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# The effect of co-solvents on the antibacterial activity of paraben preservatives

Rula M. Darwish [1], Sally F. Bloomfield *

*Department of Pharmacy, Kings College London, University of London, London SW3 6LX, UK*

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**Abstract**

The solubility of methyl and propyl *p*-hydroxybenzoate esters (parabens) in 3 M concentrations of the co-solvents ethanol, propylene glycol and glycerol was investigated. The co-solvents at 3 M concentrations increased the solubility of parabens. The increased solubility of parabens in the co-solvents over their aqueous solubility was associated with increased antibacterial activity. The extent of this effect was different for different co-solvents, and increased with increasing hydrophobicity of the co-solvent. Two potentially useful paraben/co-solvent combinations were identified and their activity compared with two commercially used paraben combinations. Further studies indicate that addition of co-solvents to oil/water systems is associated with reduced partitioning of the parabens into the oil phase which may be associated with increased preservative activity within an emulsion formulation.

*Keywords:* Paraben; Co-solvent; Solubility; Preservation; Hydrophilic/hydrophobic balance

## 1. Introduction

The antimicrobial activity of many of the preservative types used in pharmaceuticals depends on their ability to move freely in the aqueous phase, and yet be lipophilic enough to partition through the microbial outer cell envelope (where present) and the protoplasmic membrane to reach their site/s of action. Hansch et al. (1972) demonstrated the effects of the hydrophilic/hydrophobic balance on the antibacterial activity of the esters of *p*-hydroxybenzoic acid (parabens). Although these and other studies (Eklund, 1980; El-Falaha et al., 1983; Russell and Gould, 1988) show that the activity of parabens increases with increasing ester chain length, in practice the usefulness of the higher homologues as preservatives is limited by their decreasing water solubility which in turn limits their bioavailability within the system. One approach to this problem has been to use combinations of paraben esters in which the components of the combination are used at saturation levels, and the overall effect is the combined effect of the components. Another approach is to use solubilizing agents, co-solvents, etc, to facilitate increased aqueous solubility of the preservatives.

* Corresponding author.

[1] Present address: Faculty of Pharmacy, University of Jordan, Amman, Jordan.


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Reports in the literature (Data information sheet, Nipa Laboratories Ltd, Pontypridd; Yalkowsky, 1981; Anon, 1989) show that parabens are freely soluble in a range of co-solvents. Investigations described in this paper were carried out to determine the effects of ethanol (EtOH), propylene glycol (PG) and glycerol (GLY) co-solvents on the solubility and preservative efficacy of the methyl and propyl parabens (MHB, PHB). A co-solvent concentration of 3 M was chosen because it represents the concentration normally used in pharmaceutical products. The object of this study was to identify systems which might be used to achieve more effective preservation of pharmaceuticals as compared with paraben preservative systems currently used.

## 2. Materials and methods

### 2.1. Bacterial test suspensions

*Staphylococcus aureus* NCTC 10788 and *Pseudomonas aeruginosa* NCTC 06750 were grown overnight at 37°C on nutrient agar containing (g $l^{-1}$) Lab-lemco, 1; yeast extract, 2.0; peptone 5.0; NaCl, 5.0; agar no. 3, 15.0; pH 7.4. Organisms were harvested, washed twice with peptone water, containing (g $l^{-1}$) peptone, 1; NaCl, 5; pH 7.2, and diluted to contain the required number of colony forming units/ml (cfu $ml^{-1}$). All media bases were obtained from Oxoid Ltd, Basingstoke.

### 2.2. Inactivation of the antimicrobial agent

The effect of the antimicrobial agents was inactivated either by diluting 1:100 with 1/4 strength Ringer's solution or neutralized peptone water containing (g $l^{-1}$) peptone, 1; NaCl 8.9; lecithin (BDH Chemicals Ltd, Dorset), 3.0; and Tween (BDH Chemicals Ltd, Dorset), 30.0. Preliminary experiments showed that addition of aliquots of 1 ml of the reaction mixtures to 99 ml of 1/4 strength Ringer's solution or neutralised peptone water was sufficient to neutralise the effect of the antimicrobial agent.

### 2.3. Preparation of test solutions

Solutions of co-solvents were prepared by adding weighed quantities of ethanol (Hayman Ltd, Essex) propylene glycol (Fisons plc, Loughborough) and glycerol BP (MacCarthy Ltd, Romford) to sterile distilled water. To prepare solutions of parabens, weighed quantities of methyl *p*-hydroxybenzoate (Sigma Chemical Co. Ltd, Dorset) and propyl *p*-hydroxybenzoate (BDH Chemicals Ltd, Dorset) to give 0.18, 0.25 and 0.36% of MHB and 0.04, 0.06 and 0.1% of PHB, respectively, in the final test mixture were added to sterile distilled water or co-solvent solution and dissolved using sonication and/or gentle heat when necessary. Quantities of Nipasept and Nipastat (Nipa Laboratory Ltd, Pontypridd) accurately weighed to give 0.1% concentration in the test mixtures, were dissolved in sterile distilled water. A series of supersaturated solutions of parabens were prepared by dissolving weighed amounts of parabens in a minimum amount of ethanol to give concentrations of up to 0.36% of MHB and 0.1% of PHB in the test mixture. Sterile distilled water was then added to produce the required concentration of parabens in the final test mixture. It was noticed that finely divided flakes of the solute precipitated when sterile distilled water was added.

### 2.4. Solubility determination

Identical quantities of MHB and PHB well in excess of their solubility were added to solutions of identical volumes of water and 3 M concentrations of the co-solvents. The flasks were closed and equilibrated in a shaking water bath at 25°C for 24 h. Solutions were filtered and analysed for paraben esters using a UV spectrophotometer (Cecil Instruments UV Spectrophotometer, Model CE 2092) at $\lambda_{max}$ 255 nm.

### 2.5. Evaluation of bactericidal activity

Test solutions and test suspensions were placed in a water bath at 25°C to equilibrate. At zero time the bacterial test suspension was added to

each test solution to give an inoculum size of $10^5$ to $10^8$ cfu $ml^{-1}$ in the test mixture. Samples were withdrawn at predetermined time intervals, neutralised by dilution, and viable counts estimated by plating (in duplicate) 50 $\mu$l aliquots onto nutrient agar plates using a Model D spiral plater (Don Whitley, Shipley). All plates were incubated at 37°C for 24 h. Colonies were counted using the laser counter (Model 500 Bacterial Colony Counter, Don Whitley, Shipley). The microbicidal effect (ME value) was calculated by subtracting the log number of cfu $ml^{-1}$ at the sample time, from $N$, the log number of cfu $ml^{-1}$ at time zero. Where samples gave no detectable survivors (NDS) the actual number of survivors in the final dilution is $< 2 \times 10^3$ cfu $ml^{-1}$ (i.e., less than 1 cfu in 0.05 ml). In this case the log reduction was expressed as $> \log N - \log 2 \times 10^3$ cfu $ml^{-1}$.

### 2.6. Effect of the co-solvent solutions on the partition coefficient of methyl and propyl parabens

The lipid phase was 1-octanol (Aldrich Chemical Co. Ltd, Dorset). Accurately weighed quantities of MHB and PHB were dissolved in measured volumes of 1-octanol in specimen tubes by sonication. The same volumes of water or co-solvent solutions (3 M) were then added. The tubes were stoppered and inverted 20 times/min for 48 h at room temperature. The mixtures were then centrifuged and allowed to settle. The aqueous layer was removed and the concentration of parabens was determined by spectrophotometric analysis at 255 nm from which the partition coefficient was calculated as the ratio of the final concentration of parabens in the oil phase to that in the aqueous phase.

### 2.7. Bactericidal activity of co-solvents, parabens, and combinations of co-solvents and parabens in macrogol cream

Samples of preserved creams were prepared by dissolving accurately weighed amounts of MHB and PHB in melted cetomacrogol emulsifying ointment (BP). Quantities of water or co-solvent solutions were added to give 3 M concentrations in the final cream. The mixtures were stirred continually while cooling until hardened. The creams were left to equilibrate at 25°C for 24 h to ensure partitioning of parabens between the aqueous and oily phase. Samples of cream were then inoculated with suspensions of *S. aureus* to give an inoculum size of $10^6$–$10^8$ cfu $ml^{-1}$ of cream. Samples of unpreserved cream were also prepared as above using cetomacrogol emulsifying ointment and sterile distilled water. Samples were removed at 0, 2, 4 h and added to neutralized peptone water in sterile stomacher bags. The contents of the stomacher bags were homogenized using a stomacher Lab Blender 80 (Model No. BA6020, Seward Medical, London), for 30 s and viable counts determined as described above.

## 3. Results and discussion

The results of this study show that pharmaceutical co-solvents can be used to increase the aqueous solubility of paraben preservatives, but the extent of this effect is different for different co-solvents. The results in Table 1 indicate that the least polar co-solvent of those under study, EtOH at 3 M, was the most effective giving a solubility of 0.45 and 0.09% for MHB and PHB, respec-

Table 1
Solubility of methyl and propyl *p*-hydroxybenzoate in water and aqueous solutions of co-solvents

| Co-solvent | Solubility g% MHB replicate results | | Solubility g% PHB replicate results | |
|---|---|---|---|---|
| | 1 | 2 | 1 | 2 |
| Water | 0.22 | 0.21 | 0.025 | 0.03 |
| Ethanol (3 M) | 0.42 | 0.44 | 0.083 | 0.09 |
| Propylene glycerol (3 M) | 0.42 | 0.42 | 0.072 | 0.075 |
| Glycerol (3 M) | 0.13 | 0.12 | 0.029 | 0.035 |

Table 2
Log reduction in viable counts of *Staphylococcus aureus* in aqueous solutions of ethanol (3 M), propylene glycol (3 M), glycerol (3 M), methyl *p*-hydroxybenzoate (0.18%) and in combinations of 3 M concentrations of the co-solvents with methyl *p*-hydroxybenzoate (0.18, 0.25 and 0.36%)

| Co-solvents (3 M) | Time (min) | | Log viable count at time zero (N) and log reduction in viable count (ME) for | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Co-solvent alone replicate results | | MHB 0.18% alone replicate results | | Co-solvent +0.18% MHB replicate results | | Co-solvent +0.25% MHB replicate results | | Co-solvent +0.36% MHB replicate results | |
| | | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Ethanol | 0 | N | 6.36 | 6.39 | 6.18 | 5.46 | 6.37 | 6.35 | 6.33 | 6.36 | – | – |
| | 30 | ME | 0.46 | 0.31 | – | – | 0.32 | 0.50 | 0.69 | 0.86 | | |
| | 60 | ME | 1.20 | 1.27 | – | – | 0.96 | 1.20 | 2.32 | 2.40 | | |
| | 120 | ME | > 3.06 | > 3.09 | 0.20 | 0.14 | > 3.07 | > 3.05 | > 3.03 | > 3.06 | | |
| | 240 | ME | > 3.06 | > 3.09 | 0.82 | 0.60 | > 3.07 | > 3.05 | > 3.03 | > 3.06 | | |
| Propylene glycol | 0 | N | 6.37 | 6.40 | | | 6.35 | 6.37 | 6.28 | 6.32 | 6.39 | 6.44 |
| | 30 | ME | 0.10 | 0.09 | | | 0.04 | 0.10 | 0.02 | 0.04 | 0.47 | 0.33 |
| | 60 | ME | 0.11 | 0.12 | | | 0.11 | 0.25 | 0.17 | 0.20 | 1.40 | 0.65 |
| | 120 | ME | 0.15 | 0.20 | | | 0.34 | 0.58 | 0.88 | 0.63 | > 3.09 | > 3.14 |
| | 240 | ME | 0.18 | 0.30 | | | 1.20 | 1.51 | 2.58 | 2.62 | > 3.09 | > 3.14 |
| Glycerol | 0 | N | 6.33 | 6.42 | | | 6.41 | 6.44 | 6.34 | 6.35 | 6.27 | 6.24 |
| | 30 | ME | 0.08 | 0.09 | | | 0.12 | 0.01 | 0.03 | 0.05 | 0.01 | 0.01 |
| | 60 | ME | 0.10 | 0.05 | | | 0.15 | 0.15 | 0.08 | 0.05 | 0.15 | 0.27 |
| | 120 | ME | 0.12 | 0.02 | | | 0.19 | 0.20 | 0.20 | 0.06 | 0.21 | 0.40 |
| | 240 | ME | 0.30 | 0.10 | | | 0.16 | 0.21 | 0.44 | 0.39 | 0.47 | 1.00 |

Table 3
Log reduction in viable counts of *Staphylococcus aureus*, in aqueous solutions of ethanol (3 M), propylene glycol (3 M), glycerol (3 M), 0.04% propyl *p*-hydroxybenzoate (PHB) and combinations 3 M concentrations of the co-solvents with propyl *p*-hydroxybenzoate (0.04, 0.06 and 0.1%)

| Co-solvents (3 M) | Time (min) | | Log viable count at time zero (N) and log reduction in viable count (ME) for | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Co-solvent alone replicate results | | PHB 0.04% alone replicate results | | Co-solvent + 0.04% PHB replicate results | | Co-solvent + 0.06% PHB replicate results | | Co-solvent + 0.1% PHB replicate results | |
| | | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Ethanol | 0 | N | 6.36 | 6.39 | 5.63 | 5.77 | 6.29 | 6.50 | 6.26 | 6.42 | 6.43 | 6.25 |
| | 30 | ME | 0.46 | 0.31 | – | – | 0.27 | 0.20 | 1.38 | 1.22 | > 3.13 | > 2.95 |
| | 60 | ME | 1.20 | 1.27 | – | – | 1.31 | 1.11 | > 2.9 | > 3.12 | > 3.13 | > 2.95 |
| | 90 | ME | > 3.06 | > 3.09 | 0.05 | 0.10 | 2.58 | 2.42 | > 2.96 | > 3.12 | > 3.13 | > 2.95 |
| | 120 | ME | > 3.06 | > 3.09 | 0.20 | 0.30 | > 2.99 | > 3.20 | > 2.96 | > 3.12 | > 3.13 | > 2.95 |
| Propylene glycol | 0 | N | 6.37 | 6.40 | | | 6.40 | 6.05 | 6.54 | 6.35 | 6.47 | 6.27 |
| | 30 | ME | 0.01 | 0.09 | | | 0.01 | 0.03 | 0.09 | 0.12 | 0.30 | 0.50 |
| | 60 | ME | 0.11 | 0.12 | | | 0.03 | 0.05 | 0.22 | 0.39 | > 3.17 | > 2.97 |
| | 120 | ME | 0.15 | 0.20 | | | 0.37 | 0.44 | 0.90 | 0.87 | > 3.17 | > 2.97 |
| | 240 | ME | 0.18 | 0.30 | | | 2.00 | 2.30 | 2.83 | 3.05 | > 3.17 | > 2.97 |
| Glycerol | 0 | N | 6.33 | 6.42 | | | 6.46 | 6.29 | 6.42 | 6.16 | > 3.17 | |
| | 30 | ME | 0.08 | 0.09 | | | 0.02 | 0.05 | 0.05 | 0.08 | | |
| | 60 | ME | 0.10 | 0.05 | | | 0.04 | 0.07 | 0.07 | 0.11 | | |
| | 120 | ME | 0.12 | 0.02 | | | 0.07 | 0.12 | 0.10 | 0.19 | | |
| | 240 | ME | 0.30 | 0.10 | | | 0.20 | 0.32 | 0.38 | 0.45 | | |

tively. By contrast, the most polar co-solvent glycerol (3 M) was the least effective giving solubilities of 0.13 and 0.035% for MHB and PHB, respectively. Studies by Yalkowsky (1981) indicate that the solubility of a drug depends on its polarity with respect to the co-solvent, the best solvent for a particular solute being the one which matches its polarity. EtOH is less polar than PG, which is less polar than GLY. Parabens are non-polar solutes, therefore, they are more soluble in EtOH than PG, and more soluble in PG than GLY.

Although the solubility values given in Table 1, which were obtained using accepted methods, are in agreement with literature values quoted by Yalkowsky (1981) it was found that, by using heat or sonication, solutions of higher concentrations could be prepared which were stable over at least 48 h. Using solutions prepared in this way it was possible to compare the effects of the three co-solvents on the activity of MHB and PHB at concentrations up to 0.36 and 0.1%, respectively.

The results in Tables 2–4 show that aqueous solutions of MHB and PHB at saturation concentrations of 0.36 and 0.04%, respectively, had little or no activity against *S. aureus* over 4 h, but PHB 0.04% in aqueous solution had some effect on *Ps. aeruginosa* producing a 2–3 log reduction in 2–4 h. The co-solvent solutions PG and GLY at 3 M concentration had little or no activity against both *S. aureus* and *Ps. aeruginosa* over 4 h but 3 M EtOH produced total kill, i.e., no detectable survivors, for both test organisms within 2 h. Further studies (results not shown) indicate that PG produced total kill of both test organisms at 24 h, but GLY had little effect.

The results in Tables 2–4 demonstrate that the bactericidal activity of both MHB and PHB in co-solvent solution increased as the paraben concentration was increased above the aqueous solubility. The increase in activity over and above that produced by the co-solvent solution alone was however markedly different for the different co-solvents. Results in Tables 2 and 3 show that, for *S. aureus*, the increase in ME values for combinations of MHB and PHB with co-solvents was greater for EtOH compared with PG which in turn was greater than for GLY; saturated solutions of 0.1% PHB and 0.36% MHB in 3 M PG produced total kill within 1 and 2 h, respectively, whilst a combination of 0.1% PHB with EtOH produced no detectable survivors after 30 min.

Table 4 shows that the ME values for combinations of PHB/co-solvents against *Ps. aerugi-*

Table 4
Log reduction in viable counts of *Pseudomonas aeruginosa*, in aqueous solutions of ethanol (3 M), propylene glycol (3 M), glycerol (3 M), 0.04% propyl *p*-hydroxybenzoate and combinations of 3 M concentrations of the co-solvents with propyl *p*-hydroxybenzoate (0.04, 0.06 and 0.1%)

| Co-solvents (3 M) | Time (min) | | Log viable count at time zero (N) and log reduction in viable count (ME) for | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Co-solvent alone replicate | | MHB 0.18% alone replicate | | Co-solvent +0.18% MHB replicate | | Co-solvent +0.25% MHB replicate | | Co-solvent +0.36% MHB replicate | |
| | | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Ethanol | 0 | N | 7.04 | 7.16 | 6.15 | 6.24 | 6.55 | 5.77 | 6.24 | 5.68 | 6.10 | 5.85 |
| | 1 | ME | – | – | – | – | > 3.25 | > 2.47 | > 2.94 | > 2.38 | > 2.80 | > 2.55 |
| | 2 | ME | > 3.74 | > 3.86 | 2.00 | 1.55 | > 3.25 | > 2.47 | > 2.94 | > 2.38 | > 2.80 | > 2.55 |
| | 4 | ME | > 3.74 | > 3.86 | 2.84 | 2.19 | > 3.25 | > 2.47 | > 2.94 | > 2.38 | > 2.80 | > 2.55 |
| Propylene glycol | 0 | N | 5.60 | 6.12 | | | 6.42 | 5.67 | 6.29 | 5.68 | 6.01 | 5.88 |
| | 1 | ME | – | – | | | 2.00 | 1.98 | 2.00 | 1.98 | 3.20 | > 2.58 |
| | 2 | ME | 0.39 | 0.30 | | | 0.87 | 0.93 | 2.24 | 2.49 | > 2.71 | > 2.58 |
| | 4 | ME | 0.51 | 0.44 | | | 0.98 | 1.20 | 3.02 | 3.00 | > 2.71 | > 2.58 |
| Glycerol | 0 | N | 6.57 | 5.72 | | | 6.53 | 5.59 | 6.52 | 5.57 | 6.43 | 5.98 |
| | 1 | ME | 0.04 | 0.05 | | | 0.09 | 0.10 | 0.25 | 0.45 | 0.55 | 0.62 |
| | 2 | ME | 0.09 | 0.06 | | | 0.25 | 0.37 | 0.38 | 0.68 | 0.81 | 0.95 |
| | 4 | ME | 0.09 | 0.13 | | | 0.26 | 0.43 | 0.43 | 0.70 | 1.50 | 2.25 |

Table 5
Log reduction in the viable counts of *Staphylococcus aureus* in aqueous solutions of propyl *p*-hydroxybenzoate (0.04%), combination of propyl *p*-hydroxybenzoate (0.1%)/propylene glycol (3 M), Nipasept (0.1%), Nipastat (0.1%) and mixture of 0.18% MHB and 0.04% PHB

| Time (min) | | Log viable count at time zero (N) and log reduction in viable count (ME) for | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PHB 0.04% replicate | | PHB 0.1% PG(3 M) replicate | | Nipasept 0.1% replicate | | Nipastat 0.1% replicate | | 0.18% MHB + 0.04% PHB replicate | |
| | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 0 | N | 5.89 | 5.77 | 6.04 | 6.47 | 5.97 | 6.01 | 5.99 | 6.12 | 6.02 | 6.15 |
| 15 | ME | – | – | 0.25 | 0.30 | 0.03 | 0.02 | 2.00 | 1.79 | 0.10 | 0.23 |
| 30 | ME | – | – | 0.53 | > 3.17 | 0.03 | 0.05 | 2.99 | 3.00 | 0.20 | 0.23 |
| 60 | ME | – | – | > 2.74 | > 3.17 | 0.12 | 0.10 | > 2.69 | > 2.82 | 1.97 | 2.00 |
| 120 | ME | 0.05 | 0.01 | > 2.74 | > 3.17 | 0.18 | 0.15 | > 2.69 | > 2.82 | > 2.72 | > 2.85 |

*nosa* also increased with increasing concentrations of PHB. This increase was more pronounced for combinations of PHB/PG than for combinations of PHB/glycerol, combinations of 0.1% PHB with EtOH and PG producing no detectable survivors at 30 mins and 1–2 h respectively against *Ps. aeruginosa*. By contrast the ME values of solutions up to 0.1% PHB in GLY were actually less than that of 0.04% saturated aqueous solution of PHB against *Ps. aeruginosa*.

It might be suggested that the greater increase in activity for parabens in EtOH compared with parabens in PG, and for parabens in PG compared with parabens in GLY, is due to the difference in the antibacterial activity of the co-solvents. but this does not appear to be the case since, over the 4 h period of the experiment, neither GLY nor PG had any significant bactericidal activity when used alone. Further investigations by Darwish (1992) and Darwish and Bloomfield (in preparation) indicate that the increased activity of paraben/co-solvent combinations as compared to the individual compounds or co-solvents is related to their combined effects on the integrity of the cell membrane. These results indicate as might be expected that the effects of the different co-solvents on membrane integrity increased as the polarity of the co-solvent decreased, i.e. EtOH > PG > GLY.

From the results of this study a number of potentially advantageous preservative systems can be identified. The results suggest that saturated solutions of parabens in 3 M EtOH and PG have potential advantages for preservation of pharma-

Table 6
Log reduction in the viable counts of *Pseudomonas aeruginosa* in aqueous solutions of propyl *p*-hydroxybenzoate (0.04%), combination of propyl *p*-hydroxybenzoate (0.1%)/propylene glycol (3 M), Nipasept (0.1%), Nipastat (0.1%) and mixtures of 0.18% MHB and 0.04% PHB

| Time (min) | | Log viable count at time zero (N) and log reduction in viable count (ME) for | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PHB (0.04%) replicate | | PHB (0.1%) replicate | | Nipasept (0.1%) replicate | | Nipastat (0.1%) replicate | | 0.18% MHB + 0.04% PHB replicate | |
| | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 0 | N | 5.97 | 6.24 | 6.13 | 6.47 | 6.38 | 6.42 | 6.12 | 6.03 | 6.10 | 6.20 |
| 15 | ME | – | – | 1.78 | 1.89 | 0.04 | 0.06 | 1.47 | 1.52 | – | – |
| 30 | ME | – | – | 1.98 | 2.02 | 0.10 | 0.12 | 2.03 | 1.95 | – | – |
| 60 | ME | 1.50 | – | > 2.83 | > 3.17 | 0.15 | 0.13 | 2.89 | 2.55 | 3.79 | 2.00 |
| 120 | ME | 2.00 | – | > 2.83 | > 3.17 | 0.48 | 0.36 | > 2.82 | > 2.73 | > 2.80 | > 2.90 |

ceuticals as compared with saturated aqueous solutions. Using MHB (0.36%) in 3 M PG, it was found that the time required to achieve 3 log reduction in *S. aureus* and *Ps. aeruginosa* was reduced to 2 h as compared with 24 h for saturated aqueous solution of MHB (0.18%). Using PHB (0.1%) in 3 M PG the time required to achieve a 3 log reduction in *S. aureus* and *Ps. aeruginosa* was reduced to 1 h as compared with > 4 h for 0.04% PHB in aqueous solution. Using PHB in 3 M EtOH the time required to achieve 3 log reduction in *S. aureus* and *Ps. aeruginosa* was reduced from 2 to 1 h.

In practical terms for preservation of pharmaceuticals, the use of EtOH may be limited because of its volatility and therefore poor shelf stability. Of the PG systems – 0.36% (0.023 M) MHB in 3 M PG and 0.1% (0.0055M) PHB in 3 M PG – the latter may also be considered more advantageous because the use of the higher molar concentrations of parabens increases the possibility of toxic side effects. Additionally it is known that higher concentrations of parabens may have a marked taste when used in oral preparation.

In further experiments the activity of 0.1% PHB in 3 M PG was compared with two commercially available paraben combinations, Nipastat and Nipasept. The results (Tables 5 and 6) show that 0.1% PHB in 3 M PG was much more effective than solutions of 0.1% Nipasept against both *S. aureus* and *Ps. aeruginosa*, although the relative concentrations of methyl, ethyl and propyl parabens in the Nipasept are unknown. The activity of 0.1% PHB in PG was similar to although slightly less than that of Nipastat. This preparation contains butyl as well as methyl, ethyl and propyl parabens and is not generally used for oral preparations although adverse oral toxicity has never been established. The activity of 0.1% PHB in 3 M PG was also compared with that of an aqueous solution of MHB and PHB at saturation concentrations (0.18 and 0.04%) and found to be similar or marginally higher.

Experimental evidence indicates that the addition of agents having a co-solvency or solubilising effect may affect the activity of paraben preservatives in a number of ways according to the nature of the agent. Investigations by Van Doorne et al. (1988) together with preliminary studies in our own laboratories (unpublished) indicate that al-

Table 7
Log reduction of viable counts of *Staphylococcus aureus* in supersaturated solutions of methyl *p*-hydroxybenzoate

| | Log reduction in viable count [a] in methyl *p*-hydroxybenzoate | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0.1% replicate | | 0.18% replicate | | 0.25% replicate | | 0.3% replicate | | 0.36% replicate | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Supersaturated aqueous solution | 0.13 | 0.15 | 0.15 | 0.10 | 0.38 | 0.20 | 0.28 | 0.20 | 0.24 | 0.18 |
| Solution in 3 M propylene glycol | 0.48 | 0.75 | 1.17 | 1.35 | 2.17 | 2.86 | 2.32 | 2.62 | > 2.64 | > 2.88 |
| | Log reduction in viable count [a] in propyl *p*-hydroxybenzoate | | | | | | | | | |
| | 0.02% replicate results | | 0.04% replicate results | | 0.05% replicate results | | 0.075% replicate results | | 0.1% replicate results | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Supersaturated aqueous solution | 0.05 | 0.02 | 0.21 | 0.09 | 0.15 | 0.12 | 0.14 | 0.03 | 0.10 | 0.14 |
| Solution in 3 M propylene glycol | 0.40 | 0.54 | 1.38 | 1.88 | 2.10 | 2.00 | > 2.62 | > 3.02 | > 2.65 | > 3.10 |

[a] Inoculum size $10^6$–$10^8$ cfu $ml^{-1}$.

though cyclodextrins can be used as co-solvents to increase the solubility of the paraben esters, the nature of the complexation is such that this is associated with a marked decrease in preservative efficacy. Higuchi and Kuramoto (1954), Guttman and Higuchi (1957) and Patel and Kostenbauder (1958) demonstrated that phenols tend to form molecular complexes with agents, such as polyethylene glycol used in pharmaceutical preparations which are associated with increased aqueous solubility, but this is also associated with loss of activity. From the chemical structure of the parabens and the co-solvents EtOH, PG and GLY it would be expected that the interaction between these molecules would be largely hydrophobic. In agreement with this, studies in our laboratory (results not shown) indicated no change in the UV spectrum of parabens following the addition of the co-solvents EtOH, PG and GLY to the aqueous solution, i.e., no complex formation involving significant charge transfer.

Previously reported studies with some low solubility substituted phenols (Bloomfield, 1970) showed that increased activity with increasing concentrations above the aqueous solubility could be produced by using insignificant amounts of an intermediate solvent to produce supersaturated solutions of the antimicrobial agent. In this situation the additional material is seen as a finely divided precipitate. Studies with paraben esters (Table 7) show that, when supersaturated solutions of 0.1–0.36% MHB and 0.04–0.1% PHB in water were prepared by adding a minimal quantity of a concentrated solution of MHB and PHB in alcohol, there was no increase in activity against *S. aureus*, thus indicating that increased activity of parabens at concentrations above aqueous solubility can only be achieved if the parabens is brought about by the use of suitable agent which in this case was the co-solvent.

Since the co-solvents are found to increase the aqueous solubility of the parabens, this might also be expected to result in a decrease in lipophilicity. Determination of o/w partition coefficients for aqueous solutions of MHB and PHB (Table 8) gave values of 77.20 and 670.12 which are in agreement with values quoted by Hansch et al. (1972). The addition of co-solvents to the paraben solutions produced a decrease in the o/w partition coefficient and, as expected, it was found that in general the partition coefficient for parabens in least polar co-solvent EtOH was lower than the partition coefficient for parabens in PG which in turn was lower than the partition coefficient for parabens in GLY, although there was some discrepancy in the results for MHB in EtOH and PG.

From these results it was realised that the presence of co-solvents might have an effect on the efficacy of the parabens in oil/water formulations. Experience has shown (Van Doorne and Dubois, 1980) that although the activity of preservative agents, such as parabens, increases with increasing hydrophobicity, i.e., increase in oil/water partition coefficient, their effectiveness in cream formulations is compromised by the fact that a significant percentage of the preservative partitions in the oily phase, thereby decreasing the concentration of the preservatives in the aqueous phase.

Table 8
o/w partition coefficients for methyl and propyl *p*-hydroxybenzoate solutions in water and co-solvent solutions

| Aqueous phase | Methyl *p*-hydroxybenzoate | | Propyl *p*-hydroxybenzoate | |
|---|---|---|---|---|
| | Initial concentration in aqueous phase % w/v | Partition coefficient | Initial concentration in aqueous phase % w/v | Partition coefficient |
| Water | 0.18 | 77.20 | 0.04 | 670.12 |
| Glycerol (3 M) | 0.18 | 52.48 | 0.04 | 480.20 |
| Propylene glycol (3 M) | 0.18 | 39.81 | 0.04 | 236.40 |
| Ethanol (3 M) | 0.18 | 49.00 | 0.04 | 233.40 |

The results in Table 9 demonstrate the reduction in viable counts for *S. aureus* inoculated into cetomacrogol creams preserved with MHB, PHB or co-solvents alone, compared with paraben/co-solvent combinations. Although there was some discrepancies between ME values obtained from replicate experiments, the results indicate that the presence of a co-solvent produced an increase in the preservative activity of the parabens, the extent of the effect depending on the nature of the co-solvents and the oil/water partition coefficient of the preservative. Results showed that combinations of parabens with EtOH were the most effective combinations giving no detectable survivors after 2 h. PHB (0.2%)/PG (3 M) and MHB (0.4%)/PG (3 M) were also effective combinations giving no detectable survivors after 2–4 h. By contrast, however, it was found that the combinations of the most polar co-solvent GLY with PHB 0.2% showed relatively poor activity which was less than that achieved by PHB 0.2% alone. For MHB/GLY combinations conflicting results were obtained whereas combinations of 0.2% MHB with GLY also showed poor activity, MHB 0.4% with GLY gave no detectable survivors.

From this it is suggested that the co-solvents produce an increase in activity of paraben preservatives by causing a decrease in the oil/water partition coefficient, thereby increasing the concentration of the free parabens in the aqueous phase of the formulation. As predicted, the least polar co-solvent, EtOH, in combination with parabens produced the highest activity whilst the most polar co-solvent, glycerol, produced the lowest activity which was no greater than, and for PHB slightly less, than that achieved by the PHB alone. Alternatively, it may be suggested that the observed activity may result from the combined effects of the co-solvents and parabens on the bacterial cell membrane, such that, whereas the individual effects of the co-solvents and parabens produced relatively little membrane damage, their combined effects were sufficient to cause a significant and rapid loss of viability.

Overall the results of this investigation suggest that it should be possible, by altering the o/w phase distribution properties of membrane active antibacterial agents such as the parabens, to optimise the preservation of pharmaceutical or cosmetic products whilst minimising the total quantity of added preservative.

Table 9
Effects of combinations of parabens and co-solvents on the viability of *Staphylococcus aureus* ($10^6$–$10^8$ cfu $ml^{-1}$) in cetomacrogol cream

| Co-solvent | Time (h) | Log viable counts in cetomacroyol cream containing: | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Replicate | | 0.2% methyl *p*-hydroxybenzoate replicate | | 0.4% methyl methyl *p*-hydroxybenzoate replicate | | 0.2% propyl methyl *p*-hydroxybenzoate | |
| | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| None | 0 | | | 7.39 | 8.09 | 6.69 | 7.01 | 7.26 | 7.35 |
| | 2 | | | 7.05 | 7.24 | 6.11 | NDS | 5.30 | 6.34 |
| | 4 | | | 6.04 | – | – | – | NDS | 5.78 |
| Glycerol (3 M) | 0 | 7.33 | 7.33 | 7.34 | – | 7.25 | 7.32 | 7.45 | 7.31 |
| | 2 | 7.44 | 7.36 | 7.29 | – | NDS | NDS | 7.13 | 7.16 |
| | 4 | 7.49 | 7.22 | 6.94 | – | NDS | NDS | 6.30 | 6.83 |
| Propylene glycol (3 M) | 0 | 7.42 | 7.27 | 7.43 | – | 7.25 | 7.35 | 7.14 | 7.40 |
| | 2 | 7.20 | 6.83 | 5.30 | – | 4.30 | 5.30 | 5.30 | NDS |
| | 4 | 6.04 | 5.99 | 5.30 | – | NDS | NDS | NDS | NDS |
| Ethanol (3 M) | 0 | 7.37 | 7.34 | 7.33 | – | 5.30 | 5.30 | 5.78 | 7.40 |
| | 2 | 6.26 | 7.72 | NDS | – | NDS | NDS | NDS | NDS |
| | 4 | 5.60 | 6.36 | NDS | – | NDS | NDS | NDS | NDS |

NDS, no detectable survivors.

## References

Anon, Preservatives. In Reynolds, J.E.F. (Ed.), *The Extra Pharmacopoeia*, 29th Edn, Pharmaceutical Press, London, 1989, pp. 1355–1364.

Bloomfield, S.F., Studies in the mode of action of bis 2-hydroxy-5-chlorophenyl sulphide (Fentichlor). Ph.D thesis, University of Nottingham (1970).

Darwish, R., The effect of co-solvents on the activity of preservatives in oral aqueous dosage forms. Ph.D thesis, University of London (1992).

Eklund, T., Inhibition of growth and uptake processes in bacteria by some chemical food preservatives. *J. Appl. Bacteriol.*, 48, (1980), 423–432.

El-Falaha, B.M.A., Russell, A.D. and Furr, J.R., Sensitivities of wild type and envelope-defective strains of *Escherichia coli* and *Pseudomonas aeruginosa* to antibacterial agents. *Microbios*, 38 (1983) 99–105.

Guttman, D. and Higuchi, T., Possible complex formation between macromolecules and certain pharmaceuticals. X. The interaction of some phenolic compounds with polyethylene glycols, polypropylene glycols, and polyvinyl pyrrolidone. *J. Am. Pharm. Assoc.*, 45 (1957) 659–664.

Hansch, C., Coubeils, J.L. and Leo, A., The antimicrobial structure activity relationship in esters of 4-hydroxybenzoic acid. *Chim. Ther.*, 6 (1972) 427–433.

Higuchi, T. and Kuramoto, R., Study of possible complex formation between macromolecules and certain pharmaceuticals. *J. Am. Pharm. Assoc.* 43 (1954) 398–401.

Patel, N.K. and Kostenbauder, H.B., Interaction of preservatives with macromolecules. I. Binding of parahydroxybenzoic and esters and polyoxyethylene 20 Sorbitan Monooleate (Tween 80). *J. Am. Pharm. Assoc.*, 47 (1958) 289–295.

Russell, A.D. and Gould, G.W., Resistance of Enterobacteriacae to preservatives and disinfectants. *J. Appl. Bacteriol. Symp. Suppl.*, (1988) 167S–195S.

Van Doorne, H. and Dubois, F.L., The preservation of Lanette wax cream (FNA). *Pharm. Weekbl. Sci. Ed.*, 2 (1980) 271–275.

Van Doorne, H., Bosch, E.H. and Leek, C.F., Formation and antimicrobial activity of complexes of β-cyclodextrin and some antimycotic imidazole derivatives. *Pharm. Weekbl. Sci. Ed.*, 10 (1988) 80–85.

Yalkowsky, H.S., *Techniques of Solubilization of Drugs*, Dekker, New York, 1981.