Influence of Clear Liquid Shampoo Components on Preservative Activity of Parabens

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Abstract

Four ingredients, sodium lauryl ether sulfate, coconut fatty acid diethanolamide, aloe gel and disodium ethylenediaminetetraacetic acid, used as clear shampoo components, were tested for their effects on preservative activity of parabens. Each of them was formulated in various concentrations with parabens and the effectiveness of the preservative studied was determined by modifying the standard method of USP 23 using four microorganisms,i.e., *Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger* and *Candida albicans.* It was demonstrated that disodium ethylenediaminetetraacetic acid increased the effectiveness of parabens against all tested microorganisms. On the contrary, the three others components decreased the effectiveness of parabens depending on types and concentrations of the shampoo components and types of microorganisms.

1. Introduction

The parabens are the most frequently used antimicrobials found in commercial shampoo [1,2]. The activity and availability of antimicrobial preservatives in pharmaceutical preparations are greatly influened by a variety of formulating additives. Surfactants in low concentrations potentiate the activity of such preservatives by lowering the interfacial tension at microbial surface and hence facilitate the adsorption of preservative molecules on the surface of the cells. At high concentrations, they solubilize the preservatives resulting in reduction in the concentration of free preservatives available to react with the microorganisms [3]. However, nonionic surfactants form micelles in aqueous solutions at very low concentrations. For this reason, being used either as emulsifiers or solubilizers, nonionic surfactants are always present at concentrations above their critical micelle concentration. Loss of preservative activity, particularly the phenolic preservatives such as the parabens due to solubilization may be anticipated [4,5]. It has been reported that the macromolecules without surface active properties also reduce the efficacy of preservatives [1,3,6]. For instance, polyvinylpyrroridone binds the parabens to a greater extent than the polyethylene glycols, methyl cellulose, carboxymethyl cellulose or tragacanth [4]. This degree of binding, although of a far lesser order than that which occurs with nonionic surfactants, might lower some condition which necessitate the addition of a supplementary amount of preservative. Natural products are difficult to preserve because they provide such a good nutrient media for the growth of microorganisms [1,4].

Some common sequestering agents including EDTA and its salts, citric acid, and tripolyphosphates, which form soluble complexes with metal ions, are introduced to improve the activity of antimicrobial agents and to avoid discoloration of the product [7].

This article reports the effect of type and concentration of some clear liquid shampoo components on the preservative activity of parabens.

2. Materials and Methods

2.1 Test samples

Clear liquid shampoo components, including surfactant : Texapon N 8000® (Henkel;sodium lauryl ether sulfate 26.5-

28.5%), foam stabilizer : Comperlan KD® (Henkel ; coconut fatty acid diethanolamide 90 %), aloe gel (contained fresh aloe gel 100.0 g, ascorbic acid 0.12 g, citric acid 0.06 g. and sodium chloride 0.075. g) or disodium EDTA (Farmitalia Carloerba) were preserved with paraben concentrate (methyl paraben (USP/BP grade)10 %, propyl paraben(USP/BP grade) 2%, propylene glycol(USP grade) q.s. 100 %). Eleven samples were prepared for preservative challenge tests. The components of the prepared formulations are tabulated in Table 1. Purified water was used as a control.

2.2 Microbiological test

Each of the tested formulations was inoculated with each of the following microorganisms, *Staphylococcus aureus* (ATCC No.6538), *Pseudomonas aeruginosa* (ATCC No.9027), *Aspergillus niger* (ATCC No.16404) *and Candida albicans* (ATCC No.10231), using the test procedure modified from United States Pharmacopeia [8]. The bacteria were cultivated on soybean casein digest agar slants (Difco Laboratories)for 18-24 hours at 30-35°C, while the yeast and fungal strains were cultivated on sabouraud dextrose agar(Difco Laboratories) at 20-25°C for 48 hours and 1 week, respectively. All microorganisms were harvested by washing the cells from the slants with sterile saline. The growth colony in terms of colony forming units per milliliter of each organism was determined.

Twenty milliliters of the samples were placed into sterile glass bottles, one for each organism, and inoculated with the test organism (microoraganism 10^5-10^6 colonies or spores/ml sample). The containers were incubated at room temperature (30-35°C) for a total of 28 days with periodic examination. Examination was made initially and after 3, 7, 14, 21 and 28 days of inoculation. Plate counts were performed using media corresponding to those in primary cultivation.

2.3 Stability test at ambient condition

Four clear liquid shampoos were formulated for stability test. The components of the prepared formulations are tabulated in Table 2. The samples in glass containers were stored at room temperature (30-35°C) for five months. After the storage, the preservative efficacy was tested. The physical properties such as pH, viscosity, odour, colour and texture of the tested samples were also observed in comparison to the initial ones.

2.4 Interpretation

From USP 23, the preservative is effective in the examined product if (a) the concentrations of viable bacteria are reduced to not more than 0.1% of the initial concentrations by the fourteenth day, (b) the concentrations of viable yeasts and molds remain at or below the initial concentrations during the first 14 days; and (c) the concentration of each tested microorganism remains at or below these designated levels during the remainder of the 28-day test period.

Table 1 The components of formulations for microbiological test.

Ingredients	concentration used in formula No.(%)											
	1	2	3	4	5	6	7	8	9	10	11	12
Texapon N 8000®	10	20	30	40	-	-	-	-	-	-	-	-
Comperlan KD®	-	-	-	-	2	3	4	5	-	-	-	-
Aloe Gel	-	-	-	-	-	-	-	-	10	-	-	-
Disodium EDTA	-	-	-	-	-	-	-	-	-	1	-	-
Paraben Concentrate	1	1	1	1	1	1	1	1	1	1	1	-
Water qs	100	100	100	100	100	100	100	100	100	100	100	100

Table 2 The components of formulations for stability test.

Ingredients	Concentration of formula No.(%)			
	13	14	15	16
Texapon N 8000®	40	40	40	40
Comperlan KD®	2	2	2	2
Aloe Gel	10	10	10	10
Disodium EDTA	1	-	1	-
Paraben Concentrate	1	1	2	2
Water qs	100	100	100	100

Table 3 Plate counts of samples No.10-13 and 15

Sample No.	Micro organisms	Microorganism Count Per Milliter					
		Initial	3 day	7 day	14 day	21 day	28 day
10	S.a.	3.00×10^5	0	0	0	0	0
	P.a.	9.40×10^5	0	0	0	0	0
	C.a.	3.55×10^5	0	0	0	0	0
	A.n.	2.11×10^5	270	0	0	0	0
11	S.a.	6.25×10^5	20	8	0	0	0
	P.a.	6.55×10^5	5	0	0	0	0
	C.a.	2.43×10^5	6.65×10^3	0	0	0	0
	A.n.	1.00×10^5	9.00×10^3	2.90×10^3	4.70×10^3	6.40×10^2	6.70×10^2
12	S.a.	1.64×10^6	1.19×10^7	8.95×10^6	2.80×10^6	2.19×10^5	8.70×10^5
	P.a.	1.09×10^6	6.70×10^6	2.63×10^6	1.15×10^6	1.37×10^5	5.10×10^5
	C.a.	1.17×10^6	9.85×10^5	3.90×10^4	2.25×10^5	1.86×10^5	5.70×10^4
	A.n.	1.48×10^5	6.45×10^4	5.45×10^4	8.50×10^4	4.60×10^4	3.60×10^4
13	S.a.	3.20×10^5	0	0	0	0	0
	P.a.	1.63×10^6	0	0	0	0	0
	C.a.	2.69×10^5	0	0	0	0	0
	A.n.	2.76×10^5	8.70×10^4	<10	0	0	0
15	S.a.	3.00×10^5	0	0	0	0	0
	P.a.	1.76×10^6	0	0	0	0	0
	C.a.	1.99×10^5	650	0	0	0	0
	A.n.	2.09×10^5	1.28×10^5	<10	0	0	0

3. Results and Discussion

Parabens in samples with all tested concentrations of Texapon N 8000® were effective in eliminating *S. aureus* and *C. albicans*, but failed to protect from *Ps. aeruginosa* growth. Flawin, et al.(1973) showed that *Pseudomonas sp.* was able to utilize anionic detergents as the sole carbon and sulphur sources by virtue of their inducible sulphatase enzymes [9]. Although parabens effectively controlled the mold (*A. niger*) in samples with Texapon N 8000®20-40%, their effectiveness was lower than the sample without Texapon N 8000®. Moreover, parabens in sample with 10% Texapon N 8000® failed to control the mold.

Parabens in samples with all tested concentrations of Comperlan KD® failed to control all microorganisms except *A.niger*. However, parabens in sample with 5% Comperlan KD® failed to inhibit the mold growth while others with 2-4% Comperlan KD® (samples No.5-7) required longer time to reduce mold than the sample without Comperlan KD®. It was probable that the nonionic surfactant formed micelles in very low concentration. The preservative activity directly relates to the concentration of free uncombined preservative. It is expected that the range of concentrations used in the experiments be higher than the critical micelles concentration. There is a tendency for association between nonionic surfactant and parabens [4]. In addition, the effectiveness of parabens decreased because pH value of the sample with Comperlan KD® was higher than 8 [10]. Not only an appropiate preservative should be chosen, but the pH of the formulation in order to maintain the preservative effectiveness should be considered.

Aloe gel is a good nutrient for the growth of microorganisms. Parabens in sample with aloe gel failed to control *Ps.aeruginosa* and *A. niger*. Although parabens effectively controlled *S. aureus* and *C. albicans* they required longer time to reduce these micro-organisms than the sample without aloe gel.

In formulation of shampoo, EDTA in small amounts is commonly added to maintain clarity. The inherent lack of broad spectrum bactericidal activity and incompleteness of total bacterial killing effect strongly limit the use of EDTA as the sole preservative in cosmetics. Several workers have demonstrated that EDTA removes Mg^{+} ion and considerable amount of lipopolysaccharide from the outer membrane of gram negative bacteria [11]. Greater control of gram negative bacteria, especially *Ps. aeruginosa* is obtained when EDTA is used in conjunction with the parabens [2,7]. However, very few studies have been done on the system of EDTA-paraben mixtures [12]. It has been demonstrated that EDTA potentiate the activity of methyl ester against *Ps. aeruginosa* and *E. coli* [11]. The synergistic potentiation of parabens by EDTA against gram negative bacteria, gram positive bacteria, mold and yeast are shown in Table 3.

After storage for five months, only samples No.13 and 15 were physically stable. There was precipitation and mold growth observed in samples No.14 and 16. It was remarkably demonstrated that EDTA synergized the activity of preservative in samples No.13and15. Although both samples containing the components (Texapon N 8000® 40%, Comperlan KD® 2% and aloe gel 10%), affected the efficacy of parabens, disodium EDTA enhanced the activity of parabens without necessity to increase the concentration of the preservative. The mechanism was thought via decreasing complex formation between coconut fatty acid diethanolamine and parabens [4]

4. Conclusion

At 1%, Paraben concentrate (methyl paraben 10% and propyl paraben 2%) was effective against microorganisms in water. Its effectiveness,however, altered when it was used in shampoo. The efficient decrease of parabens depended on concentration and type of the components of shampoo. EDTA synergized the preservative activity of parabens. In choosing the appropiate concentration of parabens for drug and cosmetic preparations, the reaction/compatibility of this type of preservative with the components in a formula is likely to be considered. When the components, which are expected to reduce the parabens activity such as surfactant, natural product are employed in the formulation, the efficacy of the antimicrobial preservative should be evaluated. Sometimes, it is necessary to

increase the concentration of preservative or use EDTA to potentiate the preservative system without increasing the concentration of preservative as shown in this study.

5. Acknowledgement

This study was supported by a grant from Prince of Songkla University. The authors would like to thank Dr. M Chulasiri for providing the microorganisms and Dr. D Farongsang and Mr. W Wongpuwarak for helpful correcting menuscripts.

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