Some Observations on the Extraction of Triphenylmethane Dyes by Ion-Pair Formation with Amines as Counterions

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Triphenylmethane dyes such as xylenol orange, glycine cresol red, and methylthymol blue by formation of ion pairs with diphenylguanidine, Amberlite LA-2, and tri-*n*-octylamine have been extracted into several solvents. The influence of the amine/solvent system, the concentration of amine, and the presence of electrolytes and the distribution parameters are discussed. As an application, the extraction of glycine thymol blue with Amberlite LA-2 solved into 1-pentanol as the most suitable extracting system was carried out. © 1989 Academic Press, Inc.

INTRODUCTION

Triphenylmethane analytical reagents such as xylenol orange (XO), glycine cresol red (GCR), and methylthymol blue (MTB) are characterized by the presence of polar subtituent groups, which enhance their water solubility. For this reason, liquid–liquid distribution of these dyes is not favored because of the existence in solution of anionic species in a wide range of pHs. Ion pairing is an interesting possibility in performing the extraction of this type of water soluble organic reagents (*1, 2*). The molecular size of the triphenylmethane skeleton ensures in many cases the formation of ion associates if a suitable voluminous counterion is selected, and moreover a proper solvent is available, too. Some correlations exist between the dielectric constant (D_c) and extractability (*2, 3*), and the right choice of solvent is one of the most important factors which must be taken into account for elaborating an analytical extraction procedure.

Amine type large molecules with high molecular weight, such as some long chain aliphatic amines, and quaternary ammonium salts have been used as counterions to extract anionic compounds (*3, 5*).

In this paper, a study of liquid–liquid distribution for aqueous solutions of XO, GCR, and MTB is presented. In the literature data can be found about the extraction of these triphenylmethane reagents together with their metal quelates in order to achieve improved analytical methods for the determination of metal ions. However, attention has not been given to the specific conditions for extracting these dyes in order to determine their behavior as feasible counterions. Some triphenylmethane dyes like crystal violet (*6*), malachite green (*7*), brilliant green

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(8), and rhodamine B (9) have been used as cations because of their ion-pairing ability. For this purpose, amine types were chosen as counterions, since the formation of the ion pair can be more selective because the amine protonation is dependent on the pH extraction value. Diphenylguanidine (DPG), Amberlite LA2 (LA2), and tri-*n*-octylamine (TOA) were solved in several organic media with the aim of disposing of a higher amine concentration than the one expected in a water medium because of the slight solubility of these amines.

The solvents chosen: chloroform (D_c = 4.81),1-pentanol (D_c = 13.9), and benzene (D_c = 2.28) ensure not only a high amine concentration, but also the low values of the dielectric constant of the medium that favor the formation of the ion pairs and their subsequent extraction.

EXPERIMENTAL

Apparatus. A Pye Unicam SP8-200 spectrophotometer equipped with 1-cm glass cells was used to obtain the visible absorption specta. Infrared spectra were recorded using a Perkin-Elmer 599B spectrophotometer equipped with KBr variable path length cells. For pH measurements, a Crison Digital 501 pH meter was used with an Ingold 10/402/3253 combined glass calomel electrode.

Reagents. Analytical reagent-grade chemicals were used without further purification. 1.0×10^{-3} *M* stock solutions of xylenol orange (Merck), glycine cresol red (Fluka), and methylthymol (Fluka) were prepared in water. All of the solutions were standardized against a standard gallium (III) solution by spectrophotometric measure.

Buffer of pH 2.2–8.0 was prepared by mixing appropriate volumes of 0.2 *M* citric acid with 0.4 *M* disodium hydrogen phosphate, both of them solved in water.

Organic solutions of 1,3-diphenylguanidine sym. (Fluka), Amberlite LA-2, as chloride form (Serva), and tri-*n*-octylamine (Merck), in chloroform, in 1-pentanol, and in benzene were prepared daily.

Procedure. The sample solution containing 1.0 ml of 1.0×10^{-3} *M* aqueous solution of dyestuff and 5.0 ml of citrate–phosphate buffer was diluted with water to 25.0 ml. Then, 4.5 ml of this solution was placed in a stoppered centrifuge tube and shaken with 4.5 ml of an extracting solution composed of either 0.1 *M* solution of DPG in chloroform or 1-pentanol (if benzene is used the concentration of DPG is limited to about 0.07 *M*), or of 2:13 (v/v) solution of LA2 in each one of the three solvents, or of 0.1 *M* solution of TOA in the same solvents. Shaking for 30–60 s was enough to reach the equilibrium. After removing the organic phase with a previous centrifugation, this layer was dried with anhydrous sodium sulfate. Spectrophotometric measurements were carried out at several wavelengths in 1-cm glass cells, versus a spectrophotometric blank prepared in the same conditions. In all cases the remainder concentration of the dye in the aqueous phase was determined to obtain the corresponding extraction parameters.

RESULTS AND DISCUSSION

Preliminary Extraction Tests

A variety of organic solvents of the amines considered was used to obtain the

extraction of XO, GCR, and MTB. The most favorable results were only achieved when chloroform, 1-pentanol, and benzene were used. Mixtures of these solvents between themselves and with other ones were not studied. Every selected solvent shows a slight water solubility and a high ability for solving the amines considered. However, working in the presence of water soluble amines at higher concentrations such as hexamine or short aliphatic amines did not allow the extraction.

Effect of the Solvent and the Amine

Figure 1 shows the influence of the amine on the variation of the extraction percentage (%E), as a function of pH_{ex} (pH value of the aqueous phase after extraction), for the three dyes studied. All the resulting experimental possibilities of combining the nine chemicals in this study were considered.

$E(\%)$ has been calculated from the following expression:

$$E(\%) = ([D]_o/[D]_t) \times 100 = (<[D]_t - [D]_w>/[D]_t) \times 100,$$

where $[D]_o$ is the molar concentration of any dye extracted into the organic phase, $[D]_t$ is the analytical molar concentration of the same dye solved in the aqueous phase before the extraction, an $[D]_w$ is the molar concentration of the dye that remains in the aqueous phase after extraction.

From the above expression and taking into account Beer's law, the next expression has been obtained,

$$E(\%) = (1 - <A^\lambda/\epsilon^\lambda b[D]_t>) \times 100,$$

where A^λ is the absorbance value of the aqueous phase after the extraction, ϵ^λ is the molar absorptivity of the dye in aqueous solution at the wavelength chosen to carry the experimental measurements, and b is the path length in centimeters. Values of ϵ^λ, corresponding to the spectrophotometric data of aqueous solutions of the dye studied at a molar concentration equal to $[D]_t$, are obtained from calibration plots $A^\lambda = f(pH)$ at the pH_{ex} value. The measures were carried out at the following wavelength values: 440 and 580 nm for XO, 450 and 602 nm for GCR, and 442 and 590 nm for MTB. The choice of one of the two values is dependent on the pH_{ex} value. In all cases, the aqueous solutions were prepared using solvent satured water. Each extraction was carried out by duplicate.

In Fig. 1 it can be observed that (i) the extraction is different for every dye/amine/solvent system tested; (ii) horizontal comparison of the plots indicates the influence of the solvent employed; e.g., for extracting GCR, chloroform and 1-pentanol seem to be the most efficient solvents. The active role played by the amine in the extraction is also evident, and (iii) a vertical comparison between the plots shows the different behavior of the dyes when the amine/solvent extracting systems remain constant; e.g., if 1-pentanol is employed, the three dyes are easily extracted taking into account every amine studied.

Influence of Amine Concentration

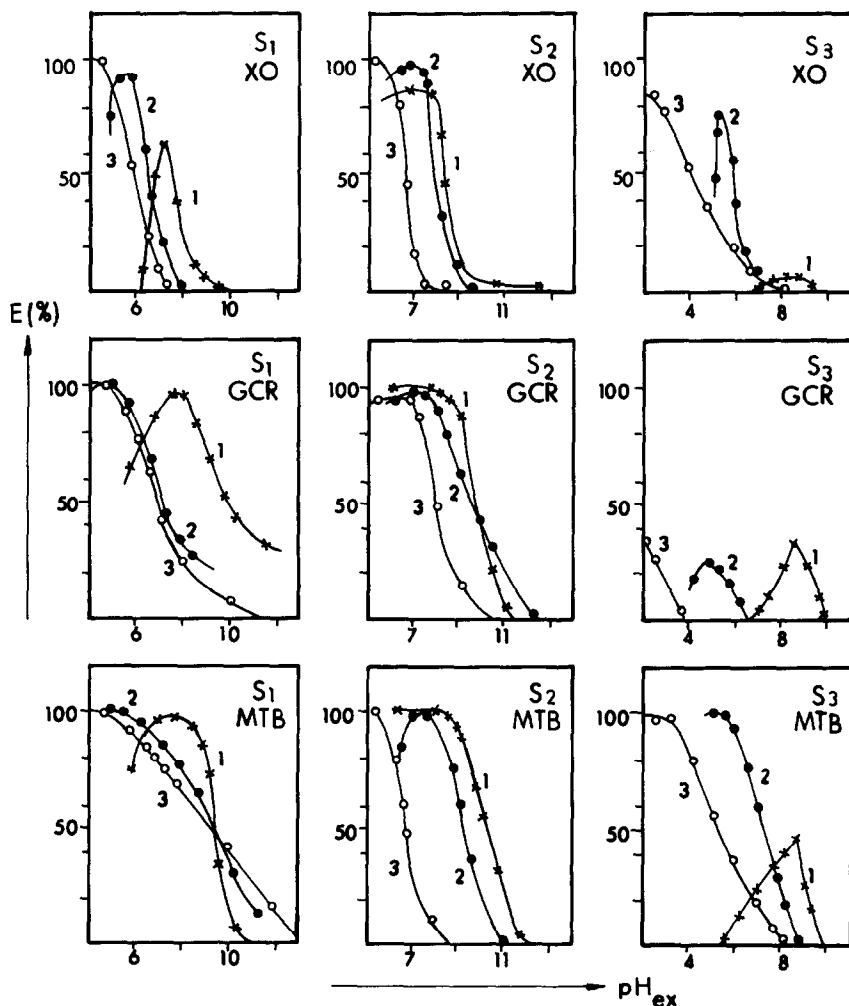To know the influence of the amine concentration on the extraction process,

FIG. 1. Percentage extraction of XO,GCR, and MTB as a function of pH_{ex}. Analytical concentration of dye: 4.0×10^{-5} M. The concentration of amine is according to the procedure. S_1, chloroform; S_2, 1-pentanol; S_3, benzene. 1, DPG; 2, LA2; 3, TOA.

partition experiments were carried out at pH values lower than the pH maximum extraction, according to the results explained above. Extraction modifies the buffered pH of the aqueous phase, because of the slight solubility of the amine in water. So, it is necessary to operate on aqueous solutions of pH 4.5 if a pH_{ex} value of 7.5 is desired when 0.1 M DPG solution into chloroform is used. Furthermore, it must be taken into account that an increase of amine concentration compels the use of a proportional increase of buffer.

In Table 1, the $E(\%)$ values obtained are listed. Complete extraction of every dye is reached when the extracting systems are 4:13 (v/v) LA2/chloroform, 4:13 (v/v) LA2/1-pentanol, and 0.3 M TOA/chloroform. Other compositions of the extraction systems can be taken into account to reach very efficient extraction for

TABLE 1

Percentage Extraction $E(\%)$ of the Dyes with Different Concentrations of Amine into the Three Solvents from Aqueous Buffered Solutions

S	Dye	pH_{ex}	DPG (mol/liter)			pH_{ex}	LA-2 (v/v)			pH_{ex}	TOA (mol/liter)		
			0.2	0.1	0.05		4:13	2:13	1:13		0.3	0.1	0.05
S1	XO	7.3	97.5	64.5	58.3	5.5	100.0	96.0	88.3	4.5	100.0	98.7	98.2
S1	GCR	7.8	99.0	98.0	64.7	5.0	100.0	100.0	93.9	4.5	100.0	73.8	68.4
S1	MTB	7.7	98.2	96.2	86.7	5.0	100.0	100.0	100.0	4.5	100.0	100.0	99.6
S2	XO	7.0	94.3	86.0	80.0	7.0	100.0	99.0	97.5	5.5	100.0	98.7	98.3
S2	GCR	7.0	98.7	98.5	81.8	7.2	100.0	99.3	95.8	6.5	95.8	90.8	87.4
S2	MTB	7.0	100.0	100.0	100.0	7.3	100.0	100.0	98.4	5.5	100.0	100.0	100.0
			DPG (mol/l)										
			>0.07	0.07	0.03								
S3	XO	8.4	Sat.	8.7	7.3	5.3	99.4	78.0	72.4	2.5	94.4	86.0	48.5
S3	GCR	8.5	Sat.	34.8	23.6	5.0	75.0	25.0	16.7	2.2	73.7	34.0	20.9
S3	MTB	8.4	Sat.	46.6	35.6	5.2	100.0	100.0	87.5	2.5	100.0	98.5	95.0

Note. S1, chloroform; S2, 1-pentanol; S3, benzene. Sat., saturated solution at $C_{DPG} > 0.07$ M.

each particular dye. Deficient results only were obtained when DPG was solved into benzene, since other minor results were due to low concentration of amine in the extracting system.

Absorption Spectra and Stability of the Extracts

Absorption spectra of all dye aqueous solutions and organic extracts were studied at several pH_{ex} values in order to determine the effect due to the extracting systems. In Table 2, the wavelengths of the absorption maxima found are listed. In the organic extracts, the value of λ_{max} is not dependent on pH_{ex}, since pH_{ex} only affects the intensity of the band. However, in aqueous medium, the acidity shifts the acid–base equilibrium between conjugated species of the dye, and the two feasible maxima of these kinds of dyes are maintained, with the most intense maximum being the one which corresponds to the predominant ionic species in solution at a defined pH value (10–12).

The absorption of the organic solutions at 425–470 nm seems to show a tendency toward the preferential extraction of the less dissociated species of the dye, which can form the ion pair more easily. However, in the XO/amine/1-pentanol

TABLE 2

Wavelengths of Absorption Maxima (nm) in Spectra of XO, GCR, and MTB Solutions

Dye	H_2O buffer	Chloroform			1-Pentanol			Benzene		
		DPG	LA-2	TOA	DPG	LA-2	TOA	DPG	LA-2	TOA
XO	440 580	470	440	460	440 580	450 585	450 590	—	440	440
GCR	450 602	450	440	450	440	450	450	—	430	425
MTB	442 590	460	425	440	430	430	450	—	440	430

systems this tendency was not observed, since two absorption maxima were found, which can show the extraction of two conjugated ionic species as ion pairs. In these cases, the intensity of the maximum at 580–590 nm decreases according to the sequence DPG–LA2–TOA, and this behavior seems to indicate that TOA forms the ion pair easily with the less dissociated ionic species of XO. When DPG/benzene was used as an extracting system, no maxima were observed, since $E(\%)$ was very low (Table 1). The extracts were found to be stable for at least 1 week at room temperature for all systems listed in Table 2.

Infrared Spectra

After the extraction was carried out, organic solutions containing amine/solvent and dye/amine/solvent systems were studied by recording their IR specta (KBr cells) in order to determine how the ion-pairing had taken place. Comparison of the spectra allow one to observe one strength band about 1575 cm^{-1}, which only appears in the spectra of the dye containing solutions. This band can be assigned to the asymmetrycal stretch vibration of the carbonyl group of the carboxylic groups of the dye. The wavenumber indicates the presence of dissociated carboxyl groups, and as a result it can be suggested that the cationic species of the amine predominantly neutralizes the negative charge of the carboxylic group, thereby allowing the extraction of ion pairs: $(\text{Dye}^{n-})(\text{amineH}^{+})_{n}$.

Effect of the Presence of Electrolytes in the Aqueous Phase

Since one of the extracting systems is capable of obtaining any dye at an extraction percentage of 100% when a solution of 4:13 (v/v) of LA2 in chloroform is used and when operating near pH_{ex} 6.0 (Table 1), the discussion about this effect is restricted to this system because LA2 reagent is used as the chloride form that makes the extraction less favorable, from the beginning.

The presence of sodium perchlorate, sodium nitrate, and potassium nitrate in the concentration range 0.01–0.5 M was studied. In Table 3 the values of $E(\%)$ found are listed. It can be noted that a comparatively large concentration of strong electrolytes hinders a complete extraction. Salting-out does not improve the extraction of this kind of anionic reagent.

TABLE 3
Influence of Strong Electrolytes on the Percentage Extraction $E(\%)$

M	XO			GCR			MTB		
(mol/liter)	NaClO$_4$	NaNO$_3$	KNO$_3$	NaClO$_4$	NaNO$_3$	KNO$_3$	NaClO$_4$	NaNO$_3$	KNO$_3$
0	100	100	100	100	100	100	100	100	100
0.01	100	100	100	100	100	100	100	100	100
0.05	100	100	100	100	97.0	96.2	100	99.0	100
0.10	100	100	100	100	90.0	90.0	100	97.5	100
0.25	100	97.5	97.5	85.0	87.5	82.5	100	96.3	95.0
0.50	73.8	61.3	61.3	85.0	55.0	57.5	100	96.3	95.0

TABLE 4

Extraction Data for GTB at Optimal pH_{ex} Values

Solvent	DPG		LA-2		TOA	
	pH_{ex}	$E(\%)$	pH_{ex}	$E(\%)$	pH_{ex}	$E(\%)$
Chloroform	7.9	72.0	5.5	90.9	3.3	81.9
1-Pentanol	7.8	87.9	6.9	96.1	4.0	95.5
Benzene	8.4	33.6	5.5	67.0	2.4	63.7

Influence of the Extraction Ratio

The influence of the volume ratio of the phases, $R = V_w/V_o$, on the liquid–liquid distribution of XO, GCR, and MTB from aqueous solutions, at pH_{ex} near 6.0 in the range of $R = 1$–15, and 4:13 (v/v) LA2/chloroform solutions was studied. $E(\%)$ has been calculated by measuring the absorbance of the organic phases at 460 nm (Table 2), using as calibration reference the absorbance value of organic extracts when $R = 1$ and $E(\%) = 100$ (Table 1). The aqueous phases were previously saturated with chloroform. As it can be expected, the $E(\%)$ values for the three dyes decreased slightly with the volume of the aqueous phase. This behavior can be expressed by the following empirical equations, which have been obtained by linear regression from the experimental data:

$$\text{XO: } E(\%) = 100.7\text{–}0.6R \text{ (correlation coefficient} = 0.909)$$
$$\text{GCR: } E(\%) = 100.1\text{–}2.2R \text{ (correlation coefficient} = 0.928)$$
$$\text{MTB: } E(\%) = 100.6\text{–}1.2R \text{ (correlation coefficient} = 0.943).$$
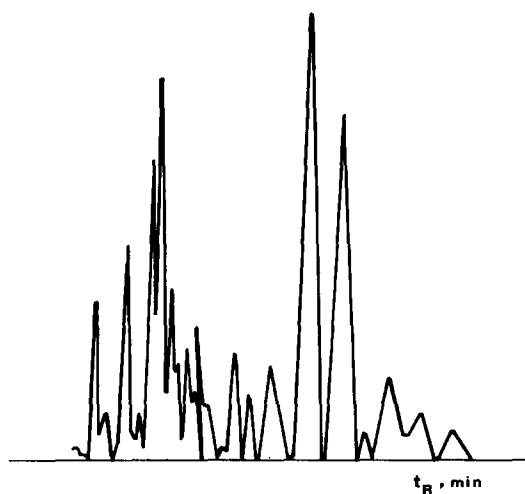

FIG. 2. HPLC chromatogram of impure GTB obtained by using a linear gradient elution. Conditions: Column; nucleosil 5 C_{18}, 150 × 4 mm; mobile phase; a linear gradient (0–100% of B) in 10 min, where A is a 0.1 M phosphate buffer (pH 2.9) and B: 0.1 M phosphate buffer (pH 2.9)–methanol (40:60); flow rate 1.5 ml/min. UV detection: 254 nm at 0.02 aufs.

Extraction of Glycine Thymol Blue

As an application of the study herein explained, the extraction of another tri-phenylmethane dye was carried out in order to check if the suggested extracting systems are suitable for a wider application. Glycine thymol blue (GTB) is a dye related to MTB, and also GCR is structurally connected to XO(*13*). However, its analytical use has been little recommended due to the low degree of purity of the isolated dyestuff. We have used "Standard Fluka" GTB, only suitable as a com-plexometric indicator, at the same analytical concentration as the other dyes studied and GTB was treated under the conditions given in the procedure. In Table 4 the results obtained for every amine/solvent system used are listed, as well as the pH_{ex} values.

The samples were prepared in aqueous medium in the pH range covered by the buffer. The spectrophotometric measures of the remainder aqueous phase were carried out at 440 and 580 nm, applying the above-mentioned equations.

In Table 4, the influence of the amine/solvent system on the extraction of GTB can be observed. It can be noted that the 100% value for $E(\%)$ was not reached in any case. This behavior may be due to no ion-pairing impurities in GTB if the extraction is related to those of MTB (Table 1). A 2:13 (v/v) LA2/1-pentanol solution can be suggested as the most suitable extracting system for GTB. This is according to the results shown in Table 1 for this extracting system. To corrob-orate the possible impurities in GTB a chromatographic—high performance liquid chromatography (HPLC)—study using a C_{18} column and a UV detector was car-ried out. The conditions are given in Fig. 2. In this figure, three predominant peaks and other minor important peaks at several retention times (t_R) were ob-tained, which explains the lack of extraction of GTB in the presence of various solvent/amine systems.

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