DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 13(8), 1429-1446 (1987)

A COMPARATIVE STUDY OF THE EFFECTIVENESS OF PRESERVATIVES IN TWELVE ANTACID SUSPENSIONS

E. Vanhaecke[1], J.P. Remon[2], J. Pijck[1], R. Aerts[1], J. Herman[2]

[1]Laboratorium voor Farmaceutische Microbiologie en Hygiëne
[2]Laboratorium voor Farmaceutische Technologie
Rijksuniversiteit Gent, Harelbekestraat 72, 9000 Gent, Belgium

ABSTRACT

In this study the effectiveness of preservatives in twelve currently used antacid suspensions was evaluated. In eight of the twelve formulations, hydroxybenzoates were used as preservative. Only four of these products met the existing USP XXI requirements. One of the discarded products systematically contained a contaminant belonging to the Pseudomonas group. Of the remaining four products with other preservatives, two failed the USP XXI challenge tests. The main cause of all failures was the incompatibility of pH with the preservatives choosen. Especially the challenge test with the Ps. aeruginosa strain posed the greatest difficulties. However the Pseudomonas results were not always easy

to interprete, partially due to the lack of interpretation criteria at the seventh and the twenty first day. Therefore we propose a three percent bacterial concentration level as an additional pass-fail criterion for the seventh and twenty first day.

INTRODUCTION

The importance of preservatives in sterile multidose preparations is generally recognized (7, 13, 14). Their function is to maintain sterility in order to ensure the safety of use of the preparation.

Oral antacid suspensions belong to the so-called non-sterile drugs. They are not dispensed as sterile products but nevertheless bacterial contamination has to be avoided. This could indeed lead to deterioration of the product and form a potential danger for the patients health (9, 13).

Good microbiological quality of the product is first of all achieved by respecting the GMP-rules. In addition, the incorporation of a preservative should prevent excessive proliferation of bacterial or fungal contaminants, introduced during manipulation of the product by the patient.

The choice of the preservative depends on several factors, such as compatibility with other ingredients and pH, the latter possibly being of substantial importance in antacid suspensions. It is also being described that certain substances such as magnesium trisilicate and bentonite, present in antacid formulations, are counteracting the activity of certain preservatives (10, 12). Therefore, once a suitable

preservative is found, a challenge test should determine whether the added substance is really active in the given formulation. However, legal European directions on the testing of the effectiveness of antimicrobial preservatives are absent. This motivated us to examine twelve currently used oral antacid suspensions for the effectiveness of their preservatives, using the challenge test as described by the USP XXI.

MATERIALS AND METHODS

1. Test Organisms.

The following microorganisms were used for the challenge test as recommended by the USP XXI : *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Staphylococcus aureus* ATCC 6538.

2. Media.

Tryptone soya broth (TSB, Oxoid, Basingstoke, England), tryptone soya agar (TSA, Oxoid, Basingstoke, England), bacto-peptone (Difco, Detroit, USA), sabouraud dextrose agar (Gibco, Paisley, Scotland), sabouraud chloramphenicol agar (Institut Pasteur, Marnes la Coquette, France), MacConkey agar (Gibco, Paisley, Scotland).

3. Inoculum Preparation.

The freeze-dried cultures were rehydrated according to the instructions of the supplier (ATCC, Rockville, USA) and subcultured on TSA or on sabouraud

slants. For the initial cultivation, bacteria were grown overnight in TSB at 32°C, Candida albicans was grown during 48 hrs in liquid sabouraud medium at 25°C and Aspergillus niger was grown for 1 week at 25°C on a sabouraud with chloramphenicol slant. Bacteria and Candida albicans were harvested from their liquid media by centrifugation at 3000 rpm and washed twice with sterile saline. Stock suspensions of the bacteria were obtained by making a one-by-fifty dilution of the washed suspension. In the case of Candida albicans, a tenthfold dilution served as stock suspension.

Aspergillus niger was harvested by washing the cells from the slant with 10 ml of sterile saline containing 0.05 % polysorbate 80. Subsequent washing of the suspension and reconstitution to the original volume yielded the Aspergillus niger stock suspension.

4. Test Products.

Twelve currently used oral antacid suspensions were checked on effectiveness of their preservatives (tables 1 and 2).

5. Test Procedure.

Fifteen gram aliquots of the test suspensions were aseptically dispensed in sterile 20 ml tubes with screw caps. For the bacteria and Candida albicans, 100 µl of the stock suspension was added to each separate test tube. As for Aspergillus niger, 200 µl of the stock suspension was used.

Immediately after inoculation, the test tubes were mixed on a Vortex mixer for 10 sec. and the number of

TABLE 1
Active Substances and Package-form

Product	Active substances								Package-form
	Aluminium hydroxide	Magnesium hydroxide	Magnesium trisilicate	Aluminium phosphate	Calcium carbonate	Sodium alginate	Sodium bicarbonate	Dimeticone	
1	+	+							multi-dose
2	+	+							multi-dose
3	+	+							multi-dose
4	+	+							multi-dose
5	+	+						+	multi-dose
6			+		+			+	multi-dose
7	+	+							single-dose
8						+	+		multi-dose
9	+	+							single-dose
10	+	+							single-dose
11								+	single-dose
12				+					single-dose

TABLE 2
Preservatives and pH

Product	Preservatives								pH
	Methyl paraben	Propyl paraben	Butyl paraben	Sodium benzoate	Chlorhexidine	Sorbic acid	Potassium sorbate	Bronopol	
1			+						8.6
2				+					7.6
3	+	+							8.0
4				+	+				7.8
5	+		+						8.5
6	+	+					+		8.8
7			+						8.0
8	+	+							7.9
9	+	+							8.0
10	+	+							7.6
11		+					+		5.8
12						+		+	5.6

viable microorganisms present at time zero was determined by the plate-count procedure.

For one product however, due to its high viscosity, it was necessary to mix the microorganisms with the test product in a mortar. Hereafter, the inoculated product was transferred in a 20 ml test tube with screw cap.

The test tubes were then incubated at room temperature (about 21°C) for four consecutive weeks. At each one-week interval, the number of surviving microorganisms was determined by the plate-count procedure.

For the interpretation of the results, the criteria of the USP XXI (15) were followed. However interpretation criteria at the seventh and twenty first day are lacking in the USP XXI. Therefore, based on the given values at the fourteenth and twenty eight day, we propose a three percent bacterial concentration level as pass-fail criterion at the seventh and twenty first day (fig. 1).

6. Identification of the Contaminants.

For the identification of the contaminating germs an API-NE identification system (API System S.A., Montalieu-Vercieu, France) was utilized.

7. pH Measurement.

The pH of the test products was measured with a DIGI 520 (W.T.W., F.R.G.), respecting a fifteen minutes equilibration time under magnetic stirring.

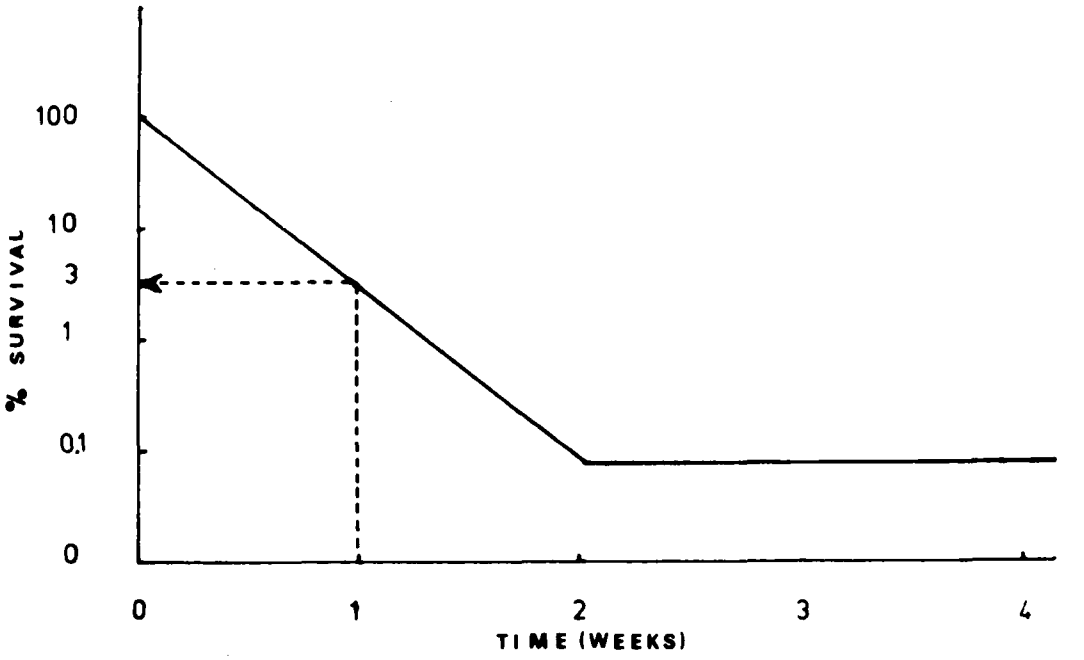

FIGURE 1

Extrapolation of the interpretation criteria for the seventh and twenty first day.

RESULTS

As can be seen from table 1, multi-dose as well as single-dose preparations were included in the study. The most common active substances were aluminium hydroxide and magnesium hydroxide : they were present in eight of the twelve products. In only four of the twelve products other preservatives than hydroxybenzoates were used. Ten of the twelve preparations had a pH higher than 7.5. Only product eleven and twelve showed a pH lower than 6.

Figures 2 and 3 show representative survival curves of the microorganisms in four of the twelve

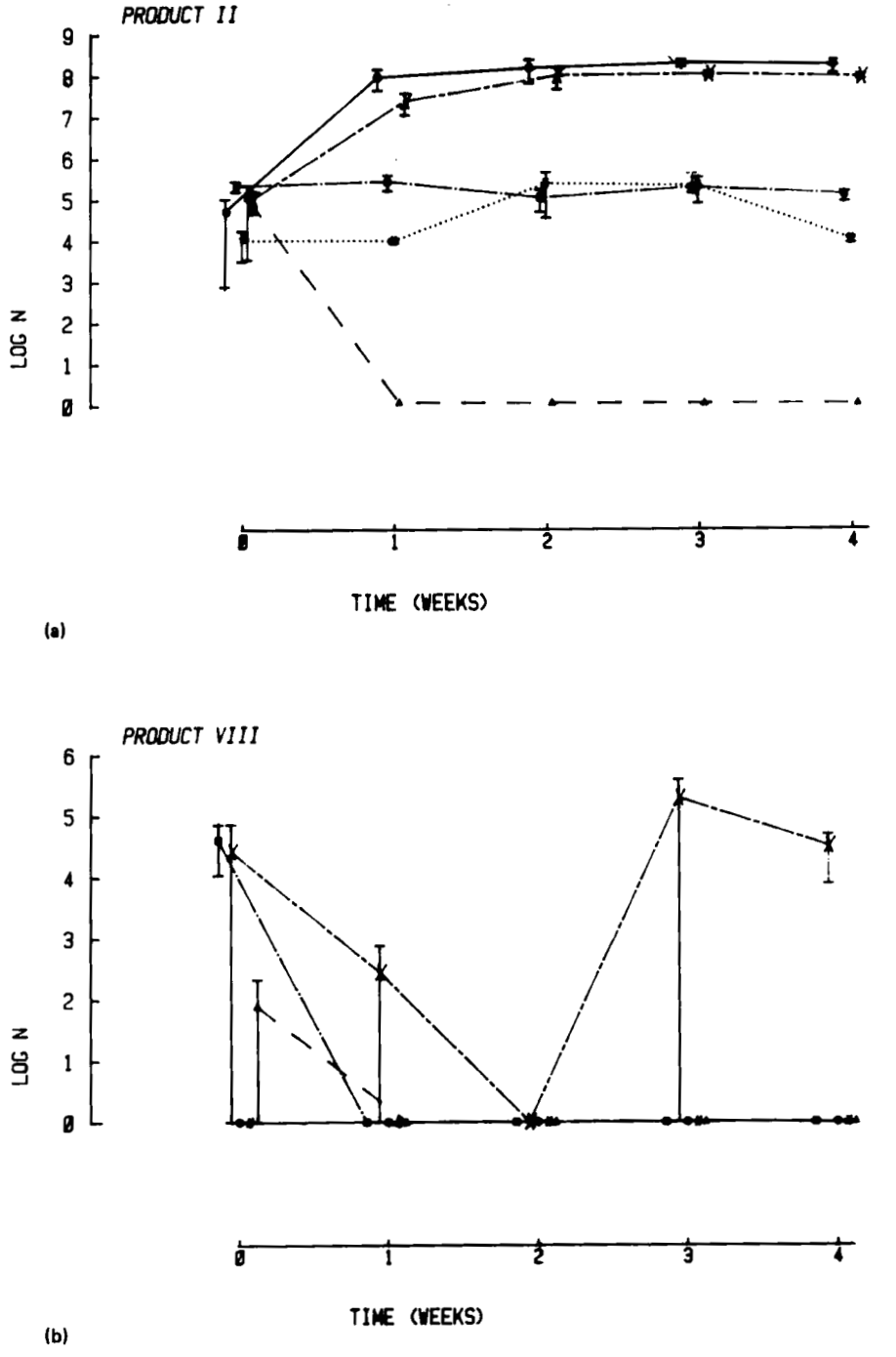

FIGURE 2

Survival curves of the microorganisms in product 2 and 8. ——·——, × : Pseudomonas aeruginosa; ——·——, □ : Aspergillus niger; —— ——, △ : Staphylococcus aureus; ——————, ○ : Escherichia coli; ·····, ✱ : Candida albicans. Log N represents the logarithmic value of the number of viable microorganisms.

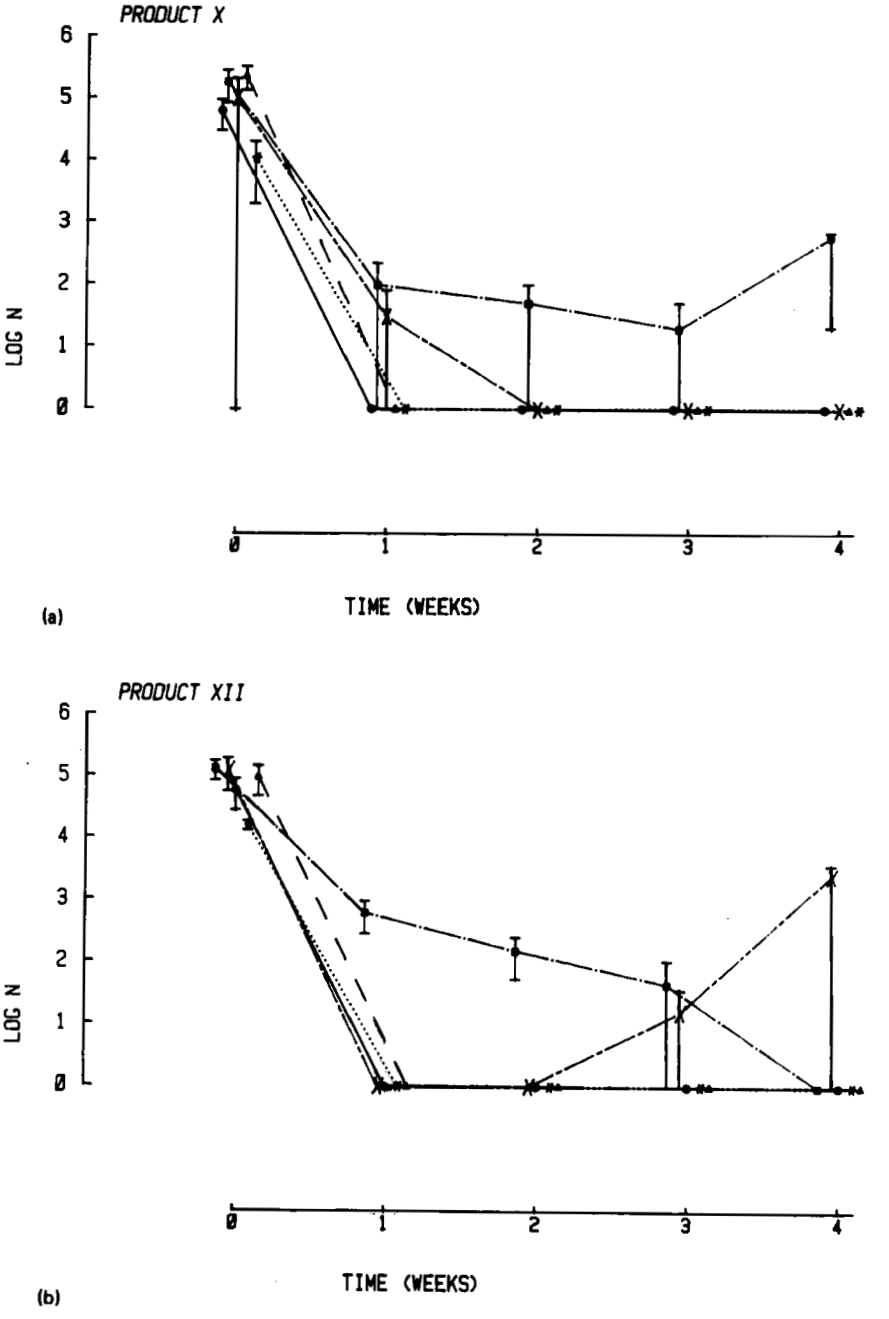

FIGURE 3

Survival curves of the microorganisms in product 10 and 12. ——- ——, × : Pseudomonas aeruginosa; ——· ——, □ : Aspergillus niger; —— ——, △ : Staphylococcus aureus; ——— , ○ : Escherichia coli; -·····, * : Candida albicans. Log N represents the logarithmic value of the number of viable microorganisms.

products. Each value represents the mean of four independent observations. Product six could not be evaluated on the effectiveness of its preservatives towards bacteria and Candida albicans due to the systematic presence of a contaminant (three different batches tested). An exact identification of the germ could not be achieved neither by the API-NE system, nor by classical biochemical reactions. However, it can be described as a non-fermenting species belonging to the Pseudomonas group.

The survival pattern of Staphylococcus aureus was identical for all (other) products. After already one week, no viable bacteria could be observed.

All products met the requirements of the USP XXI concerning the challenge test with Aspergillus niger.

The test with E. coli was not successful for the product number two and seven.

Product two definitely failed the Candida albicans test at the fourteenth day. A clear decision at the 28th day however could not be made. After three weeks, the total number of viable microorganisms was higher than the initial number in three of the four cases. However in two of these cases the observation at the 28th day was lacking, whereas one of the available observations at the 28th day met the requirements of the USP XXI and the other did not.

As can be seen from table 3, five of the remaining eleven products definitely failed the Ps. aeruginosa challenge test. In two cases, product eight and nine, no clear decision could be made at the 28th day.

TABLE 3
Evaluation of the Challenge Test

Product	Staphylo-coccus aureus		E. coli		Ps. aerug.		Candida albicans		Asperg. niger		Final evaluation
	A	B	A	B	A	B	A	B	A	b	
1	+	+	+	+	-	-	+	+	+	+	-
2	+	+	-	-	-	-	-	c	+	+	-
3	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	-	-	+	+	+	+	-
5	+	+	+	+	-	-	+	+	+	+	-
6	b	b	b	b	b	b	b	b	+	+	-
7	+	+	-	-	-	-	+	+	+	+	-
8	+	+	+	+	+	a	+	+	+	+	+
9	+	+	+	+	+	a	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+	+
11	+	+	+	+	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+	+	+	+	+

A : evaluation after fourteen days
B : evaluation after twenty eight days
+ : meets the requirements of the USP XXI
- : fails the requirements of the USP XXI
b : the test product systematically appeared to be contaminated with a Pseudomonas species
a (c) : after three weeks the total number of viable organisms was equal to (higher than) the initial number in two (three) of the four cases. However in two of those cases the observation at the 28th day was lacking, whereas the one known observation at the 28th day met the requirements of the USP XXI (and the other did not).

DISCUSSION

In seven of the twelve products, parabens were the only preservatives included in the formulation. Three of these products, product one, five and seven, definitely failed the Ps. aeruginosa challenge test.

Product seven also failed the challenge test with E. coli. The pH of these products was 8.0 or higher. At this pH, besides a possible ester hydrolysis, the parabens are partially appearing in their unactive ionized form, implicating a loss of preservative activity (2). Therefore, as it is being described that parabens are not very active against gram-negative microorganisms, their failure in these tests is not unexpected (4, 11, 13, 16, 17).

In contrast herewith, products three, eight, nine and ten pass the challenge tests, despite the not optimal pH for the parabens. However, assuming a pKa of about 8.3 (1, 2, 10), the ionization level is not as pronounced as in the above mentioned products. Furthermore in these products, butyl hydroxybenzoate is replaced by the more water soluble methyl derivate. This permits the use of a higher preservative concentration in solution, implicating a higher preservative activity, being more effective against gram-negative microorganisms.

Nevertheless, the bactericidal activity against Pseudomonas aeruginosa is not very convincing. As can be seen from figures 2 and 3, viable organisms could systematically be observed after a previous negative result (11). Bad sampling should be excluded as possible error source as this appeared with all four individual experiments. Gram stain and colonial

morphology on TSA and MacConkey medium also excluded the possibility of contamination. We believe that the negative results were obtained due to the lack of recovery of stressed microorganisms within the media used. The long lag-phase of these microorganisms could well surpass the 48-72 hours incubation period (11, 12).

Typically this phenomenon only appeared with Ps. aeruginosa and this with six of the twelve products tested. It is generally recognized that Ps. aeruginosa exhibits an adaptive or resistant mechanism towards chemical agents (5, 8, 9, 11).

For instance the ability of certain Ps. aeruginosa strains to metabolize hydroxybenzoate esters has been described by Beveridge and Hart (3). Therefore, as the total number of bacteria continually increases after a previous negative result, an adaptability of the strain towards the preservatives could well be the cause of this phenomenon.

As the rate of adaptability should not be considered as very predictable or highly reproducible, the extent of the standard error of the mean should not be interpreted as a lack of accuracy in performing the tests. On the contrary, it underlines the need of performing the Ps. aeruginosa challenge test a number of times.

In some products, the parabens are combined with other preservatives. In product eleven propyl hydroxybenzoate is associated with sorbic acid. Especially due to the low pH (5.8) and the absence of major interfering substances, the preparation meets the requirements of the USP XXI. The data of the

Aspergillus test underlines the more fungistatic activity of sorbic acid.

Product six also has potassium sorbate as preservative, next to methyl and propyl hydroxybenzoate. However these preservatives are badly choosen as at the pH of 8.8 the activity of the parabens and the sorbic acid salt is strongly reduced (16, 17). Due to this high pH, the negative effect of the magnesium trisilicate compound is probably of minor importance. Therefore the absence of excessive growth of Aspergillus niger is probably due to the unsuitable pH of the preparation. Apart from the bad choice of the preservatives, product six was also found to be systematically contaminated with a Pseudomonas species, indicating a neglecting of the GMP-rules.

As in product six, effective preservatives are also lacking in product two. Sodium benzoate, which has bacteriostatic properties below pH 5, is most likely unactive at the pH of 7.6 (13, 16, 17). The failure in the E. coli, Ps. aeruginosa and Candida albicans test is therefore to be expected. Nevertheless the Staphylococcus aureus and Aspergillus niger challenge tests were successful, in agreement with all other challenge results with these microorganisms. This suggests the influence of other factors, such as pH of the medium and nutrient facilities, on the viability of those two microorganisms in these pharmaceutical preparations.

In product four, the activity of sodium benzoate is strongly reduced due to the pH of 7.8. Because of the presence of chlorhexidine, positive results are obtained with E. coli, Candida albicans and

Staphylococcus aureus. However the preparation fails the Ps. aeruginosa test, despite the optimal pH for chlorhexidine. This should not be totally unexpected as resistance towards chlorhexidine is being described (8).

In product twelve, the combination of bronopol and sorbic acid at pH 5.6 proves to be efficient as preservative. A remarkable fact should however be stressed : although no resistance towards bronopol is described in the literature (11), viable microorganisms could be observed after a negative result was obtained with Ps. aeruginosa. However this does not interfere with the requirements of the USP XXI.

As can be seen from table 1 and 3, the majority of positive results (four of the six successful products) were obtained with preparations in single-dose package-form. However, bearing the other factors in mind, we do not believe this was a major advantage for succeeding the challenge tests.

Finally throughout this study two criteria were lacking. Although viable counts have to be performed at each one-week interval, no criteria are set up in the USP XXI for the 7th and the 21st day. As the fraction of survivors decreases exponentially with time (6), the three percent bacterial concentration level could here well be adopted (figure 1). For the viable yeasts and molds the already existing criterion at the 14th and 28th day can be used. Adopting these criteria, products three, eight and nine would additionally fail the challenge test. As their preservative activity towards Ps. aeruginosa is not convincing, this would create a more clear situation.

In conclusion, this study stresses the need for an adequate preservative policy towards oral antacid suspensions. Six of the twelve products definitely do not meet the USP XXI requirements. Therefore the choice of the preservatives should be made as a function of pH and comptability with other substances. Special attention should be made to the preservative activity towards Ps. aeruginosa. This microorganism caused major problems. The incorporation of an interpretation criterion for the 7th and the 21st day would be a substantial improvement.

REFERENCES

1. M.C. Allwood, A. Lamikanra, and S. Boydell, J. Pharm. Pharmacol., 27, 21P (1975).
2. M.C. Allwood, Int. J. Pharm., 11, 101 (1982).
3. E.G. Beveridge, and A. Hart, Pharm. J., 202, 730 (1969).
4. D.N. Entrekin, J. Ph. Sci., 50, 743 (1961).
5. F.I.P., Pharm. Ind., 34, 932 (1972).
6. M.S. Karabit, T.O. Inneskans, and P. Lundgren, Acta Pharm. Suecc., 22, 281 (1985).
7. S.R. Kohn, L. Gershenfeld, and M. Barr, J. Ph. Sci., 52, 967 (1963).
8. A.R. Longworth in "Inhibition and Destruction of the Microbial Cell", W.B. Hugo, eds., Academic Press, London, 1971, p. 95-105, p. 338-360.
9. R. Nakamori, J. Ph. Sci., 64, 1071 (1975).
10. J.A. Myburgh, and T.J. McCarthy, Ph. Weekbl., 2, 1405 (1980).
11. D.S. Orth in "Cosmetic and Drug Preservation", J.J. Kabara (eds.), Marcel Dekker Inc., New York and Basel, 1984, p. 31-77, p. 403-421.

12. J.J. Perkins in "Principles and Methods of Sterilization in Health Sciences", 2nd ed., Charles Thomas, Springfield, 1969, p. 56.
13. E.F. Reynolds in "Martindale : The Extra Pharmacopeia", 28th ed., The Pharmaceutical Press, London, 1982, p. 1281.
14. S. Turco, and R.E. King in "Sterile Dosage Forms", 2nd ed., Georg Thieme Verlag, Stuttgart, 1979, p. 370.
15. United States Pharmacopeia, XXI ed., 1985, p. 1151.
16. K.H. Wallhäusser in "Sterilisation, Desinfektion, Konservierung", 2nd ed., Lea and Febiger, Philadelphia, 1978, p. 359.
17. B. Wickliffe, and D.N. Entrekin, J. Ph. Sci., 53, 769 (1964).
18. R.T. Yousef, M.A. El-Nakeeb, and S. Salama, Canad. J. Ph. Sci., 8, 54 (1973).