Molecular Association of K-Vitamins with Biologically occurring molecules. Part 1. A Study of the Association Properties of the Quinone Moiety

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The *p*-benzoquinone (*p*BQ) moiety is known to be involved in many biochemical processes and since this moiety occurs in the structure of the K-vitamins, it was felt necessary to study the molecular association of the *p*BQ moiety. This paper deals with the stability constants and the nature of the binding in some molecular associations of *p*BQ with carboxylate ions in an aqueous medium and of 1,4-naphthaquinone with resorcinol in dioxane. We observed that *p*BQ in water is strongly hydrated and that carboxylate ions slowly displace the water molecules in the hydration sphere and bind to *p*BQ in a 1:1 stoichiometry. The n–π^* band of *p*BQ in water is shown to be blue-shifted (from the Franck–Condon principle) when a *p*BQ–carboxylate association is formed. 1,4-Naphthaquinone, which is sparingly soluble in water (like the K-vitamins) forms a 1:1 π–CT complex with resorcinol in a dioxane medium. The stoichiometries have been determined spectrophotometrically using the Benesi–Hildebrand equation and Job's continuous variation method. The formation constants of the ion–molecule and molecule–molecule associations are reported.

A quinonoid ring is common to many biomolecules, *e.g.*, ubiquinone and the K-vitamins.[1] The former acts as a coenzyme and the latter occurs in blood prothrombin, having an important, but not completely understood, role in blood coagulation.[2] In the Michaelis–Menten mechanism [3] of enzyme catalysis, the stability constant of the enzyme–substrate (ES) association is an important parameter in determining the rate of enzyme-catalysed reactions. It is also known that some enzymes (E), known as 'apoenzymes', catalyse biochemical group-transfer reactions only in combination with some 'coenzymes' (C) or 'prosthetic groups' (P) and the initial step is the reversible formation of the molecular association (EC or EP); E + C (or P) $\rightleftharpoons$ EC (or EP).

According to Heathcote and Slifkin,[4] the EP association is much stronger than the EC. The stability constant of the molecular associations is therefore important not only for a distinction to be made between coenzymes and apoenzymes but also for a detailed kinetic study and proper understanding of enzymatically-catalysed reactions. To this end we have determined the stability constants of both *p*-benzoquinone–carboxylate ion associations in an aqueous medium and of the water-insoluble 1,4-naphthaquinone (part of the K-vitamin structure with a *p*BQ moiety) associated with resorcinol in a dioxane medium.

The study of *p*BQ adducts is also biologically important, *e.g.*, Pryor *et al.*[5] have shown that among the four carcinogenic cigarette tars the predominant one is a complex between *p*BQ and hydrocarbon groups in a polymeric matrix. *p*BQ is also reported[6,7] to act as a cyclic dienophile in the Diels–Alder reaction, where a charge-transfer (CT) complex is a probable intermediate in a benzene medium. These facts have drawn the attention of recent theoretical chemists with a view to investigating the ground and excited states of *p*BQ.[8]

We have been interested in studying the *p*BQ-adducts in water because almost all biochemical reactions occur in aqueous media. The 1,4-naphthaquinone adduct has been studied in dioxane because, like the K-vitamins, it is almost insoluble in water (fat soluble), and our ultimate aim is to study the molecular association of K-vitamins with some biologically-occurring molecules.

Experimental

*p*BQ, resorcinol and 1,4-naphthaquinone were commercial reagents from E. Merck and were purified by sublimation just before use. G.R. grade sodium acetate (E. Merck) was used without further purification. Sodium formate and sodium propionate were freshly crystallised before use. The concentrations of the prepared salt solutions were determined by conductometric titration with standard H_2SO_4 solution. Dioxane, a solvent for 1,4-naphthaquinone, was purified by refluxing the A.R. grade material with KOH pellets and then distilling just before use. Spectral measurements were performed on a Hilger (UVSpek) model spectrophotometer.

For determining the stability constants and stoichiometries of the ion–molecule and molecule–molecule associations, the Benesi–Hildebrand[9,10] (BH) equation was used in a modified form. In the case of *p*BQ-acetate ion association, Job's continuous variation method[11] was attempted.

Results and Discussion

Case 1. pBQ Carboxylate Ion Associations.—In an attempt to determine the stability constant of quinhydrone, a known molecular complex of *p*BQ and hydroquinone, by a potentiometric method using varying concentrations of *p*BQ and hydroquinone in sodium acetate–acetic acid buffer solutions of differing pH values, the emf data were found to be inexplicably scattered and time dependent. The colour of the solutions was also found to gradually deepen towards reddish brown. To examine which species in the buffer was responsible for this, *p*BQ solutions were mixed with the buffer solution and its components, *i.e.*, acetic acid and sodium acetate, separately, using the same molar concentration of *p*BQ. After 2 h it was found that the intensity of the reddish-brown colour was at a maximum in the sodium acetate solution, intermediate in the buffer solution and practically negligible in the acetic acid solution. This indicated that the acetate ion must be responsible for the development of colour.

*p*BQ is known to be reduced to semiquinone radical[12] in alkali media. There is also considerable evidence to suggest that *p*BQ is photochemically reduced.[13–17] However, the colour

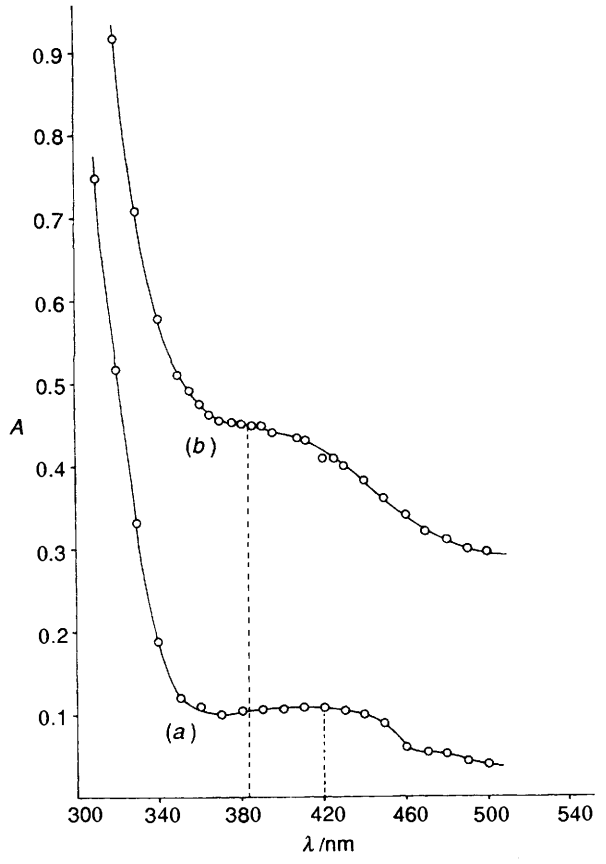

Fig. 1 Spectra of aqueous solutions of (*a*) *p*BQ at 2.7104×10^{-3} mol dm^{-3} and (*b*) *p*BQ at 2.7104×10^{-3} mol dm^{-3} + CH_3COONa at 8.1312×10^{-3} mol dm^{-3}

Table 1 Absorbances of mixtures of *p*BQ (A) and sodium formate (D) in aqueous media at 26 °C

$C^{\circ}_A/10^{-3}$ mol dm^{-3}	$C^{\circ}_D/10^{-2}$ mol dm^{-3}	Absorbance 500 nm	470 nm	450 nm
2.1924[a]	20.110 32	0.191	0.195	0.195
	17.875 84	0.177	0.180	0.180
	15.641 36	0.172	0.175	0.179
	13.406 88	0.162	0.163	0.168
	11.724 00	0.149	0.154	0.158
	8.937 92	0.127	0.132	0.135
4.3848[b]	17.875 84	0.366	0.372	0.381
	15.641 36	0.346	0.352	0.360
	13.406 88	0.322	0.328	0.336
	11.724 00	0.294	0.300	0.307
	8.937 92	0.260	0.265	0.272
	6.703 44	0.218	0.222	0.229

[a] At 500 nm, slope $S = 2.4253 \pm 0.107$, intercept $I = -7.7657 \pm 0.675$, correlation coefficient $C = 1.00$; at 470 nm, $S = 2.6500 \pm 0.106$, $I = -8.7830 \pm 0.651$, $C = 1.00$; at 450 nm, $S = 2.8343 \pm 0.186$, $I = -9.6104 \pm 1.120$, $C = 0.99$. [b] At 500 nm, $S = 5.0338 \pm 0.127$, $I = -8.2340 \pm 0.443$, $C = 1.00$; at 470 nm, $S = 5.1484 \pm 0.122$, $I = -8.2962 \pm 0.416$, $C = 1.00$; at 450 nm, $S = 5.3436 \pm 0.131$, $I = -8.5045 \pm 0.438$, $C = 1.00$.

reported in the present paper develops in acidic media and also in the absence of reducing substances in the dark. Fig. 1 shows the spectra of a *p*BQ solution and a *p*BQ–sodium acetate mixture in aqueous media against water as a reference. The mixture shows a new absorption band with a shoulder at 390 nm.

Absorbances at a number of wavelengths measured under BH conditions, *e.g.*, [salt] ≫ [*p*BQ], are given in Tables 1–3 for mixtures of *p*BQ with sodium formate, sodium acetate and sodium propionate, respectively. A rearranged form of the BH equation (1), which is known to be valid for 1:1 molecular

$$1/C^{\circ}_D = K\varepsilon C^{\circ}_A l/A - K \quad (1)$$

complexes,[18] was tried with the experimental data. In eqn. (1), C°_D is the initial concentration of the salt and C°_A that of *p*BQ in the mixture; A is the absorbance of the mixture measured against the aqueous *p*BQ solution as reference; ε is the difference in molar extinction coefficients of the molecular complex and *p*BQ; l is the cell-length and K is the stability-constant of the molecular association. Fig. 2 shows that the plot of $1/C^{\circ}_D$ *vs.* $1/A$ for the *p*BQ–sodium acetate system is linear. Similar linear plots are obtained for all the systems studied. The slopes, intercepts and correlation coefficients are shown in the Tables. We observed that the intercepts are almost independent of the initial concentrations of *p*BQ and also of the wavelengths of measurement. This demonstrates that only 1:1 complexes are

Table 2 Absorbances of mixtures of *p*BQ (A) and sodium acetate (D) in aqueous medium at 26 °C

$C^{\circ}_A/10^{-3}$ mol dm^{-3}	$C^{\circ}_D/10^{-2}$ mol dm^{-3}	Absorbance 500 nm	470 nm	450 nm
2.4574[a]	4.753 40	0.321	0.335	0.345
	4.159 23	0.287	0.295	0.299
	3.565 05	0.272	0.282	0.290
	2.970 88	0.235	0.241	0.249
	2.376 70	0.202	0.207	0.210
3.6861[b]	4.159 23	0.356	0.370	0.381
	3.565 05	0.380	0.396	0.415
	2.970 88	0.315	0.329	0.350
	2.376 70	0.267	0.277	0.283
	1.782 52	0.225	0.230	0.238

[a] At 500 nm, $S = 11.6066 \pm 0.522$, $I = -15.4073 \pm 2.060$, $C = 1.00$; at 470 nm, $S = 11.5632 \pm 0.677$, $I = -13.8870 \pm 2.592$, $C = 1.00$; at 450 nm, $S = 11.2990 \pm 1.124$, $I = -11.7292 \pm 4.192$, $C = 1.00$. [b] At 500 nm, $S = 16.8718 \pm 2.019$, $I = -19.8965 \pm 6.912$, $C = 0.98$; at 470 nm, $S = 16.8213 \pm 1.979$, $I = -17.8007 \pm 6.555$, $C = 0.98$; at 450 nm, $S = 16.8628 \pm 2.338$, $I = -15.9191 \pm 7.467$, $C = 0.97$.

Table 3 Absorbances of mixtures of *p*BQ (A) and sodium propionate (D) in aqueous medium at 26 °C

$C^{\circ}_A/10^{-3}$ mol dm^{-3}	$C^{\circ}_D/10^{-2}$ mol dm^{-3}	Absorbance 470 nm	450 nm
1.726 315[a]	16.0256	0.604	0.710
	12.6344	0.568	0.685
	11.3999	0.551	0.648
	10.4717	0.536	0.630
	8.3335	0.495	0.582
	4.7619	0.386	0.454
	4.2135	0.362	0.426
3.452 63[b]	10.4717	1.072	1.110
	8.3335	1.010	1.025
	4.7619	0.773	0.800
	4.2135	0.725	0.750
	2.1929	0.483	0.500
	1.8907	0.435	0.450
	1.6129	0.386	0.400

[a] At 470 nm, $S = 15.8406 \pm 0.002$, $I = -19.9873 \pm 0.003$, $C = 1.00$; at 450 nm, $S = 18.3849 \pm 0.307$, $I = -19.4666 \pm 0.548$, $C = 1.00$. [b] At 470 nm, $S = 31.5422 \pm 0.139$, $I = -19.6626 \pm 0.246$, $C = 1.00$; at 450 nm, $S = 32.8008 \pm 0.000$, $I = -20.0008 \pm 0.001$, $C = 1.00$.

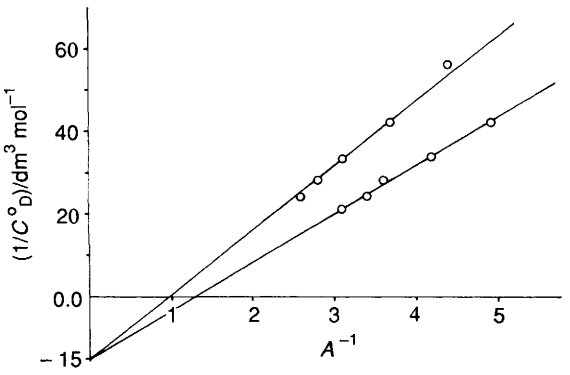

Fig. 2 Benesi–Hilderbrand plot of the *p*BQ–CH_3COONa system at 500 nm at two initial concentrations of *p*BQ

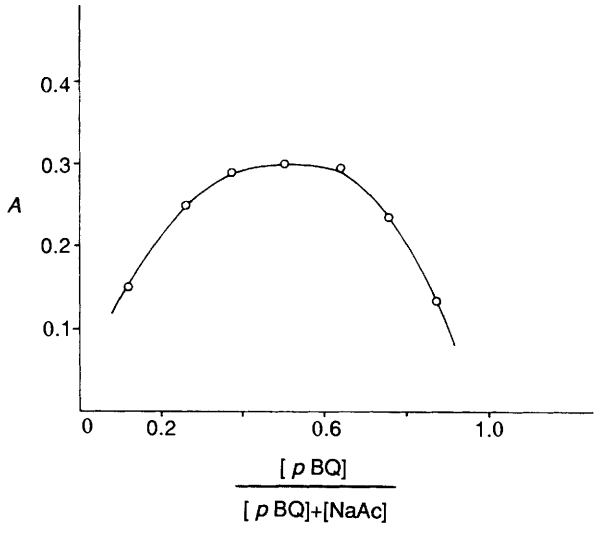

Fig. 3 Continuous-variation plot of the *p*BQ–CH_3COONa system at 500 nm; [*p*BQ] + [CH_3COONa] = 1.084 16 × 10^{-2} mol dm^{-3}

Table 4 Data of continuous variation experiment with a mixture of *p*BQ and sodium acetate (NaAc). [*p*BQ] + [NaAc] = 1.084 16 × 10^{-2} mol dm^{-3}

$\frac{[p\text{BQ}]}{[p\text{BQ}] + [\text{NaAc}]}$	Absorbance (500 nm)
0.125	0.158
0.250	0.253
0.375	0.292
0.500	0.313
0.625	0.296
0.750	0.264
0.875	0.175

Table 5 Stability constants and molar extinction coefficients of the complexes of *p*BQ with carboxylate ions in aqueous medium

Carboxylate ion	K/dm^3 mol^{-1} (wavelength in nm)	ε/dm^3 mol^{-1} cm^{-1} (wavelength in nm)
Formate	8.0 (500), 8.5 (470), 9.0 (450)	142 (500), 142 (470), 134 (450)
Acetate	17.6 (500), 15.8 (470), 14 (450)	258 (500), 286 (470), 320 (450)
Propionate	19.8 (470), 19.7 (450)	464 (470), 533 (450)

formed between *p*BQ and carboxylate ions in aqueous solution under the experimental conditions. Fig. 3 shows the Job curve

Table 6 UV spectral data for 1,4-naphthaquinone and resorcinol[31]

Compound	Solvent	λ/nm (log ε)
Resorcinol	MeOH	276.5 (3.33), 283 (3.26), 237 (3.85)
	EtOH	277 (3.34)
1,4-Naphthaquinone	MeOH	250 (4.6), 330 (3.8)
	EtOH	246 (4.34), 251 (4.34), 260 (4.05), 338 (3.5)
Dioxane		249 (4.3), 327 (3.5) liquid phase
		180 (3.8) vapour phase

for the *p*BQ–sodium acetate system, which clearly establishes the 1:1 stoichiometry of the molecular association between *p*BQ and acetate ions. Experimental data for the continuous variation experiment are shown in Table 4. Values of stability constants and molar extinction coefficients at a number of wavelengths are given in Table 5.

Proof that the carboxylate ion associates itself with *p*BQ is further provided from the fact that when *ca.* 0.5 g of *p*BQ is dissolved in 50 cm^3 of 1 mol dm^{-3} acetic acid solution, the conductance of the solution slowly rises at room temperature, the change being observable at intervals of 30 min. The association of acetate ions to *p*BQ shifts the dissociation equilibrium of acetic acid to the right and releases H_3O^+, thus increasing the conductance.

The stability constants of the molecular associations as found from the BH plots increase with increasing chain length of the carboxylate ion. Moreover, the extinction coefficients also increase with increasing stability constant. These facts[19] suggest that the new absorption bands that appear on mixing the components might be of charge-transfer type. However, the centres of the shoulders observed for the three carboxylates are almost at the same position (393 nm for formate and 389 nm for propionate) and do not change systematically with the ionisation potentials of the carboxylate ions (which should run parallel to the pK_a values of the corresponding acids) as required by Mulliken's theory.[20] Therefore the observed new bands are the blue-shifted n–π* bands of *p*BQ rather than CT bands. The force binding the carboxylate ion to the *p*BQ molecule in the ground state is presumably ion-induced dipole attraction as stressed by Briegleb.[21,22] In the excited state the π-electron density at different carbon atoms in *p*BQ changes and a different orientation of the carboxylate ion is needed to stabilise the excited state. But this is not possible in the time the electronic excitation occurs, according to the Franck–Condon principle. The orientation of the carboxylate ion with respect to the *p*BQ molecule thus stabilises the ground state but destabilises the excited state[23] and a blue-shift of the n–π* band of *p*BQ results.

Although most of the molecular complexes so far reported in the literature are formed almost instantaneously on mixing the components, there is also evidence[24] of slow formation. The n–π* band of *p*BQ in aprotic media such as acetone has a maximum at 455 nm but in aqueous solution the band shifts to 420 nm. This is clearly a Franck–Condon shift resulting from ground-state hydration of *p*BQ through hydrogen bonding as reported by Brearly and Kasha.[25] The slowness of the formation of the ion–molecule association under study may be due to slow desolvation of the aquated molecules of the components before interaction; a thorough kinetic study, however, is necessary to support this view. The formation of a Meisenheimer type of complex should not be ignored, but this is hardly possible because such a species is known[26–28] to form between very strong acceptors (like *s*-trinitrobenzene derivatives) and very strong electron donors (such as ethoxide ions).

Table 7 Optical data for spectrophotometric determination of stability constant and calculated results. Cell length = 1 cm; T = 30 °C; [1,4-naphthaquinone] = 1.349 87 × 10^{-3} mol dm^{-3}

	Absorbance		K/dm^3 mol^{-1}		ε/dm^3 mol^{-1} cm^{-1}	
[Resorcinol]/mol dm^{-3}	350 nm[a]	360 nm[b]	350 nm	360 nm	350 nm	360 nm
0.291 45	0.166	0.123				
0.255 02	0.156	0.114				
0.218 59	0.150	0.106	4	4	231	168
0.182 16	0.131	0.096				
0.145 72	0.115	0.089				
0.109 29	0.095	0.068				

[a] At 350 nm, S = 1.2505 ± 0.037, I = −4.0069 ± 0.289, C = 1.00. [b] At 360 nm, S = 0.8939 ± 0.062, I = −3.7687 ± 0.665, C = 0.99.

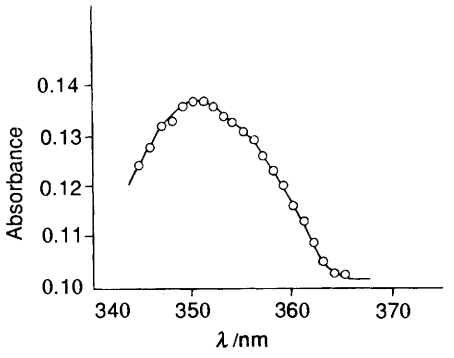

Fig. 4 Spectrum of a mixture of resorcinol and 1,4-naphthaquinone in dioxane. [Recorcinol] = 0.291 45 mol dm^{-3}; [1,4-naphthaquinone] = 0.1114 × 10^{-2} mol dm^{-3}.

Case 2. 1,4-Naphthaquinone–resorcinol Association in Dioxane.—Fig. 4 shows the charge–transfer band of a resorcinol–1,4-naphthaquinone mixture in dioxane. The blank used in the measurement was a solution of 1,4-naphthaquinone in dioxane at the same molar concentration as in the mixture. None of the three components resorcinol, dioxane and 1,4-naphthaquinone had any absorption peak in the 345–370 nm region as shown in Table 6. This means that the band shown in Fig. 4 with an absorption maximum at 350 nm is a new band characteristic of the mixture. It may not be regarded as the λ = 327 nm band of 1,4-naphthaquinone in dioxane perturbed by resorcinol because the intensity of the 327 nm peak of 1,4-naphthaquinone in dioxane (ε = 3162) is *ca.* 25 times higher than that of the 350 nm peak of the mixture (apparent ε, obtained from the BH plot, =123). It is, therefore, quite reasonable to believe that this new peak is due to a charge–transfer type of transition since quinones are known to act as good electron acceptors[29] and recorcinol as donors.[30]

The graphical plot of experimental data given in Table 7 is linear according to eqn. (1) and the slopes and intercepts at the different wavelengths of measurement are also given in this Table. The results suggest that a 1:1 molecular association is definitely formed between resorcinol and 1,4-naphthaquinone, and the intercept gives the stability constant, K.

The constancy of K (at nearly 4 dm^3 mol^{-1}) with respect to changes in wavelength is expected since it is a purely thermodynamic quantity and points to the fact that molecular adducts of stoichiometries other than 1:1 are not formed in this case. The small value of K (as compared to 10^{10} to 10^{20} for inorganic complexes) and the low free energy of formation (ΔG° = −3.493 kJ mol^{-1}) indicates that the association is quite loose, characteristic of EC or EP bindings.

The present work may be regarded as an initial study which points to the possibility of similar involvement of the quinonoid moiety of K-vitamins in forming molecular association with carboxylate ions and other π-type donors *in vivo*.

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