

Aggregate Formation of *p*-Hydroxybenzoic Acid Esters in Aqueous Solution

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The aggregate formation of *p*-hydroxybenzoic acid esters (parabens) in aqueous solutions was studied by ultrafiltration and light scattering. The permeability of parabens in aqueous solution passing through an ultrafiltration membrane decreased with an increase in carbon number in the alkyl group from methyl- to butyl-paraben. Dissociation of the phenolic hydroxyl group and the addition of ethanol made possible the free passage of butyl-paraben through the membrane. An increase in the concentration of butyl-paraben was responsible for the steep rise in light scattering intensity plots. Undissociated molecules of parabens in aqueous solution are thus shown to form aggregates by hydrophobic intermolecular force. The membrane permeability of butyl-paraben decreased and increased with concentration and the cutoff molecular weights of the membranes, respectively. Plots of surface tension against concentration of butyl-paraben showed a linear decrease but no inflection point. Paraben molecules possessing long alkyl chains may thus be concluded to form non-micellar type aggregates in aqueous solution, which differ from the clear-cut aggregate formation of conventional surfactants.

Key words hydroxybenzoic acid ester; non-micellar aggregate; self association; hydrophobic interaction; surface activity

Many drugs exhibit surface activity in aqueous solution by intermolecular hydrophobic interaction. The self-association ability of drugs in aqueous solution is a surface active characteristic reported in antiacetylcholins,^{1,2)} antihistamines,³⁾ prostaglandins⁴⁾ and antiseptics.⁵⁾ Surface active drugs may bind hydrophobically to proteins and other biological substrates, and the accumulation, concentration and action of the drugs on biological receptors may be determined in part by the surface active characteristics. *p*-Hydroxybenzoic acid esters (parabens) are widely used for aqueous based formulations as an antibacterial preservative. An ultrafiltration membrane separates macromolecules from the solution but allows the free passage of solutes of low molecular weight. We found that the membrane permeability of methyl-, ethyl-, propyl- and butyl-parabens decreased with the carbon number in the alkyl group. Parabens have simple structures and small molecular weights compared with previously identified self-associating drugs. The physicochemical characteristics of parabens should be determined for clarification of their biological functions. The association behavior of paraben molecules in aqueous solution was examined in this study.

Experimental

Materials Methyl, ethyl, propyl and butyl *p*-hydroxybenzoate (MP, EP, PP and BP, respectively) were of JP XII grade. All other reagents and solvents were of analytical reagent grade.

Sample Preparation The parabens were dissolved in aqueous solution with ethanol (0–70% (v/v)) or a universal buffer solution for UV spectrophotometry⁶⁾ (pH 5–10). Their concentrations were adjusted to ca. 0.0001–0.01% (w/v) as *p*-hydroxybenzoic acid (7.24×10^{-6} – 7.24×10^{-4} M), these being less than the solubilities in water,⁷⁾ and they were regarded as the undissociated form of the phenolic hydroxyl group since the pK_a of *p*-hydroxybenzoic acid esters (8.4)⁸⁾ exceeded the pH of the solutions (ca. 5–6).

Ultrafiltration and Determination of Parabens A polysulfone-made ultrafiltration cell (50 ml, model 8050, Amicon Co.) and various ultrafiltration membranes of cellulosic polysaccharide (43 mm i.d., Diaflo YM

series, Amicon Co.) were used. Unsymmetric pore membranes of the YM series, YM1, YM5, YM10, YM30 and YM100, were rated at cutoff molecular weights of 1000, 5000, 10000, 30000 and 100000 as spherical proteins, respectively. After being poured into an ultrafiltration cell of 50 ml, aqueous paraben solutions were filtered under constant pressure (3 kg/cm²) at 25 °C. The filtrate (ca. 5 ml) was collected to determine the concentration, rejecting the first 20 ml of filtrate due to adsorption of the paraben onto the ultrafiltration apparatus.⁹⁾ The used membrane and cell were washed, following each filtration, with aqueous ethanol solution (30% (v/v)) and water. Paraben concentrations in the feed solution and ultrafiltrate were determined spectrophotometrically at 256 nm using a spectrophotometer (model UV-2100, Shimadzu Co.).

Measurement of Static and Dynamic Light Scattering Static and dynamic light scattering of aqueous BP solution was measured at 25 °C using a light scattering spectrophotometer (model DSL-7000, Otsuka Electronics Co., Osaka) equipped with a helium-neon laser (633 nm) or argon laser (488 nm), respectively. Aqueous BP solution filtered through a membrane filter (pore size 0.2 μm) was used for static light scattering at scattering angles of 40–140°. The refractive index increment of BP was measured at 25 °C using a differential refractometer (model DRM-1021, Otsuka Electronics Co.) at 633 nm. The light scattering intensity of aqueous BP solution was given by Eq. 1:

$$R_\theta = A(n^2 I / n_b^2 I_0) \quad (1)$$

where R_θ is the Rayleigh ratio, A the calibration constant of the apparatus, n and n_b the refractive indices of water and toluene used for calibration, and I and I_0 the intensities of scattered and introduced laser light, respectively. The apparent molecular weight of BP aggregates, M , was determined according to the one concentration method by using Eq. 2:

$$\begin{aligned} K(C - C_0) / (R_\theta - R_0) - 2B_2(C - C_0) \\ = 1/M \cdot (1 + 16\pi^2 n^2 S^2 \sin^2(\theta/2) / (3\lambda^2)) \end{aligned} \quad (2)$$

$$K = 4\pi^2 n^2 (dn/dc)^2 / (\lambda^4 N_A)$$

where K is the optical constant, R_θ and R_0 the Rayleigh ratio at BP concentration, C , and critical micelle concentration (CMC), C_0 , respectively, B_2 the second virial coefficient, S^2 the mean-square radius of gyration, θ the scattered angle, λ the wavelength, dn/dc the refractive index increment, and N_A Avogadro's number. The aggregation number, N , was determined as

$$N = M/M_M \quad (3)$$

where M_M is the molecular weight of the monomer.

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Aqueous BP solution filtered through a membrane filter (pore size $0.1\ \mu\text{m}$) was used for dynamic light scattering at scattering angles of 45° . A first-order correlation function, $G^1(\tau)$, was determined from the second-order correlation function of scattered light intensity, $G^2(\tau)$, according to the homodyne method as follows:

$$G^2(\tau) = \alpha[1 + \beta|G^1(\tau)|^2] \quad (4)$$

where α and β are constants and τ is correlation time. $G^1(\tau)$ was determined according to the cumulant method by using Eq. 5:

$$G^1(\tau) = \exp[-\Gamma\tau + (-\mu_2/2)\tau^2 \dots] \quad (5)$$

$$\mu_2 = (\Gamma - \bar{\Gamma})^2$$

$$\Gamma = q^2 D$$

$$q = 4\pi n/\lambda \sin(\theta/2)$$

where Γ and $\bar{\Gamma}$ are the decay rate of the field correlation function and its average, μ_2 is the second-order cumulant related to size distribution, q the magnitude of scattering vector, and D the diffusion coefficient. The hydrodynamic radius of BP aggregates, r , was determined from the Stokes-Einstein equation:

$$r = kT/(6\pi\eta D) \quad (6)$$

where k is Boltzmann's constant, T the absolute temperature and η the viscosity of water. Size distribution is given by μ_2/Γ^2 .

Measurement of Surface Tension The surface tension of aqueous BP solutions prepared by purified water passed through a Milli-Q system (Nihon Millipore Co.) was measured at 25°C using a modified Wilhelmy-type surface tensiometer (model ST-1, Shimadzu Co.).

Results and Discussion

Self-Association of Parabens in Aqueous Solution

Figure 1 shows the effects of BP concentration on light scattering intensity, *i.e.* the Rayleigh ratio (R_θ), of the aqueous solution. A rise in R_θ at 3.62×10^{-4} – 5.43×10^{-4} M is evidence of the aggregate formation of BP molecules, as was also observed for surfactants showing an increased apparent mass above well-defined CMC. Table 1 shows the permeability of parabens while passing through an ultrafiltration membrane. Although MP passed freely through YM5 membrane, the permeability of other parabens decreased with the carbon number in the alkyl group. A decrease in paraben permeability passing through the ultrafiltration membrane suggests that the apparent molecular weight of the paraben increases somewhat with carbon number in the alkyl group.

Figure 2 shows the effects of pH and ethanol on the

membrane permeability of BP. This permeability increased rapidly near pK_a (8.4),⁸⁾ followed by free passage at pH 9 or above. An increase in ethanol was also the cause for enhanced BP permeability, and at 70% (v/v) ethanol, the BP concentration in the filtrate and feed solution was the same. Lessening these interactions may possibly be due to an increased affinity for an aqueous or aqueous ethanol phase by dissociation of the phenolic hydroxyl group or the much greater solubility in ethanol (95% (v/v)),⁷⁾ respectively.

Generally, the solute concentration in the ultrafiltrate, except for the first filtrate, must be to equal that in the feed solution. The molecular weights of parabens (152.15

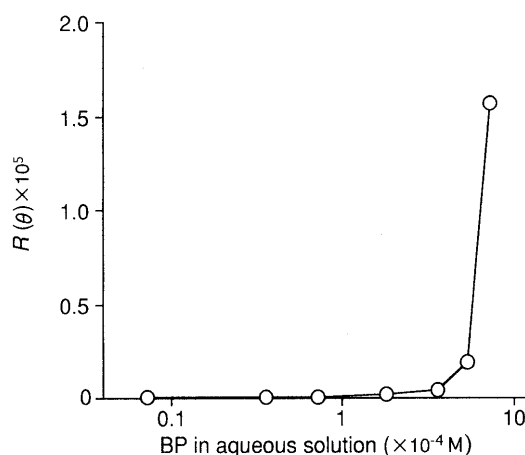


Fig. 1. Effects of BP Concentration on Light Scattering Intensity, R_θ , at 90°

Table 1. Permeability of Paraben in Aqueous Solution during Passage through a YM5 Membrane

Paraben	Concentration in ultrafiltrate ($\text{M} \times 10^4$)	Membrane permeability ^{a)} (%)
MP	7.24	100.0
EP	7.19	99.3
PP	7.11	98.2
BP	6.68	92.3

a) Percent permeability of paraben in the ultrafiltrate to initial aqueous solution.

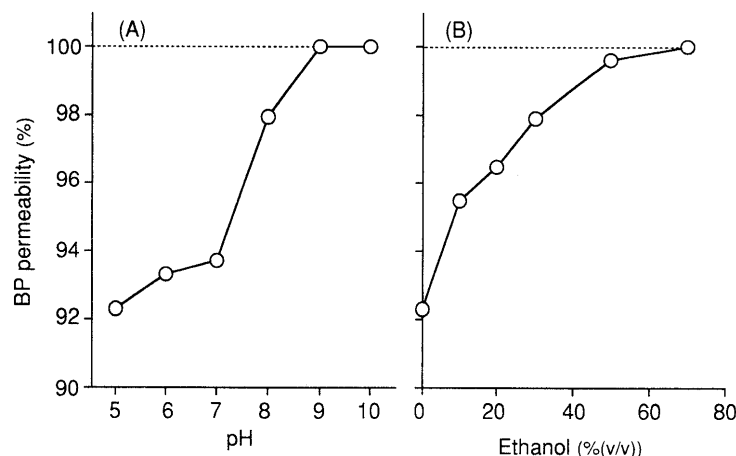


Fig. 2. Effects of pH (A) and Ethanol (B) on BP Permeability of YM5 Membrane

Ordinate: percent permeability of BP in the ultrafiltrate to initial aqueous solution with 7.24×10^{-4} M.

of MP to 194.23 of BP) are rather small compared with the cutoff molecular size of the YM5 membrane (5000). The paraben molecule consists of hydrophobic (an alkyl and a phenyl group) and hydrophilic parts (a phenolic hydroxyl and a carbonyl group). The BP molecule in particular possesses large hydrophobicity due to its long alkyl chain. The hydrophobicity of parabens may very likely contribute to their self-association in aqueous solution, resulting in the membrane permeability of parabens being dependent on the carbon number in the alkyl group.

Characteristics of Paraben Association Characteristic parameters of BP aggregates obtained by static and dynamic light scattering are summarized in Tables 2 and 3, respectively. The apparent molecular weight of the BP aggregate and aggregation number were essentially the same as those of the surfactants.¹⁰⁾ The hydrodynamic radius of the aggregates was much larger than that of normal surfactants.¹¹⁾

Figure 3 shows the effects of BP concentration on surface tension and BP permeability during passage through a YM5 membrane. Plots of surface tension against concentration decreased linearly; however, no inflection point of the slope, such as the CMC of sur-

factants, was observed at a BP concentration close to the solubility limit. BP permeability decreased with concentration. An increase in cutoff molecular weights of various membranes resulted in a gradual increase in BP permeability during passage through the membranes, as shown in Fig. 4.

The absence of an inflection point in the surface tension-concentration curve suggests that BP association differs from clear-cut aggregate formation of conventional surfactants. Molecules of phenothiazine hydrochlorides form aggregates considered to be true micelles in consideration of the clear inflection point in light scattering and conductivity plots, which are composed of about 10 molecules vertically stacked by intermolecular forces.¹⁾ Pavatrine hydrochloride molecules have self-associating ability, possibly of the non-micellar type, since the molecules exhibit concentration dependence of association.²⁾ The non-micellar-type association of BP is supported by the concentration dependence of surface tension and BP permeability. BP molecules appear to exist as a mixture from a monomer to greater aggregates of various sizes in aqueous solution, resulting in that the BP permeability varies with the cutoff size of an ultrafiltration membrane.

Table 2. Static Light Scattering Data for Aqueous Solution with BP of 7.24×10^{-4} M

dn/dc^a (ml/g)	M^b (g/mol)	$(S^2)^{1/2c}$ (Å)	N^d
0.2083	63590	1057	327

a) Refractive index increment. b) Apparent molecular weight was calculated assuming a CMC of 5.43×10^{-4} M, which was the inflection point in Fig. 1. c) Root-mean-square radius of gyration. d) Aggregation number.

Table 3. Dynamic Light Scattering Data for Aqueous Solution with BP of 7.24×10^{-4} M

r^a (nm)	D^b ($\text{cm}^2/\text{s} \times 10^8$)	μ_2/Γ^2c
47	4.63	0.329

a) Hydrodynamic radius. b) Diffusion coefficient. c) Size distribution index.

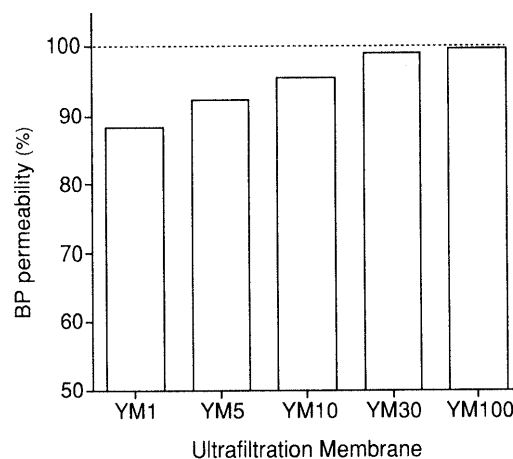


Fig. 4. Comparison of BP Permeability for Various Membranes Differing in Cutoff Molecular Weight

Ordinate: percent permeability of BP in the ultrafiltrate to initial aqueous solution with 7.24×10^{-4} M.

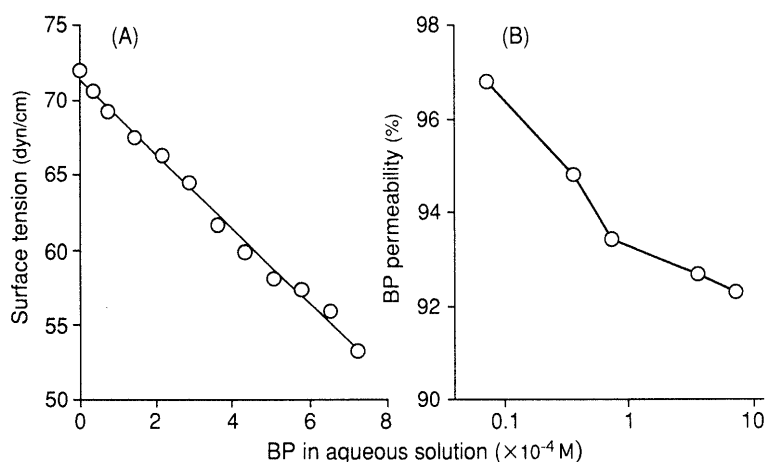


Fig. 3. Effects of BP Concentration on Surface Tension of Aqueous BP Solution (A) and BP Permeability of YM5 Membrane (B)

(B) Ordinate: percent permeability of BP in ultrafiltrate to initial aqueous solution.

Based on the present results, aggregates of paraben molecules may be considered to be bound loosely owing to hydrophobic interactions between molecules, and the association of the molecules may be of the non-micellar type.

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