statistical differences between the average values of rate constants or the standard deviations for the two sets of experiments.

More complete data will be reported on this and other systems in the future.

LITERATURE CITED

(1) S. N. Deming and H. L. Pardue, *Anal. Chem.*, **43**, 192 (1971).
(2) E. C. Toren, R. N. Carey, G. S. Cembrowski, and J. A. Schirmer, *Clin. Chem.* (*Winston-Salem, N.C.*), **19**, 1114, 1122 (1973).
(3) D. Sanderson, J. A. Bittikofer, and H. L. Pardue, *Anal. Chem.*, **44**, 1934 (1972).
(4) K. R. O'Keefe and H. V. Malmstadt, *Anal. Chem.*, **47**, 707 (1975).
(5) S. N. Deming and S. L. Morgan, *Anal. Chem.*, **45**, 278A (1973).
(6) J. E. Davis and E. D. Schmidlin, *Chem. Instrum.*, **4**, 169 (1973).
(7) B. G. Willis, J. A. Bittikofer, H. L. Pardue, and D. W. Margerum, *Anal. Chem.*, **42**, 1340 (1970).
(8) A. Savitsky and M. J. E. Golay, *Anal. Chem.*, **36**, 1627 (1964).
(9) P. R. Bevington, "Data Reduction and Error Analysis for the Physical Sciences", McGraw-Hill, New York, 1969, Chap. 11.
(10) A. I. Vogel, "Quantitative Inorganic Analysis", 3rd ed., J. Wiley and Sons, New York, 1961.
(11) I. M. Kolthoff, E. B. Sandell, E. J. Mcehan, and S. Bruckenstein, "Quantitative Chemical Analysis", 4th ed., The Macmillan Company, London, 1969, p 982.
(12) J. E. Stewart, Durrum Application Notes No. 4, "Flow Deadtime in Stopped-Flow Measurements".
(13) R. E. Thiers, A. H. Reed, and K. Delander, *Clin. Chem.* (*Winston-Salem, N.C.*), **17**, 42 (1971).
(14) H. L. Pardue, A. McDowell, D. Fast, and M. J. Milano, *Clin. Chem.* (*Winston-Salem, N.C.*), **21**, 1192 (1975).
(15) J. A. Nelder and R. Mead, *Computer J.*, **7**, 308 (1965).
(16) Anders Ringbom, "Complexation in Analytical Chemistry", Interscience, New York, 1969, pp 128–139, 287–289.

Linearizing the Calibration Curve in Determination of Sulfate by the Methylthymol Blue Method

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The linearization of the calibration curve of the automated methylthymol blue (MTB) procedure for the determination of sulfates is described. This is accomplished by altering the barium-to-MTB molar ratio of the barium–MTB reagent used. Because of the low purity of the commercial dye, barium-to-MTB molar ratios of 0.9:1 or lower are usually needed for obtaining linear calibration curves. A linear relation between absorbance and sulfate concentration with a correlation coefficient of 0.9995 or better is obtained. The relative standard deviation among replicates, in the range of 0 to 100 μg/ml, is 1.4 to 0.4%. The sensitivity for this range is 4 μg/ml SO_4^{2-}.

Routine determination of sulfate in a large number of water and air samples is usually performed by a variety of automated wet chemical methods. The automated methylthymol blue method developed by Lazrus et al. (*1*) is gradually displacing all the other methods, and currently most of the Federal and State water and air pollution agencies have adopted this method for the determination of sulfates.

The method involves displacement of methylthymol blue (MTB) from a barium–MTB complex by sulfate and colorimetric measurement of the freed MTB in highly basic solution.

$$Ba^{2+} + MTB^{6-} \rightleftharpoons BaMTB^{4-} \qquad (1)$$

$$BaMTB^{4-} + SO_4^{2-} \rightleftharpoons BaSO_4 + MTB^{6-} \qquad (2)$$

The absorption maximum of the barium–MTB complex is at 610 nm and that of the free MTB is at 460 nm. Therefore, the free MTB can be measured at 460 nm without major interference from the barium–MTB complex. As Equation 2 shows, the concentration of MTB is directly related to the concentration of sulfate, which permits determination of the sulfate by measuring the concentration of the displaced MTB.

One of the most unwanted characteristics of this method has been the nonlinear relationship between the output of the instrument (absorbance) and the sulfate concentration. The plot of absorbance vs. concentration appears to be a parabolic function which becomes linear above a certain concentration of sulfate. Thus, in most cases, a calibration curve may be assumed as consisting of two linear portions of different slopes. The first linear portion corresponds to low sulfate concentrations (about one fifth of the total range), and the second to higher sulfate concentrations. The slope of the first linear portion of the calibration curve is lower by at least a factor of two from the slope of the second portion. Currently, two different approaches are used for the utilization of the nonlinear calibration curves for determination of sulfates. In the first approach, the output signals corresponding to each point of the calibration curve are linearized electronically with respect to the standard sulfate concentration, and thus the entire range can be utilized. In the second approach, only the linear portion of the calibration curve corresponding to higher sulfate concentrations is used for the analysis of samples. Both approaches have several disadvantages. For the electronic linearization, additional equipment and some extra effort to set the calibration are required. In the other approach, the sensitivity of the method is reduced by more than 20% and, therefore, low sulfate samples cannot be analyzed unless a larger or more concentrated sample is used.

In our laboratory, the MTB method is used extensively for determination of sulfate in environmental samples. To avoid all the disadvantages which arise from the nonlinear calibration curves, we investigated the chemistry involved in this system with the hope of linearizing the calibration curves by modifying the original analytical procedure.

In this paper, we wish to report the results of our research which enabled us to obtain linear response with respect to sulfates without any electronic linearization of the resulting signals.

EXPERIMENTAL

Instruments and Apparatus. A Technicon AutoAnalyzer II connected to an Automatic Sampler IV was used throughout this study. The flow diagram used in the present study was similar to the one recommended by Technicon for sulfate analysis in waste water (*2*). In this, the sample containing sulfate in the range of 4 to 100 μg/ml
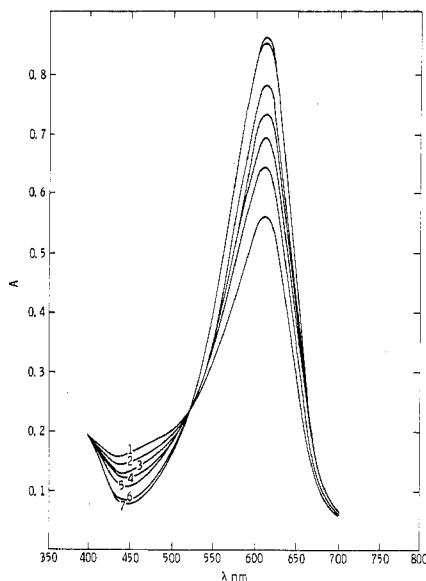
Figure 1. Absorption spectra of the barium-methylthymol blue complexes at various barium-to-MTB molar ratios

[Ba²⁺]/[MTB] for spectra 1, 2, 3, 4, 5, 6, and 7 was equal to 0.89, 1.07, 1.25, 1.43, 1.78, 2.32, and 2.94, respectively. pH = 12

is first diluted (1:9.7) with water, then passed through a cation exchange column to remove all the interfering cations, and finally reacted with a reagent containing barium ion and MTB (pH ~2.4) in a 20-turn mixing coil followed by addition of the appropriate amount of 0.18 N NaOH in a second 20-turn mixing coil to bring the pH to about 12. The absorbance of the resulting solution is monitored continuously in the colorimeter at 460 nm. Other, more sensitive, concentration ranges can be established by changing the sample dilution. Samples containing sulfate in the range of 0.5 to 10 μg/ml can be analyzed by eliminating the dilution and passing the sample directly into the ion-exchange column.

Reagents. Methylthymol blue (J. T. Baker, Baker grade) was used without further purification. The rest of the chemicals were reagent grade and were used without any further purification.

All the reagent solutions were prepared as described in the Technicon procedure (2) except the methylthymol blue reagent solutions, which were prepared in the following manner: a 6.796×10^{-3} M Ba²⁺ solution was prepared by dissolving 1.6601 g of BaCl₂ and diluting to 1 l. with distilled water. A methylthymol blue solution of the same molarity was prepared by dissolving 0.5193 g of MTB in 100 ml of distilled water. These two solutions were used for the preparation of the Ba–MTB reagent with varying barium-to-MTB molar ratios by adding: 12.4 ml aliquots of the BaCl₂ solution to each of several 250-ml volumetric flasks, then adding volumes of the MTB solution ranging from 9.0 ml (for [Ba²⁺]/[MTB] = 1.39) to 16.0 ml (for [Ba²⁺]/[MTB] = 0.78). Two ml of 1 N HCl, the appropriate amount of distilled water to bring the volume to 50 ml, and a small amount (1.5 ml) of wetting agent (Brij-35) were then added, and finally the solution were diluted to 250 ml with ethanol. The solutions were refrigerated overnight before they were used in the AutoAnalyzer II with the usual flow arrangement for the 4–100 μg/ml sulfate range.

The nominal [Ba²⁺]/[MTB] ratio of each reagent can be calculated from the volumes of the respective solutions used to prepare the particular reagent. Reagents of barium-to-MTB molar ratios above 1.39 or below 0.78 may also be prepared using a similar procedure.

Procedure. Nine-point calibration curves covering the operating range of 4 to 100 μg/ml SO₄²⁻ (4, 8, 12, 16, 20, 40, 60, 80, and 100 μg/ml, respectively) were run with each methylthymol blue reagent solution of varying barium-to-dye molar ratios. The instrumental parameters were kept constant throughout all these runs and they were as follows: standard calibration adjustment at 4.0, wash-to-sample ratio 1:3 and 30 samples/h sampling rate. The baseline was adjusted as necessary. After each reagent was run with the nine standards, the heights of the obtained peaks were measured and corrected for the baseline. The standard curve parameters (3) of slope (b) and intercept (a) were calculated from the known sulfate concentrations and the heights of the resulting peaks by using the linear least squares equation. The standard error of estimate

$$Sy = \sqrt{(\Sigma Y^2 - a \Sigma Y + b \Sigma YX)/n} \tag{3}$$

and the coefficient of correlation,

$$r = \frac{(\eta \Sigma XY - \Sigma X \Sigma Y)}{\sqrt{[n \Sigma X^2 - (\Sigma X)^2][n \Sigma Y^2 - (\Sigma Y)^2]}} \tag{4}$$

were calculated also for each set of data. Using the standard error and the coefficient of correlation as criteria, an optimum [Ba²⁺]/[MTB] ratio can be selected for the preparation of MTB reagent suitable for routine use. Using the selected [Ba²⁺]/[MTB] ratio, the weight of MTB dye for 1 l. of reagent can be calculated. This weight corresponds to the particular lot of commercial MTB and it can be used thereafter each time Ba–MTB reagent is prepared from the same lot of MTB dye. For each new lot of MTB, a linearity study should be performed to establish the optimum barium-to-MTB ratio.

Spectrophotometric Determination of the Purity of the Dye. The purity of the commercial dye can be estimated spectrophotometrically by measuring the absorbance of solutions of known concentrations and using the known molar absorptivity of MTB at that wavelength. The molar absorptivity at 435 nm for aqueous MTB solutions of pH 1.8 to 5.4 has been measured by Yoshino et al. (4). The reported value is 1.89 l. mol⁻¹ cm⁻¹. An MTB solution was prepared by dissolving 0.1947 g MTB in 50 ml 0.001 N HCl, which gives a calculated concentration of 5×10^{-3} M. This MTB solution was diluted 1:100 with 0.001 N HCl and the absorbance at 435 nm was recorded with a Cary-14 spectrophotometer using a 1-cm cell. The purity was calculated from the relation:

$$\% \text{ purity} = 105.82 A_{435\,nm} \tag{5}$$

where $A_{435\,nm}$ is the measured absorbance at 435 nm.

Spectrophotometric Investigation of the Formation of Binuclear Complexes. The change in the absorption spectra of solutions containing constant concentrations of MTB and varying amounts of barium was used to demonstrate the formation of binuclear complexes of barium with MTB. For this purpose 5.0 ml of a Ba–MTB reagent (3.8×10^{-5} M MTB) of known [Ba²⁺]/[MTB] ratio and appropriate amounts of BaCl₂ solution to yield the desired [Ba²⁺]/[MTB] ratio were added in 50-ml volumetric flasks followed by 3 ml of 0.18 N NaOH. Then the solutions were all diluted to the mark with distilled water and absorption spectra were recorded immediately with a Cary-14 spectrophotometer using a 1-cm cell.

RESULTS AND DISCUSSION

Methylthymol blue (MTB) is a derivative of thymol sulfonphthalein with two N,N'-di(carboxymethyl)amino-methyl groups attached to its 3,3' position (1).
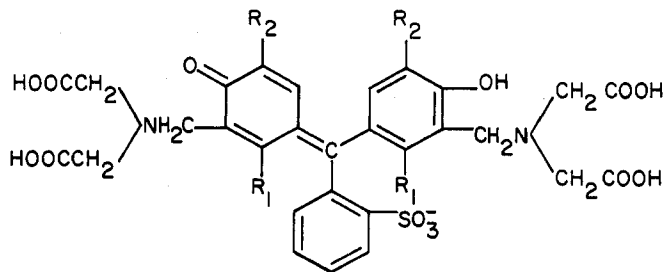
Because of these two groups, MTB behaves as a ligand capable of chelating with a metal atom at either one of the two end groups. However, because of the stereochemical hinderance of the bulky sulfonphthalein structure, the participation of both groups in the coordination sphere of a single metal ion is not feasible (4). Thus, the second group remains vacant and available for coordination with a second metal ion.

Formation of binuclear complexes of MTB with metal ions such as Co(II), Ni(II), Cu(II), and Zn(II) has been studied (4–6). It has been found that the absorption maximum of the binuclear complex is at the same wavelength (610 nm) as the 1:1 complex, but the molar absorptivity of the binuclear complex is significantly larger. The spectra of Figure 1 show the formation of the binuclear barium–MTB complex in alkaline solutions. It can easily be seen that the molar absorptivity at 610 nm increases as the barium-to-dye ratio increases up to about 2:1 indicating that the amount of barium in excess of that required for formation of the 1:1 complex forms further complexes. When the barium-to-dye ratio becomes higher
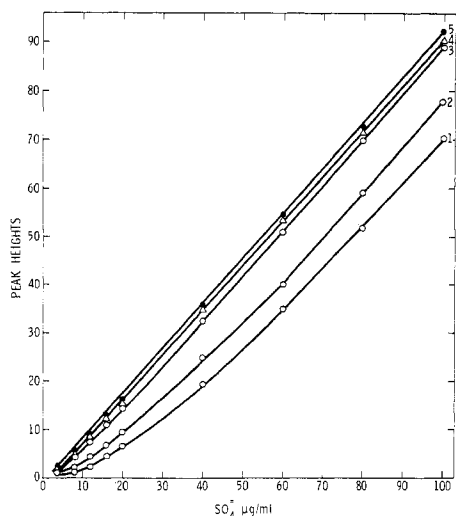
Figure 2. Change of the calibration curve with the barium-to-MTB ratio

$[Ba^{2+}]/[MTB]$ for curves 1, 2, 3, 4, and 5 was 1.39, 1.25, 1.00, 0.83, and 0.78, respectively

Figure 3. Change of the coefficient of linearity of the calibration curve with the barium-to-MTB molar ratio

than 2:1, the molar absorptivity does not increase further. It is, therefore, rather evident that MTB forms binuclear complexes with excess of barium ions.

Commercial methylthymol blue, as most dyes, is not 100% pure. In fact, the purity of MTB is even lower than many other dyes because the monosubstituted derivative of sulfonphthalein (semimethylthymol blue, SMTB), is produced along with MTB during preparation. SMTB can be separated from MTB by column chromatography (7).
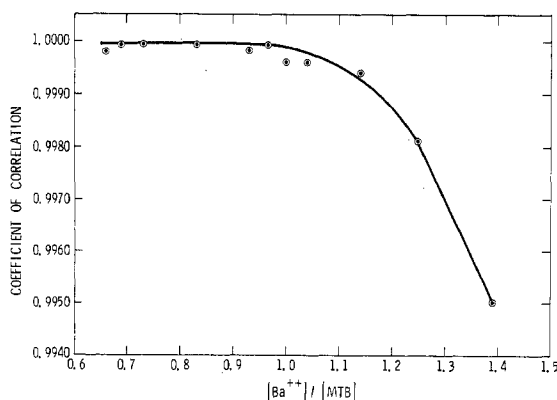
As described in the previous section, the purity of MTB can be determined spectrophotometrically. This technique gives essentially a measure of the existing impurities which do not absorb at 435 nm. Impurities such as SMTB or unsubstituted sulfonphthalein which absorb at 435 nm cannot be determined. The amount of nonabsorbing impurities in commercial dye preparations has been shown to vary from lot to lot ranging from 10 to 50%.

This low purity of the MTB dye is the main reason for the nonlinear calibration curves. For example, the composition of the MTB reagent solution recommended by Lazrus et al. (1) and in the Technicon procedure (2) contains a barium-to-dye molar ratio equal to 1:1, but due to the low purity of the commercial dye, the actual ratio is usually considerably higher than that. Binuclear species are, therefore, formed along with the mononuclear complexes and the removal of small amounts of barium ions by the sulfate (at low sulfate concentration) results in the transformation of the binuclear to mononuclear complexes (Equation 6).

$$Ba_2MTB^{2-} + SO_4^{2-} \rightleftharpoons BaSO_4 + BaMTB^{4-} \qquad (6)$$

The molar absorptivity of the binuclear complex at 460 nm is lower than that of the mononuclear (Figure 1) and, therefore, under the above experimental conditions, the absorbance of the solutions will be increased only slightly with added sulfate. With higher sulfate additions, the 1:1 complex is converted to free MTB (Equation 2) resulting in a higher change in absorbance per mole of added sulfate.

The formation of binuclear complexes can be eliminated by changing the barium-to-dye molar ratio. Figure 2 shows the effect of the barium-to-dye molar ratio on experimentally obtained calibration curves. It can be seen that the deviation from the linearity increases as the barium-to-dye molar ratio becomes greater. This deviation from the linearity is shown better in Figure 3 where the coefficient of correlation of the calibration curve is plotted vs. the barium-to-MTB ratio.

Table I. Determination of Sulfate in Synthetic Solutions with the Modified MTB Procedure

SO_4^{2-} $\mu g/ml$			
Taken	Found[a]	Std dev	%
5.00	4.90	0.07	1.4
10.00	10.08	0.08	0.8
20.00	20.08	0.29	1.4
40.00	41.14	0.36	0.9
60.00	59.95	0.24	0.4
80.00	80.04	0.43	0.5
100.00	100.58	0.38	0.4

[a] Average of five runs.

These coefficients of correlation have been calculated from the 9-point calibration curves as described above, with five of these points located at the lower part of the calibration curve (4 to 20 $\mu g/ml$) where the curvature usually occurs. The coefficient of correlation approaches unity when sufficient excess of dye prevents the formation of binuclear species. Thus, by changing the concentration of MTB, the optimum barium-to-dye ratio can be established for any given lot of commercial dye preparation and then used thereafter for routine preparation of the reagent.

The spectrophotometrically determined purity of MTB correlates very well with the optimum barium-to-dye ratio established experimentally as it is described above. The commercially available dye usually contains inorganic salts as well as small amounts of SMTB and other organic compounds. However, the nonabsorbing species constitute the larger part of the commercial dye impurity and, therefore, the estimated spectrophotometrically purity of the dye can be used for establishing the barium-to-dye ratio required for the preparation of the MTB reagent. This is based on the assumptions, that 1) MTB forms mononuclear complexes when it is mixed with equimolar amounts of barium and 2) the concentration of organic impurities absorbing at 435 nm is very small. These two assumptions appear to be substantially supported by the experimental results. The value of the barium-to-dye molar ratio required for the preparation of the MTB reagent in most cases was found to be in the range of 0.8 to 0.9 which indicates dye purity of 80 to 90%. However, in some cases, commercially available dye preparation may be found to contain larger amounts of impurities. For example, a recent lot of dye was found to contain 51.3% absorbing species by the spectrophotometric method described above, and the linearity study yielded an optimum $[Ba^{2+}]/[MTB]$ molar ratio of 0.513. Therefore, the optimum barium-to-dye ratio might be defined using the spectrophotometrically de-

termined purity of the commercially available dye. However, the actual measurement of the correct barium-to-MTB ratio, by constructing calibration curves using various ratios, is preferred because of possible impact of the presence of absorbing impurities during the spectrophotometric determination.

Table I shows typical results obtained with the modified MTB procedure for determination of sulfate. For these, the instrument was calibrated independently with standard solutions, and slope and intercept of the calibration curve were determined by the linear least squares equation. The relative standard deviation for replicate standards was found to be between 0.4–1.4%. The sensitivity for this range (0 to 100 μg/ml) was found to be equal to about 4 μg/ml. Higher or lower sensitivities may be obtained by modifying the flow diagram of the Technicon AutoAnalyzer as described above. In our laboratory, we were able to obtain sensitivities of 0.5 μg/ml in a range up to 10 μg/ml by such modifications. Even with this sensitive range, the calibration curves remain linear when the MTB reagent is prepared using the weight of MTB dye calculated from the results of the linearity study.

Both the high (0–100 μg/ml) and low (0.5–10 μg/ml) ranges are used in our laboratory routinely for determination of sulfate, and the results obtained are always comparable to those described here.

LITERATURE CITED

(1) A. L. Lazrus, K. C. Hill, and J. P. Lodge, "Automation in Analytical Chemistry, Technicon Symposia, 1965", Mediad, 1966, pp 291–293.
(2) Technicon Industrial Method No. 118-71 W/tentative, Technicon Industrial Systems, Tarrytown, N.Y., 1972.
(3) T. Yamane, "Statistics, An Introductory Analysis", Harper and Row, New York, 1967, pp 340, 419–423.
(4) T. Yoshino, H. Imada, S. Murakami, and M. Kagewa, Talanta, **21**, 211 (1974).
(5) V. N. Tikonov, Zh., Anal. Khim., **22**, 658 (1967).
(6) O. A. Tataev, S. A. Akhavedov, and B. A. Magomeciova, Zh. Anal. Khim. **25**, 1229 (1970).
(7) T. Yoshino, H. Imada, M. Kagewa, and K. Iwasa, Talanta, **16**, 151 (1969).

Application of Thermal Analytical Methods in the Characterization of Carbonaceous Materials

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Thermal analytical methods are applied to a study of the reactivities toward O_2 and CO_2 of three kinds of carbonaceous materials which give different rate expressions with respect to the fractional completion of the reaction. It is demonstrated that a pseudo-third-order reaction can give a bifurcated peak in the DTA curve and, therefore, the notion of the existence of two kinds of carbonaceous materials based on a bifurcated peak, as has often appeared in the literature, should be asserted with caution.

The thrust of developing more efficient energy systems from coal has renewed a great interest in the application of thermal analytical methods to the study of reactivities of carbonaceous materials toward gases. In the field of catalysis, meanwhile, the employment of differential thermal analysis (DTA) to examine the catalysts poisoned by carbonaceous materials deposited on the surface has attracted wide attention and application. More specifically, in the studies by Rode and Balandin (1, 2) on the nature of the carbonaceous deposits on chromia catalyst, the DTA curve produced by combustion of the deposits showed bifurcation, or two peaks, of the exothermic peak in some instances. The authors interpreted this as an indication of two types of carbonaceous materials on the surface. Thereafter, a bifurcated peak in the DTA curve has been quite commonly related to the notion of the existence of two kinds of carbonaceous materials (3, 4). Furthermore, it has been suggested that, by matching with a synthesized bifurcated DTA peak of a standard reference mixture consisting of two known carbonaceous materials, an unknown carbonaceous material could be analyzed quantitatively (5).

In this work, thermal analytical methods are used to study the reactivities of three widely different carbonaceous materials. The quantitative relationship between a single DTA

curve and the kinetic parameters for a "first-order" heterogeneous reaction has been previously developed (6). The qualitative relationship between the general form of the rate expression and the shape of its corresponding DTA curve is examined in this study for two higher-order gas–carbon reactions. More importantly, it will be shown that a pseudo-third-order reaction can also produce a bifurcated peak in the DTA curve. Therefore, caution must be taken in relating such a peak to the existence of two kinds of carbonaceous materials.

KINETIC ANALYSES OF DTA CURVES

Extraction of kinetic information from DTA curves is, in general, limited to reactions with simple rate expressions (6, 7). For reactions with more complicated rate expressions, the approximate methods outlined by Kissinger (8) and by Yang and Steinberg (6) can also be applied; but the results would be lengthy and the mathematics tedious, and therefore such analyses would not be of practical interest.

In this section, it is not intended to extract the kinetic parameters from a DTA curve. Rather, the qualitative relationship between the rate expression and the general shape of the DTA curve is to be explored. More specifically, the possible number of peaks in a DTA curve will be predicted from the order of the reaction.

The rate of a heterogeneous reaction is expressed in the conventional fashion:

$$\text{Rate} = r = -\frac{1}{m}\frac{dm}{dt} \quad (1)$$

where m is the instantaneous mass of the reacting solid and t is the time.

The rate of a gas–carbon reaction at steady state, or at constant burnoff (x) is normally expressed as a function of the concentrations of the gases involved. However, in the DTA