCxT	8	170.03	21.25	0.86	ns
SxFxT	6	36.55	6.09	0.25	ns
CxFxT	24	97.52	4.06	0.16	ns
SxCxFxT	24	111.35	4.06	0.19	ns
Error	240	5908.88	24.62		
Total	359	42936.38			

S: fibre type; C: conc of elastane; F: tightness factor; T: take-down tension; **: highly significant; ns: non-significant

maximum fabric strength at 7% elastane feed, followed by C_3, C_2, C_1 and control (C_0), with their respective values as 101.32, 97.79, 91.12, 85.25, 85.10 and 80.75 lbs (Table 4b). All these values differ significantly from each other. The present results depicted a direct relationship between feed of elastane and fabric strength, indicating that more the concentration of elastane, more the strength of the fabric. It has been reported that the elastomeric yarns may be used where excessive elasticity and grip are required, such as in welt of hose or half hose, elastic stockings, corsets and brassiers (Chamberlain, 1951). The strength of the fabric is also dependent upon the yarn, and on the length of the loop, fabric weight, yarn breaking strength, whereas yarn breaking elongation are the major parameters that affect the bursting strength of the plaink-nitted fabrics (Ertugrul and Nuray, 2000). Lycra provides a superb route for achieving surface effects in both single and double jersey fashion knits through the techniques for differential collapse.

Table 4b. Comparison of individual means for fabric strength (lbs)

Lycra percentage (C)	Tightness factor (F)	Take-down tension (T)	Fabric construction (S)
80.75 e	89.60 c	91.95	85.25
85.10 d	90.49 bc	91.30	97.18
91.12 c	91.69 ab	90.39	
97.79 b	93.08 a		
101.32 a			

values with different alphabets significantly different from each other at p = 0.05 (Duncan's multiple range test)

The individual comparison of mean values regarding tightness factor indicates that the maximum fabric strength was recorded at F_4, followed by F_3, F_2 and F_1, with their respective values as 93.08, 91.69, 90.49 and 89.60 lbs (Table 4b). All these values differ significantly from each other. These results indicate that fabric strength of a knit is directly proportional to the tightness factor. The reason is that at lower stitch length, the number of the loops in unit area is greater and consequently more strength will be required to burst all loops. As the stitch length increases the tightness factor decreases and the bursting strength of the fabric also decreases for both states of fabric relaxation (full relaxation and finished relaxation).

The comparison of mean values for fabric strength showed that at different levels of take-down tension T_1, T_2 and T_3, the fabric strength was 91.95, 91.30 and 90.39 lbs. These results show that the fabric strength is independent of the take-down tension. Jeon *et al.* (2003) investigated the mechanical properties of warp-knitted fabrics which had differences in the knitting structure, knitting density and yarn composition. Fabric weight showed the tendency of gradually decreasing as the number of abrasion cycles increased. It was due to the fact that pill was removed after the abrasion cycles. Tensile strength to rupture decreased with increasing the number of abrasions. The arrangement of yarn input to the machine principally affected this property.

Comparative study of individual mean values for the fabric strength under rib and plain knit fabrics revealed, 85.25 and 97.19 lbs strength, respectively. These results depicted that the bursting strength of the rib fabric was less than that of the plain knit fabric. The 1x1 rib-knitted fabrics have been shown to be slacker structures in comparisin with plain-knitted fabrics (Jong and Postle, 1977), that is, the yarn interlocking forces were generally lower ($PrL^2/B = 3.3$), as compared with the plain-knitted structure ($PL^2/B = 7$). The 1x1 rib structure is, therefore, generally knitted to a greater tightness factor than those used for plain-knitted fabrics. The 1x1 rib structure is naturally jammed between the ribs with a force ($RL^2/B = 2.25$) in interlocking region, the yarn within the 1x1 rib fabric cross each other at an angle much closer to 90° and consequently the force of distribution are much more peaked than those for the plain-knitted fabrics.

Table 4c represents the interaction study of the fabric type and elastane share (SxC). It depicts that under rib fabrics, the maximum fabric strength, 98.11 lbs, was achieved at the maximum percentage of elastane, at the combination S_1xC_4, whereas the minimum value of fabric strength (73.77 lbs) was observed for combination S_1xC_0. Similarly, for single jersey fabric, the maximum fabric strength (104.54 lbs) was noted

under combination S_2xC_4, whereas the minimum fabric strength (87.73 lbs) was observed for combination S_2xC_0. However, the overall best combination was S_2xC_4 with 104.54 lbs fabric strength, whereas combination S_1xC_0 represented the minimum fabric strength (73.77 lbs).

Table 4c. Interactions (SxC) of fabric type (S) and concentration (C) of elastane for fabric strength (lbs)

	C_0	C_1	C_2	C_3	C_4
S_1	73.77 g	77.90 f	83.13 e	93.34 c	98.11 b
S_2	87.73 d	92.30 c	99.11b	102.23 a	104.54 a

S_1: rib fabric; S_2: single jersey

Table 4d represents the interaction study of the fabric type and take-down tension (SxT). This interaction observations indicate that the best combination was S_2xT_2 of 97.82 lbs fabric strength, whereas the combination S_1xT_3 represented the minimum fabric strength (83.65 lbs).

Table 4d. Interactions (SxT) of fabric type (S) and take-down tension (T) for fabric strength (lbs)

	T_1	T_2	T_3
S_1	87.31 b	84.78 c	83.65 c
S_2	96.60 a	97.82 a	97.13 a

S_1: rib fabric; S_2: single jersey

Conclusions

The results of this study depicted a direct relationship between feed of elastane and fabric strength, indicating that more the concentration of elastane the more will be the strength of the fabric. The spirality was found to be independent of elastane concentration in the fabric, and a direct relationship was recorded between the concentration of the elastane with fabric density, thus indicating that more the elastane concentration the greater will be the density of the fabric. The weight per unit area of the fabric increased with the addition of elastane. This was so because of the fact that addition of elastane draws both the courses and the wales closer.

The bursting strength of the rib fabric was less than that of the plain-knitted fabric. The 1x1 rib-knitted fabrics were shown to be of slacker structure in comparison with plain-knitted fabrics. The results also showed that rib fabrics were less spiral than the plain-knit fabrics. In fact, 1x1 rib was balanced by alternate wales of face loops on each side. The plain-knitted fabrics, made from single cotton yarn, were the most prone to spirality.

The strength of both the rib and plain-knitted fabrics was directly proportional to the tightness factor. The take-down tension was found to be responsible for most of the lengthwise distortions of the fabric; more the take-down tension, the higher was the spirality for both types of fabrics (rib and single jersey). The results also showed an inverse relationship between the tightness factor and spirality, indicating that more the tightness factor, the lower will be the spirality. A direct relationship between the tightness factor and the fabric density was also observed, indicating that if tightness factor was more, the more was the weight per unit area of the knitted fabric.

References

Abou-iiana, M.N. 1998. Spandex yarn knitting. *Pak. Text. J.* **47:** 34-35.

ASTM Committee. 1997a. Standard method for test for weight, bursting strength, shrinkage, bow and skewness of the knitted fabric. In: *American Society for Testing and Material Standards*, ASTM Designation D: 231; D: 3787-89; D: 1905; and D: 3882-90, part-33, 34th edition, American Society for Testing and Materials, Philadelphia, USA.

ASTM Committee 1997b. Standard method of test for spirality of the knitted fabrics. In: *American Society for Testing and Material Standards*, ASTM Designation D: 3882, part-33, 34th edition, American Society for Testing and Materials, Philadelphia, USA.

ASTM Committee 1997c. Standard method of test for weight of the knitted fabric. In: *American Society for Testing and Material Standards*, ASTM Designation D: 231, part-33, 34th edition, American Society for Testing and Materials, Philadelphia, USA.

Banerjee, P.K., Alaiban, T.S. 1988a. Geometry and dimensional properties of the plain loops made of rotor spun cotton yarns. Part-1. Outline of problems and experimental approach. *Text. Res. J.* **58:** 123-128.

Banerjee, P.K., Alaiban, T.S. 1988b. Geometry and dimensional properties of the plain loops made of rotor spun cotton yarns. Part-III. Spirality of the wale line. *Text. Res. J.* **58:** 287-290.

Black, D.H. 1974. Shrinkage control for cotton blend knitted fabric. *Text. Res. J.* **44:** 606-611.

Brackenbury, T. 1992. *Quality Control of the Knitted Garments, Knitted Cloth Technology*, pp. 158-175, 2nd edition, Blackwell Scientific Publications, Oxford, UK.

Buehler, G., Haid, H. 1986. Dimensional stability of the knitted fabrics from the point of view of research and development, *Knitting Technol.* **22:** 134-137.

Chamberlain, J. 1951. *Principles of Machine Knitting*, pp. 25-26, The Textile Institute, Manchester, UK.

Corbman, B.P. 1983. *Textile Fibre to Fabrics*, pp. 104-123, 1st edition, McGraw Hill, Singapore.

Ertugrul, S., Nuray, R. 2000. Predicting bursting strength of cotton plain-knitted fabrics using intelligent techniques. *Text. Res. J.* **70:** 845-851.

Freed, R.D. 1992. *M-Stat. Micro Computer Statistical Program*, pp. 20-44, 1st edition, Michigan State University of Agriculture, Norway 324-B, Agriculture Hall, East Lamsing, Michigan, USA.

Gill, U.D. 2000. Influence of Yarn Count and Some Knitting Variables Upon the Quality of Double Knit Fabrics. *M.Sc. Thesis*, Department of Fibre Technology, University of Agriculture, Faisalabad, Pakistan.

Higgins, L., Anand, S.C., Hall, M.E., Holmes, D.A. 2003. Factors during tumble drying that influence dimensional stability and distortion of cotton knitted fabrics. *Inter. J. Clothing Sci.Technol.* **15:** 126-139.

Jeon, Y.H., Jeong, W.Y., Park, J.W., Ann, S.K. 2003. The mechanical properties and abrasion behavior of warp knitted fabrics for footwear. *Fibers and Polymers* **4:** 151-155.

Jong, D.E., Postle, R. 1977. An energy analysis of the mechanics of weft knitted fabrics by means of optimal-control theory. Part-III. The 1x1 rib knitted structure. *J. Text. Inst.* **47:** 324-329.

Mumtaz, A. 2001. A Comparative Study on Blending Techniques at Different Stages of Fibre to Yarn Spinning. *M.Sc. Thesis*, Department of Fibre Technology, University of Agriculture, Faisalabad, Pakistan.

Raz, S. 1993. *Flat Knitting Technology*: *Basic Knit Structures*, pp. 55-73, 2nd edition, C.F. Rees Gmbh, Druck Repro, Verlage, Heidenheim, Germany.

Saleem, F. 2003. Comparison of Knitted Fabric from Air-Jet and Ring Spun Yarn by Selecting Multiple Blend Ratios of Cotton and Polyester. *M.Sc. Thesis*, Department of Fibre Technology, University of Agriculture, Faisalabad, Pakistan.

Steel, R.G.D., Torrie, J.H. 1984. *Principles and Procedures of Statistics*, 2nd edition, McGraw-Hill Book Company, London, UK.

Tariq, M. 1998. Influence of Some Knitting Variables Upon Dimensional and Physical Characteristics of Single Jersey Fabric for Carded and Combed Yarn. *M.Sc. Thesis*, Department of Fibre Technology, University of Agriculture, Faisalabad, Pakistan.

Pak. J. Sci. Ind. Res. 2005 **48**(2) 143-148

Castor Oil: A Vital Industrial Raw Material

D. S. Ogunniyi
Department of Chemistry, University of Ilorin, Ilorin, Nigeria

(received September 2, 2002; revised September 20, 2004; accepted December 12, 2004)

Abstract. Even though castor oil is inedible, it has for long been an article of commerce. This is in large measure due to the versatility of the oil. This review discusses the extraction of castor oil and its refining methods, while emphasis is placed on the review of the industrial applications of the oil. Since castor oil is not edible, it could be substituted in many industrial application areas where edible oils are used. An awareness of the various applications of the oil can be used to emphasize the need for an increase in its production as a vital raw material for the chemical industries.

Keywords: castor oil, raw material, industrial oil

The trade of castor oil as an item of commerce dates back to antiquity. The oil is obtained by extracting or expressing the seeds of *Ricinus communis,* or red castor Family Eurphorbiacae (Kirk-Othmer, 1979). It is also obtained from the seeds of another plant, *R. sanguineous*. Castor oil is a viscous, pale yellow, non-volatile and non-drying oil, with bland taste. It is sometimes used as a purgative. It has a slight characteristic odour while the crude oil tastes slightly acrid with a nauseating after-teste. Relative to other vegetable oils, it has a good shelf-life and it does not turn rancid unless subjected to excessive heat. The objective of this paper is to highlight the various uses of castor oil in the chemicals production industries. Being a non-edible oil, its increased use can reduce the demand of some edible oils used in the chemical industry, for its availability for human consumption.

Producing countries. Castor oil is a vegetable oil used as a major industrial raw material. The world's major producers of castor oil are India, Brazil, China and Russia. The importance of castor oil to the economy of many African countries is evident from the fact that many companies in the continent sponsor the cultivation of castor plant. Although reliable production statistics are hard to come by, it is noteworthy that a substantial amount of the oil is produced in Zimbabwe, which is expected to exceed 1000 tonnes in the near future (Chamunorwa, 1995). The total world production of seeds is estimated at 1 million tonnes and the oil expressed from these is about 500,000 tonnes. The breakdown of production is shown in Table 1 (www.ciara.com.ar/estadize.htm).

Cultivation of castor plant. The castor plant grows in the wild in large quantities mostly in the tropical and sub-tropical countries. It is a herbaceous plant with a height between 1 m and 2 m when fully developed. Some varieties are perennials with the size of small trees, whereas some other varieties are dwarf types and are grown as annuals. Generally, the plant is able to grow on most soils, except those that are compacted, such as heavy clays. For best yields, it requires moderate soil fertility, warm conditions, and average rainfall between 600-700 mm (Weiss, 1971). However, the plant is able to tolerate varying weather conditions and it has been adapted to semi-arid conditions of Brazil, making the country to be a major producer of castor seeds. High soil fertility enables the castor plant to grow vegetatively, while humid and wet conditions may promote the development of capsule moulds. On the other hand, high temperatures affect pollination and seed set (Woodend, 1993). A disadvantage of the cultivation of castor plants is the presence of the castor bean white fly population that has the characteristics of developing into a severe pest hazard potential. Castor seeds are about the size of a bean and sometimes the seed is referred to as a bean although it is not a legume. Other informations on the agricultural production of castor oil (Woodend, 1993; Roetheli *et al.*, 1991) and other biological aspects of the plant have been published (Weiss, 1983; 1971).

There are different varieties of the castor seeds, but on the average, they contain about 46 to 55% oil by weight (Weiss, 1983). Castor seeds are poisonous to humans and animals, because they contain ricin, ricinine and certain allergens that are lethally toxic. If a seed is ingested accidentally, it causse abdominal pain, vomiting and diarrhoea. In fact, it is claimed that as little as one milligram of ricin can kill an adult (Woodend, 1993). Mascolo *et al.* (1996) have reported that administration of castor oil at 2 ml/rat produced diarrhoea in all the rats they tested; similar effects are also expected in humans.

Extraction of castro oil. The extraction of oil from castor seeds is done by one or a combination of mechanical pressing and solvent extraction procedures. In mechanical pressing, the seeds are crushed and then adjusted to low moisture content by warming in a steam-jacketed vessel. Thereafter, the seeds are loaded into a hydraulic press and the seeds are pressed by mechanical means to extract oil. The oil obtained through the from mechanical pressing procedure has light colour and low free fatty acids (Kirk-Othmer, 1979). However, mechanical pressing removes only about 45% of the oil present and the remaining oil in the cake can only be recovered by solvent extraction. In the solvent extraction method, the crushed seeds are extracted with a solvent in a Soxhlet extractor or a commercial extractor. Solvents used for extraction include heptane, hexane and petroleum ether.

Refining of castor oil. As in other vegetable oils, it is usual to refine the crude oil obtained, either from mechanical pressing or solvent extraction. The main aim of refining is to remove impurities, such as colloidal matter, free fatty acids, colouring matter, and other undesirable constituents, thus making the oil more resistant to deterioration during storage. The general method of refining oils is also applicable to castor oil. Refining includes the following stages:

- removal of solid and colloidal matter by settling and filtration, and neutralization of the free fatty acids by alkali treatment,
- removal of colouring matter by bleaching,
- only edible oils are deodourized by treatment with steam at high temperatures and under low pressure, this step is thus redundant for castor oil.

Properties and chemistry of castor oil. Castor oil, like all other vegetable oils, has different physical and chemical properties that vary with the method of extraction. The average fatty acids composition of castor oil is, ricinoleic acid 86%, linoleic acid 3.5%, stearic acid 2%, and dihydrostearic acid 2% (Kirk-Othmer, 1979). Cold pressed castor oil has low acid value, low iodine value and a slightly higher saponification value than the solvent extracted oil, and it is lighter in colour. Also, the oil is insoluble in alcohol. Typical properties of castor oil are given in Table 2.

The chemistry of castor oil is centred on its high content of ricinoleic acid. Castor oil consists mainly of esters of 12-hydroxy-9-octadecenoic acid (ricinoleic acid), thus the presence of hydroxyl groups and double bonds makes the oil suitable for many chemical reactions and modifications (Fig. 1). The ricinoleic acid comprises over 80% of the fatty acids of the oil. Castor oil is characterized by high viscosity, although this is unusual for a natural vegetable oil, which is largely due to hydrogen bonding of its hydroxyl groups. It is a unique naturally occurring polyhydroxy compound.

Dehydration. Castor oil is classified as a non-drying oil, but it can be dehydrated to give semi-drying or drying oil. The treatment involves the removal of water from the fatty acid portion of the oil. Being a polyhydroxy compound, its hy-

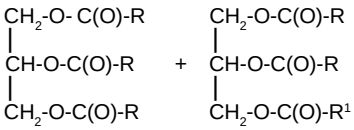
Table 1. Production volume (x000 tonne) of castor oil by major producers*

Major producers	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000
India	192	239	232	242	271	333	344	278	304	294	324
Peoples Republic of China	77	86	93	97	97	82	73	83	80	91	105
Brazil	77	73	54	28	28	22	21	43	21	19	52
Thailand	18	18	19	18	16	14	10	9	9	7	5
European Union	20	16	14	12	14	11	9	10	7	8	8
Others	54	52	49	41	20	21	22	19	20	23	23
Total	438	484	461	438	446	483	479	442	441	442	517

*source: www.ciara.com.ar/estadize.htm

Table 2. Characteristics of castor oil grades

Properties	Cold-pressed oil	Solvent-extracted oil	Dehydrated oil
Specific gravity (at 30 °C)	0.961-0.963	0.957-0.963	0.926-0.937
Acid value (KOH, mg/g)	3	10	6
Iodine value (Wijs)	82-88	80-88	125-145
Saponification value (KOH, mg/g)	179-185	177-182	185-188



where:

R: $-(CH_2)_7-CH=CH-CH_2-CH(OH)-(CH_2)_5-CH_3$

R^1: other fatty acid derivatives

Fig. 1. Constitution of castor oil.

droxyl functionality can be reduced through dehydration or increased by interesterification with a polyhydric alcohol. The dehydration process is carried out at about 250 °C in the presence of catalysts, such as concentrated sulphuric acid, activated earth, and under an inert atmosphere or vacuum. Under this condition of dehydration, the hydroxyl group and an adjacent hydrogen atom from the C–11 or C-13 position of the ricinoleic acid portion of the molecule is removed as water (Fig. 2). This yields a mixture of two acids, each containing two double bonds, but in one case they are conjugated. The presence of an acid containing conjugated double bond results in the oil resembling tung oil in some of its properties. Thus, castor oil, which is non-drying, can be treated and converted into a semi-drying/drying oil, commonly known as dehydrated castor oil.

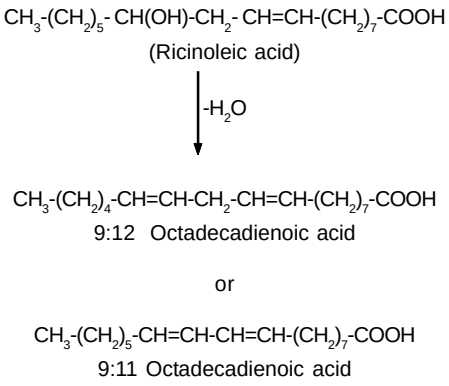


Fig. 2. The dehydration of ricinoleic acid.

Industrial uses. Although the oil is not edible, it is widely used as a starting material for many industrial products because of its unique structure. It is one of those vegetable oils that have found usage in many chemical industries; it is a raw material for paints, coatings and a variety of other products.

Because of its hydroxyl functionality, the oil is suitable for use in isocyanate reactions to make polyurethane elastomers (Quipeng *et al.*, 1990), polyurethane millable (Kirk-Othmer, 1979), castables (Lyon and Garret,1973; Heiss, 1960), adhesives and coatings (Yeadon *et al.*, 1959), interpenetrating polymer network from castor oil-based polyurethane (Patel and Suthar, 1988), and polyurethane foam (Ogunniyi *et al.*, 1996; Ehrlich *et al.*, 1959). Some semi-rigid foams that have potential uses in thermal insulation were produced when castor oil/polyether mixture was reacted with toluene diisocyanate (Ogunniyi *et al.*, 1996).

Sebacic acid is manufactured by heating castor oil to high temperatures (about 250 °C) with alkali. This treatment results in saponification of the castor oil to ricinoleic acid that is then cleaved to give capryl alcohol (2-octanol) and sebacic acid (Fig.3). Although the sebacic acid yields are low, this route has been found to be most competitive. Sebacic acid is used in the production of nylon-6,10. The esters of sebacic acid are also used as plasticizers for vinyl resins and in the manufacture of dioctyl sebacate, a jet lubricant and lubricant in air-cooled combustion motors. Also, capryl alcohol is used in plasticizers in the form of dicapryl esters of various dibasic acids.

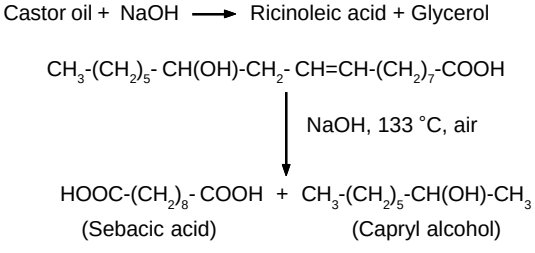


Fig. 3. Production of sebacic acid and capryl alcohol from castor oil.

As shown in Fig. 4 the pyrolysis of castor oil at 700 °C under reduced pressure has been used to obtain heptaldehyde and undecylenic acid (Das *et al.*, 1989). Undecylenic acid and heptaldehyde are important intermediates in the preparation of perfume formulations. When undecylenic acid is mixed with isobutylamine, an insecticidal synergist is obtained. Heptaldehyde can be further hydrogenated to produce alcohol for use as a plasticizer. In addition, undecylenic acid is used in preparing the athlete's foot remedy.

In order to obtain ω-aminoundecanoic acid (Saunders, 1988; Brydson, 1975), the castor oil is subjected to methanolysis to yield the methyl ester of ricinoleic acid, for which the route shown in Fig. 4 is used. In the first step, the pyrolysis of methyl ricinoleate is carried out at about 500 °C to give *n*-heptaldehyde and methyl undecylenate. The methyl undecylenate is hydrolysed to give undecylenic acid, which is

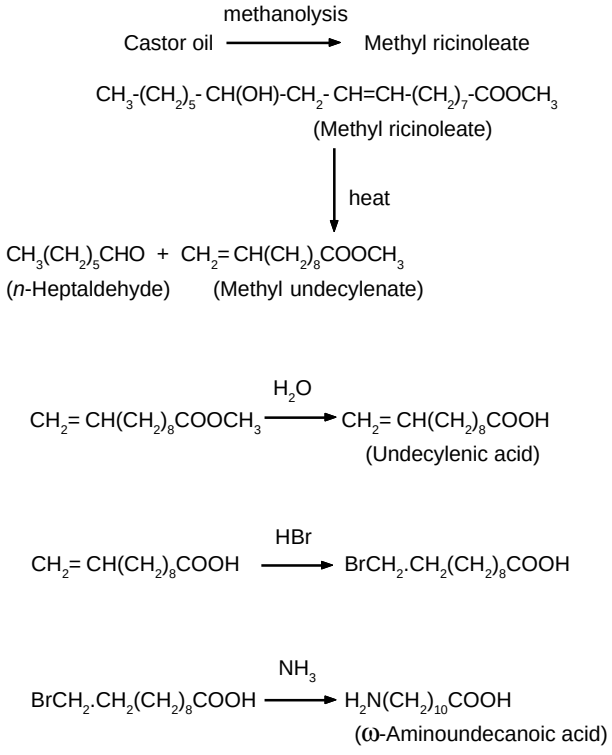


Fig. 4. Production of ω-aminoundecanoic acid from castor oil.

treated with hydrogen bromide in a non-polar solvent in the presence of peroxide. Under these conditions, reverse Markownikoff addition occurs and the main product is ω-bromoundecanoic acid. This product is then treated with ammonia to give ω-ami-noundecanoic acid, which is a crystalline solid. Amino-undecanoic acid is the starting material for nylon-11. It is claimed that a French company produces nylon-11 from this route (Kovaly, 1982).

Another characteristic of castor oil is the hydrogen bonding of its hydroxyl group. This confers high viscosity to the oil, which makes the oil useful as a component in blending lubricants and as an ingredient in fuels (Kirk-Othmer, 1979).

The castor cake is mainly used as a fertilizer. It is unsuitable as an animal feed because of the presence of toxic protein called ricin and toxic allergen, often referred to as CBA (castor bean allergen). Some methods reported for the detoxification of the cake include treatment with ammonia, caustic soda, lime and heat (Horton and Williams, 1989; Kirk-Othmer, 1979; Weiss, 1971; Gardener, 1960). When the cake is steamed, the ricin is detoxified and the allergen is inactivated. Although the use of detoxified cake as cattle feed has been reported (Woodend, 1993), extreme caution and experimentation are desirable before the cake is fed to farm animals. Interestingly, however, some people in parts of South-Eastern Nigeria have long developed a method for treating and detoxifying the unextracted seed that is subsequently used as food seasoning (Okagbue, 1993; Weiss, 1971).

Castor oil was previously used as plasticizer for celluloid and in lacquers, however, the blown oil has been discovered to perform better. Blown or oxidized castor oil is prepared by blowing into it air or oxygen at temperatures of 80-130 °C, with or without a catalyst, to obtain oils of varying viscosity. The blown oil is used widely as plasticizer in lacquers, artificial leathers, hydraulic fluids, and adhesives (Kirk-Othmer, 1979; Weiss, 1971).

Castor oil can also be modified by reduction with hydrogen to produce hydrogenated castor oil, which is a wax like material with melting point of 86 °C. Hydrogenated castor oil is used in cosmetics, hairdressing, ointments, preparation of hydrostearic acid and its derivatives, and in certain cases as wax substitutes and for polishes. Sometimes, hydrogenated castor oil is used as a paint additive, solid lubricant, and as pressure mould release agent in the manufacturing of formed plastics and rubber goods (Kirk-Othmer, 1979; Weiss, 1971).

Another product formed from the modification of castor oil is sulphated castor oil (also known as "Turkey red oil"). Sulphated castor oil is prepared by adding concentrated sulphuric acid to castor oil at 25-30 °C, allowed to stand for several hours, followed by washing and neutralizing with sodium hydroxide solution. It is an active wetting agent. As such, it is used extensively in dyeing and in finishing of cotton and linen. The action of sulphuric acid on castor oil also produces a useful emulsifier for certain insecticidal oils (Kirk-Othmer, 1979; Weiss, 1971).

Even though a small amount is involved, about 0.7 parts per hundred of rubber of castor oil is added to latex or wet rubber to promote crumbling and thus produce the crumb rubber grade (RIMK, 1966).

Dehydrated castor oil (DCO) is used in the preparation of alkyd resins (Ogunniyi and Njikang, 2000) that are in turn used for paints, enamels, lacquers and varnishes with high gloss, good adhesion, and wetting qualities. It has advantages over tung oil because it is non-yellowing (Weiss, 1971). The vulcanization of DCO with sulphur has been reported (Botros and Meinecke, 1987), the resulting product, factice, has been found to be a rubber additive with antiozonant and good flow properties.

Other miscellaneous applications in which castor oil is used include the preparation of brake fluids, the formulation of cathartic, the formulation of contraceptive creams,

the preparation of bland emollient to treat skin diseases, and for inducing labour in pregnancy. It is also used as an ingredient of soaps and polishes. Castor oil-based synthetic detergents are less prone to foaming and the disposal of the detergent is hastened since microbiological breakdown is simplified. Since DCO contains unsaturated acids, it is probable that DCO can be epoxidized (Kirk-Othmer, 1979; Weiss, 1971). If DCO is epoxidized, the product can be evaluated in polyvinyl chloride compounds as a plasticizer/stabilizer with the possibility of serving as an alternative to epoxidized soybean oil that is currently being used.

In addition, stems from castor plant can be used as fibre for making ropes while the cellulose obtained from the stem can be used for making cardboard and paper. The leaves in the growing plant are also said to be able to repel flies and mosquitoes. An assessment of the use of the castor plant in agroforestry has also been reported (Muchena, 1998).

Conclusion

The various uses of castor oil have been outlined but it must be realized that several other chemicals that can be produced from castor oil have not been mentioned. In many countries, with little or no petrochemical feedstock, castor oil can prove to be a useful renewable resource. This oil, with a variety of uses, is a suitable substitute for industrial applications where many edible oils are being used currectly. Generally, it is desirable that non-edible oils should be exploited for such purposes, as far as possible, so that edible oils can become available for human consumption. This is especially important in developing countries where food security poses a serious challenge and renewable chemical feedstocks are not available.

References

Botros, S.H., Meinecke, E. 1987. Effect of brown vulcanized vegetable oil on ozone resistance, aging and flow of rubber compounds. *Rubber Chem. Technol.* **60:** 159 -175.

Brydson, J.A. 1975. *Plastic Materials*, p. 394, Newnes-Butterworths, London, UK.

Chamunorwa, S. 1995. *Financial Gazette*, Harare, December 21, 1995, Harare, Zimbabwe.

Das, G. Trivedi, R.K., Vasishtha, A.K. 1989. Heptaldehyde and undecylenic acid from castor oil. *J. Am. Oil. Chem. Soc.* **66:** 938 - 941.

Ehrlich, A., Smith, M.K., Patton, T.C. 1959. Castor polyols for urethane foams. *J. Am. Oil. Chem. Soc.* **36:** 149-154.

Gardener, H.K. 1960. Detoxification and deallergenisation of castor beans. *J. Am. Oil. Chem. Soc.* **37:** 142-148.

Heiss, H.L. 1960. Low durometer cast urethane elastomers. *Rubber Age* **88:** 89-97.

Horton, J., Williams, M.A. 1989. A cooker-extruder for deallergenation of castor bean meal. *J. Am. Oil. Chem. Soc.* **66:** 227-231.

Kirk-Othmer. 1979. *Encyclopaedia of Chemical Technology*, vol. **5**, John Wiley & Sons, New York, USA.

Kovaly, K.A. 1982. *Biomass ⇔ Chemicals, Chemtech.* (August), pp. 486-489.

Lyon, C.K., Garret, V.H. 1973. New castor oil-based urethane elastomers. *J. Am. Oil. Chem. Soc.* **50:** 112-114.

Mascolo, N., Autore, G., Borrelli, F., Izzo, A.A., Capasso, F. 1996. Diarrhoea and mucosal injury evoked by castor oil are independent events. *Pharm. Biol.* **34:** 91-95.

Muchena, S. 1998. An assessment of alternative perennials for use in agroforestry systems of small holder farmers. *Trans. Zimbabwe Sci. Assn.* **72:** 66-68.

Ogunniyi, D.S., Njikang, G.N. 2000. Preparation and evaluation of alkyd resins from castor oil. *Pak. J. Sci. Ind. Res.* **43:** 378-380.

Ogunniyi, D.S., Fakayejo, W.R.O., Ola, A. 1996. Preparation and properties of polyurethanes from toluene diisocyanate and mixtures of castor oil and polyol. *Iranian Polym. J.* **5:** 56-59.

Okagbue, R.N. 1993. Microbiological activity in traditional food processing in Nigeria. In: *Proc. International Conf. Uniting African World Scholars and Communities: Global Realities and Social Transformation*, July 8-30 August, 1993, Ghana National Theater, Accra, Ghana.

Patel, P., Suthar, B. 1988. Interpenetrating polymer network from castor oil based polyurethanes. *Polym. Eng. Sci.* **28:** 901-905.

Quipeng, G., Shixia, F., Qingyu, Z. 1990. Polyurethanes from 2,4-toluene di-isocyanate and a mixture of castor oil and hydroxyether of bisphenol A. *Eur. Polym. J.* **26:** 1177-1180.

RIMK. 1966. *Planter's Bulletin: Specifications for Castor Oil for Use in the Production of Hevea Crumb Rubbers*, Rubber Institute of Malaysia, Kualar Lumpur, Malaysia.

Roetheli, J.C., Glaser, L.K., Brigham, R.D. 1991. Castor: assessing the feasibility of US product. In: *Summary of Workshop*, Plainview, Texas, USDA Office of Agriculture and Texas A & M University, Texas, USA.

Saunders, K.J. 1988. *Organic Polymer Chemistry*, **11**, 2nd edi-

tion, Chapman and Hall, London, UK.

Weiss, E. 1971. *Castor, Sesame and Safflower*, Chapter 11, Leonard Hill, London, UK.

Weiss, E. 1983. *Oilseed Crops, Tropical Agriculture Series*, pp. 530-564, Longman Scientific and Technical Publications, London, UK.

Woodend, J. J. 1993. Genetic improvement and commercialization of the African perennial castor (*Ricinus communis)* plant. *Zimbabwe Sci. News* **27:** 42-45.

Yeadon, D.A., McSherry, W. F., Goldblatt, L.A. 1959. Preparation and properties of castor oil urethane foams. *J. Am. Oil. Chem. Soc.* **36:** 16-20.

INSTRUCTIONS TO AUTHORS

Pakistan Journal of Scientific and Industrial Research publishes original research in all scientific and technological fields in the form of **research articles, short communications and critical reviews**. All manuscripts are evaluated by subject experts. However, authenticity of the contents remains the sole responsibility of the authors. Manuscripts should be submitted with a covering letter, giving names and addresses of all the authors, indicating the corresponding author and certifying that the paper has neither been, nor will be sent elsewhere for publication. Names and addresses of four experts in the relevant field should also be provided.

Authors may submit electronic copies, followed by two legible double spaced typed copies of the manuscript. The manuscript should contain, in order, Running Title (not more than 60 characters), Title of the article, full names, addresses, telephone numbers, fax numbers, e-mail addresses of all the Authors, Abstract (not more than 150 words), three or more Keywords, Introduction, Materials and Methods, Results and Discussion, Illustrations, and References. All illustrations including figures, drawings and photographs should be of good quality, suitably labelled, captioned, numbered and referred to in the text along with tables and references. The drawings should be in black ink, on art or tracing paper, drawn to fit in single or double columns (8 or 16.5 cm) on reduction. Letterings and symbols should be of sufficient size so as to be clearly legible on reduction. Use IUPAC rules for units and their abbreviations. Two sets of original coloured or black & white photographs, mounted on light weight white cardboard, should be provided. In case of coloured photographs, 50% printing expenses would have to be borne by the authors. Two hard copies of the paper should be sent along with an electronic copy in MS Word, Excel, FreeHand. Responsibility for any error/omission in the paper lies with the author.

References should be cited in the text by the last name of the author (both authors if only two, the first author and *et al* when more than two) followed by the year, in descending chronological order. All references in the bibliography should be listed in alphabetical order of the authors' last names followed by date of publication and other complete details as given below.

Articles in Journal

(In "References")

Park, S.H. 2005. Fine tuning and cross-talking of TGB-β signal by inhibiting Smads. *J. Biochem. Mol. Biol.* **38:** 9-16.

Aksu, Z., Kabasakal, E. 2004. Batch adsorption of 2,4-dichlorophenoxyacetic acid (2,4-D) from aqueous solution by granular activated carbon. *Separation Purification Technol.* **35:** 223-240.

Evans, W.J., Johnson, M.A., Fujimoto, Cy. H., Greaves, J. 2000. Utility of electrospray mass spectrometry for the characterization of air-sensitive organolanthanides and related species. *Organometallics* **19:** 4258-4265.

(In "Text")

Park (2005), Aksu and Kabasakal (2004) and Evans *et al.* (2000)

(Park, 2005; Aksu and Kabasakal, 2004; Evans *et al.*, 2000)

Books

Cinar, A., Parulekar, S.J., Undey, C., Birol, G. 2003. *Batch Fermentation: Modeling, Monitoring, and Control,* Marcel Dekker, Inc., NY, USA.

Chapters in Edited Books

Newby, P.J., Johnson, B. 2003. Overview of alternative rapid microbiological techniques. In: *Rapid Microbiological Methods in the Pharmaceutical Industry,* M.C. Easter (ed.), pp. 41-59, 1st edition, Interpharm/CRC, Boca Raton, Florida, USA.

Articles in Proceedings of Conferences, Symposia, Seminars, Workshops

Marceau, J. 2000. Innovation systems in building and construction, and the housing industry in Australia. In: *Proc. Asia-Pacific Sci. Technol. Mangmt. Sem. National Innovation systems: How to Maintain a Sustainable Growth of the Asia-Pacific Region,* 6th, pp. 129-156, Japan Int. Sci. Technol. Exchange Centre, Saitama, Japan.

Technical/Department Reports

SIC-PCSIR. 2002. *Biannual Report, 2000-2001; 2001-2002,* Scientific Information Centre, Pakistan Council of Scientific & Industrial Research, PCSIR Laboratories Campus, Off University Road, Karachi, Pakistan.

Thesis

Saeed, A. 2005. Comparative Studies on the Biosorption of Heavy Metals by Immobilized Microalgal Cultures, Suspended Biomass and Agrowastes. *Ph. D. Thesis*, pp. 1-248, University of the Punjab, Lahore, Pakistan.

Patents

Young, D.M. 2000. Thermostable Proteolytic Enzymes and Uses Thereof in Peptide and Protein Synthesis, US Patent No. 6,143,517, 7th November, 2000.

All correspondence should be addressed to:

Executive Editor, Pakistan Journal of Scientific and Industrial Research, Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

Phone: (92-21)-4651739, 4651740, 4651741-3

Fax: (92-21)-4651738

E-mail: pcsirsys@super.net.pk; pcsir-sic@cyber.net.pk