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LC studies on the potential interaction of paraben preservatives with sorbitol and glycerol*

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Abstract: Pharmaceutical formulations often contain one or more paraben preservatives in conjunction with a polyol such as sorbitol or glycerol. In one of these experimental formulations a number of unknown polar peaks have been detected near the solvent front by reversed-phase LC. These degradation products were not attributable to the active drug component or a hydrolysis product. The possibility of an interaction between the polyols and paraben preservatives has been explored using a three-variable, two-level, factorial design to determine the relative significance of the factors involved in the formation of these unknown peaks. The factors examined were pH, temperature and the ratio of polyol to paraben. This study has shown that pH and temperature are key factors affecting the formation of these unknown peaks. On the basis of these results suitable conditions can be suggested for minimizing the production of these unknown peaks. It seems clear that a number of pharmaceutical formulations containing a polyol and a paraben would present potential problems for assay validation on storage owing to the formation of these degradation products, particularly if the drug component is polar and elutes near the solvent front.

Keywords: *Parabens; sorbitol; glycerol; hydrolytic degradation; reversed phase-LC; trans-esterification reaction; factorial design.*

Introduction

The alkyl esters of *p*-hydroxybenzoic acid (methyl, ethyl, propyl or butyl) are often known as parabens and are widely used as antimicrobial preservatives in the pharmaceutical, cosmetic and food industries. They are often used as combinations of two or more alkyl esters because together they display enhanced antimicrobial activity. They degrade by hydrolysis under alkaline and acidic conditions to form *p*-hydroxybenzoic acid (PHBA), which has little preservative action. This reaction is well documented [1, 2].

When experimental dosage forms containing methyl and propyl parabens with sorbitol and glycerol were analysed by reversed-phase liquid chromatography (LC), several unknown, polar peaks were observed near the solvent front (M.L. Robinson, unpublished data). It was postulated that the peaks could arise from degradation involving an interaction between the drug, the parabens or PHBA with the sorbitol and/or glycerol. Therefore a series of experiments was designed to investigate the principal factors responsible for the formation of these degradation products.

The key objectives of the present work were to confirm the degradation profile observed in the experimental formulation, and to establish that the degradation products were not related to either the drug candidate or the PHBA hydrolysis product, using a drug-free solution of the same formula stored at 60°C. Further studies were conducted with methyl paraben (MP) alone to confirm the degradation profile in the presence and absence of either sorbitol (S) or glycerol (G). Then a factorial design was established to examine the effects of pH, temperature and concentration ratio of polyol to paraben in order to assess the significance of each of these factors and their potential interaction.

Experimental

Materials

The following were used as received: glycerol (May and Baker, Dagenham, Essex, UK); sorbitol (Sigma Chemical Co., Poole,

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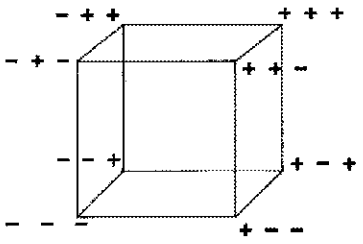
UK); methyl paraben (Aldrich Chemical Co. Ltd, Dorset, UK); HPLC grade methanol (Fisons Scientific Equipment, Loughborough, UK); potassium dihydrogen phosphate (BDH Laboratory Supplies, Poole, UK); citric acid (May and Baker, Dagenham, Essex, UK); sodium hydroxide (BDH Laboratory Supplies, Poole, UK); propyl paraben (BDH Laboratory Supplies, Poole, UK) and sodium chloride (May and Baker). Samples of saccharin and trisodium citrate dihydrate were donated by Bristol–Myers Squibb.

Experimental design

Before embarking on this study, a real formulation (without the drug) was prepared in accordance with the formula used for an experimental oral formulation at pH 6.0 containing methyl paraben (1 g l^{-1}), propyl paraben (0.2 g l^{-1}), sorbitol (500 g l^{-1}) and glycerol (100 g l^{-1}) together with saccharin, sodium chloride, citric acid and trisodium citrate dihydrate. This solution was incubated at 60°C for 2 weeks in order to establish whether any polar degradation products were formed in the absence of the drug. Samples of 0.5 ml were taken initially and again after 2 weeks and were analysed by gradient LC as described below.



Effects

$$A = 1/4\,((Y_1 + Y_2 + Y_3 + Y_4) - (Y_5 + Y_6 + Y_7 + Y_8))$$
$$B = 1/4\,((Y_1 + Y_2 + Y_5 + Y_6) - (Y_3 + Y_4 + Y_7 + Y_8))$$
$$C = 1/4\,((Y_1 + Y_5 + Y_6 + Y_7) - (Y_2 + Y_4 + Y_6 + Y_8))$$

Interactions

$$AC = 1/4\,((Y_1 + Y_2 + Y_7 + Y_8) - (Y_3 + Y_4 + Y_5 + Y_6))$$
$$BC = 1/4\,((Y_1 + Y_4 + Y_5 + Y_8) - (Y_2 + Y_3 + Y_6 + Y_7))$$
$$AB = 1/4\,((Y_1 + Y_3 + Y_6 + Y_8) - (Y_2 + Y_4 + Y_5 + Y_7))$$

Figure 1
Diagram showing the distribution of experiments for a two-level, three-variable factorial design with the equations used to assess the effects and interactions between variables.

Using a simplified model formulation based on a citrate buffer, a three-variable, two-level factorial design was developed (Fig. 1) using eight solutions each for sorbitol and glycerol

Table 1
Composition of the solutions used for the factorial design

(A) Sorbitol

Solution no.	Concentration: Methyl paraben (mg ml^{-1})	Concentration: Sorbitol (g ml^{-1})	Ratio sorbitol:methyl paraben	pH*	Temp.† (°C)	
1	0.3	0.15	500:1	8	60	+ + +
2	0.3	0.15	500:1	8	25	+ + −
3	0.3	0.15	500:1	3	60	+ − +
4	0.3	0.15	500:1	3	25	+ − −
5	1.5	0.15	100:1	8	60	− + +
6	1.5	0.15	100:1	8	25	− + −
7	1.5	0.15	100:1	3	60	− − +
8	1.5	0.15	100:1	3	25	− − −

(B) Glycerol

Solution no.	Concentration: Methyl paraben (mg ml^{-1})	Concentration: Glycerol (g ml^{-1})	Ratio sorbitol:methyl paraben	pH*	Temp.† (°C)	
1	0.5	50	100:1	8	60	+ + +
2	0.5	50	100:1	8	25	+ + −
3	0.5	50	100:1	3	60	+ − +
4	0.5	50	100:1	3	25	+ − −
5	1.0	50	50:1	8	60	− + +
6	1.0	50	50:1	8	25	− + −
7	1.0	50	50:1	3	60	− − +
8	1.0	50	50:1	3	25	− − −

*pH measured at ambient temperature.
†Temperature ± 3°C.

(Table 1). The MP and the appropriate amount of polyol were dissolved in citric acid–sodium hydroxide buffer in order to cover the pH range 3–8 [3]. The final pH was adjusted and the solutions were diluted to volume (10.0 ml) with double-distilled water. Samples were taken of each solution before incubation and then after 1, 2, 7, 14, 21 and 28 days, respectively. Suitable control solutions containing MP alone, or sorbitol, or glycerol, or PHBA under each of the specified conditions of temperature and pH were also prepared and incubated.

Chromatography

The samples were analysed using isocratic or gradient LC, as required, using the HP-1090 LC with diode-array detector (DAD) (Hewlett–Packard GmbH, Waldbronn, Germany). The DAD was used in order to assess peak purity for validation purposes. Data were acquired and evaluated using the HP-9000 series Workstation (with HPLC ChemStation software).

Method development

On the basis of a three-variable, two-level factorial design involving pH (3, 7), percentage (v/v) of methanol (10%, 30%) and buffer concentration (0.01 M, 0.1 M), it was established that the dominant parameters for the separation of the two poorest resolved degradation peaks (observed in a test solution of glycerol and methyl paraben in phosphate buffer at pH 8.0 stored at 60°C for 14 days) were pH and the percentage of organic modifier. A modified simplex design based on these parameters (Fig. 2) showed the optimum eluent composition to be that described below. However the more complex group of degradation products observed in the sorbitol–methyl paraben experiments (carried out under conditions similar to those described above) could not be separated under isocratic conditions within an acceptable analysis time. Therefore a gradient system was developed for this part of the study.

Chromatographic conditions. For the glycerol–MP combination isocratic analysis was employed with the following eluent: methanol–potassium dihydrogen phosphate (pH 3.0; 0.05 M), (18:82, v/v). For the sorbitol–MP combination gradient elution was used: solvent A, methanol; solvent B, potassium dihydrogen phosphate (pH 3.0, 0.05 M); the linear gradient employed was 0–40% solvent A in 30 min.

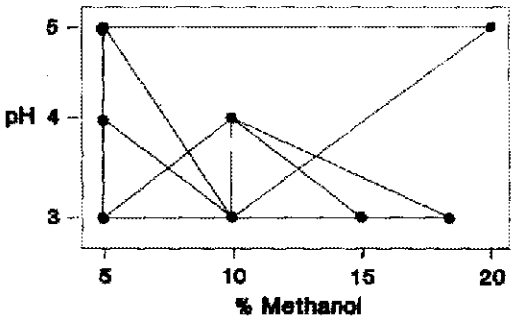


Figure 2
Modified simplex procedure employed to develop the mobile phase for the analysis of the glycerol–methyl paraben solutions.

The 150 × 4.6 mm i.d. column was packed with 5-μm ODS Hypersil (Chromex, Cheshire, UK); the flow rate was 1 ml min^{-1}; the sample volume was 20 μl. Detection was effected at 254 nm and the column temperature was ambient (approximately 21°C).

Evaluation of chromatograms for factorial design. The chromatograms of the samples collected at 28 days were integrated and the peak areas were analysed using the factorial design equations [4] to assess the effect of each variable on each chromatographic peak of significance and to detect any interaction between variables.

Results and Discussion

The drug-free solution stored at 60°C for 4 weeks at pH 6.0 displayed several unknown peaks (Fig. 3), with retention times corresponding to those in earlier data obtained for an experimental formulation (M.L. Robinson, unpublished data). It can therefore be assumed that these peaks are not attributable to the active drug present in the original experimental formulation.

The assays developed for MP and PHBA were validated for linearity of response and precision. Several standard solutions of MP and PHBA were injected in triplicate under both the gradient and isocratic conditions. For the isocratic conditions the response was found to be linear for 0.2–1.5 mg ml^{-1} of MP, the regression equation being $y = 0.12x + 0.012$

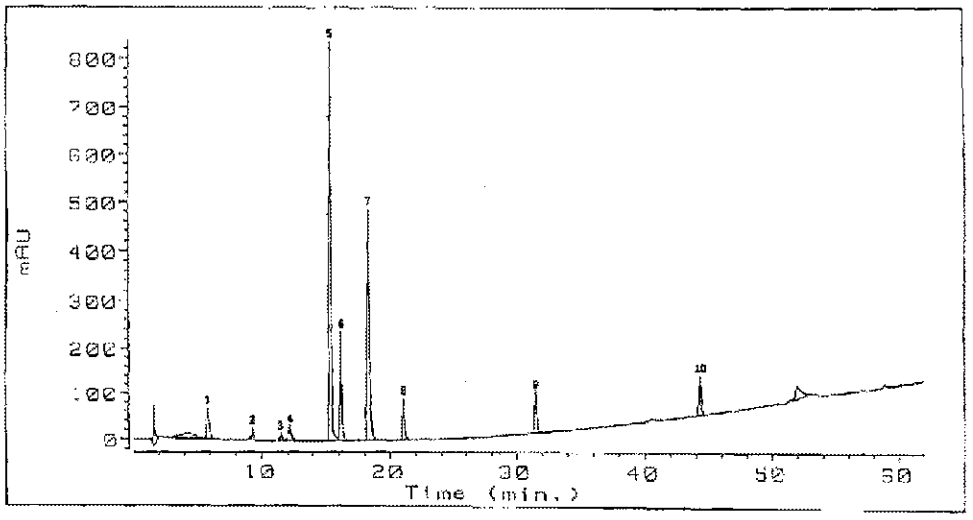


Figure 3
Chromatogram of a drug-free experimental formulation used to establish whether the unknown peaks were attributable to the drug candidate. Peaks 1–4, 6 and 8 are unknowns. Peak 5, saccharin; peak 7, HBA; peak 9, methyl paraben; peak 10, propyl paraben.

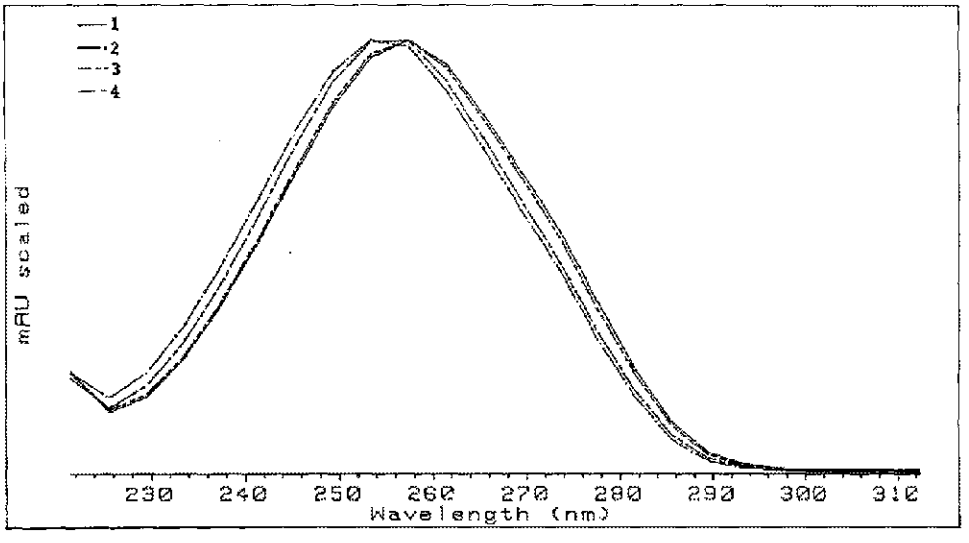


Figure 4
Normalized UV spectra obtained by diode-array detection of the peaks observed in Fig. 6. 1, unknown 1; 2, PHBA; 3, unknown 2; 4, MP.

($r = 0.9999$, $n = 7$); for 2–20 μg ml^{-1} of PHBA, the regression equation was $y = 0.14x + 0.004$ ($r = 0.9999$, $n = 9$). For the gradient elution method the response was found to be linear for the same concentration ranges of MP and PHBA. For MP the regression equation was $y = 0.15x + 0.02$ ($r = 0.9999$, $n = 7$). For PHBA the regression equation was $y = 0.15x + 0.015$ ($r = 0.9999$, $n = 9$). As the other two peaks observed in the chromatogram from the glycerol system have not yet been identified, validation experiments could not be carried out. However, DAD data were used to show the close similarity of the UV spectra of each peak obtained (Fig. 4). This finding shows how closely related are the UV spectra of the MP and the degradation products, which indicates structural similarities and comparable molar absorptivities.

The precision of these methods was assessed using 10 replicate injections for each compound under each set of conditions. For MP by the isocratic method the RSD was 0.34% at 1.0 mg ml^{-1}; for PHBA the RSD was 0.32% at

10 μg ml $^{-1}$. For MP by the gradient method the RSD was 0.52% at 1.0 mg ml^{-1} and for PHBA the RSD was 0.53% at 10 μg ml^{-1}.

The factorial design equations [4] enable assessment of the effects and interaction of pH, temperature and concentration ratio on the formation of the degradant peaks, based on peak areas. In the case of sorbitol (Fig. 5), five degradation peaks were detected, including that for PHBA, whereas three peaks were found in the case of glycerol (Fig. 6) including that for PHBA. In both the glycerol and sorbitol studies the degradation of the MP was also assessed using the factorial design equations. For each set of experiments the MP and PHBA peaks were identified by their retention times by running standards separately; co-chromatography was used in the initial validation.

In both the glycerol and sorbitol studies every sample showed a reduction in the peak area for MP compared with the initial sample ($t = 0$) and an increase in the hydrolysis product.

The results from the factorial design equations are shown in Figs 7 and 8. It can be

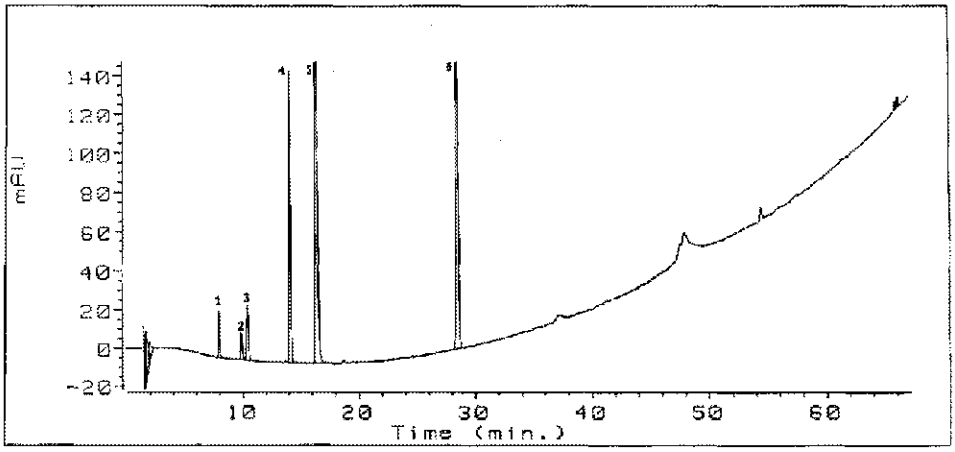


Figure 5
Chromatogram of a solution of methyl paraben and sorbitol after storage at 60°C for 4 weeks. Mobile phase as described for gradient elution in the text. Peaks 1–4 are the unknowns assessed using the factorial design equations. Peak 5, PHBA; peak 6, methyl paraben.

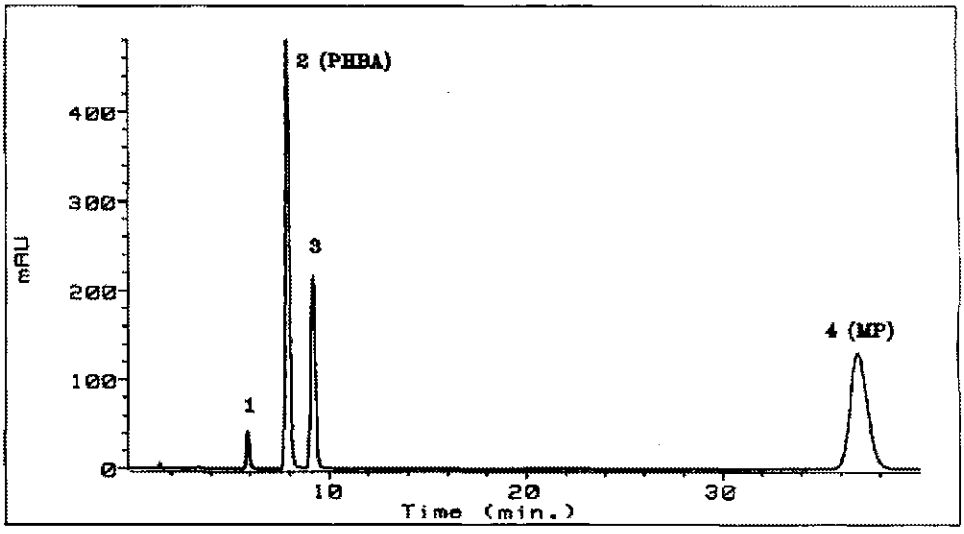


Figure 6
Chromatogram of a solution of methyl paraben and glycerol after storage at 60°C for 4 weeks. Mobile phase as described for isocratic analysis in the text. Peaks 1 and 3 are the unknowns assessed by the factorial design equations. Peak 2, PHBA; peak 4, methyl paraben.

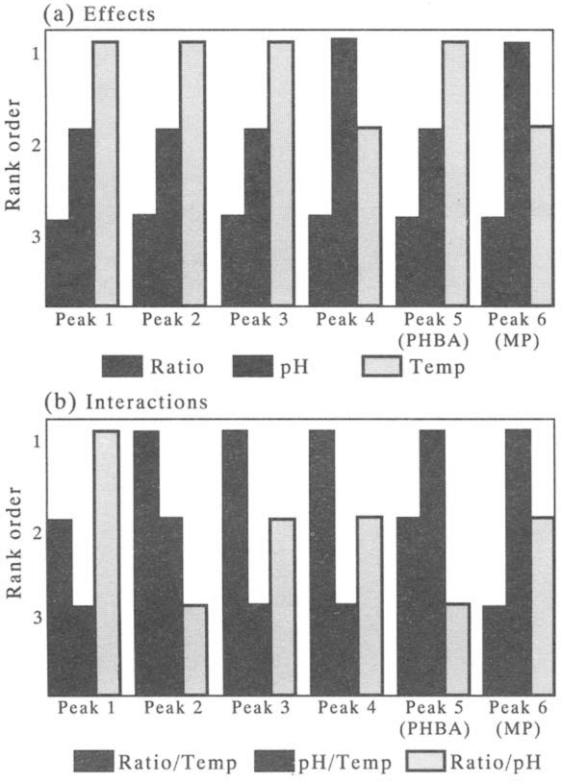


Figure 7
Diagram showing the rank order of factors for effects and interactions in the sorbitol system.

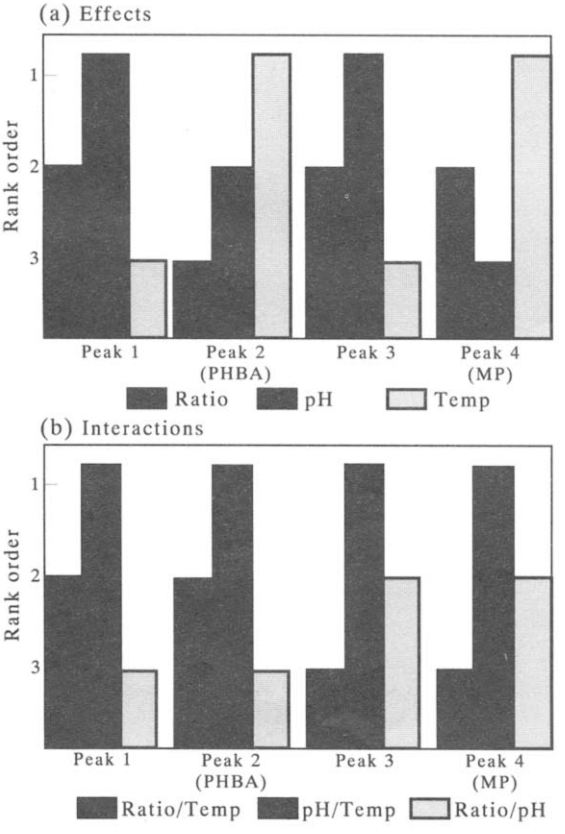


Figure 8
Diagram showing the rank order of factors for effects and interactions in the glycerol system.

seen from these that the rank order of the effects for each variable differ with respect to the degradation of MP and the formation of the unknown peaks.

In the case of sorbitol, four of the six peaks assessed show the same rank order of variables where temperature is the most important and the molar ratio of sorbitol to MP is the least. The remaining two peaks show a different rank order with pH as the dominant factor and the ratio of polyol to paraben as the least important.

For glycerol, two peaks show the same order of priority: pH is the most influential factor and the molar ratio of glycerol to MP is the least. The other two peaks assessed show different rank orders, but both show temperature to be the key factor in their formation. When the factorial design equations are used to assess the interactions between the variables, the sorbitol system shows a complex picture. Three of the six assessed peaks show the major interaction to be between the molar ratio of sorbitol to MP and temperature; two show pH and temperature to interact and the other shows an interaction between molar ratio and pH. For glycerol, however, it is clear that pH and temperature interact most strongly with respect to all four peaks.

Examples of the graphs obtained for peak area plotted against time for the glycerol system can be seen in Figs 9–12. In Fig. 9 for solution 1, where the molar ratio of glycerol to MP is low, but pH and temperature are high, the extent of degradation of MP is very high. After 4 weeks' storage only approximately 20% of the MP remains, under the most extreme conditions of temperature and pH, for the glyerol system and only 30% for the sorbitol system. The main degradant is PHBA but significant levels of other polar compounds are seen. These increase progressively, then it would appear that they themselves degrade after about 20 days' storage.

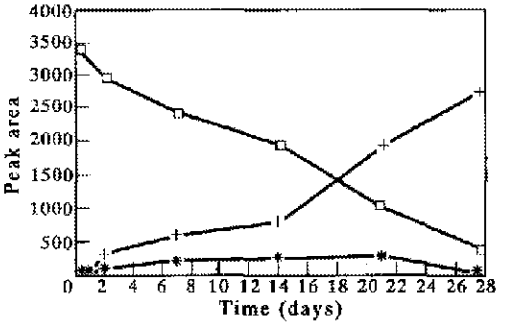


Figure 9
Graph showing peak areas against time for the factorial design solution 1 for glycerol (ratio low, pH 8, 60°C). 1, MP; 2, PHBA; 3, peak 3 (unknown); 4, peak 1 (unknown).

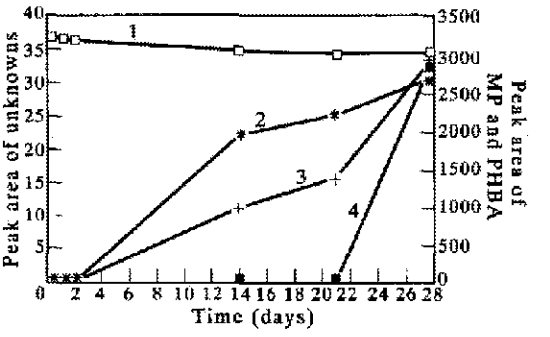


Figure 10
Graph showing peak area against time for the factorial design solution 2 for glycerol (ratio low, pH 8, 25°C). 1, MP; 2, peak 3 (unknown); 3, PHBA; 4, peak 1 (unknown).

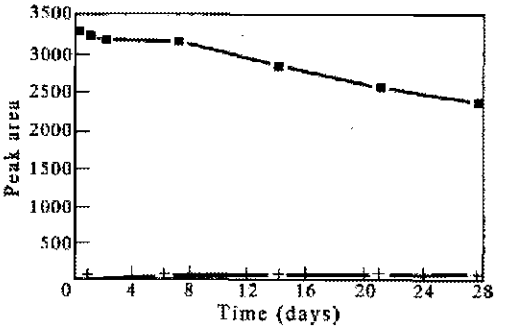


Figure 11
Graph showing peak areas against time for the factorial design solution 3 for glycerol (ratio low, pH 3, 60°C). 1, MP; 2, PHBA.

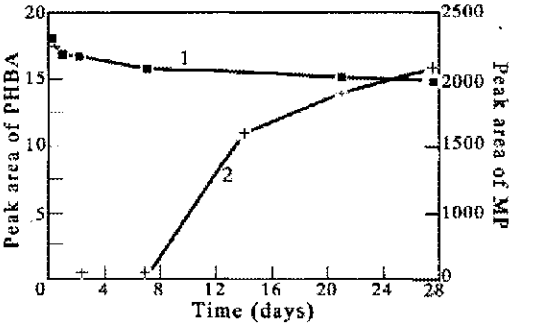


Figure 12
Graph showing peak areas against time for the factorial design solution 4 for glycerol (ratio low, pH 3, 25°C). 1, MP; 2, PHBA.

In Fig. 10, where the ratio of glycerol to MP is also low and the pH high, but the temperature is only 25°C, a more gradual degradation

of the MP peak is observed, together with much lower levels of the other peaks. However, two additional unknown peaks are produced together with the PHBA. For solutions 3 and 4 at low pH (Figs 11 and 12) a different profile is observed; here the only degradant seen in detectable quantities is PHBA. In solution 3 (where the temperature is 60°C) the extent of degradation of the MP is greater than that for solution 4, where the temperature is 25°C. Solutions 5–8 all have a high polyol to paraben ratio. When graphs of peak area against time were plotted for these solutions, similar graphs to those already obtained for solutions 1–4 were observed. This shows that the molar ratio of polyol to paraben has little effect on the degradation.

These results indicate that pH and temperature are the principal factors in the reaction. Additional work is ongoing to study further the sorbitol system, in a similar manner to that applied to the glycerol system, in order to elucidate further the rank order of the factors for each of the degradation products.

One mechanism that has been postulated for this reaction is a trans-esterification between the paraben and the polyols [5]. In theory this would be expected to yield six trans-esters for sorbitol and two for glycerol. In the present work two unknown peaks were indeed observed for glycerol but only four for sorbitol. From the relative areas of the peaks observed it would appear that the unknown peaks are formed at different reaction rates and may themselves be subject to further degradation. One study on this trans-esterification between sorbitol and parabens has been reported [5] and other work has also been reported on this type of reaction in similar compounds [6].

Conclusions

The results of these experiments confirm the formation of unknown peaks in the presence of parabens and sorbitol or glycerol. Work using the factorial design indicated the key factors that affect the formation of these peaks. Further work on one of these systems, together with identification of the peaks by mass spectrometry, should enable the mechanism of this process to be clarified.

References

[1] N.N. Raval and E.L. Parrott, *J. Pharm. Sci.* **56**, 274–275 (1967).
[2] S.M. Blaug and D.E. Grant, *J. Soc. Cosmet. Chem.* **25**, 495–506 (1984).
[3] D.D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control*. Chapman and Hall, London (1974).
[4] J.C. Berridge, *Techniques for the Automated Optimization of HPLC Separations*. John Wiley, London (1985).
[5] B. Runesson and K. Gustavii, *Acta. Pharm. Suec.* **23**, 151–162 (1986).
[6] W.J. Irwin, Q.N. Masuda and A. Li Wan Po, *Int. J. Pharm.* **21**, 35–50 (1984).

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