*Food Chemistry* **50** (1994) 9–11

# Distribution of benzoic acid in an emulsion

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(Received 21 April 1993; accepted 4 May 1993)

The affinity of benzoic acid for the oil phase of a sunflower oil-in-water emulsion stabilised by whey protein is significantly greater than can be accounted for by oil–water partitioning alone. It is suggested that benzoic acid binds to the protein adsorbed at the interface. The binding isotherm is obtained up to 6 mM benzoic acid in the aqueous phase, when 0.28 mg of the preservative is bound per square metre of interface.

## INTRODUCTION

The antimicrobial effect of benzoic acid in foods depends on it being significantly soluble in both water and non-aqueous phases (e.g. oil) (Ecklund, 1989). Thus, the preservative is available in the aqueous phase where microorganisms exist, yet the undissociated acid is sufficiently non-polar to traverse microbial cell membranes by passive transport. When added to a mixture of oil and water, benzoic acid tends to partition between the two phases. Such partitioning is well characterised for bulk two phase systems (Wedzicha, 1988), but the behaviour of solutes in dispersed oil–water systems has received little attention despite its importance in relation to foods. Here we describe the oil–water partitioning behaviour of benzoic acid in a whey protein-stabilised sunflower oil-in-water emulsion.

## MATERIALS AND METHODS

### Bulk oil–water experiments

Equal volumes of sunflower oil containing benzoic acid (10–100 mM) and acidified water (15 mM HCl, pH 2.5±0.1) were allowed to reach equilibrium in a water bath at 25.00±0.05°C. The benzoic acid concentration in the aqueous phase was measured at 270 nm after diluting in HCl solution (0.1 M) and the concentration of benzoic acid remaining in the oil was obtained by difference.

### Emulsion experiments

Emulsions of 20% (v/v) sunflower oil (containing 12.5–300 mM benzoic acid) in citrate buffer (6 mM, pH 2.5) containing whey protein (1% w/v, whey protein 4034, batch PSR 04199, New Zealand Dairy Board, Wellington) were prepared using a laboratory homogeniser (model S500, Shields Instruments, York). Droplet size distribution, and the total droplet surface area per ml of oil, in the emulsions, were determined using the Mastersizer (Malvern Instruments, Malvern).

Emulsions (25 ml) were placed in Visking dialysis tubing which was immersed in aqueous citrate buffer (175 ml, 6 mM, pH 2.5). The system was allowed (20 h) to come to equilibrium at 25.00±0.05°C with constant stirring and the benzoic acid concentration in the aqueous phase was determined at 270 nm after diluting in HCl (0.1 M). The concentration of benzoic acid associated with the oil fraction of the emulsions was obtained by difference.

The oil content of emulsions was determined by measuring their density, by weighing in specific gravity bottles (100 ml) at 25.00°C. Possible interactions between benzoic acid and the Visking tubing or whey protein were investigated by placing water (25 ml) or whey protein solution (25 ml, 1% w/v) in the tubing and dialysing against a benzoic acid solution (4.8 mM, 150 ml) in citrate buffer (pH 2.5, 5 mM). The concentration of benzoic acid in the solution outside the dialysis tubing was measured as before.

## RESULTS AND DISCUSSION

The distribution of a solute $i$ between an aqueous and an oil phase is expressed in terms of the partition coefficient $P$,

$$P = a_i^{oil}/a_i^{aq}$$

where $a$ denotes the activity of the solute in each phase (Wedzicha, 1988). When given in terms of concentration, the expression for $P$ may be used to determine the way in which the relative activity of the solute in each phase changes with composition. In general, carboxylic acids are much more soluble than their salts in organic

*Food Chemistry* 0308-8146/94/$07.00 

phases (Rekker, 1977), and it is normal to neglect the distribution of the anions in oil–water systems. The pH chosen for the present investigation, pH 2.5, ensured that most (98%) of the benzoic acid was in its undissociated form ($pK_a$ 4.18) to obtain the greatest extent of partitioning between oil and water, and to avoid as far as possible any electrostatic interactions between the anion and positively charged groups on the protein used to stabilise emulsions. The pH chosen also represents the lowest value that might be expected of a food emulsion. It was not intended that the model should refer to any specific food product but that it represent a means of demonstrating the behaviour of a food preservative in a dispersed system under practical conditions.

It was decided to use whey protein to emulsify and stabilise the oil phase in preference to low-molecular weight surfactants such as Tween or SDS. It is known that such micelle-forming surfactants tend to bind benzoic acid strongly (Wedzicha *et al.*, 1991). On the other hand, preliminary experiments involving the dialysis of whey protein against benzoic acid solution showed that there was no detectable interaction between the two at pH 2.5 and at the concentrations found in the aqueous phase of the emulsion experiments reported here. A 1% (w/v) solution of whey protein was sufficient to give a stable emulsion containing 20% (v/v) sunflower oil; this concentration of oil was chosen to provide a sufficiently concentrated emulsion to ensure that significant partitioning of benzoic acid between the two phases would be observed.

Measurement of the density of emulsions and of the oil phase gave the oil content of all emulsions prepared as 20±0.25%; individual compositions were used to calculate partition coefficients. Separate experiments showed that the concentration of benzoic acid in the dialysate, when emulsions were dialysed against citrate buffer, reached equilibrium within 20 h. Also, the dialysis tubing did not bind benzoic acid at the concentrations found in the present experiments. The weighted mean diameter $d(3,2)$ of droplets in 15 emulsion preparations was in the range 0.53–0.73 $\mu$m. Droplet surface areas were in the range 9.9–13.3 $m^2$ per ml oil.

The measured partition coefficient is referred to here as the apparent partition coefficient, $P_{app}$, because it is obtained using the total analytical concentration of benzoic acid species in the two phases. This includes any dimers which may be formed in the oil phase (Rubio *et al.*, 1986).

Figure 1 shows the effect of benzoic acid concentration in the aqueous phase on $P_{app}$ measured for emulsions and in experiments where bulk oil and aqueous phases were used. The results from emulsion experiments are collected together here regardless of the droplet size parameters of the emulsions. The value of $P_{app}$ increases as the concentration of benzoic acid in the system is increased. In the absence of whey protein, the increase is simply due to solute–solute interactions in the oil phase leading to dimerisation of the acid. The true partition coefficient and the dimerisation constant

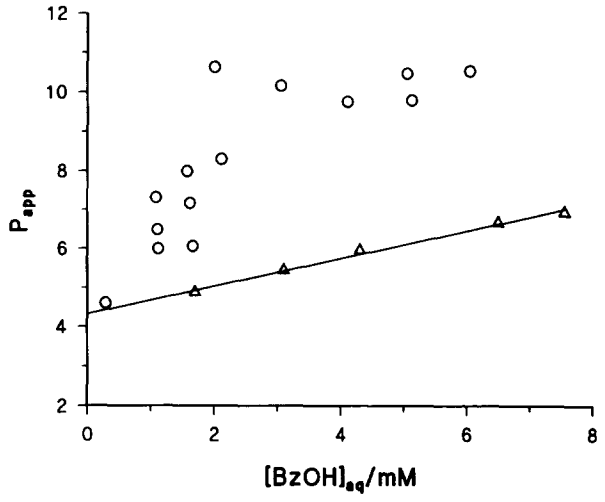


**Fig. 1.** Effect of benzoic acid concentration in the aqueous phase, $[BzOH]_{aq}$, on the apparent partition coefficient, $P_{app}$, of benzoic acid between sunflower oil and water when measured for bulk oil and water phases Δ, and when the oil was emulsified with whey protein ○. All measurements were at 25°C. Emulsions contained aqueous citrate buffer (6 mM, pH 2.5), whey protein (1% w/v) and oil (20% v/v). Bulk phase experiments were in 15 mM HCl, pH 2.5.

for benzoic acid in oil, $K_d$, are related to $P_{app}$ and benzoic acid concentration in the aqueous phase, $[BzOH]_{aq}$, by (Rubio *et al.*, 1986),

$$P_{app}=P+2P^2K_d[BzOH]_{aq}$$

The data in Fig. 1 give $P$ = 4.3, which is close to that ($P$ = 5.3) reported by Garrett and Woods (1953) for benzoic acid in peanut oil and water and $K_d$ = 9.82 $mol^{-1}$ litre.

It is seen that the oil phase of emulsions has a greater affinity for benzoic acid than expected from bulk-phase experiments. It is expected that benzoic acid would be much less surface-active than the whey protein and any benzoic acid which might be adsorbed at the oil–water interface should, therefore, be displaced by the protein. The oil droplets are very large on the scale of molecular size and it is unlikely that the activity coefficient of benzoic acid in these microscopic environments would be significantly different from that in bulk oil, at the same concentration. The activity coefficients of non-ionic solutes which do not take part in specific solute–solute or solute–solvent interactions are independent of concentration to at least several per cent (mol/mol) (Hildebrand & Scott, 1964). This would be true of benzoic acid in the aqueous phase, and the activity coefficient of benzoic acid is likely to remain constant throughout the set of experiments reported. The only suggestion for the excess of benzoic acid associated with the oil droplets is that it is bound to the adsorbed interfacial layer.

If all the excess benzoic acid were located at the interface, the surface excess could be calculated by estimating the theoretical amount of benzoic acid present in the oil phase as a result of oil–water partitioning, using the values of $P$ and $K_d$, and subtracting this from the measured amount associated with the oil phase. This excess is expressed in terms of interfacial area

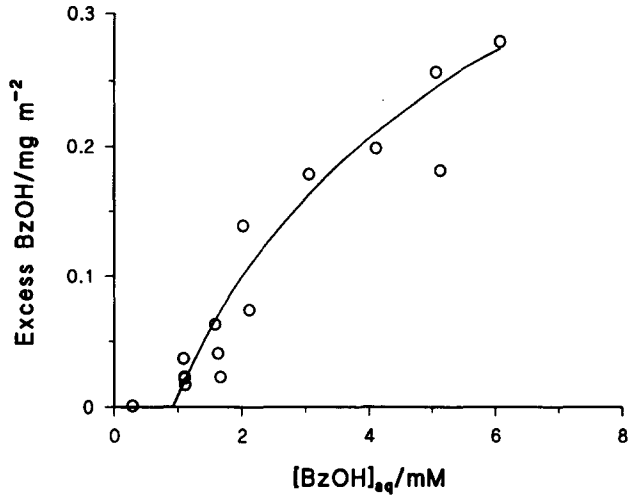


**Fig. 2.** Effect of benzoic acid concentration in the aqueous phase, $[BzOH]_{aq}$, on the excess benzoic acid associated with the oil phase of whey protein-stabilised sunflower oil-in-water emulsions, expressed in terms of the area of the oil–water interface. Data on benzoic acid partitioning used to calculate the excess are those given in Fig. 1.

in Fig. 2 and it is seen that the maximum amount of benzoic acid associated with the interface reaches 0.28 mg $m^{-2}$ when the equilibrium concentration in the aqueous phase is 6 mM. The graph shows only slight curvature and the capacity of the emulsion in question for benzoic acid is, therefore, somewhat greater than 0.28 mg $m^{-2}$.

Any explanations for the apparent tendency of benzoic acid to bind to the interfacial layer can only be speculative. One can rule out ionic interactions between the preservative and charged groups on the protein because, at pH 2.5, benzoic acid is essentially unionised and no interaction between whey protein and benzoic acid could be identified in the absence of the oil phase. It is suggested, therefore, that the observed behaviour is the result of hydrophobic interactions between benzoic acid and structures created when the protein is adsorbed at an oil–water interface. In some respects, these interactions may resemble those which are responsible for the binding of benzoic acid to micelle-forming surfactants (Wedzicha *et al.*, 1991). It is, however, not possible to speculate whether the benzoic acid bound in the emulsion is within the oil or aqueous phases.

This paper is the first report of the possible binding of a food preservative molecule to an adsorbed protein at an oil–water interface. Consider an emulsion with an equilibrium benzoic acid concentration in the aqueous phase of 3 mM. This arises when the total benzoic acid content is about 1 g per litre of emulsion. Thus, given a typical interfacial area of 10 $m^2$ per ml oil, 1 litre of the emulsion containing 20% (v/v) oil has a surface area of 2000 $m^2$ and will bind 2000 × 0.17 = 340 mg benzoic acid. Under these conditions, one-third of the preservative is found to be associated with the binding sites on the emulsion droplets.

It is reasonable to suggest that similar behaviour could be observed when other carboxylic acid preservatives (e.g. sorbic acid) and esters (e.g. *p*-hydroxybenzoates) are used in emulsified foods. Such binding tends to reduce the activity of the preservative in the aqueous phase but this behaviour should also be examined in relation to possible effects on chemical reactivity in appropriate situations: for example, when sorbic acid is the food preservative. It is established that some micelle-forming surfactants (Wedzicha & Zeb, 1990) and bovine serum albumin (Wedzicha & Zeb, 1991; Wedzicha & Picard, 1993) have a marked effect on the reactivity of sorbic acid towards thiols. The nitrosation of secondary amines is similarly enhanced by micelle-forming surfactants and dispersed cell fragments (Okun & Archer, 1977). This behaviour has been explained in terms of the association of the reactants with hydrophobic and hydrophilic sites on the surfactant or protein. Similar behaviour may be possible within the adsorbed protein layer at the oil–water interface.

## ACKNOWLEDGEMENTS

We are grateful to the Agricultural and Food Research Council for a Research Fellowship to one of us (S.A.) and to École Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, Dijon, for an appointment as Professeur Visité to B.L.W.

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