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Effect of Flavoring Oils on Preservative Concentrations in Oral Liquid Dosage Forms

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Abstract □ The partitioning of methyl-, ethyl-, propyl-, and butylparabens into flavoring oils from aqueous systems was studied. The partitioning is dependent on the concentration of the flavoring oil, the pH of the aqueous medium, and the nature and concentration of additives to the aqueous medium.

Keyphrases □ Flavoring oils—effect on preservative concentrations in oral liquid dosage forms, partitioning of parabens □ Preservatives—effect of flavoring oils on concentrations in oral liquid dosage forms, partitioning of parabens □ Parabens—partitioning from aqueous systems, effect of flavoring oils on concentrations in oral liquid dosage forms □ Interactions—parabens–flavoring agents in aqueous medium, effect on preservative concentrations, oral liquid dosage forms

Although the search for effective preservatives continues unabated, esters of *p*-hydroxybenzoic acid are still the most frequently used preservatives in oral pharmaceutical preparations. Several recent articles discussed the conditions in which various preservatives are effective and various conditions or agents that reduced or destroyed their effectiveness (1–3).

The effect of partitioning on the antimicrobial activity of preservatives was discussed (4). In a mixed system containing an oil phase, emulsified or as a separate layer, the concentration of antimicrobial agent required was higher than that in a completely aqueous system (5). Higher concentrations of preservatives are required to compensate for the quantities made unavailable for antimicrobial action due to adsorption, binding, or solubilization by nonionic surfactants or partitioning into a nonaqueous phase (6–11).

The theory behind partitioning of a preservative in an oil–water system was discussed (12). Several reports (7, 8, 10, 13) also described the partitioning of a preservative between two phases and the equilibria involved in partitioning and binding of a preservative to surfactants. Patel and Romanowski (12) further showed microbiologically the partitioning and binding effect of the oils and the surfactants on the preservatives. It has been noted that creams and emulsions are more difficult to preserve than aqueous solutions (14–18). The significant part played by partitioning of the preservatives between aqueous and nonaqueous phases, allowing availability of only a portion of the total quantity of the preservatives in the aqueous phase where contaminants normally multiply, has been stressed (19–22).

Flavoring agents are used in most oral liquid pharmaceutical preparations. These flavoring agents are composed of aromatic oils, natural or synthetic, and other ingredients to make them compatible with aqueous systems.

Preliminary observations in this laboratory showed that the solubility of parabens in these flavoring agents was extremely high. Since parabens are used in low concentrations, their high solubility in flavoring agents may cause depletion of the preservatives from the aqueous phase, with a consequent reduction in overall preservative activity. The purpose of this paper is to report the interaction between parabens and flavoring agents in an aqueous medium.

EXPERIMENTAL

General Partitioning Study—A 0.15% solution of methylparaben was prepared in a buffer solution of specified pH. Fifty-milliliter aliquots of the solution were transferred to 125-ml erlenmeyer flasks, and 0.5 ml of flavoring oil (1% of the total volume) was added to each flask. Respective blanks were also prepared containing flavoring oil but no methylparaben in the buffer solutions. The flasks were mounted on a wrist shaker[1] and shaken for 12 hr. The insoluble oily component in the mixture was then separated by a combination of centrifugation and column filtration technique.

The mixture was centrifuged at 10,000 rpm, corresponding to approximately 12,000 rcf. in a high-speed analytical centrifuge[2] for about 30 min. After centrifugation, the aqueous layer was separated from the floating oily layer using a Pasteur pipet. Aqueous layers from different tubes corresponding to one sample were pooled. This aqueous portion was then passed through an acid-washed kieselguhr column. The columns were prepared as follows.

A 10-ml disposable plastic syringe was plugged with a wad of glass wool of about 10-mm constant thickness. Two grams of acid-washed dry kieselguhr was weighed into the syringe. The column was lightly tapped down to a 35-mm column height, corresponding to the 6-ml mark on the plastic syringe, and the mixture was passed through the column. The first 5 ml of the effluent was re-

[1] Burrell.

[2] I.E.C. international centrifuge model H-T; rcf. = reciprocal centrifugal force.

Table I—Partitioning of Propylparaben from Acetate Buffer into Oils (1.0%)

	Concentration of Propylparaben in Aqueous Phase, mg/100 ml	
	Before Partitioning	After Partitioning
Orange oil	73.3	66.0
Lemon oil	73.3	55.6
Anise oil	73.3	48.9
Peppermint oil	73.3	12.6
Spearmint oil	73.3	0.2

Table II—Solubility (in g/100 ml) of Parabens in Distilled Water, Peppermint Oil, and Spearmint Oil

Paraben	Distilled Water	Peppermint	Spearmint
Methyl	0.25	15.68	20.54
Ethyl	0.17	19.97	23.93
Propyl	0.05	25.56	32.51
Butyl	0.02	44.17	45.59

jected, and the next 25–30 ml was collected for further work. The solutions serving as blanks contained parabens but no flavoring oil or contained flavoring oil but no parabens, and they were treated the same way.

Paraben Assay—The solutions containing parabens were assayed spectrophotometrically. The filtrates or the standard aqueous solutions were diluted first with methanol containing 20% 0.1 *N* HCl. These solutions were then further diluted with pure methanol. The absorbance of the final solution was measured[3] at 256 nm, the λ_{max} for undissociated parabens, against a corresponding blank solution. Intermittently, UV spectra of these solutions were obtained[4] to check the absence of any interference from extraneous matter as well as to be sure that parabens were present in the undissociated form. Interfering species capable of absorbing in the UV region were acetate ion (λ_{max} 205 nm), spearmint oil (λ_{max} 245 nm), and peppermint oil (λ_{max} 252 nm).

The sample solutions needed a minimum of a 3:100 dilution with acidic methanol. At this dilution the contribution to absorbance from the interfering moieties was minimal. The absorbance measurements of the sample solutions also were made against blank solutions containing the same ingredients except flavoring oil or paraben, as the case may be, in exactly the same concentrations and treated identically to sample solutions. This procedure compensated for the possible contribution in absorbance measurements from interfering moieties.

Solubility of Paraben in Flavoring Oils—Ten milliliters of spearmint oil and peppermint oil was saturated individually with methylparaben, ethylparaben, propylparaben, and butylparaben at room temperature. These solutions were shaken in the presence of an excess of solute for 24 hr on a wrist shaker. The flasks were then transferred to a 25° thermostated shaker bath for further shaking for 24 hr. The solutions were filtered through a fine-porosity sintered-glass funnel, and the filtrates were adequately diluted with pure methanol. The spectrophotometric absorbances of the solutions were then measured at 256 nm against the proper blanks.

In another experiment, the solubility of propylparaben in lemon, orange, spearmint, and peppermint oils was compared in a similar way. The solubilities of all four esters in water also were determined in a similar way except for cutting the shaking time to 8 hr to avoid possible degradation of the compounds in water. Degradation was not controlled, nor was it accounted for in the final results.

pH–Partition Studies—Acetate, phosphate, and borate buffers, pH 4–9, were prepared. Solutions of propylparaben in a concentration of approximately 20 mg/100 ml were prepared in buffer solution. Spearmint oil and peppermint oil were added to these solutions in quantities to obtain concentrations of 0.25 and 0.4%, respectively. The mixtures were equilibrated by shaking on the wrist shaker for 16 hr at room temperature, and the aqueous layer was then separated and assayed for propylparaben. Buffer solutions containing propylparaben but no oil were shaken for the same length of time to account for any possible pH-dependent degradation of propylparaben in aqueous solutions.

Effect of Salt on Partitioning—Sodium chloride or sugar was added to solutions of 0.02% propylparaben in pH 4.7 acetate buffer in concentrations ranging from 0.25 to 3.0 *M* in salt or sugar. These solutions were then equilibrated with 0.15% spearmint oil for 16 hr at room temperature. The separation of the aqueous layer and the assay of propylparaben in it followed.

[3] Beckman DU spectrophotometer.
[4] Cary model 15 recording spectrophotometer.

Table III—Effect of Concentration of Peppermint Oil on Partitioning of Propylparaben from 0.025% Solution in Acetate Buffer

Peppermint Oil, %	Concentration of Paraben, mg/100 ml
0.00	73.6
0.10	49.4
0.20	27.0
0.40	13.6
0.60	8.5
0.80	6.7

Table IV—Effect of pH on Partitioning of Propylparaben from 0.025% Solution in Buffer into 0.25% Spearmint Oil

pH	Concentration of Paraben in Aqueous Phase, mg/100 ml		C_{oil}/C_{aq}[a]
	Before Partitioning (B.P.)	After Partitioning (A.P.)	$\frac{\text{B.P.} - \text{A.P.}}{\text{A.P.}}$
5.60	79.8	9.3	7.61
6.74	79.8	11.1	6.18
7.35	79.6	13.6	4.84
7.45	79.6	14.7	4.41
8.56	79.1	39.8	0.99
9.01	79.7	55.1	0.45

[a] No volume adjustments were made.

RESULTS AND DISCUSSION

The partitioning of propylparaben from a pH 4.7 acetate buffer into flavoring oils added in a concentration of 1.0% is shown in Table I. All of the oils showed some partitioning effect. However,

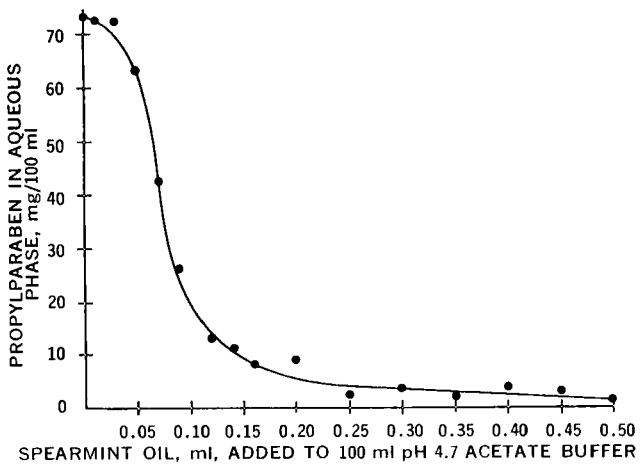

Figure 1—*Partitioning of propylparaben from solution in pH 4.7 acetate buffer into spearmint oil as a function of concentration of spearmint oil.*

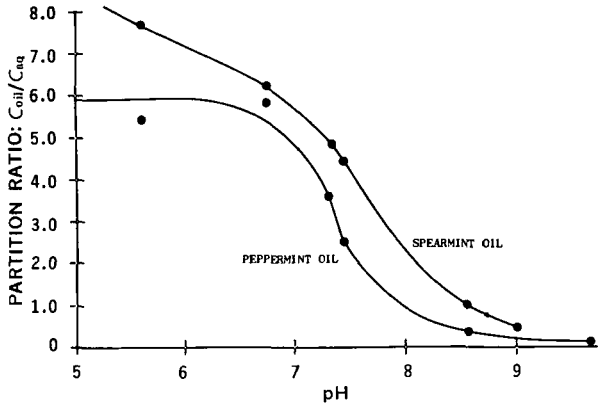

Figure 2—*Partitioning of propylparaben from solution in aqueous buffer into 0.4% peppermint oil and 0.25% spearmint oil as a function of pH of aqueous buffer solution.*

the effect was observed best with peppermint oil and spearmint oil, so further work was confined to them.

The solubilities of four esters of *p*-hydroxybenzoic acid in distilled water, peppermint oil, and spearmint oil are given in Table II. As reported in the literature, the solubility in water decreases and the solubility in oil increases with the increase in the chain length of the ester.

The effect of the concentration of the flavoring oil on the partitioning of propylparaben from its solution in acetate buffer is shown in Table III. Even at 0.1% oil concentration, almost 30% of the propylparaben partitioned into the oil. The same effect of the concentration of oil on partitioning was observed with spearmint oil (Fig. 1).

The effect of pH on the partitioning of propylparaben from a 0.025% solution in buffers into 0.25% spearmint oil is shown in Table IV. No attempt was made to calculate the true partition coefficients since the volume adjustments would give tremendously high numbers. Only ratios of concentrations of paraben in oil and aqueous phase are given in Table IV. It can be observed from the data that as the pH of the propylparaben solution is raised, the concentration of ionized species in relation to unionized species increases. Since the solubility of ionized species in the nonaqueous solvents is negligible, the amount partitioning into the oil layer decreases steadily with the increase in pH. The same observation was made with peppermint oil. The results for the effect of pH on the partitioning are shown graphically in Fig. 2 for both peppermint oil and spearmint oil. The sigmoidal curve seen for peppermint oil with its inflection point at pH 7.4 corresponds to the titration curve of a buffer species with a pKa of 7.4.

The effect of the length of the side chain in the ester linkage was investigated by determining the ratio of the concentration of the four esters in an aqueous acetate buffer and a nonaqueous dispersed phase of mixtures containing a fixed amount of added flavoring oil—0.2% spearmint oil or 0.2% peppermint oil (Tables V and VI). A plot of the partition ratio against the molecular weight of the esters in the spearmint and peppermint oils is shown in Fig. 3. From the plot it can be observed that a longer chain length causes higher partitioning into the oil. Thus, butylparaben partitions the most into the oil followed by propyl-, ethyl-, and methylparaben, in that order. This order follows the order of solubility of the

Table V—Partitioning of Parabens Using a Constant Quantity of Spearmint Oil (0.2%) in Acetate Buffer

Paraben	Concentration of Parabens in Aqueous Phase, mg/100 ml: Before Partitioning (B.P.)	After Partitioning (A.P.)	$\frac{C_{oil}/C_{aq}\ \text{B.P.} - \text{A.P.}}{\text{A.P.}}$
Methyl	59.2	36.6	0.62
Ethyl	69.1	19.2	2.60
Propyl	68.7	1.4	48.1
Butyl	47.1	0.0	—

Table VI—Partitioning of Parabens Using a Constant Quantity of Peppermint Oil (0.2%) in Acetate Buffer

Paraben	Concentration of Parabens in Aqueous Phase, mg/100 ml: Before Partitioning (B.P.)	After Partitioning (A.P.)	$\frac{C_{oil}/C_{aq}\ \text{B.P.} - \text{A.P.}}{\text{A.P.}}$
Methyl	58.8	55.7	0.06
Ethyl	74.0	51.4	0.44
Propyl	64.9	26.0	1.50
Butyl	60.1	9.1	5.77

esters in oil themselves. The longer chain length of the ester linkage imparts the characteristic of higher solubility in the nonaqueous phase (Fig. 3).

Since any solution in need of preservation would contain ionic and nonionic materials, the effect of their presence on partitioning of propylparaben from a 0.05% solution into a 0.15% spearmint oil was studied. Sodium chloride was added as an ionic material, and sucrose was added as a nonionic material (Tables VII and VIII). As the concentration of sodium chloride in solution increased, the amount of propylparaben partitioning into oil increased. This effect was conspicuously absent with sugar.

The increased partitioning of parabens in the presence of sodium chloride may be explained on the basis of the salting out of oil; the amount of oil separating increased with an increased concentration of salt in the solution. The inherent solubility of these flavoring oils is fairly low. In the presence of a strong electrolyte, some of the dissolved oil or its components—terpenes—would come out of solution. This oil would then disperse throughout the aqueous phase as a nonaqueous organic phase, exerting its full partitioning effect and causing depletion of parabens from the aqueous phase.

In the foregoing experiments, the concentration of oils used was 4–10 times the normal concentrations used as flavoring agents. The exaggerated conditions were necessary to discover the possible interaction of the flavoring oils at their normally lower levels. The solubility of the flavoring oils is dependent on the concentration of other additives, particularly ionic compounds. As noticed in the effect of concentration of sodium chloride on the partitioning of propylparaben, the ionic compound would increase the partitioning of propylparaben into oil or cause salting out of the oil from the solution which, in turn, would be available for partitioning of more

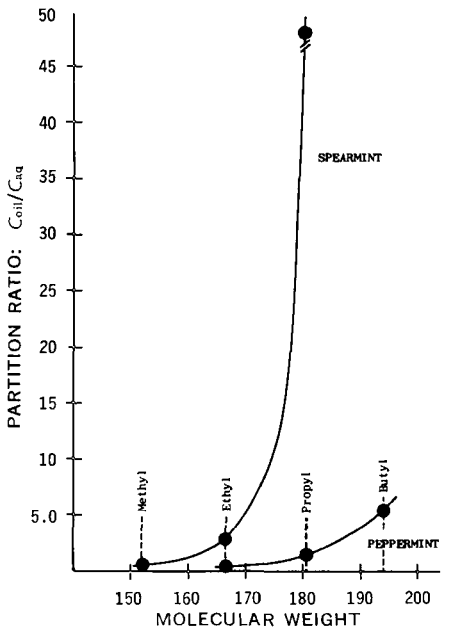

Figure 3—*Partitioning of parabens from solution in pH 4.7 acetate buffer solution into peppermint oil and spearmint oil as a function of molecular weight of parabens.*

Table VII—Effect of Sodium Chloride Concentration on Partitioning of Propylparaben from 0.05% Solution into 0.15% Spearmint Oil

Molarity of Salt in Acetate Buffer	pH	Concentration of Paraben in Aqueous Phase, mg/100 ml		$\frac{C_{oil}/C_{aq}}{\frac{B.P. - A.P.}{A.P.}}$
		Before Partitioning (B.P.)	After Partitioning (A.P.)	
0.00	4.60	77.2	52.1	0.48
0.25	4.50	77.2	46.9	0.65
0.50	4.45	77.2	38.4	1.01
0.75	4.40	77.2	33.3	1.32
1.00	4.40	77.2	23.1	2.34
2.00	4.30	77.2	9.8	6.87
3.00	4.28	77.2	2.4	31.18

paraben. The overall effect is depletion of parabens from the aqueous phase.

A higher pH would decrease the amount of parabens partitioned into the oils because of the higher concentration of dissociated paraben molecules in the aqueous medium. The dissociated molecule does not have the preservative action, so adjustment of pH to reduce partitioning would not be helpful.

An experimental antacid formulation was prepared containing a combination of methylparaben (1.0 mg/ml) and propylparaben (0.5 mg/ml) as a preservative. This formulation was flavored with anise oil, and another similar formulation was flavored with peppermint oil. The concentration of anise oil was 5.5 times that of peppermint oil.

Both formulations were challenged with *Pseudomonas aeruginosa* and *Paracolobactrum coliforme* separately with 10,000,000 and 500,000 organisms/ml. A mixed culture of the two was also used as a challenge inoculum at two levels. Over a 4-week testing period during which seeded bottles were incubated at 25 and 37° and plated on growth media, the formulation containing peppermint oil did not show growth, indicating adequacy of the preservative system. However, the preparation containing anise oil as a flavoring agent showed growth of *Pseudomonas* in the 2nd week. When the concentration of propylparaben in the anise oil formulation was raised to 0.7 mg/ml, the formulation withstood the microbial challenge test and was comparable to a control formulation. Therefore, it was concluded that in the presence of a small concentration of flavoring oils, the partitioning of parabens into flavoring oil does occur, causing a decrease in the preservative action. The antimicrobial action of the flavoring oils makes quantitative microbial evaluation of this partitioning effect in pharmaceutical formulations very difficult. A method for such evaluation is under consideration.

Table VIII—Effect of Sucrose Concentration on Partitioning of Propylparaben from 0.025% Solution into 0.1% Spearmint Oil

Molarity of Sucrose in Acetate Buffer	Concentration of Paraben in Aqueous Phase, mg/100 ml	
	Before Partitioning	After Partitioning
0.00	48.5	48.5
0.25	48.5	49.5
0.50	48.5	49.6
0.75	48.5	53.2
1.00	48.5	49.2
2.00	48.5	45.8

CONCLUSION

This study demonstrates that parabens used as preservatives in oral liquid pharmaceuticals partition into flavoring oils. The partitioning effect depends upon the concentration of the flavoring oils, the pH of the aqueous medium, and the nature of other additives. The depletion of parabens from the aqueous phase may lower the concentration of the preservatives below the critical required level for preservative action.

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