Detection and Identification of Side Reactions of Halogenated Hydrocarbon Solvents with Amines of Pharmaceutical Interest by Secondary Processes to the Neutralizations of Sulphonphthaleinic Dyes with These Amines

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Abstract □ The neutralization reactions between amines and diprotic acid dyes in organic solvents generate {dye⁻, amineH⁺} and {dye²⁻, (amineH⁺)₂} ion associates that show two absorption bands in the visible spectrum. An unidentified third absorption band; which appears with a high amine concentration, proves that halogenated hydrocarbon solvents (dichloromethane, chloroform, 1,2-dichloroethane, and carbon tetrachloride) give side reactions with amines (atropine, tropine, quinine, ephedrine, and ajmaline) that generate a quaternary ammonium salt, *N*-halogenalkylammonium halide ({N⁺–RX,X⁻}). The molecular weight of the quaternary ammonium salt is the sum of the amine and that of the solvent. The {N⁺–RX,X⁻} ion associated reacts with {dye²⁻, (amineH⁺)₂} by substitution reactions, forming {dye²⁻, amineH⁺, N⁺–RX} and {dye²⁻, (N⁺–RX)₂} ion associates that justify the third absorption band. The amine–solvent side reactions are of first order with respect to the amine, being very slow processes with rate constant values from 399.4 h^{-1} (tropine–dichloromethane reaction) to 15.8 h^{-1} (atropine–1,2-dichloroethane reaction). Rate constants increase with the basicity of the amine measured in the halogenated hydrocarbons employed. Rate constants also increase with a reduction in the number of the halogen atoms present in the halogenated solvent. The new visible absorption band that appears in the amine–dye neutralization gives a quick colorimetric test to bring to light this kind of side reaction in these solvents.

Introduction

The two-phase photometric titration of amines of pharmaceutical interest with sulphonphthaleinic dyes is an important method for the analysis of pharmacologically active substances.[1-6] In previous papers, the reaction between homatropine, ajmaline, atropine, and tropine with dyes such as bromocresol green (BCG) or bromophenol blue (BPB) in dichloromethane, (CH_2Cl_2) has been described, and it was established that amine neutralizes the protons of the dye forming {dye⁻, amineH⁺} and {dye²⁻, (amineH⁺)₂} ion associates.[7-9] Unidentified spectral changes in dye–amine mixtures with a high concentration of amine made evident the existence of side reactions of the halogenated hydrocarbon solvents employed. The explanations given about these side reactions did not justify the observed spectral changes.[7-9] Although the discovery of these side reactions was accidental, it is very important to study them because they indicate unknown properties of universally used solvents.

In this paper, we give an explanation that permits the justification of the above-mentioned spectral changes. We also describe and quantify the side reactions of various halogenated hydrocarbon solvents with a group of amines. Atropine (AT), tropine (TP), quinine (Q), ephedrine (EF), and ajmaline (AJ) were used as the amines, BCG and BPB were used as the dyes, and CH_2Cl_2, chloroform, 1,2-dichloroethane, and carbon tetrachloride were used as solvents. A specially detailed study was carried out with AT and CH_2Cl_2 that showed that AT reacts with this solvent, generating a quaternary ammonium compound (*N*-chloromethylatropinium chloride, {AT^+-CH_2Cl,Cl^-}) with a molecular weight of 374.36 g/mol, the sum of the molecular weights of AT and CH_2Cl_2. This product was obtained in the solid state by direct precipitation in a solution of AT (1 M) and characterized by ^1H and ^{13}C NMR, mass spectrometry, IR spectrometry, etc. The addition of {AT^+CH_2Cl,Cl^-} to a dye–AT solution generated in the visible absorption spectrum an identical change to that observed in the solution with a large excess of AT.

A kinetic study was also carried out on the quaternization reaction of AT, TP, Q, AJ, and EF in carbon tetrachloride, chloroform, CH_2Cl_2, and 1,2-dichloroethane. Determination of the rate constants of each case established the physicochemical properties of the amines that influence their quaternization in these solvents. The sequence of the reactivity of each solvent was also established. Because the *N*-halogenalkylammonium halide ({N⁺–RX,X⁻}) has a great capacity for ionic interchange, it reacts spontaneously with the {dye²⁻, (amineH⁺)₂} ion associates forming {dye²⁻, amineH⁺, N⁺-RX}, which is a type of mixed ion associate or ternary ion associate. This type of ion associate was proposed for quantitative determinations of benzethonium and benzalkonium chloride,[10,11] which can be determined spectrophotometrically without interference from various other quaternary ammonium salts and alkylamines. Recently, papers about new types of ternary ions of analytical character[12,13] and also of basic chemistry[14] have been published.

Experimental Section and Results

Reagents—The reagents used were CH_2Cl_2 (*probus* HPLC, with <0.02% water content), carbon tetrachloride and chloroform (*Merck* spectrophotometric grade), 1,2-dichloroethane (*Carlo Erba* analytical grade), methanol (*Probus* HPLC), dimethylsulfoxide, (DMSO; *Merck* spectrophotometric grade), hydrochloric acid, (*Merck* analytical grade), BCG and BPB (*Merck*), AT, TP, EF, and AJ (*Sigma*), Q, (Fluka-Chemie), sodium hydroxide (*Probus*) , and phosphorous pentoxide, (*Panreac*). All the solid materials were of analytical grade and all the amines were used as free bases without further purification after storage in a desiccator with phosphorous pentoxide.

Apparatus—The ^1H and ^{13}C NMR spectra were recorded on a *Bruker AM-300 Spectrometer* with DMSO as solvent. Mass spectra were recorded on a *Hewlett-Packard HP-5988-A* by electronic-impact ionization by the direct injection probe (D.I.P) method at 280 °C. All vis–UV spectrophotometric measurements were made on a *Perkin-Elmer Lambda-5* spectrophotometer, with a *B. Braun AG* model *Frigomix* and *thermomix 1441* thermostat with a ±0.2 °C accuracy. Quartz cells of 1 cm with Teflon stoppers were also used. The IR spectra were made on a *Perkin-Elmer 298* IR spectrophotometer with potassium bromide pellets. The KBr had been previously dried in an oven at 200 °C. A *Radiometer Copenhagen PHM64 Research pH* meter was used for the pH measurements. All calculations were made with an *Amstrad PC1512SD* computer. The thermal differential and thermogravimetric analyses were carried out in a *Rigaku Thermoflex TA10* model with a heating velocity of 5 °C min in a static atmosphere of air. Some of the substances were dissolved with the help of a *Selecta ultrasons* ultra sound apparatus.

Dye–AT Reaction when AT:Dye Mole Ratios Are <100—The general guidelines of the spectrophotometric study of the new absorption

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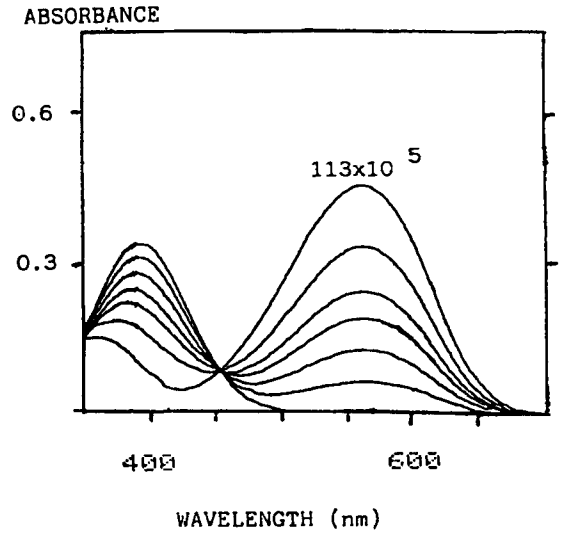


Figure 1—Visible—UV spectra of dichloromethanic solutions of BCG (2 × 10^{-5} M) and AT base (2.00, 2.79, 6.00, 10.50, 15.60, 30.30, and 113 × 10^{-5} M; T = 293.16 (K).

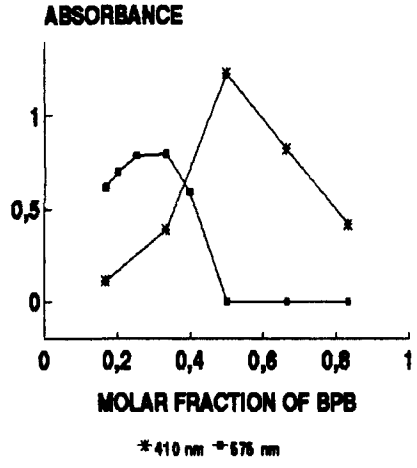


Figure 2—Job's plot for the BPB–AT reaction in CH_2Cl_2 ([BPB] + [AT] = 10^{-4} M; T = 293.16 K). Key: (*) 410 nm; (■) 575 nm.

bands observed under these conditions have been given previously,[7] where homatropine (HT) was used instead of AT, which is the most extensively studied amine used in this work.

When AT is mixed with a dye in CH_2Cl_2, new bands of absorption in the visible spectrum are observed that suggest the existence of new compounds in the solution. Figure 1 shows the spectral evolution of a solution with a constant concentration of dye and a variable and increasing concentration of AT for AT: dye ratios of <100. The spectra (Figure 1) did not change with time and showed two peaks of absorption at 410 and 575 nm with either of the dyes employed. The stoichiometry of the corresponding reaction of each of the new absorption bands has been determined by Job's continuous variations method.[15] In Job's plot (Figure 2) it can be observed that at 410 nm the stoichiometry is 1:1, whereas at 575 nm the stoichiometry is 1:2. Therefore it can be concluded that the bands of absorption at 410 and 575 nm correspond to the ion associates $\{DH^-, ATH^+\}$ and $\{D^{2-}, (ATH^+)_2\}$, respectively, obtained after the neutralization of the two protons of the dye according to the reactions depicted in eqs 1 and 2:

$$DH_2 + AT \rightleftharpoons \{DH^-, ATH^+\} \quad (1)$$

$$\{DH^-, ATH^+\} + AT \rightleftharpoons \{D^{2-}, (ATH^+)_2\} \quad (2)$$

The reaction shown in eq 1 is quantitative because the first proton of the dye is very acidic, whereas the reaction shown in eq 2 is a chemical equilibrium that is made evident by an isosbestic point observed in Figure 1. The 1:1 molar ratio of the ion associate obeys Beer's law. The molar absorptivities, (ϵ_1) that were obtained from the absorbance values at 410 nm of equimolar mixtures of dye and AT in CH_2Cl_2 were 19208 and 23750 $L \cdot mol^{-1} \cdot cm^{-1}$ for BCG and BPB, respectively, as determined by the least squares method.

The molar absorptivity of the 1:2 ion associate (ϵ_2) could not be determined directly because with a strong excess of AT, CH_2Cl_2 produces side reactions with AT that interfere with the determination. To avoid these side reactions, it is convenient for quantitative measurement that the AT:dye mole ratio not be >5 because higher molar ratios require freshly prepared solutions. These side reactions will be studied later. For this reason, the molar absorptivity of the ion associate has to be calculated from the data of the equilibrium by simultaneously determining the equilibrium constant of the reaction in eq 2 (K_2), which could be written as follows:

$$K_2 = [\{D^{2-}, (ATH^+)_2\}]/[\{DH^-, ATH^+\}] [AT] \quad (3)$$

The simultaneous calculation of K_2 and ϵ_2 is identical to that reported previously,[7] where a calculus program using the Rosseinsky's method[16] was given. According to these guidelines, solutions of combinations of dye and AT with a constant mole ratio were prepared and their absorbances measured at 575 nm at various temperatures with the object of determining the thermodynamic parameters of the reaction in eq 2 using van't Hoff's equation.[17] The initial concentrations of the dye and AT, and their absorbance values at different temperatures are then fed into a computer program. The results obtained are presented in Table 1.

The linear determination coefficients (r^2), obtained by the least squares method for the Benessi–Hildebrand[18,19] approximation or by the modified Rose Drago method[20] are presented in Table 1. These values are close to 1, indicating that the method selected by the program leads to suitable K_2 and ϵ_2 values that are good enough to start the iteration cycles in Rosseinsky's method. The final parameters, their confidence intervals for a 0.95 level, and the root mean squared deviation (rmsd) are presented in Table 1. The rmsd values indicate low errors in absorbance measurements that are small enough to accept the validity of the proposed equilibrium in the reaction shown in eq 2 from the quantitative point of view. The values of K_2 for AT in Table 1 are similar to the K_2 for HT,[7] because AT has a similar basic character as HT.[21] From the values of K_2 in Table 1 it can be deduced that the van't Hoff equation [$\ln K_2 = (\Delta S°/R) - (\Delta H°/RT)$] is obeyed given the high value of r^2, obtained by the least squares method. From the values of the slope and the intercept, the values of the thermodynamic parameters in Table 1 have been obtained. The reactions depicted in eqs 1 and 2 are acid–base reactions regardless the base employed. The value of K_2 for BCG is less than that for BPB, which means that the second proton of BCG is less basic than that of BPB.

Dye–AT Interaction at High Concentrations of AT: Obtaining a New Product, Dichloromethane–AT—When BCG or BPB reacts with highly concentrated AT (in a freshly prepared CH_2Cl_2 solution), a new third absorption zone is seen in the visible spectrum range with a maximum of 628 nm for BCG-AT and 602 nm for BPB-AT. The intensity increases with time until it reaches a maximum according to the temperature of the experiment. The spectra of a solution of BCG in a large excess of AT are presented in Figure 3 as an example. Mixtures of BCG-AT and BPB-AT in a large excess of AT and variable concentrations of BCG and BPB were also prepared, and their maximum absorption values (at 628 nm for BCG-AT and 602 nm for BPB-AT) were measured after 48 h, showing that BCG or BPB follows Beers' law. These experiments were carried out at 628 and 602 nm, respectively, and their molar absorptivities at 293.16 K were 65050 and 97000 $L \cdot mol^{-1} \cdot cm^{-1}$ obtained by the least squares method.

As indicated in the *Introduction*, these spectral changes are not due to the neutralization reactions shown in eqs 1 and 2, but rather are due to the side reactions of CH_2Cl_2, which reacts with AT at high concentrations of the latter. To interpret these changes in the absorption spectra, we obtained the product of the side reaction (i.e., the CH_2Cl_2-AT product), which is what reacts with the ion associate $\{D^{2-}, (ATH^+)_2\}$ (present in the solution in a large excess of AT), modifying its absorption band. The spectral changes described in Figure 3 suggest that the CH_2Cl_2–AT product increases its concentration with time. In these experiments, it was also observed that the spectral changes occur faster both as the concentrations of AT increases as well as with an increase in temperature.

In agreement with this and to obtain the new product of the side reaction, a highly concentrated solution of AT in CH_2Cl_2 (10 g of AT base in 40 mL of CH_2Cl_2) was prepared in a tightly closed Pyrex bottle

Table 1—Physicochemical Properties for $BCG^{2-}(ATH^{+})_2$ and $BPB^{2-}(ATH^{+})_2$ As Formed by the Reaction in Equation 2

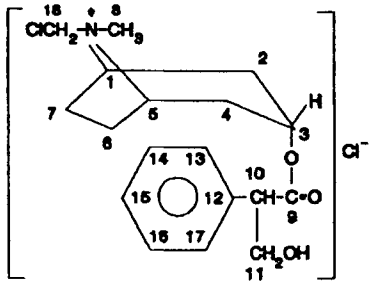
Ion Associate	T, K	K_2,[a] L·mol^{-1}	ϵ_2,[a] L·mol^{-1}·cm^{-1}	rmsd	r^2	$\Delta G°$,[a,b] kJ·mol^{-1}
$BCG^{2-}(ATH^{+})_2$[c]	288.16	9082 ± 430	29 106 ± 739	0.002	1.000	−21.835 ± 0.116
	293.16	5944 ± 697	27 663 ± 1373	0.004	0.998	−21.181 ± 0.304
	298.16	4490 ± 336	28 383 ± 1031	0.002	0.999	−20.847 ± 0.193
	303.16	2960 ± 204	27 628 ± 1092	0.001	0.999	−20.146 ± 0.180
	308.16	1495 ± 383	32 785 ± 5637	0.003	0.973	−18.729 ± 0.758
$BPB^{2-}(ATH^{+})_2$[d]	290.16	100 629 ± 15929	46 917 ± 1360	0.003	1.000	−27.789 ± 0.416
	295.66	59 024 ± 9169	47 908 ± 1861	0.003	1.000	−27.004 ± 0.415
	300.66	30 706 ± 5112	49 765 ± 2888	0.003	0.999	−25.827 ± 0.455
	306.16	21 849 ± 8940	49 341 ± 7368	0.006	0.993	−25.433 ± 1.340
	311.66	14 659 ± 7754	51 968 ± 11047	0.006	0.976	−24.856 ± 1.951

[a] Confidence intervals for a 0.95 level are given. [b] Free energy. [c] Enthalpy ($\Delta H°$), −63.396 ± 20.596 kJ·mol^{-1}; enthropy ($\Delta S°$), −144 ± 69 J·K^{-1}·mol^{-1}; obtained from the Van't Hoff plot, r^2 = 0.970. [d] $\Delta H°$, −68.132 ± 14.627 kJ·mol^{-1}; $\Delta S°$, −139 ± 49 J·K^{-1}·mol^{-1}; obtained from the Van't Hoff plot, r_2 = 0.987.

to avoid evaporation of the solvent. This solution was maintained in a water bath at 313.16 K for months. On the seventh day, white crystals appeared spontaneously, these crystals grew as the days went by, whereas the solution remained colorless. The obtained precipitate was then washed various times with pure CH_2Cl_2 to eliminate the remaining base AT. The obtained precipitate is *N*-chloromethylatropinium chloride, {AT^+CH_2Cl, Cl^-}, which is a quaternary ammonium product with molecular weight of 374.36 g/mol (sum of the molecular weight of AT and that of CH_2Cl_2). The identification of this new product by different methods is given in the next section.

When *N*-chloromethylatropinium chloride, {AT^+CH_2Cl,Cl^-}, is added to a mixture of AT-dye with the ratio AT/dye >1 and <100, a third absorption zone with an absorption maximum at 628 nm if the dye is BCG and 602 nm if it is BPB is instantaneously observed; therefore, the addition of {AT^+CH_2Cl,Cl^-} to the mixture produces a bathochromic and hyperchromic effect to the 1:2 ion associate band. In a qualitative approximation, this indicates that the observed third absorption zone (Figure 3) is produced by the reaction between {AT^+CH_2Cl,Cl^-} and the 1:2 ion associate. It seems evident that this reaction has to be a substitution reaction where ATH^+ is displaced by AT^+CH_2Cl. Although *N*-chloromethylatropinium chloride is insoluble in CH_2Cl_2, its dichloromethanic solutions can be prepared by dissolving a very small quantity in methanol and then adding CH_2Cl_2. The low content in methanol (<0.1%) makes its possible influence negligible.

Identification of the CH_2Cl_2–AT Product—The solid product obtained after the reaction between AT and CH_2Cl_2 is *N*-chloromethylatropinium chloride as will be shown subsequently. An IUPAC name proposed is endo "3-[[*O*-(±)2-phenyl-3-hydroxypropionyl]-8-chloromethyl-8-methyl-8-azabicycle-8-onium-[3.2.1] octane chloride" (1). It is a white crystalline powder, very soluble in water and methanol, and practically insoluble in CH_2Cl_2 and chloroform. Its empirical formula is $C_{18}H_{25}NO_3Cl_2$ and its molecular weight is 374.38 g/mol. The structural formula of 1 is shown below.



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Its theoretical composition is as follows: C (57.60%), H (6.67%), and N (3.73%) and that found in elemental analysis was C (57.56%), H (6.77%), and N (3.74%). Thermal differential and thermogravimetric analyses indicate that the product has no definite melting point and that its decomposition starts at 455 K. These properties are clearly distinct from those of AT, which is the substance from which this compound was obtained. The melting interval of AT is between 387.16 and 391.16 K.[22]

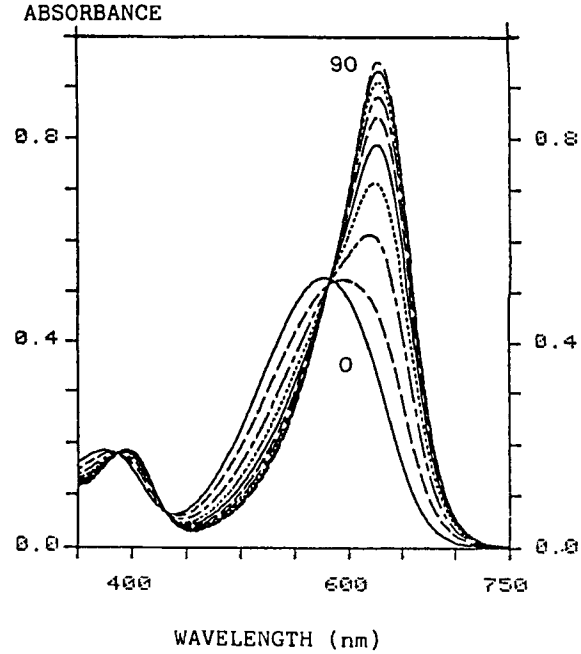


Figure 3—Visible–UV spectra of a CH_2Cl_2 solution of BCG (2×10^{-5} M) and AT (0.1 M) registered at 0, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min (T = 313.16 K).

The obtained signals in DMSO for both AT and the new product (1) are presented in Tables 2 and 3, respectively, together with their proposed assignments. Tetramethylsilane (TMS) was used as a reference standard. The ^{13}C and ^{1}H NMR spectra of AT published by some authors[23–27] were taken as the reference for this interpretation.

When assignments for AT and the new product (1) are compared, the following aspects should be pointed out with regards to the identification of the new substance.

^{13}C NMR—In the DEPT experiment of the new product the peaks corresponding to the five methylenic carbons (CH_2) appear. The peak that shows a chemical shift of 66.85 ppm corresponds to the group CH_2-Cl (carbon 18, Table 3). This peak is not present in the AT spectrum. The signals at 65.06 and 64.89 ppm that are assigned to carbons 1 and 5 in the new product (Table 3) also appear in the AT spectrum but at 58.79 and 58.72 ppm (Table 2), respectively. This shift at a lower magnetic field for the new product is due to the reduction of the screening effect caused by quaternization of the nitrogen group of AT. The signal at 54.03 ppm that is assigned to carbon 8 (N-CH_3) in the new product also appears in the AT spectrum but at 39.64 ppm. This is also due to the reduction of the screening effect caused by quaternization of the nitrogen group of AT. The total number of peaks correspond to 18 carbon in the new product.

Table 2—Nuclear Magnetic Resonance for Atropine[a]

NMR	Carbon Number[b]	Chemical Shift, ppm[c]	Area	Number of Protons
Carbon-13	C_9	171.19	—[d]	—
	C_{12}	136.32	—	—
	C_{13}, C_{17}	128.36	—	—
	C_{14}, C_{16}	127.82	—	—
	C_{15}	127.09	—	—
	C_3	67.18	—	—
	C_{11}	62.91	—	—
	C_1	58.79	—	—
	C_5	58.72	—	—
	C_{10}	54.47	—	—
	C_8	39.64	—	—
	C_2	35.54	—	—
	C_4	35.39	—	—
	C_7	25.21	—	—
	C_6	24.91	—	—
Proton	C_{13} C_{14} C_{15} C_{16} C_{17} (5 aromatic protons)	7.31 (m)	29.58	5
	C_3 (one H)	5.03 (t)	5.77	1
	C_{11} (CH_2–O*H*)	4.84 (t)	6.03	1
	C_{10} (one H)	4.00 (m)	6.10	1
	C_{11} (CH_2–OH)	3.67 (m)	12.12	2
	C_1—C_5 (1,5 bridge)	2.98(s), 2.85(t)	12.09	2
	C_8 (N–Me)	2.11 (s)	17.70	3
	C_2 C_4 C_6 C_7 (8 protons)	1.70 (m)	48.76	8

[a] The molecular structure for AT is the same as **1** except that the CH_2Cl group bonded to the nitrogen atom does not exist. [b] Groups are given in parentheses. [c] NMR conducted with DMSO; s = singlet, d = doublet, t = triplet, m = multiplet. [d] —, not applicable.

^1H NMR—A new singlet signal at 5.47 ppm with area corresponding to two hydrogen atoms is assigned to the N-CH_2Cl group in the new product (Table 3). This is not present in the AT spectrum (Table 2). The two signals at 3.67 and 3.80 (m) ppm that are assigned to two hydrogen atoms in carbons 1 and 5 in the new product also appear in the AT spectrum, but at 2.98 (s) and 2.85 (t) respectively. This shift at a lower magnetic field for the new product is due to the reduction of the screening effect caused by quaternization of the nitrogen group of AT. The singlet signal at 3.21 ppm with area corresponding to three hydrogen atoms is assigned to the N^+-CH_3 group in the new product. This also appears in the AT spectrum but at 2.11 ppm. This is also due to the reduction of the screening effect caused by quaternization of the atropine nitrogen group. The total number of the hydrogen atoms counted from the area of the peaks is 25 for the new product and 23 for atropine.

Mass Spectrum—The assignment of the ion fragments of the spectrum has been carried out taking as a reference the mass spectrum of AT base.[28] Subsequently, some of the ion fragments and the effectuated assignments are presented. The *m/e* 337 (0.5%) fragment is assigned to the positive ammonium cation [*N*-chloromethylatropinium]$^+$ after the loss of a hydrogen radical. The fragment is considered as M-1, because M is the positive ammonium cation. This peak is not present in the mass spectrum of AT. The *m/e* 319 (1%) fragment is M-1-H_2O. The *m/e* 289 (5.5%) fragment corresponds to the cation fragment [M-CH_2-Cl].$^+$. This fragment can be observed in the mass spectra of AT because it is its molecular ion. The *m/e* 124 (100%) fragment appears as the base peak of the mass spectra of the new product. It is also the base peak of the mass spectra of AT. It corresponds to the radical cation **2** (see structure).

IR and UV Spectra—The IR spectra were determined using potassium bromide pellets. Their main bands and their assignments are given in Table 4. These assignments are similar to those given for AT.[29] The UV spectra carried out in ethanol show very similar peaks to those described for AT.[30,31]

Kinetics of the AT Base–CH_2Cl_2 Reaction—As suggested before, the reaction of AT with CH_2Cl_2 is a slow process whose kinetic aspects can be studied under working conditions. Because the obtained product is *N*-chloromethylatropinium chloride, the reaction has to be a nucleophilic substitution process which can be written as:

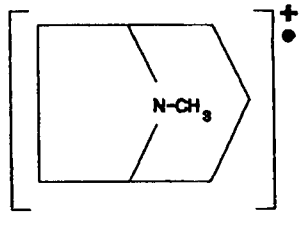
Table 3—Nuclear Magnetic Resonance for *N*-Chloromethylatropinium Chloride

NMR	Carbon Number[a]	Chemical Shift, ppm[b]	Area	Number of Protons
carbon-13	C_9	171.14	—[c]	—
	C_{12}	136.06	—	—
	C_{13}, C_{17}	128.49	—	—
	C_{14}, C_{16}	127.96	—	—
	C_{15}	127.20	—	—
	C_{18}	66.85	—	—
	C_3	66.49	—	—
	C_1	65.06	—	—
	C_5	64.89	—	—
	C_{10}	63.31	—	—
	C_{11}	62.84	—	—
	C_8	54.03	—	—
	C_2	31.48	—	—
	C_4	30.96	—	—
	C_7	23.85	—	—
	C_6	23.58	—	—
proton	C_{13} C_{14} C_{15} C_{16} C_{17} (5 aromatic protons)	7.28 (m)	21.63	5
	C_{18} (2 protons of –N^+–CH_2Cl)	5.47 (s)	7.62	2
	C_3 (one H)	5.3 (t)	4.34	1
	C_{11} (CH_2–O*H*)	5.01 (t)	4.25	1
	C_{11} (CH_2–OH)	4.12 (d)	8.67	2
	C_{10} (one H)	3.95 (m)	4.51	1
	C_1—C_5 (1,5 bridge)	3.67, 3.8(m)	4.44, 4.33	2
	C_8 (N–Me)	3.21 (s)	11.49	3
	C_2 C_4 C_6 C_7 (8 protons)	2.20 (m)	34.37	8

[a] Groups are given in parentheses. [b] NMR was conducted with DMSO; s = singlet, d = doublet, t = triplet, m = multiplet. [c] —, Not applicable.

Table 4—Principal Frequencies of Absorption of *N*-Chloromethylatropinium Chloride in Potassium Bromide Pellets

Frequency, cm^{-1}	Assignment
3090	OH (hydrogen bonded)
3000–3010	CH stretch aromatic
2970	CH stretch aliphatic
2850	N–CH_3
1730	C=O (ester)
1590, 1620	C=C aromatic
1030–1170	C–O–C ether



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$$AT + CH_2Cl_2 = \{AT^+CH_2Cl,Cl^-\} \quad (4)$$

The rate at which *N*-chloromethylatropinium chloride appears in a solution of AT in CH_2Cl_2 has been determined by extracting equal volumes of it with sodium hydroxide (0.1 M) at different times. Because the partition coefficient in CH_2Cl_2/H_2O of AT base is high,[19] in the first extraction, all the ammonium ion is extracted to the aqueous medium whereas only a minimum part of AT base can be retained in this phase.

Table 5—Rates of the Reaction between Atropine and Dichloromethane

[AT]	Rates × 10^5, mol·L^{-1}·h^{-1}			
	283.16K	293.16K	303.16K	313.16K
0.1	0.53	3.53	5.11	17.62
0.2	1.20	5.44	8.98	32.89
0.4	2.25	8.32	17.01	61.37
0.6	3.17	11.38	24.02	88.40

The AT base retained in this phase is easily eliminated by carrying out three or four successive extractions with pure CH_2Cl_2. The quantity of pure *N*-chloromethylatropinium chloride present was determined by measuring its spectrophotometric absorbance at 257.5 nm. The molar absorptivity of *N*-chloromethylatropinium chloride in NaOH at 257.7 nm is 201.6 L·mol^{-1}·cm^{-1} at 293.16 K.

The logarithms of the velocity of the formation of *N*-chloromethylatropinium chloride (log *V*) for different initial concentrations of AT (Table 5) were determined at different temperatures. It has been verified experimentally that for each of these initial concentrations of AT, the velocity (*V*) at a given temperature is constant because it does not change for at least the first 7 days. This is due to the low values of *V* and the insolubility of *N*-chloromethylatropinium chloride in CH_2Cl_2, factors which make the concentration of *N*-chloromethylatropinium chloride always negligible compared with that of AT not only for long periods of time but also at all times. For the same reasons, the concentration of CH_2Cl_2 is constant and, moreover, far greater than that of AT in any solution of AT in CH_2Cl_2. The concentration of CH_2Cl_2 in these solutions is 15.6 M. Consequently, the concentrations of AT and CH_2Cl_2 can be considered as constant for many days; this justifies that the velocity (*V*) of the reaction of AT and CH_2Cl_2 is constant within the period that the study was carried out. Taking this into account, the relationship between *V* and [AT], the equation of the velocity, can be expressed as follows:

$$\log V = \log k' + n_{AT} \log [AT] \quad (5)$$

In eq 5, k' is the apparent velocity constant of the process and n_{AT} is the order of the reaction for AT. The value of k' includes the concentration of CH_2Cl_2 and the order of the reaction for CH_2Cl_2 ($n_{CH_2Cl_2}$), both of which have constant values. Therefore $k' = k\,[CH_2Cl_2]^{n_{CH_2Cl_2}}$, where k is the true velocity constant.

The plot of log *V* against log [AT] is given in Figure 4A. From the values of the intercept and the slope determined by the least squares method, the values of k' and n_{AT} of the process at various temperatures are obtained. These values are presented in Table 6. The n_{AT} values are close to 1, suggesting that the reaction is a first order reaction with respect to AT. The lack of exactness in the values of n_{AT} and k' may be due to the lack of precision in the experimental data because the determinations take various days as a result of the slowness of the process.

The order of the reaction with respect to CH_2Cl_2 can not be determined by the same procedure because it is not possible to modify its concentration to the same magnitude as that of AT because the solubility of AT in CH_2Cl_2 is high but also limited. Therefore, the order of the reaction with respect to CH_2Cl_2 could not be obtained. However, given the molecularity of the reaction in eq 4, we suppose that it is a first-order reaction. This makes possible the calculation of the frequency factor, (*A*) from the Arrhenius equation.

The possibility of the reverse process of the reaction in eq 4 has also been investigated. So, when $\{AT^+CH_2Cl,Cl^-\}$ is dissolved in CH_2Cl_2 (it has already been pointed out that this is done by dissolving $\{AT^+CH_2Cl,Cl^-\}$ in a minimum quantity of methanol) that contains dye, neither the characteristic neutralization absorption bands (reactions in eqs 1 and 2 at 410 nm nor those at 575 nm are observed. This result demonstrates that $\{AT^+CH_2Cl,Cl^-\}$ does not substantially decompose into AT and CH_2Cl_2 in our experimental conditions and therefore the extension of a reverse process in the reaction in eq 4 is negligible. This is a logical result because as was previously indicated, during the kinetic process, the concentration of $\{AT^+CH_2Cl,Cl^-\}$ is very low given its practical insolubility in CH_2Cl_2. In Figure 4B, the values of ln k' have been plotted against 1/T. The high values of the linear determination coefficient (r^2) indicate that the values of k' obey the Arrhenius equation ($\ln k = \ln A - E_a/RT$); the values of the activation energy, E_a, and the frequency factor, *A*, obtained by the least squares method are given together with their confidence intervals in the footnote to Figure 4).

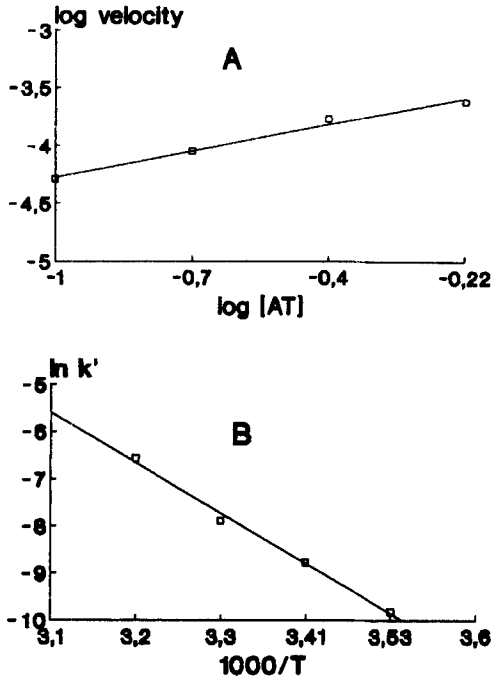

Figure 4—(**A**) Determination of the order and the rate constant of the reaction between AT and CH_2Cl_2 (T = 303.16 K). (**B**): The Arrhenius graphic plot for the DCM–AT reaction. The parameters obtained by the least squares method are ln A = 23.3 ± 12.7 (A in h^{-1}), E_a = 39.3 ± 12.4 kJ·mol^{-1}, and r^2 = 0.989.

Table 6—Order of the Reaction between Atropine and Dichloromethane, Apparent Rate Constants (k'), and Linear Determination Coefficients (r^2)[a]

Temperatures K	Order	k', h^{-1}	r^2
283.16	0.99 ± 0.22	5.46 ± 2.11	0.994
293.16	0.65 ± 0.08	15.46 ± 2.00	0.998
303.16	0.87 ± 0.07	37.60 ± 3.92	0.999
313.16	0.90 ± 0.00	140.06 ± 10.98	0.999

[a] Determined by the least squares method.

Discussion

The spectra in Figure 3 present two stages. The first is a bathochromic shift without a change in the molar absorptivity (second spectrum in Figure 3 after 10 min). The second stage is a hyperchromic shift (spectra between 20 and 90 min) that after 24 h shows a molar absorptivity that has a double value. The spectral shift in the first stage is due to the displacement of the ATH^+ group by the AT^+CH_2Cl group, thus forming the ternary ion, $\{D^{2-},ATH^+,AT^+CH_2Cl\}$. This process is useful to measure the rate of the side reaction between AT and CH_2Cl_2, as will be demonstrated later. The spectral change in the second stage is due to the second displacement of the ATH^+ group by AT^+CH_2Cl, which leads to the specie $\{D^{2-},(AT^+CH_2Cl)_2\}$. In Figure 3, time 0 is shown as a continuous line that is the spectrum of the ionic associate $\{D^{2-},(ATH_2^+)\}$ because, in a strong excess of AT, the reaction in eq 2 is complete.

The values of the rate constants of the side reaction between AT and CH_2Cl_2 are very low (Table 6), and by direct experiments like the ones described above, it is very slow and tedious to find out if the quaternization of the amine has been produced and measure its rate constant k'. Figures 1 and 3 can be registered together and they give a quick and efficient colorimetric test to determine if the quaternization of the amine has taken place and measure its rate, k'. In Figure 3, the quaternization of AT can be observed in only 30 min, whereas 24 h are needed for the

Table 7—Rate Constants, (k'), Equilibrium Constants (K_2), and Acid Dissociation Constants (pK_a) for some Amines in Various Solvents

Temperature, K	Amine	Solvent	$k' \times 10^5$, h^{-1}	K_2, $L \cdot mol^{-1}$	pK_α
298.16	Tropine	CH_2Cl_2	218	24870	11.73[a]
	Quinine	CH_2Cl_2	57	14247	8.80[b]
	Atropine	CH_2Cl_2	23	4490	9.99[b]
	Ephedrine	CH_2Cl_2	0	452	9.60[a]
	Ajmaline	CH_2Cl_2	0	4633	—[c]
303.16	Atropine	$C_2H_4Cl_2$	15.8	—	—
	Atropine	CCl_4	0.0	—	—
	Atropine	$CHCl_3$	0.0	—	—
	Atropine	CH_2Cl_2	37.6	—	—
	Tropine	$C_2H_4Cl_2$	51.4	—	—
	Tropine	CCl_4	0.0	—	—
	Tropine	$CHCl_3$	1.1	—	—
	Tropine	CH_2Cl_2	399.4	—	—

[a] Reference 30. [b] Reference 4. [c] —, Not applicable.

procedure of the experimental part. From the colorimetric test, a new determination of k' and n_{AT} for the reaction between AT and CH_2Cl_2 has been carried out, realizing a series of spectra (Figure 3) at various concentrations of AT. The rate of the reaction, V, is the rate of bathochromic change, which can be deduced from eq 6:

$$V = A'/\epsilon' t = x/t \quad (6)$$

In eq 6, A' is the increase in the absorbance at 630 nm (if BCG is used) after the lapse of time (t), ϵ' is the molar absorptivity difference between the ternary ion, $\{D^{2-}, AT^+CH_2Cl, ATH^+\}$, and $\{D^{2-}, (ATH^+)_2\}$ at 630 nm, and x is the quantity of *N*-chloromethylatropinium chloride generated in time t. The solution of eq 6 with these values of V has led to very similar values for n_{AT} and k' to those obtained by the extraction method given in the *Experimental Section*. By this second and faster method, the quaternization of the amines TP, AT, Q, AJ, and EF in the halogenated hydrocarbon solvents CH_2Cl_2, chloroform, carbon tetrachloride, and 1,2-dichloroethane has been studied. The rate constants calculated for these amines in CH_2Cl_2 are presented in Table 7 with the acid dissociation constants in aqueous media (pK_α) and the value of K_2 calculated according to the experimental method given for AT in the *Experimental Section*.

In the first part of Table 7 (data at 298.16K), the values of k' given for the different amines and CH_2Cl_2 are clearly arranged according to the K_2 value and not pK_a. This shows that the values of k' and the basic character of the amine measured in CH_2Cl_2, which is characterized by the value of K_2, have the same tendency. Although Q is more basic than AT and EF in CH_2Cl_2, in water it is the least basic of the three amines, as indicated by the pK_a values[4,30] in aqueous medium (Table 7). This inversion in the order of the basic character when the solvent is changed has been described for other kinds of organic bases. Although the basic character of the amines plays an important role in the reaction shown in eq 4, the structure of the amine is also important because AJ has similar values for K_2 to those of AT but either does not give the reaction or the reaction is not detected within a reasonably short time (~2 h). (In Table 6, a k' value of 0 is assigned when for an amine concentration of 0.1 M there is no change in the spectrum for the first 2 h.)

In the second part of Table 7 (data at 303.16 K), it can be observed that the arrangement of the k' values for the reaction shown in eq 4 is as follows: CH_2Cl_2, chloroform, and carbon tetrachloride. This order suggests that the velocity diminishes with an increase in the number of chlorine atoms present in methane. Indeed, with 1,2-dichloroethane, the velocity is slower than with CH_2Cl_2 but faster than with chloroform (Table 7).

From the k' values (Table 7), it can be deduced that 5% of TP and 0.5% of AT are quaternized at 298.16 K in 24 h in a CH_2Cl_2 solution of these amines. Although the extension of this process is not too great, it is big enough to interfere with the dye–alkaloid neutralization process as has been shown in this paper. Recent analytical methods[12-14] for the quantitative determination of quaternary ammonium compounds in which acid dyes form colored extractable compounds from aqueous solutions with this kind of solvents suggest the importance of this side reaction because the analysis of a quaternary ammonium compound could be interfered with by the quaternary ammonium generated in the employed solvent. In these cited works, the colored extractable compound is a mixed ion associate of the type {dye^{2-}, quinineH$^+$, ammonium$^+$} and the reaction has to be carried out in an excess of Q. Moreover, there are many papers showing the pharmaceutical and analytical interest of the reaction of dyes with alkaloids and quaternary ammonium compounds[1-14] using halogenated hydrocarbon solvents in which knowledge of these side reactions could be useful. The influence of the small extension in which the amine is quaternized should not be neglected because the ammonium compound generated is very reactive as a result of its great capacity for ionic interchange. We think that this can be important given that these solvents are universally used in fields of pharmacy, chemistry, biology, and medicine.

Conclusions

Halogenated hydrocarbon solvents react with amines of pharmaceutical interest forming a quaternary ammonium salt in a slow process that is a first-order reaction with respect to the amine. The reaction is a nucleophilic substitution process whose formation constant obeys the Arrhenius equation. The rate of the reaction increases with an increase in the basic power of the amine employed and diminishes as the number of halogen substituents in the series CH_2Cl_2, chloroform, and carbon tetrachloride increases. Although the quaternary ammonium salt is present at a low concentration for many days, its presence has to be taken into account because it can interfere with processes such as the dye–alkaloid neutralization reaction. The neutralization of a sulphonphthaleinic dye, DH_2, with an amine in the absence of the side reactions of the halogenated hydrocarbon solvents only produces the ion associates {DH$^-$, amineH$^+$} and {D^{2-},(amineH$^+$)$_2$}. But, if there are side reactions, ion associates of types {D^{2-}, amineH$^+$,ammonium$^+$} and {D^{2-},(ammonium$^+$)$_2$} will appear in the solution after two reactions of ionic interchange between {D^{2-},(amineH$^+$)$_2$} and the quaternary ammonium salt. The side reactions of the halogenated hydrocarbon solvents are brought to light by the presence of {D^{2-}, amineH$^+$, ammonium$^+$} and {D^{2-},(ammonium$^+$)$_2$}, which produce, respectively, a bathochromic and hyperchromic effects in the expected absorption bands after the reactions of the sulphonphthaleinic dyes with amines of pharmaceutical interest. These spectral changes constitute a quick qualitative and quantitative test to manifest the side reactions of these important solvents, permitting the establishment of the sequence in the extension of them.

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