term, and the Flory–Huggins factor. Curve D represents the contribution of the geometric mean term. Although it intersects the experimental data points and, therefore, is a good approximation by itself, it does not seem to account for the chain-length effect. The Flory–Huggins term is shown as line C, and this line is essentially parallel to the experimental values. The sums of the two terms are labeled line A. It is obvious that line A predicts a higher $\log(k_{h/o})$ than the observed values. Thus, to best describe the data, all three terms are apparently needed.

Application to Other Systems—Although the method of Prausnitz has been further extended to cover polar–polar mixtures (13), Eq. 3 cannot be applied to other solvent pairs reported previously because of the lack of means of estimating Ψ_{ni} and l_{ni} in polar mixtures. Furthermore, it was difficult to estimate reliable corrected $\log(k_{j/i})$ values from the oil–water partition data due to significant mutual solubilities of the solvents.

The introduction of the Flory–Huggins factor and the deviation term to Eq. 1 appears to be justified in at least nonpolar mixtures. In this case, the expression becomes:

$$RT \ln(\gamma_{ni}) = V_n(\lambda_n - \lambda_i)^2 + 2V_n l_{ni}\lambda_n\lambda_i + RT[\ln(V_n/V_i) + 1 - (V_n/V_i)] \quad \text{(Eq. 10)}$$

Figure 3 compares the calculated contributions from Eq. 10 and the experimental data of Cruickshank *et al.* (14). While the geometric mean term always gives nonnegative contribution to the free energy of solution, the combination of the geometric mean term and the Flory–Huggins factor seems to improve the correlation. The poor fit of cyclohexane and benzene suggests that the deviation term is an important factor for the two solutes whose molecular geometry differs greatly from that of the solvent *n*-octadecane.

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Reduction of Activity of Cyanocobalamin in the Presence of Methylparaben Sodium at Autoclave Temperature

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Abstract □ Reduction of activity of cyanocobalamin (vitamin B_{12}) due to degradation or interaction with methylparaben sodium was measured by UV spectrophotometry and microbial assay. TLC of the heated mixture showed two different spots, which indicate some alteration in the structure of the cyanocobalamin molecule in the presence of methylparaben sodium at 115° for 10 min. The losses were about 20% by UV measurements and 32% by microbial assay. The degree of loss was sufficient to suggest that methylparaben sodium might have considerable influence on the stability of pharmaceutical products containing cyanocobalamin. Methylparaben and sodium chloride had no effect on cyanocobalamin.

Keyphrases □ Cyanocobalamin—activity, effect of methylparaben sodium, autoclave temperature, pharmaceutical formulations □ Methylparaben sodium—effect on cyanocobalamin activity, autoclave temperature, pharmaceutical formulations □ Vitamins—cyanocobalamin, activity, effect of methylparaben sodium, autoclave temperature, pharmaceutical formulations

Methylparaben sodium is often used in the formulation of pharmaceutical products. A fairly extensive literature has developed on the incompatibilities of the parabens and macromolecule polymers. Patel and Kostenbauder (1) found that solubilization of parabens with nonionic surfactants involved binding effects. The interaction between methylcellulose and the parabens also was reported (2).

Table I—Losses of Cyanocobalamin in the Presence of 0.1% Methylparaben Sodium Heated at 115° and Measured by UV Spectrophotometry

Cyanocobalamin Concentration, μg/ml	Reduction, %					
	Run 1	Run 2	Run 3	Run 4	Run 5	Mean ± *SD*
45	18.23	19.12	20.45	17.78	—	18.89 ± 1.17
30	22.64	18.39	22.64	20.00	21.00	20.92 ± 1.83
22.5	16.67	18.89	14.45	17.34	—	16.83 ± 1.84
15	17.07	18.20	16.00	—	—	17.09 ± 1.10

Table II—Losses in Activity of Cyanocobalamin in the Presence of 0.1% Methylparaben Sodium Heated at 115° in an Autoclave and Measured by Microbial Assay

Cyanocobalamin Concentration, μg/ml	Reduction, %				
	Run 1	Run 2	Run 3	Run 4	Mean ± *SD*
45	25.50	32.50	37.50	—	31.83 ± 5.99
30	31.50	34.50	—	—	33.00 ± 2.12
22.50	29.50	33.50	37.50	—	33.50 ± 4.00
15	26.50	31.00	37.00	36.20	32.67 ± 4.90

Although several reviews (3) devoted considerable discussion to the incompatibility that might arise from the interaction of a paraben with the containers, the incompatibility or inactivation that might arise from the interaction or binding of parabens with drugs of a large molecular nature has not been reported. However, the binding of parabens with some molecules such as polyethylene, povidone, and gelatin has been demonstrated (4). These observations suggest that generally unrecognized incompatibilities might exist in many pharmaceutical formulations and might markedly influence the release of the drug from a dosage form and alter the stability and assay of a drug. The present study was designed to investigate the possible interaction or binding effect of methylparaben sodium with cyanocobalamin (vitamin B_{12}) at the autoclave temperature (115° for 10 min).

EXPERIMENTAL

Materials—Methylparaben sodium[1], analytical grade, which was recrystallized three times from methanol, and cyanocobalamin USP were used after analysis by TLC and IR spectroscopy.

Methods—TLC, according to the method of Stahl (5), was performed on each preautoclaved (115° for 10 min) sample on fluorescent silica gel plates. The solvent system used was methanol–water (95:5) or butanol–acetic acid–0.66 *M* KH_2PO_4–methanol (36:18:36:10). Since cyanocobalamin and the interaction product were colored, no detecting reagent was used. Methylparaben sodium spots were detected by UV light.

The potency of cyanocobalamin was measured by the microbial method described in USP XVIII (6), using *Lactobacillus leichmannii* (ATCC 7830) as a test organism.

The UV analysis[2] at 360 nm (6) was the USP XVIII method.

RESULTS AND DISCUSSION

A decrease in the potency of several concentrations of cyanocobalamin in aqueous solution in the presence of methylparaben sodium at autoclave temperature (115° for 10 min) was investigated. The TLC method showed that the autoclave temperature had no effect on an aqueous solution of cyanocobalamin but confirmed the formation of a new compound in the presence of methylparaben sodium (Fig. 1).

Table I shows the percent reduction of cyanocobalamin measured by UV spectrophotometric method after autoclaving in the presence of methylparaben sodium. The increase in concentration of cyanocobalamin had no significant effect on the amount of reduction of cyanocobalamin measured by UV spectrophotometry. Pure cyanocobalamin was used at each stage under the same conditions as a control.

Table II shows the percent reduction of cyanocobalamin potency after autoclaving at 115° for 10 min in the presence of methylparaben sodium measured by microbial assay; the results show that the concentration of cyanocobalamin had little effect on the percent reduction. A comparison of the results obtained by the UV and microbial methods shows a higher reduction by the latter method, suggesting the reliability of the microbial assay.

The interaction or degradation of cyanocobalamin on methylparaben sodium appears to be similar to an interaction of a rubber stopper with methylparaben as described by Lachman *et al.* (7). It appears that substances having large molecular weights interact with methylparaben sodium and form new compounds.

Figure 1 illustrates the effect of methylparaben sodium with cy-

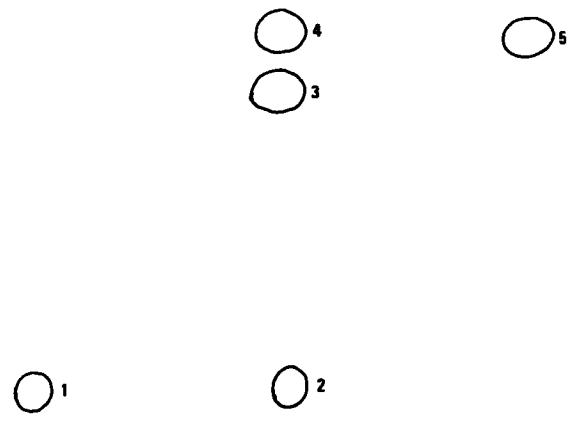

Figure 1—*Thin-layer chromatogram (silica gel) of cyanocobalamin. Key: 1, standard cyanocobalamin; 2, unbound part of cyanocobalamin; 3, bound part of cyanocobalamin with methylparaben sodium; 4, methylparaben sodium; and 5, standard sample of methylparaben sodium.*

[1] Merck.
[2] Varian-Tektron 635.

anocobalamin. The results are indicative of an interaction between these two substances and the formation of a new compound. The interaction is apparently enhanced by elevated temperatures. When the mixture was kept at room temperature for a long period, a small amount of a new compound was formed, suggesting a very low rate of deterioration of cyanocobalamin at room temperature.

Methylparaben and sodium chloride had no effect on cyanocobalamin in the same conditions, which rules out the effect of sodium ion in deterioration of cyanocobalamin.

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Enhancement of Solubility of Drug Salts by Hydrophilic Counterions: Properties of Organic Salts of an Antimalarial Drug

S. AGHARKAR, S. LINDENBAUM [x], and T. HIGUCHI

Abstract □ Judicious choice of the salt form of a drug can greatly affect the aqueous solubility and formulation of the compound. The objective of this work was to demonstrate the effect of various counteranions on the aqueous solubility of the antimalarial agent α-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol. Several organic salts of this drug were studied. The methods of synthesis, the apparent aqueous solubilities, and *in vitro* dissolution tests for these salts are reported. The lactate salt was 200 times as soluble as the hydrochloride salt. This enhanced solubility suggests that parenteral administration of this drug may now be feasible.

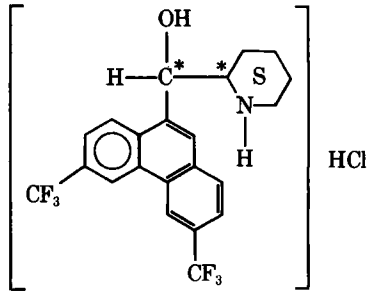
Keyphrases □ Solubility, aqueous—salts of substituted phenanthrenemethanol antimalarial agent, effect of hydrophilic counterions □ Salts—substituted phenanthrenemethanol antimalarial agent, effect of hydrophilic counterions on aqueous solubility □ Counterions, hydrophilic—effect on aqueous solubility of substituted phenanthrenemethanol antimalarial agent □ Antimalarial agents—α-(2-piperidyl) -3,6- bis(trifluoromethyl) -9- phenanthrenemethanol, salts, effect of hydrophilic counterions on aqueous solubility

It has been shown that organic acid salt forms of basic drugs, such as amines, have higher aqueous solubilities than their corresponding halide salts (1). This technique has found important application in the development of more soluble salt forms of drugs to improve their bioavailability and ease in formulation.

The objective of this work was to utilize previous findings (1) for α-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol (I). The principles employed in choosing counterions to render a high molecular weight hydrophobic drug more water soluble are generally applicable.

Compound I has been shown to exhibit significant activity against infections with strains of *Plasmodium falciparum* resistant to chloroquine, quinine, and pyrimethamine (2). Because of two optically active centers, it can exist as any one of four possible stereoisomers or mixtures thereof. The absolute configuration of all of the isomers has been established (3). The work reported here was done with one racemic pair, referred to as "isomer a" by Carroll and Blackwell (3). They also showed that all four enantiomers are potent antimalarials and, therefore, no attempt was made to resolve the racemic mixture.

The apparent solubility of the hydrochloride salt of I and its free base was measured in water at 25°. Compound I, being large and hydrophobic, is only sligtly soluble as the hydrochloride salt (Table I). This poor solubility was suspected to be the main reason for its poor bioavailability. In all previous work, I was administered orally as the hydrochloride



I

* = optically active centers