Selection of parabens as preservatives for cosmetics and toiletries

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Synopsis

Theoretical extension of Evans' application of the Ferguson principle to PRESERVATION of simple emulsions leads to the conclusion that mixtures of PARABENS are never more efficient than an equal weight of one of their components. This applies to both initial potency and capacity, the latter according "kinetic" expressions also derived from the Ferguson principle. We propose that regardless of the complexity of the product, the most efficient paraben can be identified by making direct measurements of solubilities in the complete product; the least soluble paraben is the most efficient in initial potency and, probably, in capacity as well. Microbiological and solubility data on model systems and finished COSMETICS are presented and discussed in terms of the theory.

INTRODUCTION

Many cosmetics and toiletries are preserved with mixtures of parabens, the esters of parahydroxybenzoic acid. A combination of the methyl and propyl esters in a ratio of about two to one is often used. There are many papers in the technical and trade literature in which such mixtures are recommended (1–6) but we find no clear experimental demonstration that any particular binary mixture exhibits synergism in the strict sense of being better, on an equal weight basis, than its more potent component. Neither do we find any theoretical argument that such synergy is to be expected. On the contrary, both theory and data seem to indicate that simple additivity of efficiencies is the rule.

The gist of this paper is that the Ferguson principle (7) implies that the paraben of least solubility in the product to be preserved is the best choice in both initial potency and capacity and that no combination can be better in either respect.

INITIAL POTENCY IN SIMPLE EMULSIONS

In 1965, W. P. Evans (8) showed on theoretical grounds that it is impossible for a binary mixture of parabens to be better than both of its components in a simple
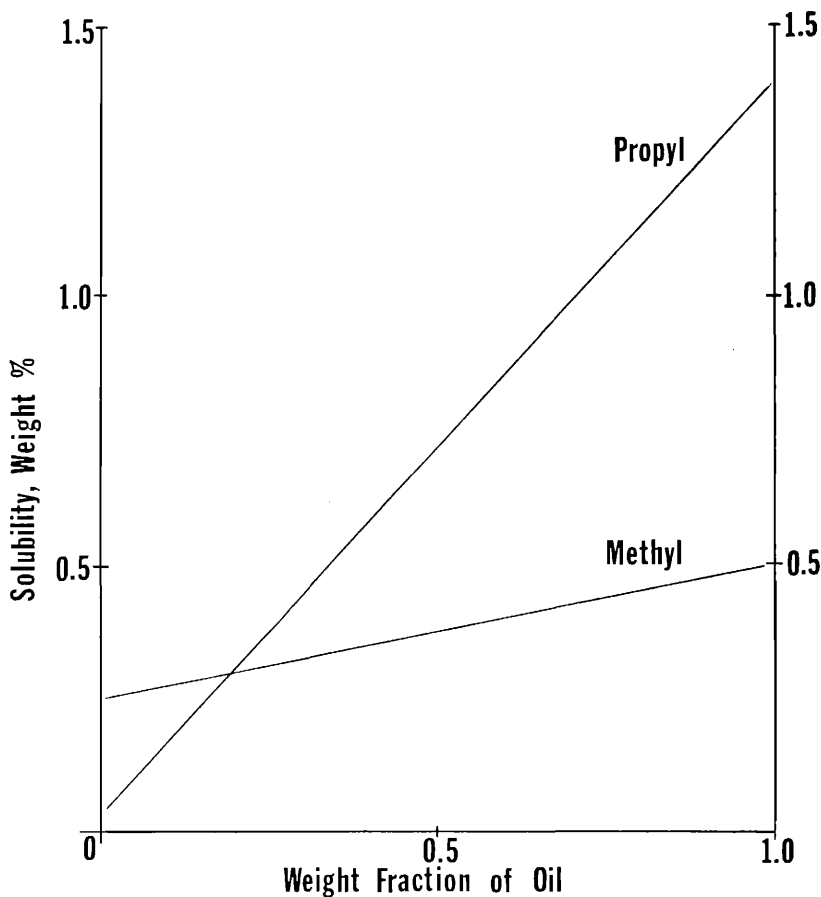
Figure 1. Solubility of methyl and propyl parabens in water/peanut oil mixtures.

emulsion—an oil/water mixture in which the only deactivation mechanism is the extraction of each paraben into the oil phase according to its own partition coefficient. Evans argued from the Ferguson principle which can be approximately paraphrased as stating that all homologous antimicrobials are equally effective, not at equal concentrations but at concentrations corresponding to the same fraction of their solubility in the medium in which the antimicrobial activity is being measured.

Evans' algebraic treatment of oil/water mixtures is re-presented in graphical form in Figure 1 which shows the apparent solubilities of methyl and propyl parabens in peanut oil/water mixtures as a function of the oil fraction. In the oil-saturated water phase, methyl paraben is much more soluble, but this is reversed in the water-saturated oil phase. Since the solubilities in all mixtures must fall on straight lines connecting the terminal points, one can calculate what Evans calls the "crossover point"—the oil fraction at which the apparent solubilities are equal.

Below the crossover point it takes less propyl paraben than methyl paraben to saturate the system and since according to the Ferguson principle the two are equally effective at saturation regardless of the concentration difference, the propyl ester is the more efficient preservative for these mixtures. By the same argument, the methyl ester is
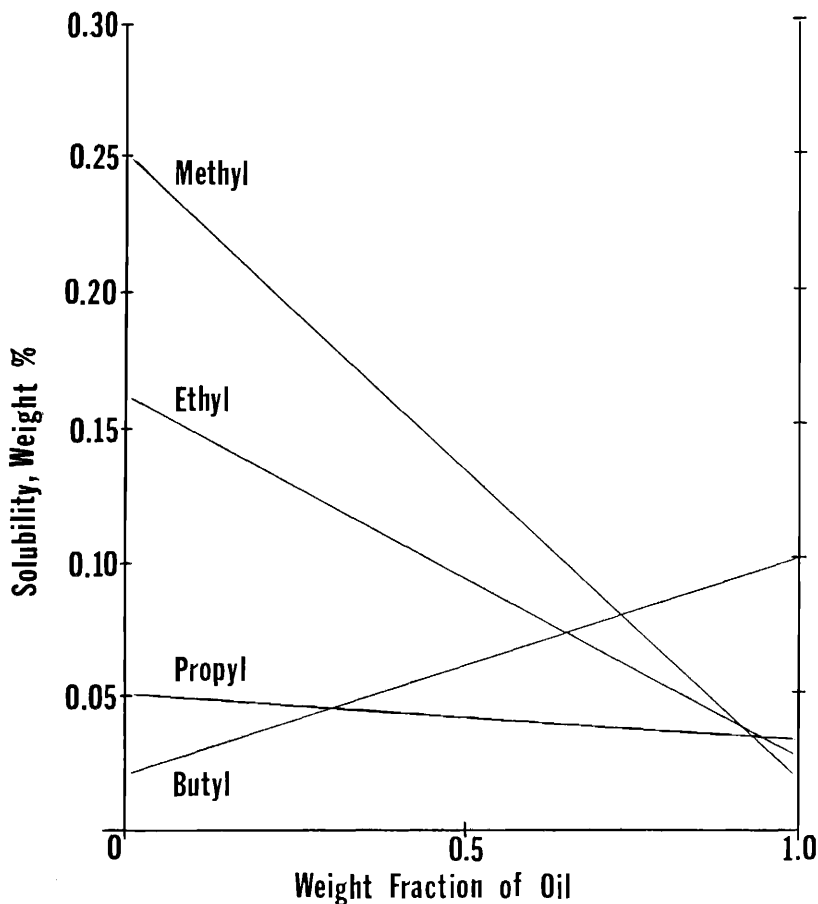
Figure 2. Solubility of parabens in water/mineral oil mixtures.

more efficient at high oil fractions. In either region—high or low oil fraction—mixtures of the two esters must have a lower cumulative saturation fraction than an equal concentration of the less soluble homologue and must be less efficient. The only exception occurs at the crossover point where the solubilities are equal; here all mixtures are as good as, but no better than, either component. Figure 2 shows similar solubility plots in mineral oil/water mixtures for the first four alkyl parabens. Without discussing each of the six crossovers in detail, one can see that at low oil fractions the butyl ester should be the most efficient preservative and that the methyl ester is best at very high fractions while the propyl ester is best at oil fractions from 0.3 to 0.9.

COMPLEX SYSTEMS

The rule emerging from Evans' analysis of simple emulsions, that the paraben of lowest apparent solubility in the emulsion is the most efficient one, can be extended to systems of any degree of complexity on grounds of the thermodynamic principle that at equilibrium the chemical potential of every component of the system is the same in all accessible phases; in particular, if the system is saturated with one of the parabens,

the chemical potential of that paraben is equal in all phases to that of the pure crystal phase at the given temperature and pressure. Since, according to the Ferguson principle, the thermodynamic activity relative to the saturated solution is the appropriate measure of antimicrobial potency, all saturated systems are equally antimicrobial.

To paraphrase this statement in practical terms, if the unpreserved product can support microbial growth it will be rendered equally antimicrobial by saturating it with any one of the alkyl parabens regardless of big differences in their saturation concentrations. It follows that the least soluble paraben is the most efficient one.

If it can be assumed that the thermodynamic activity of each paraben is equal to its saturation fraction (the ratio of concentration to saturation concentration) and that these fractions are additive in applying the Ferguson principle to mixtures, then it also follows that at any concentration the least soluble paraben is superior to all equal weight mixtures. For example, if the methyl ester is the one least soluble in the product to be preserved and if it is measurably effective at, say, 0.75 of its saturation concentration, the same efficacy will be observed with a combination of methyl paraben at half saturation plus propyl paraben at 0.25 saturation or with any three parabens at 0.25 saturation, and so forth; all such mixtures will constitute a greater total weight concentration than methyl paraben alone and will, in this sense, be less efficient.

The conclusion that at saturation the paraben least soluble in the product is the most efficient preservative follows directly from the Ferguson principle, but the argument regarding mixtures is not rigorous since thermodynamic activity is only roughly proportional to concentration and at high concentrations the deviations from proportionality can be large. It seems unlikely, however, that the forms of the deviations should differ so much from one homologue to another as to cause inversions in the order of efficacy predicted rigorously at saturation.

We propose then, as a working hypothesis, that the least soluble paraben is the most efficient one over its entire range of solubility.

ANTIMICROBIAL CAPACITY

Thus far we have discussed the efficiency of preservatives only in terms of their initial potency, but the capacity to resist many challenges or a massive inoculation must be an equally important factor because it is known that antimicrobials are decomposed or sequestered by the organisms they attack. Apart from this consideration, our working hypothesis would lead to the absurdity of recommending an infinitesimally soluble paraben such as a long-chain fatty alcohol ester for use in a simple aqueous system (although the Ferguson principle itself breaks down when applied to high molecular weight homologues, perhaps, in part because of the capacity factor). Obviously, the concentration of the antimicrobial in the phases where the microbes grow must be high enough to permit transport of a damaging dose to the surfaces of the microbes in a time comparable to their vegetative period.

Within this conservative limit, is it likely that the least soluble homologue is consumed so much more rapidly and ineffectively than a more soluble homologue or mixture of homologues as to vitiate its advantage in initial potency? We try here to answer this

question by deriving a capacity function from the Ferguson principle as interpreted in terms of the partitioning of the antimicrobial between the medium and the microbe (see, for example, Reference 9).

In its simplest form, this interpretation would predict that at any given thermodynamic activity (as defined above), all members of a homologous series should be present within the microbe "phase" at the same concentration. The data reported by Lang and Rye in 1972 (10) support this interpretation very nicely. Their radiochemical measurements show that the intracellular concentrations of methyl, ethyl and propyl parabens are the same when *Escherichia coli* are in contact with equipotent solutions. The external concentrations are quite different on a weight percentage basis but they correspond to about the same saturation fraction.

The theory and these observations can be expressed as:

$$c_i' = k^* s_i \tag{1}$$

where c_i' is the concentration in the microbe "phase" of the ith paraben and s_i is its equilibrium saturation fraction in the medium. The Ferguson principle is manifested here in the absence of a subscript on the proportionality constant, k^*, which has the same value for all homologues (for a given medium and for a particular species of microbe).

On the untested but plausible assumption that the partitioning of each homologue is independent of the presence of other homologues in both phases, we can write:

$$C' = k^* \Sigma s_i = k^* S \tag{2}$$

where C' is the total concentration of all parabens in the microbe phase and S is the equilibrium cumulative saturation fraction in the medium.

Generalizing again from the Lang and Rye data, we suppose that there is some internal cumulative concentration C' at which the microbes are incapacitated to a degree that satisfies the criteria of preservation. We can now define the capacity of any mixture of homologous preservatives as the size of the inoculum which reduces S from its initial value to that corresponding to the critical value of C' in eq 2.

Assuming first that the process leading to eq 1 is reversible, as though the inoculum were an inert "oil" phase into and out of which the chemically unchanged preservative freely diffuses, the capacity of a single preservative is given by substitution of eq 1 into the conservation equation:

$$c_i^o V = c_i V + c_i' v \tag{3}$$

where c_i^o and c_i are the initial and equilibrium values of the preservative concentration in the bulk volume, V, and v is the volume of the microbe phase.

This substitution gives the decay equation:

$$s_i = s_i^o \bigg/ \left(1 + \frac{k^*}{\sigma_i} \frac{v}{V}\right) \tag{4}$$

where σ_i is the apparent solubility of the paraben in the system.

The capacity function for a single paraben is given by rearrangement of eq 4 to:

$$\frac{v_c}{V} = \frac{1}{k^*}\left(\frac{c_i^o}{S_c} - \sigma_i\right) \quad \text{(but see the Appendix)} \tag{5}$$

where v_c is the inoculum volume which reduces s_i from its initial value to S_c, the critical value below which the preservation criteria are not satisfied.

Since v is the total volume of the microbe inoculum (Lang and Rye presumably report their internal concentration data on this basis) the ratio v/V can be converted to the number of microbes per unit volume by dividing by the volume of a single microbe.

To use eq 5 to compute capacities, we need values for the microbiological constants k^* and S_c in addition to the solubilities, σ_i. The work reported by Lang and Rye can be used to calculate consistent sets of these constants. These authors found intra- to extracellular concentration ratios of 3.5, 6.4 and 18.5 for methyl, ethyl and propyl parabens over a wide range of external concentrations equitoxic to *Escherichia coli*. According to eq 1, this ratio is given by:

$$c_i'/c_i = k^*/\sigma_i \tag{6}$$

Using the solubilities of these three parabens as given in the trade literature (2.5, 1.6 and 0.5 g/l) the corresponding values of the Ferguson constant, k^*, are 8.8, 10.2 and 9.3 g/l. The mean value, 9.4 g/l, was used in eq 5 to compute the capacities shown in Table I for a 50% reduction in the growth constant which occurred at an average external saturation fraction of 0.27 (S_c in eq 5). The cell volume of *E. coli* was taken as 5 cubic microns.

The initial concentrations chosen for Table I are the respective saturation levels for the first four alkyl parabens. Capacities for other initial concentrations are shown graphically in Figure 3. This figure shows that at an initial concentration of 1.0 g/l, for example, the capacities of methyl and ethyl paraben are about 2.6 and 4.3 $\times$ $10^{10}/cm^3$; the propyl and butyl esters would have capacities of 6.7 and 7.6 $\times$ $10^{10}/cm^3$ on the same basis if they were soluble to this extent (they are not, but we may suppose that these capacities would be realized if the excess over their solubilities were well dispersed in the medium).

Figure 4 shows the capacities predicted by eq 5 for $S_c = 0.5$ which is roughly the mean saturation corresponding to the concentrations at which the growth rate constant is zero in Lang and Rye's Figure 1 (1). Since this corresponds to complete inhibition of growth, it is closer to a practical criterion of good preservation. The slopes of the

Table I

Capacities of Alkyl Parabens for 50% Reduction of Growth Rate Constant of *E. Coli* in Water at 25°C

Paraben	Soly., g/l	$N_c \times 10^{-9}/ml$			
		Initial Conc., g/l			
		0.2	0.5	1.6	2.5
Methyl	2.5	nil	nil	73	144
Ethyl	1.6	nil	5.4	92	163[a]
Propyl	0.5	5.1	29	115[a]	186[a]
Butyl	0.2	12	35[a]	122[a]	193[a]

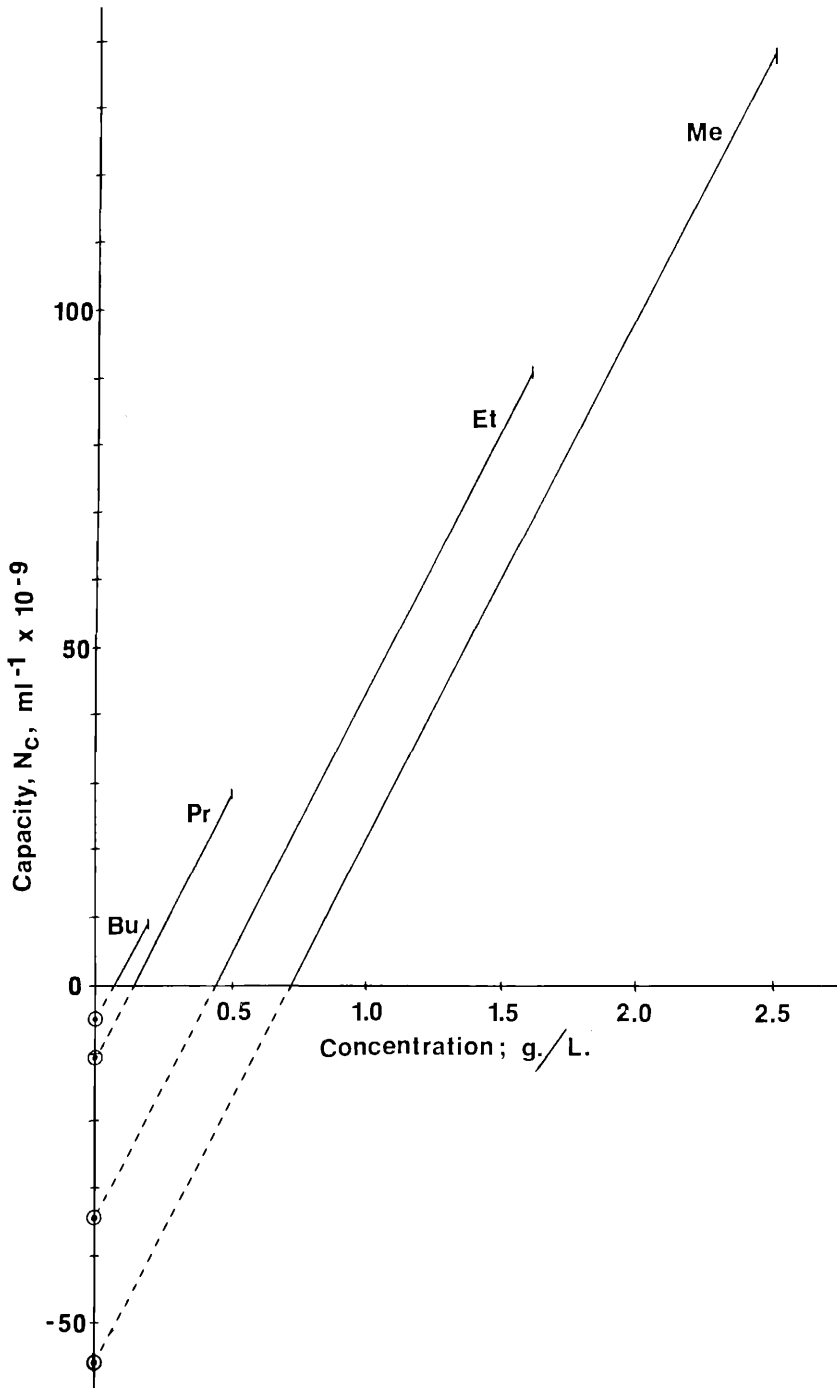[a]Initial concentration greater than solubility.

Figure 3. Capacity of parabens to cause 50% reduction in the growth rate constant of *E. coli* in water at 25°C.
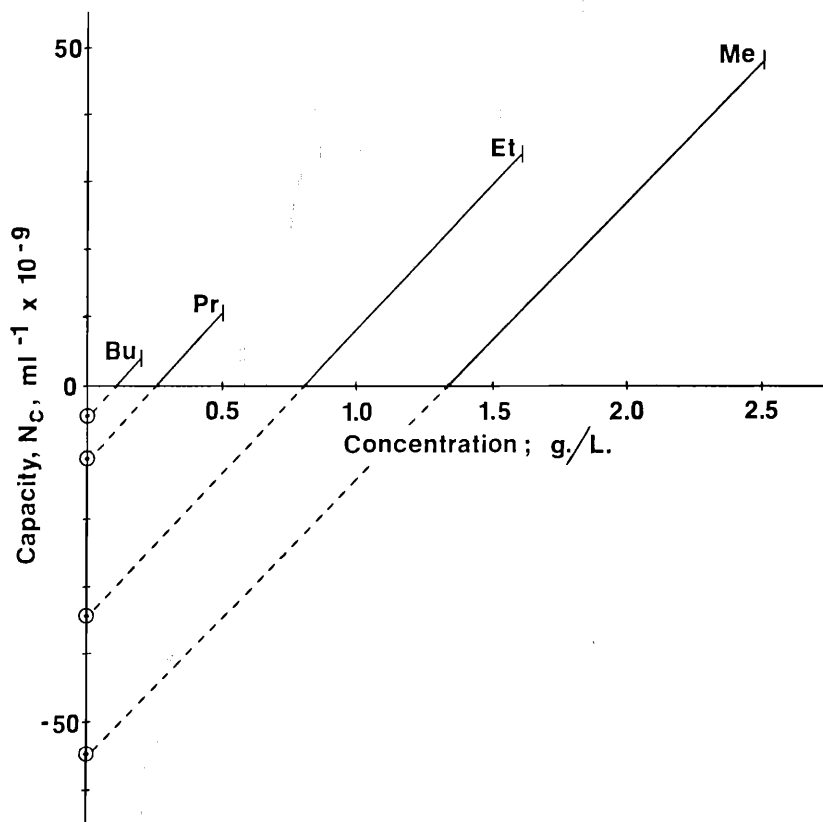
Figure 4. Capacity of parabens to reduce to zero the growth rate constant of *E. Coli* in water at 25°C.

capacity lines are about half those in Figure 3, but note that for each paraben the line comes from the same negative intercept of σ_i/k^* on the capacity axis.

As the critical saturation fraction approaches unity, the capacities of all the homologues vanish simultaneously; at any point short of this, the generalization holds that the least soluble homologue has the greatest capacity at any initial concentration within its solubility range.

The mathematics of the capacity of mixtures is more complex because substitution of eq 4 in eq 2 gives a decay function of the same order as the number of components. It can be shown by consideration of the reciprocal decay function:

$$1/s_i = \frac{\sigma_i}{c_i^o} + \frac{k^*}{c_i^o}\frac{v}{V} \qquad (7)$$

that the saturation fraction function of all binary mixtures in a plot of $1/s_i$ against v/V is bounded by the parallel straight lines representing an equal weight of the individual components. Such mixtures must then be inferior to their less soluble component for all levels of inoculation.

The reversible partitioning model predicts that the least soluble homologue is not only

superior in initial potency and capacity to all other homologues but to all possible binary mixtures as well.

In a previous publication (11) on this subject, we discussed an irreversible binding mechanism expressed as:

$$c_i' \, dv = -V dc_i \tag{8}$$

Here it is assumed that the inoculum is added incrementally and that each increment, dv, consumes an increment dc_i of the ith paraben. Substitution in eq 1 and integration gives the decay equation:

$$s_i = s_i^o \exp\left(-\frac{k^*}{\sigma_i}\frac{v}{V}\right) \tag{9}$$

(see Footnote 1) which rearranges to the capacity function:

$$\frac{v_c}{V} = \frac{\sigma_i}{k^*} \ln \frac{c_i^o}{S_c \sigma_i} \tag{10}$$

Because the decay plots for two homologues at the same initial concentration intersect as shown in Figure 5, it cannot be said unequivocally that the less soluble homologue maintains the higher saturation fraction for all degrees of inoculation. It appears, however, that unless the solubility difference is negligible, intersection occurs only after massive inoculations corresponding to near exhaustion of the preservative and for all practical cases the same solubility rule holds: the less soluble homologue has the greater capacity. In any case, as demonstrated graphically in Figure 5, no mixture of the two homologues can have a greater capacity than the better of the two, because the decay plots of all possible mixtures pass through the intersection point; mixtures can be dismissed again on the grounds that they are, at best, as good as the better of any two homologues.

PRACTICAL APPLICATION

In describing the optimum preservative system based on parabens of known solubility, we assume that the reversible partitioning model is valid. This eliminates consideration of the ambiguities in the capacities which result from the intersection of the irreversible curves. This is probably not a serious omission because even if the irreversible model is correct, it predicts different rankings only for small differences in solubility and only at the low saturation fractions corresponding to large inoculations. From our own experience in product preservation, it appears that the parabens are effective only near saturation and this is supported by Mitchell's data (12) showing that chloroxylenol is ineffective at saturation fractions below 0.9.

Having put aside the complication of intersections in the decay functions, the problem of selecting the best preservative system is reduced to that of selecting the paraben or paraben mixture with the highest initial cumulative saturation fraction; in the absence of intersections, this system will maintain the highest saturation fraction for all levels of inoculation and, therefore, it must have the greatest capacity as well.

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[1]This equation is obtained from the first term of the series expansion of eq 4.
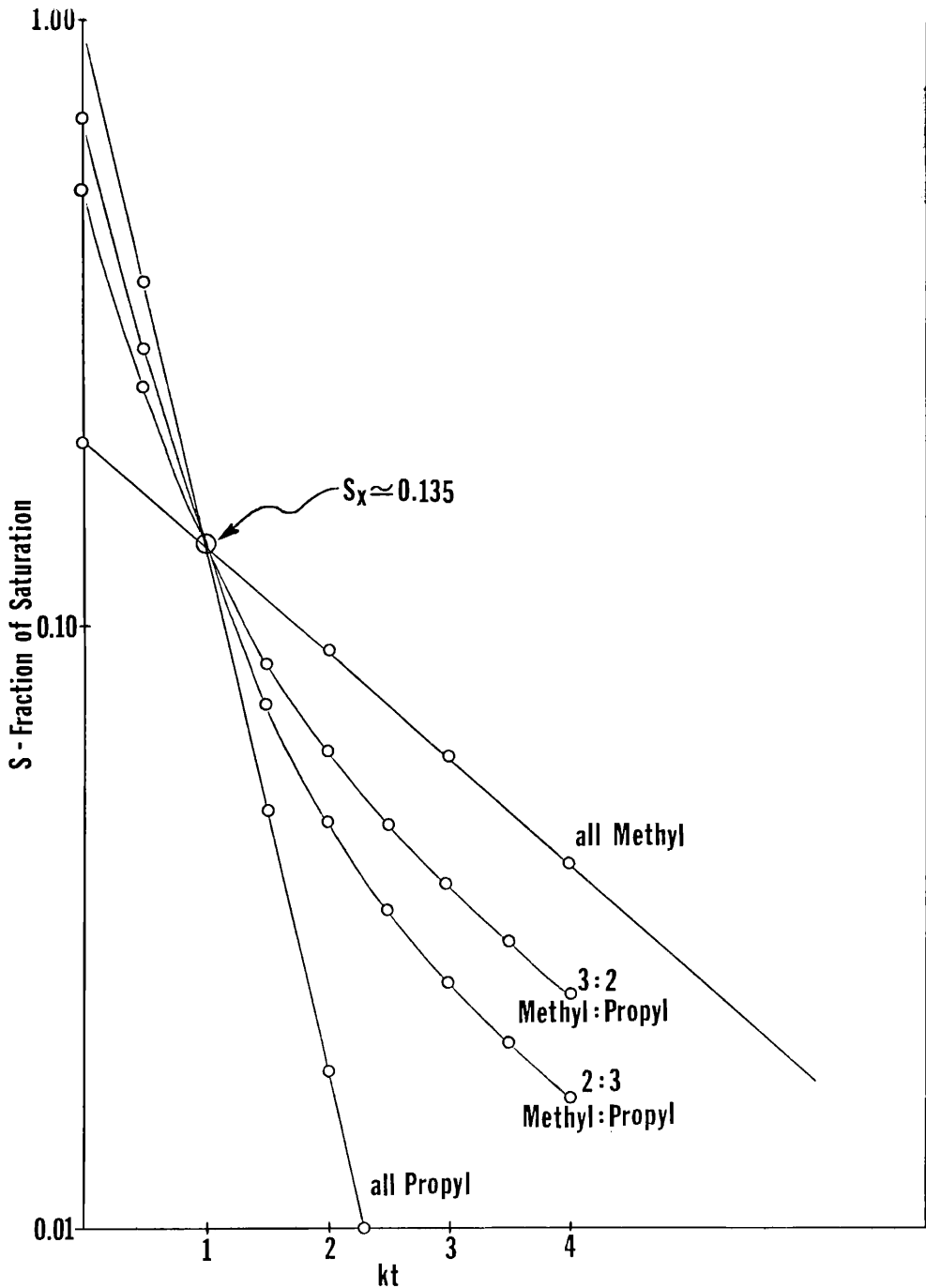
Figure 5. Decay curves of methyl and propyl parabens and mixtures at 0.5 g/l initial concentration in water at 25°C according to the irreversible, incremental model. The abscissa is in arbitrary units proportional to inoculum volume.

Assume, now, that the solubilities of the available parabens have been measured directly in the product to be preserved. If the lowest solubility is equal to or greater than the maximum permissible paraben level, which is about 8 g/l in current practice, then the most efficient system is obtained with this paraben alone; all other parabens and mixtures have been shown to be inferior. If this does not suffice, the product cannot be preserved with parabens alone as we have found to be the case with several shampoo and liquid bubble bath formulations in which we find minimum solubilities of 2 to 5%.

If, as happens less frequently, one or more of the solubilities is appreciably less than 8 g/l, the optimum system is formulated by taking the saturation concentration of the least soluble, plus the saturation concentration of the next least soluble, and so on until the total is 8 g/l. For example, in water, the solubilities of butyl, propyl, ethyl and preservation, it would be expedient for many good reasons to saturate with only two or three homologues but several precautions should be noted.

First, since the solubilities of the parabens increase rapidly with temperature, it is not possible to formulate for saturation at, say 37°C, without risking unsightly and disruptive recrystallization at low storage temperatures.

Second, the capacity factor must be considered again. Table II shows the capacity of the first four alkyl parabens at saturation in water for various assumed values of S_c ranging from 0.5 to 0.99. These values were calculated from eq 5 for the single parabens and from the quadratic equation for the capacity of binary mixtures obtained by substitution of eq 4 in eq 2.

Considering first the single parabens, it is evident that homologues much less soluble than the butyl ester would make negligible contributions to the capacity regardless of the critical saturation fraction and it would be pointless to include them.

The most striking feature in Table II is the enormous advantage of multiple saturation, especially at high values of S_c. This may appear to contradict our earlier dismissal of mixtures, but it must be emphasized that the enhancement of capacity cannot occur in equal weight comparisons; it occurs only when the system can be brought to saturation with at least one homologue which is seldom the case with modern cosmetics and toiletries. It should also be emphasized that the enhancement is not a matter of synergistic interaction; on the contrary, it follows from the assumptions of independence and simple additivity according to our extension of the Ferguson principle to cumulative saturation fractions greater than one.

Table II
Capacities of Alkyl Parabens and Mixtures at Saturation Against *E. Coli* in Water at 25°C

| | | $N_c \times 10^{-9}$ per ml | | | | |
| | | S_c | | | | |
Paraben	Soly., g/l	0.5	0.75	0.90	0.95	0.99
Methyl	2.5	53	18	5.9	2.8	0.54
Ethyl	1.6	34	11	3.8	1.8	0.34
Propyl	0.5	11	3.5	1.2	0.56	0.11
Butyl	0.2	4.3	1.4	0.47	0.22	0.043
Methyl & Ethyl	4.1	129	71	52	47	43
Propyl & Butyl	0.7	21	12	8.3	7.5	6.9

EXPERIMENTAL

We are exploring this approach in both a simple model system and in cosmetic products as they are formulated for the market.

The objective in the model system work is to test Evans' treatment of simple emulsions. The system is a simple olive oil/water emulsion aseptically prepared with a minimum level of emulsifier (to permit representative sampling after mixing). Typical compositions studied to date are given in Table III.

Table III
Composition of Oil Water Emulsions (%)

	50/50 Oil/Water			20/80 Oil/Water		
	Control	1.2% Methyl	1.2% Butyl	Control	0.6% Methyl	0.6% Butyl
Olive Oil[a]	49.90	49.35	49.35	19.98	19.86	19.86
Water	49.90	49.35	49.35	79.92	79.44	79.44
Myrj 52[b]	0.05	0.05	0.05	0.05	0.05	0.05
Brij 72[b]	0.05	0.05	0.05	0.05	0.05	0.05
Methyl Paraben[c]	0.00	1.20	0.00	0.00	0.60	0.00
Butyl Paraben[c]	0.00	0.00	1.20	0.00	0.00	0.60
	100.00	100.00	100.00	100.00	100.00	100.00

[a]Progresso.
[b]ICI, Americas Inc.
[c]Tenneco.

Methyl and butyl paraben were compared at 0.6 and 1.2%, just below the measured solubilities of the former, 0.65 and 1.25% in 20 and 50% oil mixtures respectively. In these same mixtures, the butyl paraben solubilities are much greater, 3 and 7%, and consequently this preservative is present in both comparisons at less than half its saturation concentration.

Fifty (50) gram aliquots in 125 ml Erlenmeyer flasks were inoculated with a 24-hr culture of *Pseudomonas cepacia* adjusted to give a count of either 1.8×10^3 or $1.8 \times 10^5/ml$. The capped flasks were mixed continuously on a gyratory shaker. On day 14 respective aliquots were inoculated again at 2.2×10^2 or $2.2 \times 10^4/ml$ and on day 25 at 4×10^2 or $4 \times 10^4/ml$. Standard plate counts were performed on each work day from days 0 through 31.

In both unpreserved 20% oil mixtures (low and high inoculation levels) the recoveries were 10^7 to $10^8/ml$ throughout the test period. In the unpreserved 50% oil mixtures the recovery increased to $10^8/ml$ over the first few days and remained at that level thereafter. The emulsions with methyl paraben near its saturation concentration gave no recoverable counts at any time during the test period. The undersaturated butyl paraben emulsions gave somewhat reduced counts on day one, but by day two the counts had risen to $10^6/ml$ and increased to greater than 10^7 throughout the test. Overall, the butyl paraben systems were not markedly different from the unpreserved controls.

In a similar test of the 20% olive oil emulsion with methyl and butyl parabens at levels corresponding to the same saturation fractions (as estimated from our solubility measurements), we found methyl paraben very effective (no recoveries) at 0.5 and 0.4%

and marginally effective at 0.3%; the last is below half saturation. Butyl paraben, on the other hand, gave no significant reduction in count relative to the unpreserved control even at 2.75%, or about 90% of saturation. This result is in conflict with expectation based on Evans' analysis but it supports, albeit inexplicably, our qualitative rule that the less soluble preservative is preferable.

In the standard challenge test of products being developed, we inoculate with resuspended 24-hr cultures of bacteria grown in nutrient broth and fungi grown on low pH Mycophil agar. Bacterial samples are incubated for 18–48 hr at 32°C, fungi for five days at 20–23°C. The challenge organisms include *Pseudomonas aeruginosa*, *Enterobacter hafnia*, *Aspergillus niger* and *Enterobacter agglomerans*. In some cases, we use both ATCC strains and wild strains isolated from cosmetics.

Following are summaries of observations in several cases where products in development were compared at equal weight concentrations of single and mixed parabens:

1. A heavy night cream with 0.8% methyl parben gave no recoveries at any test date in a three inoculation study over 11 weeks. The same product with the conventional 0.5% methyl paraben, 0.3% propyl paraben gave high counts in all tests after the fourth week; and with 0.8% propyl paraben high counts were recovered throughout the test.
2. An eyeliner with 0.8% methyl paraben gave no recoveries at any point in a two-inoculation study while its counterpart with 0.5% methyl paraben, 0.3% propyl paraben, gave high counts in all samples.
3. A glycerine/rose water hand cream, which gave high mold counts from the outset and high bacterial counts after the fourth week when preserved with 0.2% methyl paraben and 0.15% propyl paraben, gave only low mold counts at week four and no counts on any other test date when preserved with 0.35% methyl paraben alone.
4. A moisturizing lotion with 0.5% methyl paraben and 0.3% propyl paraben and its counterpart with 0.8% propyl paraben both gave high counts throughout a two-inoculation test. The same product with 0.8% methyl paraben gave no counts during the first three weeks of the test but gave high counts at the fourth week and thereafter.

Although we have not always succeeded in satisfactorily preserving problem products with a single paraben, we have not yet found an instance of better preservation with a mixed paraben system than with an equal weight of a single paraben.

DISCUSSION

The "problem" products are those with high levels of vegetable oils (fragrance oils probably contribute to the problem as indicated in reference 13) emulsified with nonionic surfactants and thickened with weak acid polyelectrolytes at pH's above 7.0.

The generally greater efficacy of methyl paraben in such systems is probably attributable, in part, to its lower solubility in vegetable oils as shown in Figure 1. We have also found methyl paraben much less strongly adsorbed on talc and less soluble in shampoos and liquid bubble baths, products with high concentrations of solubilizing surfactants.

Perhaps all of these observations on methyl paraben are reflections of the fact that it is the most water soluble member of the paraben series and, hence, the least susceptible to deactivation in many emulsion systems. This would suggest that a somewhat more

hydrophilic analogue would be a still more effective preservative for currently fashionable cosmetics and toiletries.

APPENDIX

The effects of methyl, ethyl and propyl parabens on the growth rate constant of *Escherichia coli* as reported by Lang and Rye (10) can be represented as:

$$k = k^\circ(1 - as_i) \tag{11}$$

where k° is the rate constant in the absence of preservatives and s_i is the saturation fraction of any one of the parabens. The dimensionless empirical constant a has the value of 2.0 from the data reported ($k = 0$ at $s_i \simeq 0.5$). For small values of the microbial volume (v in eq 4), s_i is approximately the apparent saturation fraction in the uncontaminated product; but for large values of v corresponding to large inocula or long incubation times, s_i is diminished according to eq 4 and k may then tend to return to k°. Since we assume that the parabens partition equally and reversibly into live and dead organisms, the dependence of the growth rate constant on time will depend on the inhibition mechanism invoked, whether by killing or by increasing the vegetative period. If a killing mechanism is operative, the cell volume, v, is not given by a count of recoverable viable organisms but it might be given by a measure of absorbance or turbidity if these methods respond to both dead and viable cells.

In any case, it appears that eq 5 gives only the capacity for reduction of the initial value of the growth rate constant; if this is greater than zero, the total cell volume increases without limit and any finite amount of preservative will eventually be overwhelmed (assuming, of course, that growth is not limited by exhaustion of nutrients or other external factors). In this sense, the non-zero capacity values given in Table I are fictitious.

The capacity then, appears to be rigorously definable only for high preservative levels which monotonically reduce the count of viable organisms. It also appears that there are intermediate preservative levels which will cause a temporary reduction in viable count followed by resumption of growth.

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