Ion Associates and Hydrogen Bonded Complexes of Bromocresol Green and Quinine in Dichloromethane

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Quinine base (Q) reacts with bromocresol green ($BCGH_2$) in dichloromethane forming compounds of 2:1, 1:1 and 1:2 stoichiometric balance depending on the dye–quinine molar relation. In an excess of dye, 2:1 and 1:1 ion associates are formed clearly in a chemical equilibrium, and in an excess of quinine base 1:1 and 1:2 compounds in equilibrium are also formed. The 1:2 compound is a hydrogen bonded complex (HBC) formed through hydrogen bonding between –O–H in $BCGH_2$ and amino nitrogen of quinine. Canonical resonance structures of the hydrogen bond justify the large value of the molar absorptivity, the appearance of the band in the visible region, the large value of equilibrium constant and large negative values of the heat of reaction. These values indicate that the HBC is very stable. Quinine hydrochloride (Cl^-QH^+) reacts with $BCGH_2$ forming 1:1 ion pairs in chemical equilibrium for all molar relations. The maximum absorption for 2:1, 1:1 and 1:2 compounds are at 415, 415 and 547 nm respectively. With a large excess of quinine base, a third absorption band with maximum at 620 nm appears in the solution which proves that the excess of quinine base also reacts with dichloromethane forming an ammonium quaternary compound. The presence in the solution of $BCGH_2$–ammonium associates justifies the third absorption band (620 nm). The ammonium compound generated is *N*-chloromethylquininium chloride whose molecular weight is the sum of the amine and that of dichloromethane.

The sulfonphthaleine dyes bromocresol green ($BCGH_2$) and bromophenol blue ($BPBH_2$) have been proposed for the analysis of pharmacologically active substances by the extraction spectrophotometric determination method.[1–4] Sakai[5,6] proposed a method of selective extraction for a number of quaternary ammonium ions using the 1:2 associates of $BCGH_2$ and $BPBH_2$ and quinine as a favourable substance for the quantitative extraction of the ion complex. More recent papers indicated that $BCGH_2$, $BPBH_2$ and quinine are the most important substances in order to get a selective analysis of quaternary ammonium compounds by extraction procedure.[7,8] In a previous paper,[9] the reaction of ajmaline and homatropine with $BCGH_2$ and $BPBH_2$ in dichloromethane was studied and it was established that for small differences in the dye–amine concentration, the amine neutralized the first and second proton of the dye, forming 1:1 and 1:2 (dye:amine) associates. A more recent paper shows that when the dye–amine reaction is carried out in a great excess of homatropine, the excess of homatropine also reacts with the dichloromethane forming *N*-chloromethylhomatropinium chloride, a quaternary ammonium compound.[10]

In this paper, the reactions of quinine base (Q) with bromocresol green, $BCGH_2$, are investigated, being more complicated than those of homatropine and ajmaline,[9] due to the fact that quinine is a dibasic amine while ajmaline and homatropine are monobasic amines. The $BCGH_2$–Q solutions show a regular and reversible thermochromism in dichloromethane, the study of which is now very important since a similar effect in amine–tetrabromophenolthalein ethyl ester (TPBE) solutions had interesting analytical applications.[11,12] The reaction between quinine and dichloromethane is also described for the first time showing the problems that it can generate on the analytical methods described previously in ref. 5–8. Spectral data, equilibrium constants, thermodynamic parameters and conductometric data have been obtained and used to study the nature, properties and analytical importance of the $BCGH_2$–quinine associates.

Experimental

Apparatus.—The spectrophotometer used was a Perkin-Elmer Lambda 5 with a B. Braun Melsungen AG model Frigomix 1.495 and thermomix 1.441 (±0.2 °C) thermostat. Quartz cells of 1 cm path length were employed. Other apparatus included a Hewlett-Packard 9825 A microcomputer, and a Radiometer model CDM 83 conductimeter.

Reagents.—α-(6-Methoxy-4-quinolyl)-5-vinyl-2-quinuclidinemethanol base and its hydrochloride salt (quinine base and its hydrochloride salt), Sigma analytical grade, were used without prior purification and were kept in a desiccator with phosphorus pentoxide under low pressure. Analytical grade bromocresol green ($BCGH_2$) from Merck was used without prior purification. Carlo Erba analytical grade dichloromethane was used without prior purification. Acetone and ethanol (Probus HPLC grade) were used without prior purification.

Results and Discussion

Absorption Spectra, Composition and Nature of Associates.—When quinine base was mixed with $BCGH_2$ in dichloromethane, new bands of absorption in the visible spectrum were observed which suggest the existence of new compounds in the solution. Fig. 1 shows the spectral evolution beginning from an equimolar solution of $BCGH_2$–quinine mixture and later addition of quinine base in increasing concentrations. The spectra of Fig. 1 show two absorption bands with peaks at 415 and 547 nm which are cut at an isosbestic point.

Job's method had to be used to determine the composition of the associates given that quinine possesses two basic nitrogens. Continuous variation plots (Fig. 2) between $BCGH_2$ and quinine base suggest 2:1 stoichiometry ($BCGH_2$:Q) for 415 nm and 1:2 stoichiometry for 545 nm. Accordingly, the three processes following are assumed as reactions (1)–(3), where

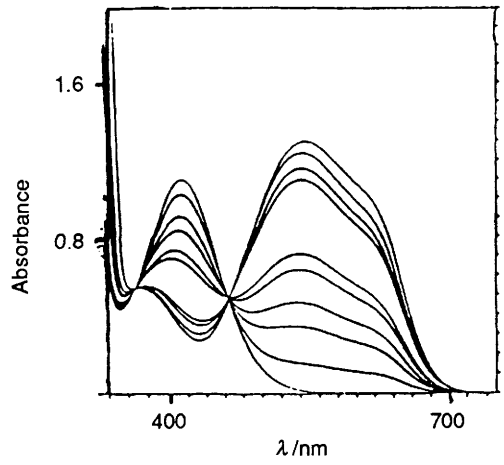


Fig. 1 VIS–UV spectra of mixed solutions of $BCGH_2$ 5.64×10^{-5} and quinine 5.64, 6.74, 8.33, 9.50, 11.50, 12.90, 25.90, 31.60, 47.00 and 79.60×10^{-5} mol dm^{-3}; $T = 293.16$ K

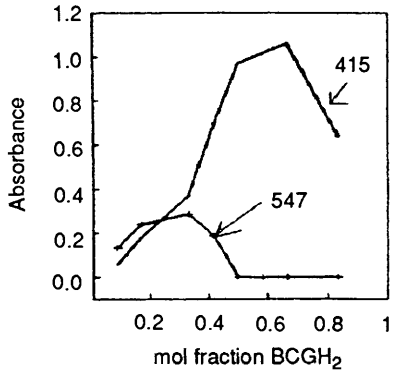


Fig. 2 Job's plot for dye–quinine base. The number inside each figure corresponds to the wavelength of the absorption maximum of each associate; $[BCGH_2] + [Q] = 10^{-4}$ mol dm^{-3}; $T = 293.16$ K

$$2\,BCGH_2 + Q \rightleftharpoons (BCGH^-)_2QH_2^{2+} \quad (1)$$

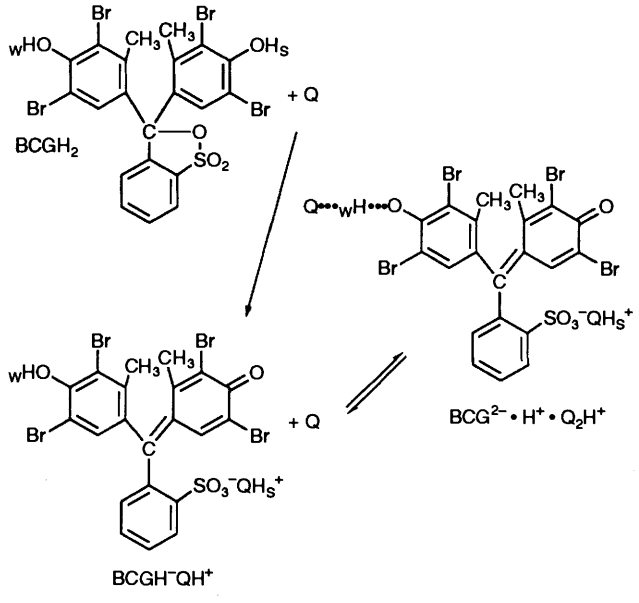
$$BCGH_2 + Q \rightleftharpoons BCGH^-QH^+ \quad (2)$$

$$BCGH^-QH^+ + Q \rightleftharpoons BCG^{2-}{-}{}^+H{-}Q_2H^+ \quad (3)$$

$BCGH_2$ is the free dye, Q quinine base, $(BCGH^-)_2QH_2^{2+}$ and $BCGH^-QH^+$ are the ion associates of 2:1 and 1:1 stoichiometry. The $BCG^{2-}{-}{}^+H{-}Q_2H^+$ associate represents a hydrogen bonded complex (HBC) of 1:2 stoichiometry. Reaction (2) is introduced because the 1:1 associate is a necessary intermediary associate between the 2:1 and 1:2 associate, as will be shown later.

The $(BCGH^-)_2QH_2^{2+}$ and $BCGH^-QH^+$ ion associates contain the monovalent anion of $BCGH_2$, $BCGH^-$, that is why they show an identical peak of absorption at 415 nm, which is assigned to the $BCGH^-$ anion. If other amines, such as ajmaline and homatropine[9] are used, the $BCGH^-$ajmalineH$^+$ and $BCGH^-$homatropineH$^+$ ion associates show the same visible absorption band. Therefore, the species QH^+ only acts as the stabilizer of the $BCGH^-$ ion in the organic solvent. On the other hand, the species $BCG^{2-}{-}{}^+H{-}Q_2H^+$ shows a maximum absorption at 547 nm, which is different if those other amines are used.[9] When ajmaline is used the maximum level appears at 562 nm, while the peak appears at 577 nm with homatropine. Consequently, absorption at 547 nm is not due to the BCG^{2-} anion but rather to a hydrogen bonded complex. Various procedures to reach the spectrum of the BCG^{2-} anion will be discussed in a later section.

Reaction (1) will be present in the solution when Q reacts with an excess of $BCGH_2$, reaction (2) in an equimolar mixture and reaction (3) in an excess of Q. Reaction (1) justifies the 2:1 stoichiometry for 415 nm given by Job's method. Reaction (3) justifies the isosbestic point in Fig. 1 and the equilibrium between the absorption at 415 nm ($BCGH^-QH^+$) and the absorption at 547 nm ($BCG^{2-}{-}{}^+H{-}Q_2H^+$), as well as the 1:2 stoichiometric balance given by Job's method, since the 1:2 complex only can be obtained from the 1:1 ion associate. Reaction (2) justifies the existence of the 1:1 ion associate in the solution and the chemical equilibrium which is established in an excess of quinine base by reaction (3). Reactions (2) and (3) at a molecular level are shown schematically in Scheme 1. The molecular structure of quinine will be shown in a later section. The binding sites of Q with $BCGH_2$ are not necessarily those shown in Scheme 1, since $BCGH_2$ is an extensively conjugated molecule and can show resonance, as will be discussed later. The hydrogens H_s and H_w in Scheme 1 are, respectively, the stronger and the weaker acidic proton of $BCGH_2$.



Scheme 1

Equilibrium Constants and Molar Absorptivities of the 2:1 *and* 1:1 *Ion Associates.*—The molar absorptivity of the $(BCGH^-)_2QH_2^{2+}$ ion associate, $\varepsilon_{2:1}$, was determined from absorbance values at 415 nm of dye–quinine base mixtures with an excess of $BCGH_2$ being 39 000 ± 2000 dm^3 mol^{-1} cm^{-1} at 293.16 K. The molar absorptivity of the $BCGH^-QH^+$ ion associate, $\varepsilon_{1:1}$, determined from absorbance values at 415 nm of dye–quinine base equimolar mixtures was 19 500 ± 400 dm^3 mol^{-1} cm^{-1} at 293.16 K. These values are in the order $\varepsilon_{2:1} = 2\varepsilon_{1:1}$, in agreement with the fact that only $BCGH^-$ is absorbent while QH^+ and QH_2^{2+} in the visible spectrum range are not absorbent. The determination of the molar absorptivity for $BCG^{2-}{-}{}^+H{-}Q_2H^+$ is the subject of the next section of this study, due to its particular difficulty and its analytical importance.

Reaction (2) is a quantitative reaction since dye–Q equimolar mixtures obey Beer's law ($\varepsilon_{1:1}$ = 19 500 ± 400 dm^3 mol^{-1} cm^{-1}). Reaction (1) is not quantitative since dye–Q mixtures in a 2:1 molar relation (dye:quinine base) show a molar absorptivity smaller than that obtained from dye–quinine mixtures with a higher dye concentration. As the quinine base has two nitrogens of very different basicity reaction (1) is in a first phase the neutralization of the more basic nitrogen of the quinine and later the neutralization of the other nitrogen. The

first phase is that already indicated by the quantitative reaction (2), and the second phase is given by reaction (4). Therefore,

$$BCGH^-QH^+ + BCGH_2 \rightleftharpoons (BCGH^-)_2QH_2^{2+} \quad (4)$$

reaction (1) is the global process sum of the reaction (2) and that of the reaction (4). The formation constant for the reaction (4) was computed using the equation: $K_4 = [(BCGH^-)_2QH_2^{2+}]/[BCGH^-QH^+][BCGH_2]$. The value of K_4 was determined from absorbance values at 415 nm of dye–Q mixtures of different concentrations in a 2:1 molar relation using the formulae (5)–(7), where A_{415} is the absorbance value at 415 nm,

$$[(BCGH^-)_2QH_2^{2+}] = (A_{415} - C_Q^\circ\varepsilon_{1:1})/\varepsilon_{1:1} \quad (5)$$

$$[BCGH^-QH^+] = C_Q^\circ - (BCGH^-)_2QH_2^{2+} \quad (6)$$

$$[BCGH_2] = C_{BCG}^\circ - C_Q^\circ - [(BCGH^-)_2QH_2^{2+}] \quad (7)$$

C_{BCG}° is the initial concentration of $BCGH_2$ and C_Q° the initial concentration of quinine base. Eqns. (5)–(7) are valid because reaction (2) is quantitative and $\varepsilon_{2:1} = 2\varepsilon_{1:1}$. These equations take into account the complete overlapping of the absorption bands of $BCGH^-QH^+$ and $(BCGH^-)_2QH_2^{2+}$. A constant value for K_4 [$\log K_4 = (5.55 \pm 0.08)$ dm^3 mol^{-1}] from an average of nine experiments was calculated.

Reaction of $BCGH_2$–Quinine in a Slight Excess of Quinine. Thermochromism of the Solution.—Fig. 3 shows the thermochromism of a dichloromethane solution containing $BCGH_2$ and quinine in a slight excess of quinine. The thermochromism is reversible since after a change in temperature, the absorbance returns to its initial value when the temperature is restored to its original value. If a lower excess of Q than that in Fig. 3 is used then the red colour (547 nm) disappears at high temperature and a colour change, from red to yellow, detectable by the eye is clearly observed. Fig. 3 also confirms that the reaction (3) is a clear chemical at the temperatures studied and it suggests the possibility of calculating the thermodynamic parameters of this reaction. Figs. 4(*a*) and (*b*) are the Coleman's test[9] from Fig. 1 which clearly shows two absorbent species whose sum of concentrations is constant in agreement with that already established. An identical result is obtained when the Coleman's test is applied to the spectral curves in Fig. 3.

The molar absorptivity of the 1:2 associate (HBC), $\varepsilon_{1:2}$, could not be determined directly because with a strong excess of Q, CH_2Cl_2 produces side reactions with Q which interfere with the determination.[10] In order to avoid these side reactions, it is convenient for quantitative measurement, that the $Q/BCGH_2$ mole ratio not be greater than 3 because higher molar ratios require freshly prepared solutions. These side reactions will be studied later. For this reason, the molar absorptivity of the BCG^{2-}–^+H–Q_2H^+ complex has to be calculated from the data of the equilibrium determining simultaneously the equilibrium constant K_3 ($K_3 = [BCG^{2-}$–^+H–$Q_2H^+]/[BCGH^-QH^+][Q]$). The simultaneous calculation for K_3 and $\varepsilon_{1:2}$ is identical to that of ref. 9 where a calculus program using the Rosseinsky's method[13] was given. According to these guidelines, solutions of combinations of dye–quinine with constant mole ratio were prepared and their absorbances measured at 547 nm at various temperatures with the object of determining the thermodynamic parameters of reaction (3) using van 't Hoff's equation. The

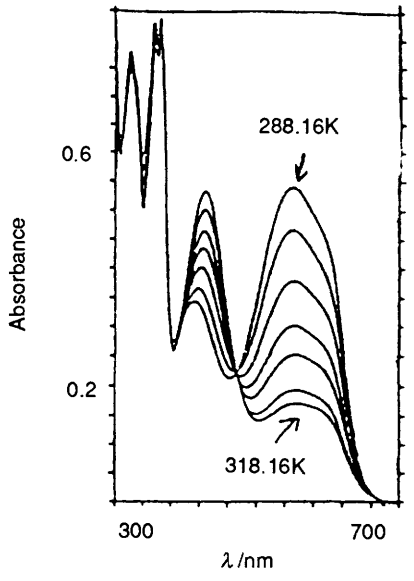


Fig. 3 VIS–UV spectra of a mixed solution of $BCGH_2$ 2.9×10^{-5} and quinine 5.8×10^{-5} mol dm^{-3} at 288.16, 293.16, 298.16, 303.16, 308.16, 313.16 and 318.16 K in dichloromethane

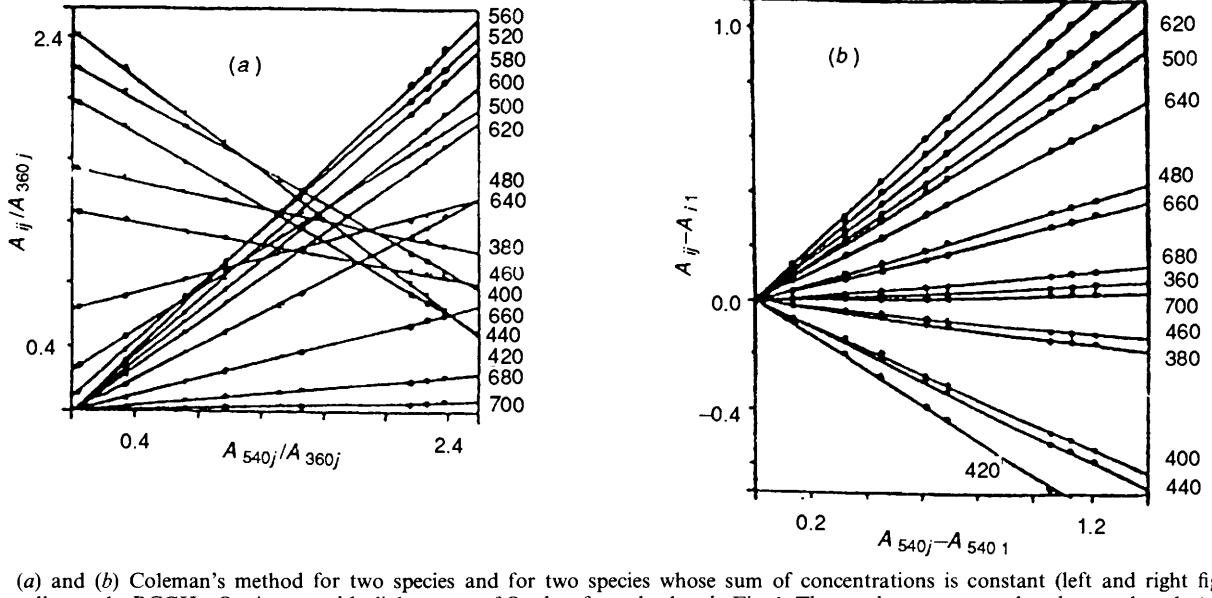


Fig. 4 (*a*) and (*b*) Coleman's method for two species and for two species whose sum of concentrations is constant (left and right figures) corresponding to the $BCGH_2$–Q mixtures with slight excess of Q taken from the data in Fig. 1. The numbers correspond to the wavelength, *i*, of the row of the matrix which has been resolved graphically according to Coleman.

Table 1 Reaction (3) for BCG^{2-}–^{+}H–Q_2H^{+}; formation constants, K_3 ($dm^3\ mol^{-1}$), molar absorptivity, $\varepsilon_{1:2}$ ($dm^3\ mol^{-1}\ cm^{-1}$) and root mean squared deviation (rmsd), determined using Rosseinsky's method[a]

T/K	K_3/$dm^3\ mol^{-1}$	$\varepsilon_{1:2}$	rmsd	r^2	$\Delta G°$
287.66	39 000 ± 5 000	24 500 ± 900	0.005	0.998	−25.3 ± 0.3
293.66	26 000 ± 3 000	24 000 ± 1 000	0.004	0.999	−24.8 ± 0.3
298.66	14 000 ± 3 000	26 000 ± 2 000	0.007	0.981	−23.7 ± 0.6
304.66	12 000 ± 2 000	23 000 ± 2 000	0.005	0.994	−23.9 ± 0.4
309.16	9 000 ± 2 000	23 000 ± 2 000	0.004	0.992	−23.4 ± 0.5
313.16	6 000 ± 1 000	26 000 ± 4 000	0.005	0.962	−22.7 ± 0.7
	$\Delta H°$ = −52 ± 12	$\Delta S°$ = −93 ± 38	0.974 (van 't Hoff plot)		

[a] The values of the determination coefficient, r^2, from Rose–Drago's method. The free energy variations, $\Delta G°$ (kJ mol^{-1}), values at each temperature. The enthalpy, $\Delta H°$ (kJ mol^{-1}), and entropy, $\Delta S°$ (J K^{-1} mol^{-1}), variations calculated using the least squares method from the van 't Hoff plot. The confidence intervals are given for a 0.95 level.

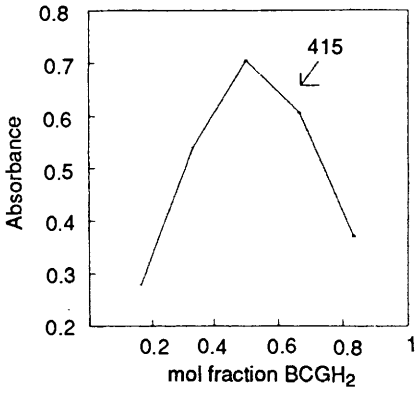


Fig. 5 Job's plot for $BCGH_2$–quinine hydrochloride. The number inside the figure corresponds to the wavelength of the absorption maximum of the ion associate; T = 293.16 K.

initial concentrations of the $BCGH_2$, quinine and their absorbance values at different temperatures are then fed into a computer program.

In Table 1 are presented the linear determination coefficients, r^2, obtained by the least squares method for the Benesi–Hildebrand[14] approximation, or by the modified Rose–Drago method.[15] These values are close to 1 indicating that the method selected by the program leads to suitable K_3 and $\varepsilon_{1:2}$ values which 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, small enough to accept the validity of the proposed equilibrium in reaction (3) from the quantitative point of view. From the values of K_3 in Table 1 it can be deduced that the van 't Hoff equation, $\ln K_3 = (\Delta S°/R) - (\Delta H°/RT)$, is obeyed given the high value of the linear determination coefficient, 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 were calculated. These data suggest some interesting points which will be discussed in a later section.

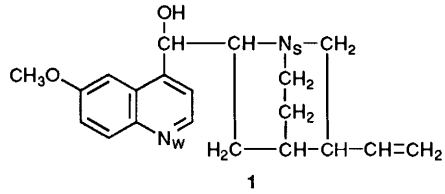
Reaction Between $BCGH_2$ *and Quinine Hydrochloride. Basicity and Acidity of* $BCGH_2$ *and Quinine.*—When $BCGH_2$ is mixed with quinine hydrochloride, $Cl^{-}QH^{+}$, an absorption band with a maximum level at 415 nm also appears. The Job plot in Fig. 5 suggests that the stoichiometric balance is 1:1. Therefore, the reaction of $Cl^{-}QH^{+}$ with $BCGH_2$ is shown in reaction (8).

$$BCGH_2 + Cl^{-}QH^{+} \rightleftharpoons BCGH^{-}Cl^{-}QH_2^{2+} \quad (8)$$

The molar absorptivity of the $BCGH^{-}Cl^{-}QH_2^{2+}$ ion associate was determined from absorbance values at 415 nm of dye–$Cl^{-}QH^{+}$ mixtures with an excess of any of the reagents (at least 20 times higher than that of the other component, $BCGH_2$ or $Cl^{-}QH^{+}$) since by reaction (8) the concentration of the reagent which is lacking is equal to the concentration of $BCGH^{-}Cl^{-}QH_2^{2+}$ ion associate. The Lambert–Beer law was satisfied at 415 nm in these experimental conditions. The molar absorptivity obtained was 20 100 ± 400 $dm^3\ mol^{-1}\ cm^{-1}$. This value is identical to that of the $BCGH^{-}QH^{+}$ ion pair in agreement with the fact that Cl^{-} and QH_2^{2+} are not absorbent.

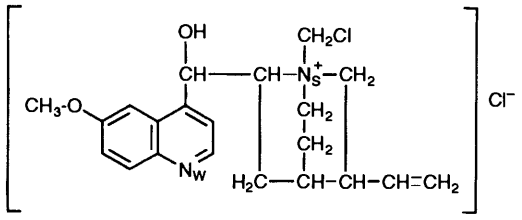
The equilibrium constant for reaction (8) can be written as: $K_8 = [BCGH^{-}Cl^{-}QH_2^{2+}]/[BCGH_2][Cl^{-}QH^{+}]$. From absorbance values at 415 nm in Fig. 5 the equilibrium constant was calculated to be $\log K_8$ = 5.4 ± 0.2 (K_8 in $dm^3\ mol^{-1}$). This value is identical to that of the reaction (4) calculated before.

It is obvious that the stoichiometric balance in dichloromethane varies depending on whether the quinine is free base or in the form of its salt. This agrees with the fact of that the nitrogens are the reactive elements of quinine. In the case of quinine hydrochloride salt one nitrogen is occupied and the second nitrogen is free to react with $BCGH_2$ forming a sole compound of 1:1 stoichiometry. In the same way, the sulfonphthaleine dye react by their protons with the nitrogens of the quinine to form the ion associates of $BCGH^{-}$ and the HBC with an increase in the concentration of quinine base. The pK_a values of quinine are 4.1 and 8.3,[16] and the pK_a values of $BCGH_2$ are −0.85 (ref. 17) and 4.7 (ref. 18) at 293.16 K. These pK_a values indicate that both, the two protons of the dye and the two nitrogens of quinine, are very different in basicity or acidity. The knowledge of basicity or acidity of these groups is useful in order to explain the values of the equilibrium constants calculated before. The molecular structure of Q is given in **1**, where N_s and N_w are, respectively, the stronger and weaker basic nitrogen of Q.



1

Accordingly, reaction (**2**) is quantitative because it is the neutralization of both the more basic nitrogen of the quinine base and the more acid proton of the sulfonphthaleine dye. Reaction (**1**), which is the sum of reaction (**2**) and (**4**), is not quantitative since reaction (**4**) is produced when the weaker basic nitrogen of quinine reacts with the more acid proton of a second molecule of the dye, neutralization which is identical to that of the reaction (**8**). This explains that reactions (**4**) and (**8**) have an identical formation constant as shown before. Both the quinine hydrochloride, $Cl^{-}QH^{+}$, and $BCGH^{-}QH^{+}$ ion



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associate have occupied the more basic nitrogen of quinine, in the first case by the hydrochloric acid group and in the second by acid dye group and the weaker basic nitrogen of both, Cl^-QH^+ and $BCGH^-QH^+$, is free to react with the acid dye to form, respectively, the $BCGH^-Cl^-QH_2^{2+}$ and $(BCGH^-)_2$-QH_2^{2+} ion associates.

Reaction (3) is the progressive formation of the hydrogen bonding between $BCGH^-QH^+$ and Q with the increase in the concentration of quinine base. Given that the dye has two protons of different acidity, the reactivity of both can be studied separately. The first phase is the one described by reaction (2) which is a quantitative reaction and the second phase, reaction (3), is a clear chemical equilibrium which is established with an excess of quinine base but not with quinine hydrochloride. This proves that in reactions (2) and (3), only the more basic nitrogen of quinine (N_s in **1**) is active to neutralize the first proton of the sulfonphthaleine dye in a first stage and later to form a hydrogen bond with $BCGH^-QH^+$ in a second stage. Therefore, the formation of the HBC requires the most basic nitrogen of Q (N_s in **1**) and the least acidic proton in $BCGH_2$ (H_w in Scheme 1). The latter is confirmed in reaction (8), between N_w and H_s, where an ion associate is formed, not a HBC. Finally, if both, the most basic nitrogen (N_s in **1**) and the most acidic proton (H_s in Scheme 1) react, then the reaction is a quantitative neutralization and ion associates are formed as it has been seen in reaction (2) or Scheme 1.

The preceding discussion suggests that the quinine base reacts in both equimolar mixtures and with an excess of quinine base as a monobasic amine and only with an excess of dye reacts as a divalent base. Similarly, $BCGH_2$ reacts in both equimolar mixtures and with an excess of dye as a monoprotic acid and only with an excess of quinine base reacts as a diprotic acid.

Reaction $BCGH_2$–Q *with Highly Concentrated Quinine.*—In a large excess of quinine base [Fig. 6(*a*)] the maximum wavelength is displaced from 547 to 620 nm. The application of Coleman's test to the data of Fig. 6(*a*) indicates two absorption species whose sum of concentrations is constant [as can be seen in Fig. 6(*b*)], one already described having a maximum at 547 nm and a new one at 620 nm. As the absorption intensity at 620 nm increases with the passage of time, different solutions with a large excess of Q and variable concentrations of $BCGH_2$ (always much less) were prepared and measured after 36 h at 293.16 K. The application of Coleman's method again suggests only one absorption species at longer time, which satisfies Beer's law with a molar absorptivity of 40 000 ± 2000 dm^3 mol^{-1} cm^{-1} at 620 nm.

The spectral change [Fig. 6(*a*)] in a large excess of quinine is only seen if the $BCGH_2$–quinine mixture is carried out with a recently prepared solution of quinine base. If the solution of the quinine base was prepared 36 h before mixture the absorption at 620 nm is immediately formed. From this it is interpreted that the quinine base reacts slowly with the solvent in the first phase forming a Q–solvent product and later this last compound reacts with BCG^{2-}–H^+–Q_2H.

In a previous paper, it has been demonstrated that a highly concentrated solution of atropine or homatropine in dichloromethane precipitates after various days generating a new compound of quaternary ammonium of type *N*-chloromethylammonium chloride with a molecular weight sum of the amine and that of the solvent.[10] Although the solution of quinine does not form a precipitate in dichloromethane, it may be assumed that Q reacts in the same way by reaction (9), where Q^+–

$$Q + CH_2Cl_2 \rightleftharpoons Q^+\text{–}CH_2Cl,Cl^- \qquad (9)$$

CH_2Cl,Cl^- is the ammonium quaternary compound generated. In this reaction the more basic nitrogen of Q is quaternized since the absorption band at 620 nm does not appear when an excess of Cl^-QH^+ is added. The structural formula of Q^+–CH_2Cl,Cl^- is shown in **2**. The existence of Q^+–CH_2Cl,Cl^- in a highly concentrated solution of Q explains the displacement to higher wavelength and the intensification of the absorption bands of BCG^{2-}–H^+–Q_2H^+ [see Fig. 6(*a*)] since Q^+–CH_2Cl replaces a protonated quinine, QH^+, and quinine hydrochloride is liberated by reaction (10), where $BCG^{2-}(Q^+\text{–}CH_2Cl)_2$ is the

$$BCG^{2-}\text{–}H^+\text{–}Q_2H^+ + 2Q^+CH_2Cl,Cl^- \rightleftharpoons BCG^{2-}(Q^+\text{–}CH_2Cl)_2 + 2Cl^-QH^+ \qquad (10)$$

new ion associate generated. This new compound justifies the bathochromic and hyperchromic shift in the absorption spectra since an identical peak at 620 nm is assigned to the $BCG^{2-}(ammonium^+)_2$ ion associates in the literature.[5-8,11,12] This displacement from 547 to 620 nm can be due to the breaking of the hydrogen bridge in reaction (10). Consequently, the peak at 620 nm of the $BCG^{2-}(ammonium^+)_2$ ion associate, would correspond to the BCG^{2-} species in dichloromethane. In Fig. 1 a shoulder can be observed at 620 nm in addition to the peaks at 415 and 547 nm. This suggests that some BCG^{2-} anions may be present even at low quinine concentrations.

In Fig. 6(*a*) at longer times there is a great excess of Q^+–CH_2Cl,Cl^- with respect to BCG^{2-}–H^+–Q_2H^+ which displaces reaction (10) completely to the right. This explains that at longer times there is only one absorption species as has already been shown.

The instantaneous speed of formation of Q^+–CH_2Cl,Cl^- product, V, is defined by $(A_t - A_{t-1})/\varepsilon' t$, where A_t is the experimental absorbance at time 't', A_{t-1} the absorbance at the previous instant, 't' the time in minutes and ε' the difference between molar absorptivities at 620 nm of $BCG^{2-}(Q^+\text{–}CH_2Cl)_2$ and BCG^{2-}–H^+–Q_2H^+ species. The value of V decreases as time passes, as it can be seen in Fig. 6(*c*). An identical result is obtained for the average speed. These facts suggest that the reaction (10) is not quantitative since the speed of formation of Q^+–CH_2Cl,Cl^- product should not decrease (Q and CH_2Cl_2 in great excess against Q^+–CH_2Cl,Cl^-) if this gives a quantitative reaction with BCG^{2-}–H^+–Q_2H^+.

Since all reactions start when Q is dissolved in dichloromethane, then the absorption measurements for kinetic studies are always carried out after reaction (9) has started and therefore reaction (10) has started, too. As this last reaction is not quantitative, all absorption measurements for kinetic studies lead to approximate results in the speed constants calculated for the reaction (9). To calculate these speed constants from the absorption measurements at 620 nm only the initial speeds, $V°$, may be taken into account owing to the fact that the formation of $BCG^{2-}(Q^+\text{–}CH_2Cl)_2$, is not quantitative. So, if only the measurements at initial times are taken into account, where the concentration of BCG^{2-}–$H^+Q_2H^+$ complex is very much higher than that of Q^+–CH_2Cl,Cl^-, then the $BCG^{2-}(Q^+\text{–}CH_2Cl)_2$ formation is indeed complete.

It has been checked that at a constant concentration of Q, the initial values of V, $V°$, are identical for different concentrations of $BCGH_2$. This clearly indicates that the dye does not take part

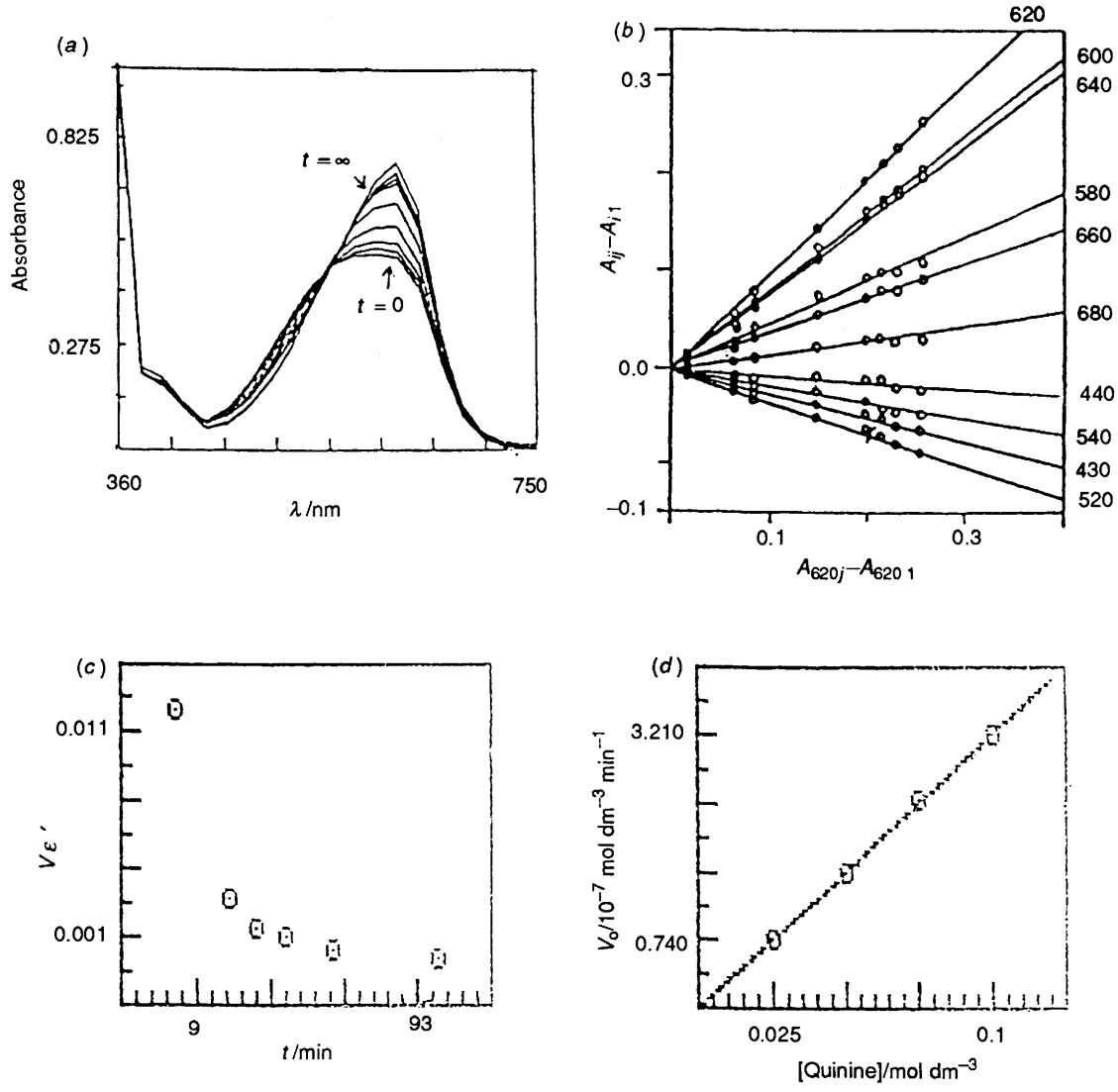


Fig. 6 (*a*) VIS–UV spectra of a mixed solution of $BCGH_2$ 2 × 10^{-5} and Q 0.05 mol dm^{-3} at 0.0, 9.3, 22.5, 55.5, 121.2, 213.0, 252.6, 312.5 and 2160 min in dichloromethane; T = 293.16 K. (*b*) Coleman's method for two species whose sum of concentrations is constant corresponding to the $BCGH_2$–Q mixture with a large excess of Q taken from the data in Fig. 6(*a*). The number corresponds to the wavelength, *i*, of the row of the matrix which has been resolved graphically according to Coleman. (*c*) Instantaneous speeds (V in mol dm^{-3} min^{-1}) multiplied by ε', $V\varepsilon'$, for different times in min, t, of a solution of $BCGH_2$ 2 × 10^{-5} and Q 0.05 mol dm^{-3} at 303.16 K. (*d*) Initial speed in mol dm^{-3} min^{-1}, $V°$, for different concentrations of Q (mol dm^{-3}) in solution of $BCGH_2$ 2 × 10^{-5} mol dm^{-3} in dichloromethane at 303.16 K.

Table 2 Rate constants of the quinine–dichloromethane reaction for different temperatures and determination coefficients, r^2, obtained by least squares method

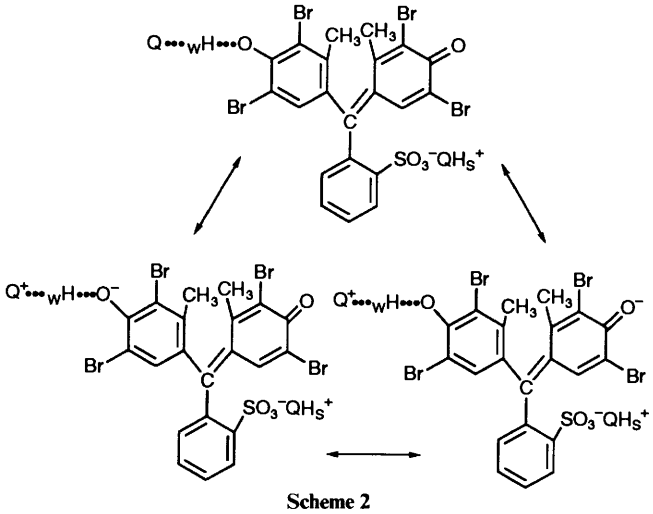
T/K	$k'/10^7$ min^{-1}	r^2
288.16	1.49	0.990
298.16	3.33	0.999
308.16	6.14	0.991
318.16	10.31	0.889

in the reaction of formation of Q^+–CH_2Cl,Cl^-, it only makes this reaction visible. In contrast, as can be seen in Fig. 6(*d*) the plot of the initial values of V for different concentrations of Q is a straight line. This indicates that reaction (9) is a first order process with respect to the quinine base, Q. As dichloromethane is in a great excess, it can be included in a rate constant of pseudo-first order, k'. So, $V = k([CH_2Cl_2] - x)([Q] - x) = k'[Q]$, where '$x$' is the concentration of Q^+–CH_2Cl,Cl^-, since 'x' is negligible with respect to both solvent and Q concentration. Therefore, the slope of the straight line in Fig. 6(*d*) is the apparent rate constant for the pseudo-first order reaction, k'. Quantitatively all the experimental results at very short times are adjusted by the equation $V° = k'[Q]$ ($V°$ = initial value) as can be seen in Fig. 6(*d*). The rate constant, k', and the determination coefficients, r^2, obtained by the least squares method for the reaction (9) are shown in Table 2. The calculated rate constants also satisfy Arrhenius' equation: $\ln k' = -E_a/RT + \ln A$; where A is the frequency factor and E_a the activation energy. By the least squared method the activation energy, E_a, is 49 ± 37 kJ mol^{-1} and the determination coefficient, r^2 is 0.998.

Solvent Effect on Absorption Band of HBC and Conductometric Results. Canonical Resonance Structures of the Associates.—In the preceding sections, the formation of a hydrogen bonded complex (HBC) between $BCGH^-QH^+$ and Q in reaction (3) (see also diagram **1**) is helped by two facts: (*a*) changes in the absorption peaks of $BCGH_2$ solutions when a slight excess of different amines is used (Q at 547 nm, ajmaline[9] at 562 nm and homatropine[9] at 577 nm; (*b*) displacement of absorption band of BCG^{2-}–H^+–Q_2H^+ (547 nm) until 620 nm when QH^+ is replaced by a quaternary ammonium cation. This absorption band (620 nm) has been assigned to the BCG^{2-}

anion, which is formed after the breaking of the hydrogen bridge. A third fact helps this interpretation, when acetone or ethanol is used a similar shift is observed, in agreement with that in more polar solvents the BCG^{2-} anion should be present. Finally, there is a fourth fact. In the recent literature, during solvent-extraction studies, Sakai[7] suggested the formation of charge transfer complexes (CTC) by a hydrogen bridge between protonated quinidine ($^+$H–Qd) and phenolic O^--group of $BCGH_2$ to explain a similar absorption band, which undergoes a bathochromic effect when $^+$H–Qd is displaced by an ammonium ion. Also an identical interpretation has been proposed to explain the thermochromism of amine–tetrabromophenolthalein ethyl ester (TBPE) solutions in 1,2-dichloroethane,[11] which is similar to that of Fig. 3.

As $BCGH_2$ is an extensively conjugated molecule, it shows resonance. Therefore, all the associates obtained can present resonance structures. So, the HBC could be represented by the resonance hybrid of the hydrogen bond as shown in Scheme 2.



Scheme 2

In Scheme 2, Q forms the hydrogen bridge by its N_s as has already been indicated. The above canonical resonance structures could be responsible for the large values for ε_{max} (547 nm), the appearance of the band in the visible region, and the numerical values of the thermodynamic parameters. This interpretation is identical to that suggested for the TBPE–amine associates[19,20] but these recent references indicate that charge transfer complexes (CTC) are involved between TBPE and amines. According to these authors the CT band may be due to the transition of the electron through the hydrogen bridge between the nitrogen of the amine and oxygen of the dye.[21]

The electric conductivity in dichloromethane for $BCGH_2$ or quinine was 0.0 μS cm^{-1}, which indicates that neither $BCGH_2$ nor Q conduct electric current in this solvent. Similarly, the electrical conductivity of dichloromethane solutions of BCG^{2-}–H^+–Q_2H^+ (8.49×10^{-5} mol dm^{-3}) was 0.0 μS cm^{-1}. On the other hand, the electrical conductivity of a dichloromethane solution of $BCG^{2-}(Q^+–CH_2Cl)_2$ (8.49×10^{-5} mol dm^{-3}) was 1.2 μS cm^{-1}. This confirms that the BCG^{2-}–H^+–Q_2H^+ species is in the molecular state while the $BCG^{2-}(Q^+–CH_2Cl)_2$ species is in the ionized state; that is to say, this second species is of ionic nature. The weak value of specific conductivity corroborates the conclusion that the second species is an ion associate in dichloromethane. The nature assigned to each species from conductometric results agrees with spectrophotometric interpretation. It also has been checked that in a highly concentrated quinine solution the specific conductivity increases with time. This also corroborates that quinine reacts with solvent generating the ammonium quaternary compound already described.

Conclusions

Quinine base reacts in dichloromethane with bromocresol green $BCGH_2$ forming compounds of 2:1, 1:1 and 1:2 ($BCGH_2$:Q) stoichiometric balance depending on the dye–quinine base molar relation. In mixtures with an excess of dye 2:1 [$(BCGH^-)_2QH_2^{2+}$] and 1:1 ($BCGH^-QH^+$) ion associates in chemical equilibrium are obtained. The 2:1 and 1:1 ion associates are generated, respectively, from dye–quinine base mixtures in a large excess of dye and dye–quinine base in equimolar mixtures. In mixtures with an excess of quinine base 1:1 and 1:2 (BCG^{2-}–H^+–Q_2H^+) associates in chemical equilibrium are formed. Spectrophotometrically this equilibrium is seen as a regular and reversible thermochromism of great analytical interest. Spectral data and thermodynamic parameters indicated that the 1:2 compound is not an ion associate but a hydrogen bonded complex (HBC) formed through hydrogen bonding between –O–H in $BCGH^-QH^+$ and the amino nitrogen of quinine. In a large excess of quinine base, dichloromethane gives a side reaction with quinine generating *N*-chloromethylquininium chloride, $Q^+–CH_2Cl,Cl^-$, in a pseudo-first order kinetic process with respect to quinine base. This new compound reacts with HBC generating $BCG^{2-}(Q^+–CH_2Cl)_2$, which is an ion associate of BCG^{2-} whose absorption spectrum corresponds to the BCG^{2-} species in this solvent. The reaction between quinine and dichloromethane can generate interferences on analytical methods proposed for selective analysis of quaternary ammonium compounds, which use this amine as a favourable substance for the quantitative extraction of the dye–ammonium ion associate.

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