J. Pharm. Pharmac., 1966, **18**, *Suppl.*, 98S–102S Received April 27, 1966

The evaluation of a molecular sieve technique to determine the interaction between a preservative and a surfactant

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The interaction between methyl *p*-hydroxybenzoate and polysorbate 80 has been determined using a molecular sieve technique. At 25° and at surfactant concentrations of 2·86 and 1·43% respectively, the ratios of total to free preservative were 2·9 and 1·9. These results are comparable with published data.

THE use of non-ionic surfactants in pharmaceutical and cosmetic formulations has increased the problem of preserving such preparations against microbial attack. Wedderburn (1964) has reviewed the subject and emphasised the significance of the interaction which can occur between the non-ionic surfactant and the preservative. Although there may be no obvious incompatibility, the thermodynamic activity of the preservative, which for dilute solutions can be equated with its concentration in true aqueous solution, may be reduced. Such a reduction in effective concentration can be studied by microbiological methods and also by physico-chemical techniques (for references see Mitchell & Brown, 1966). The results obtained by physical methods compare well with those obtained microbiologically (Pisano & Kostenbauder, 1959).

In recent years, size separation with molecular sieves has become widely used and we have used this phenomenon to study the degree of binding between a surfactant and a preservative. To evaluate the technique we re-examined the interaction between methyl *p*-hydroxybenzoate and polysorbate 80 and compared the results obtained with those available in the literature and also with the degree of interaction as revealed by a solubility study. Determination of the degree of interaction by molecular sieving with a gel involved three stages: measurement of the external volume of the gel, evaluation of the extent of adsorption of the methyl *p*-hydroxybenzoate on the gel matrix, and determination of the amount of interaction of the preservative with polysorbate 80. An experiment was also made to demonstrate that polysorbate 80 did not penetrate the gel.

Experimental

The material used was Sephadex G-25 fine grade (Pharmacia) which has a nominal water regain of 2·5 g water per g of dry gel. To prepare the swollen gel, 4 g of the dry gel was added to water (15 ml) and the gel was allowed to swell (2 hr). The relevant solutions were then added plus any additional water so that the total volume of liquid added to the gel was always 25 ml. The system was then equilibrated by shaking

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(1 hr) at 25° ± 0·1° (this was shown to be sufficient in preliminary experiments).

Binding of methyl p-*hydroxybenzoate to polysorbate* 80 *in the presence of Sephadex.* Aqueous solutions (5 ml) of known but varying concentrations of methyl *p*-hydroxybenzoate (Nipa) and 5 ml of the appropriate polysorbate (Tween) 80 (Honeywill-Atlas) solution were added to the swollen gel. After equilibration, the systems were allowed to stand at 25° for about 5 min to allow the gel to settle. About 4 ml of the supernatant liquid was pipetted into a tube and centrifuged to deposit any small particles of gel (taking supernatant liquid only has the advantage that it is not necessary to centrifuge at a controlled temperature). The supernatant liquid in the centrifuge tube was then analysed for methyl *p*-hydroxybenzoate by measuring the absorbance at 256 mμ. A correction for the absorbance due to the polysorbate 80 was obtained from a calibration graph.

The adsorption of methyl *p*-hydroxybenzoate to Sephadex was measured as above with the polysorbate 80 solution replaced by water (5 ml).

Determination of the external volume was made using the method described above but with a solution of Blue Dextran (Pharmacia) of average molecular weight 2,000,000 instead of methyl *p*-hydroxybenzoate. The concentration of this in the supernatant was found by measuring the absorbance at 620 mμ.

The degree of binding of methyl *p*-hydroxybenzoate to polysorbate 80 was also assessed by the solubility method of Patel & Kostenbauder (1958) at a temperature of 25° ± 0·1°.

The amount of polysorbate 80 in the Sephadex internal phase was found by equilibrating the swollen gel with solutions of known but varying concentrations of polysorbate 80. After 1 hr, the whole of the external phase was removed by filtering under gentle vacuum. The gel was then well washed with water to remove any surfactant and the washings analysed for polysorbate 80 content by the second method of Stevenson (1954).

Results and discussion

The grade of Sephadex used has a nominal water uptake of 2·5 ± 0·2 g per g of dry gel. This enables the volume of the external phase to be calculated as 14·2 to 15·8 ml. However, as the answer for the degree of interaction between the preservative and the surfactant is dependent on this value it has been determined with greater precision. The mean of nine determinations using Blue Dextran was 15·8 ml.

Gelotte (1960) showed that most aromatic compounds were adsorbed, reversibly, on Sephadex and that a linear adsorption isotherm was obtained. We therefore anticipated that methyl *p*-hydroxybenzoate would also be adsorbed and Fig. 1 is a plot of the concentration of methyl *p*-hydroxybenzoate in the external phase against the weight of the compound associated with the gel. This latter is the sum of that which is in solution in the internal phase and the quantity actually adsorbed on the gel matrix. It is neither necessary to distinguish between these, nor

necessary to know the volume of the internal phase since the quantity of methyl *p*-hydroxybenzoate adsorbed is dependent only on its concentration in the internal phase. This latter must be equal to the external concentration and thus the quantity of methyl *p*-hydroxybenzoate associated with the gel is dependent on the concentration of the compound in the external phase.

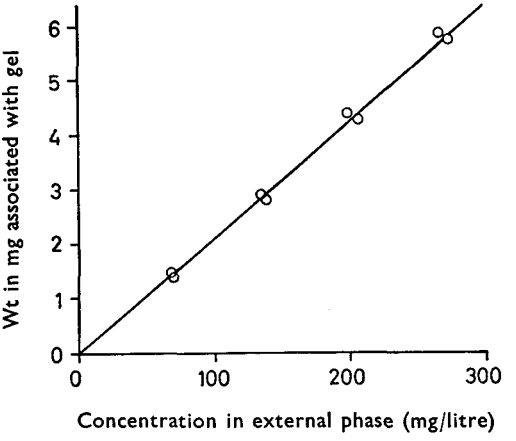

FIG. 1. Relationship between the concentration in the external phase and the weight associated with the gel (adsorbed and in solution) of methyl *p*-hydroxybenzoate at 25°.

In the presence of polysorbate 80, determination of the concentration of methyl *p*-hydroxybenzoate free and bound in the external phase enables the quantity of preservative associated with the gel to be determined, as the total amount of preservative added to the system is known. Fig. 1 gives the concentration of free methyl *p*-hydroxybenzoate in the external phase and thus the ratio of total preservative to free preservative can be calculated. These ratios for the two polysorbate concentrations used are in Table 1 and are plotted in Fig. 2. The results for the solubility studies are given in Fig. 3. Here, the concentration of free preservative is taken as its maximum water solubility under the conditions used.

TABLE 1. BINDING OF METHYL *p*-HYDROXYBENZOATE TO POLYSORBATE 80

Total methyl *p*-hydroxybenzoate/Free methyl *p*-hydroxybenzoate

Method	Molecular Sieve[1]	Solubility	Solubility[3]	Solubility[4]	Dialysis[2,4]
Temperature	25°	25°	27°	27°	30°
Polysorbate 80 conc. 2·85 %	2·86; 2·89 2·88; 2·96	3·10	2·8	2·8	3·0
1·43 %	1·94; 2·14 1·93; 1·97	2·05	1·9	1·8	2·0

1. Concentration range of free preservative was 75 to 190 mg/litre.
2. Concentration range of free preservative was 300 to 630 mg/litre.
3. Blaug & Ahsan (1960).
4. Patel & Kostenbauder (1958).

All the results obtained are given in Table 1 and are compared with those reported by previous workers. Apart from one slightly high value for each polysorbate concentration, the values given by the molecular sieve technique agree well with each other and with those obtained with other techniques by earlier workers.

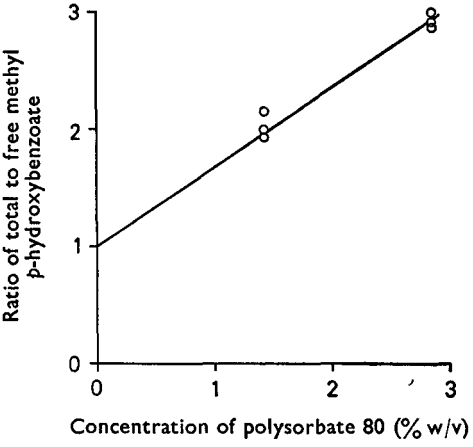

FIG. 2. A plot showing the ratio of total to free methyl *p*-hydroxybenzoate, at 25°, in aqueous solutions containing varying concentrations of polysorbate 80 as determined by a molecular sieve technique.

Although monomeric polysorbate 80 with a molecular weight of about 1300 can penetrate the gel, we considered it unlikely that much would do so in the time used for the experiment (1 hr). Experimental work

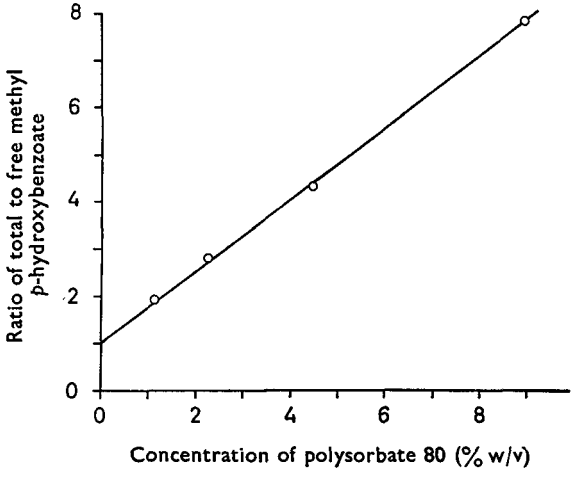

FIG. 3. A plot showing the ratio of total to free methyl *p*-hydroxybenzoate, at 25°, in aqueous solutions containing varying concentrations of polysorbate 80, as determined by a solubility method.

confirmed that for the highest surfactant concentration in the external phase (5·0%), the concentration in the gel was only 0·009%. It is probably less than this because it is not easy to achieve complete separation of the external phase.

Compared with conventional equilibrium dialysis, the use of a gel instead of a semi-permeable membrane has the advantage of rapid equilibration and ready availability of suitable materials of various pore sizes. Errors due to leaching of constituents from the membrane are avoided. Its main disadvantage is that the concentration of preservative is determined in one phase only. Consequently, any error in this determination gives an error in the quantity associated with the gel. Such errors are minimised if the volume of the external phase is chosen so that the quantity of compound in this phase is approximately equal to the quantity associated with the gel.

The method is obviously not restricted to studies of the interaction of preservatives with surfactants. In addition it could probably be used to study drug-protein binding. For this latter, it would seem to offer advantages over the column techniques used with Sephadex by Hardy & Mansford (1962) and Barlow, Firemark & Roth (1962).

Acknowledgement. We thank Mr. A. G. Fishburn for his helpful comments during the preparation of the manuscript and Mr. S. W. Thomas for the surfactant assays.

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