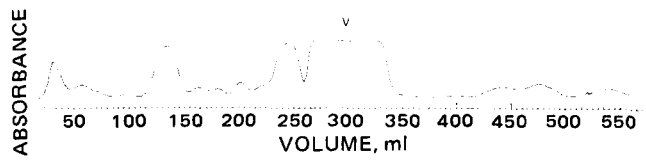

Figure 3—*Chromatogram of investigational methotrexate from a cellulose column by linear gradient elution from 0.01 to 0.4 M ammonia–ammonium bicarbonate buffer, pH 8.3.*

trexate solution after chromatographic separation. The procedure needs further explanation. The method implies that the absorptivities (on a weight basis rather than a molecular basis) of the impurities are identical to the absorptivity of methotrexate, which may not be absolutely true. However, as mentioned, the major impurities have similar UV spectra, extinction coefficients, and molecular weights. Therefore, the assumption is probably valid. Furthermore, even assuming a 25% error in the overall assumption, the absolute error in the determination of the purity of methotrexate would only be about 2% (total impurities are approximately 8%, and a 25% error would cause an absolute error of 8 × 0.25 = 2%). Therefore, this assumption, although not absolutely valid theoretically, is reasonable for practical calculations and correct for comparing various methotrexate samples if the impurities and their relative quantities are similar.

Table I—Comparative Percent Purity of Methotrexate and Quantitation of Its Impurities in Three Different Preparations

Compound	Concentration, % USP	Commercial Injection	Investigational Injection
Methotrexate (anhydrous basis)[a] (peak V)	86.0	86.2	86.7
N^{10}-Methylpteroylglutamic acid (peak VII)	4.0	5.2	—
2,4-Diamino-N^{10}-methylpteroic acid (peak IV)	1.5	2.6	—
4-Amino-N^{10}-methylpteroyl-N-methylglutamine (peak III)	1.6	1.2	—
2,4-Diamino-N^{10}-methylpteramide (peak II)	0.5	0.2	—
Unidentified impurities	1.0	0.5	—

[a] All methotrexate samples contained approximately 5% water.

Presented in Table I is the comparative percent purity of methotrexate and the identity and quantitation of its impurities in three different preparations. The percent purity of methotrexate in all three preparations was almost identical. In addition, methotrexate USP and commercial methotrexate injection had similar impurities. However, the investigational methotrexate preparation contained different impurities, which have not been identified (Figs. 1 and 3). These impurities warrant further investigation.

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Thermodynamics of Aqueous Solutions of Parabens

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Abstract □ The solubility of a related series of parabens was determined in water at four temperatures. The parabens chosen were the methyl through *n*-butyl *p*-hydroxybenzoates, and the temperature variations were 5° increments from 25 to 40°. These solutes are useful preservatives, especially combinations of the methyl and propyl ester derivatives. The chemical relationship of these compounds varied by successive linear methylene additions on the ester portion of the molecules. The thermodynamic values obtained for these aqueous systems could be related to these molecular variants since the remainder of the molecule was constant. For the overall thermodynamics, the free energy functions such as the ideal, actual, and excess were found to be smooth, nonlinear functions of the number of carbon atoms in the alkyl portion of the paraben esters. A linear relationship with the number of carbon atoms in the ester portion of these esters was found with the partial excess free energy of the solute.

Keyphrases □ Parabens, various—solubility in water at four temperatures, thermodynamic parameters related to number of carbon atoms □ Solubility—various parabens in water at four temperatures, thermodynamic parameters related to number of carbon atoms □ Thermodynamic parameters—related to solubility of various parabens in water at four temperatures □ Preservatives—various parabens, solubility in water at four temperatures, thermodynamic parameters related to number of carbon atoms

The thermodynamic parameters associated with solution phenomena were determined by studying the variation of solubility with temperature.

The well-known relationship of log mole fraction solubility *versus* reciprocal temperature allows for the determination of heats of solution, ΔH_s, and entropies of solutions, ΔS_s, from slopes and intercepts, respectively (1).

From heats of fusion, ideal mole fraction solubilities can be determined. From these basic derived quantities, mixing and excess functions also can be calculated.

Table I—Determined Mole Fraction Solubilities of the Parabens in Water

Solute	Mole Fraction Solubility $\times 10^5$			
	25°	30°	35°	40°
Methylparaben	26.0	33.0	41.1	46.1
Ethylparaben	9.6	12.2	14.9	17.2
Propylparaben	3.7	4.4	5.5	7.3
Butylparaben	2.3	3.2	4.0	4.7

Activity coefficients can be obtained from the ratio of ideal mole fraction solubility to the actual mole fraction solubility. Moreover, a partial excess free energy value can be determined that relates to an overall solution property.

The purposes of this study were to obtain information about the overall solution process in terms of ideal, actual, and excess free energies and to determine the relationship of the number of methylene groups in this normal alkyl series to these thermodynamic parameters. Because of the chemical relationship of these solutes, a relationship of thermodynamic values and chemical constitution might be observed.

With heats of fusion for these solutes available from the literature (2), the complete spectrum of thermodynamic elements could be obtained by calculation.

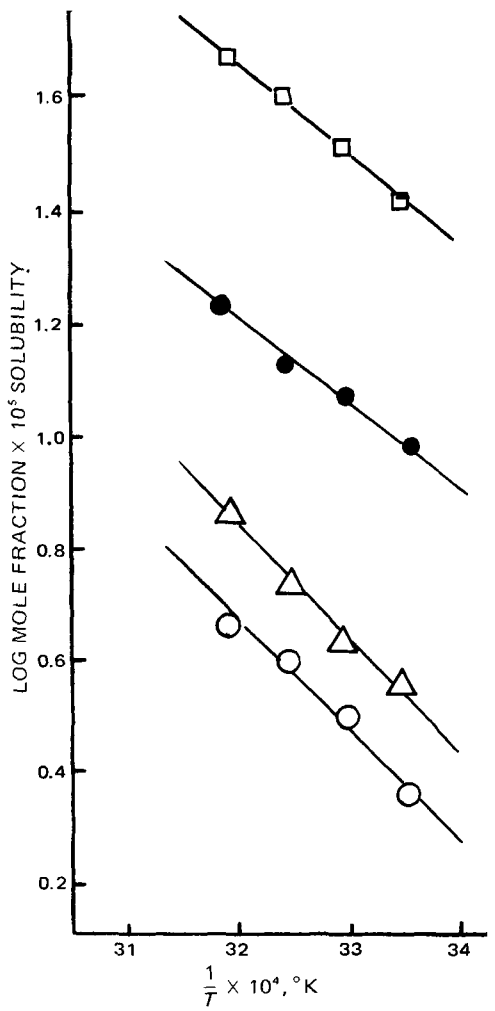

Figure 1—*Plot of the log mole fraction solubility as a function of reciprocal temperature for methylparaben (□), ethylparaben (●), propylparaben (△), and butylparaben (○).*

Table II—Ideal Mole Fraction Solubilities for the Parabens in Water

Solute	Ideal Mole Fraction Solubility			
	25°	30°	35°	40°
Methylparaben	0.157	0.178	0.200	0.224
Ethylparaben	0.189	0.213	0.239	0.267
Propylparaben	0.272	0.304	0.339	0.376
Butylparaben	0.450	0.500	0.553	0.610

EXPERIMENTAL

Equipment—A melting-point apparatus[1], a spectrophotometer[2], and a temperature-control unit[3] were used.

Materials—The chemicals were methyl *p*-hydroxybenzoate[4] (methylparaben), ethyl *p*-hydroxybenzoate[5] (ethylparaben), *n*-propyl *p*-hydroxybenzoate[6] (propylparaben), *n*-butyl *p*-hydroxybenzoate[7] (butylparaben), and distilled water. All solutes were within 1° of the literature melting-point value.

Method—Solubility was determined by a method previously described (3). The solubility of each solute was determined at least eight times at each temperature.

RESULTS AND DISCUSSION

The solubility of the parabens in water at the various temperatures is given in Table I in terms of mole fraction. The data were converted to log mole fraction solubility and plotted as a function of reciprocal temperature (Fig. 1). From these plots, the slopes and intercepts were obtained by least squares; they are related to the enthalpy of solution, ΔH_s, and the entropy of solution, ΔS_s, respectively.

The log mole fraction plots for these solutes are linear but not parallel to each other. This result implies that the enthalpy and entropy of solution vary for each solute.

Table II lists the calculated ideal mole fraction solubilities for these parabens at the four temperatures; the ideal mole fraction solubility equation was used. If the heats of fusion for the solutes are known, the ideal mole fraction solubility can be calculated. These values would hold for these solutes in any solvent system with which they form an ideal solution. These mole fractions are substantial in value and, no doubt, are far in excess of the determined or anticipated solubilities for these solutes in water. These mole fraction values vary from about 0.15 to 0.61, and one

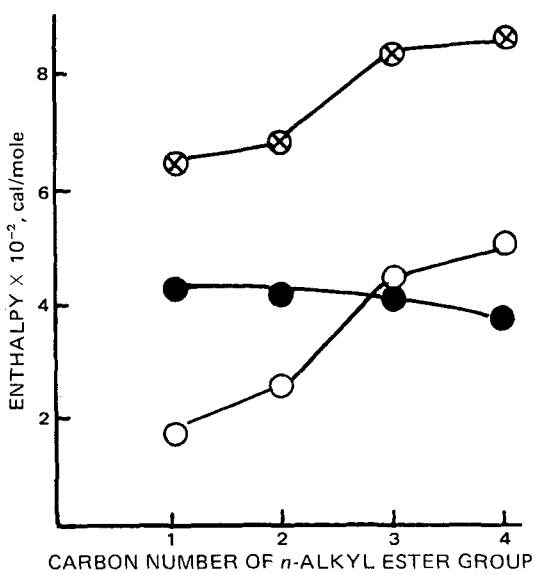

Figure 2—*Plot of the enthalpies of fusion (●), solution (⊗), and mixing (○), in calories per mole, as a function of the carbon number of the n-alkyl ester group of the parabens.*

[1] Hoover 6406, A. H. Thomas Co., Philadelphia, Pa.
[2] Cary model 16, Cary Instruments, Monrovia, Calif.
[3] Temptrol 150 Tempunit, Precision Scientific Co., Chicago, Ill.
[4] Lot 14, Matheson, Coleman and Bell.
[5] Lot EX665, Matheson, Coleman and Bell.
[6] Lot PX 1910, Matheson, Coleman and Bell.
[7] J. T. Baker Chemical Co.

Table III—Activity Coefficient for the Parabens in Water

Solute	Activity Coefficient of Solute, γ_2 25°	30°	35°	40°
Methylparaben	605	539	487	486
Ethylparaben	1,970	1,746	1,604	1,552
Propylparaben	7,352	6,909	6,164	5,151
Butylparaben	19,565	15,625	13,825	12,979

can consider a change in the "nature" of these binary mixtures at the higher values.

The solubilities in water varied in value from about 2.3×10^{-5} to 46.1×10^{-5} in terms of mole fraction. The activity coefficients calculated from the ratio of the ideal mole fraction solubilities to the actual mole fraction solubilities would be expected to be very large in magnitude. These "activity coefficients" were calculated (Table III). The magnitude of these values is illustrative of the fact that these systems of parabens in water are many orders of magnitude below the ideal solubilities and behave as highly nonideal solutions.

Table IV lists the thermodynamic elements for these solutes in water for the enthalpies of fusion, solution, and mixing, for the entropies of fusion, solution, and mixing, and for the ideal, actual, and excess free energies.

The heats of fusion for these compounds occur in a regular order, methylparaben possessing the highest heat of fusion and butylparaben possessing the lowest heat of fusion, with increasing differences as a function of carbon number.

The heat of solution and mixing both increase in magnitude with increasing nonpolarity, as evidenced by the increase in the alkyl portion of the ester group. These increases would be expected in aqueous systems as the molecular size increases.

The enthalpies of fusion, solution, and mixing were plotted as a function of the carbon number of the *n*-alkyl ester group on the molecule (Fig. 2). In this series of molecular variants, the differences in the determined properties between compounds are directly due to the incremental increase in the methylene groups since the remainder of the molecule remains constant. There is no pattern of the various enthalpies as a function of the carbon number of the alkyl ester portion of these molecules.

The entropies of fusion, solution, and mixing also were plotted as a function of the carbon number as previously described (Fig. 3). The entropies of fusion are quite similar in magnitude and are essentially invariant with increasing molecular size (Table IV). The entropies of solution and mixing also change with no apparent relationship to polarity or molecular size. Again, there seems to be no pattern of these individual

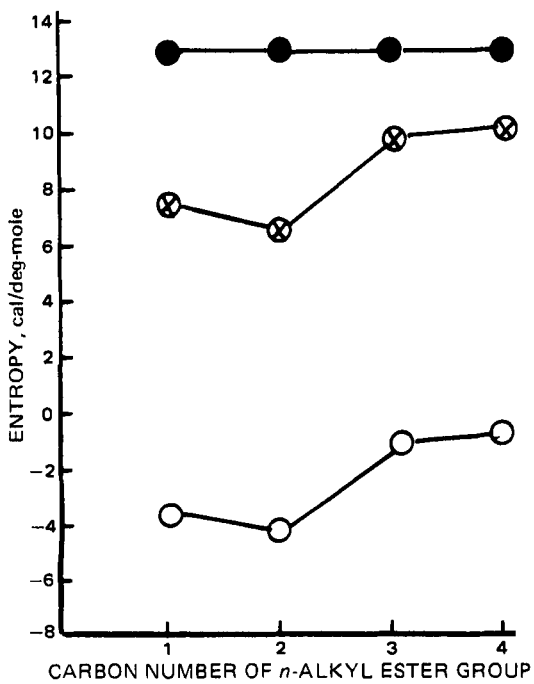

Figure 3—*Plot of the entropies of fusion (●), solution (⊗), and mixing (○), in calories per degree-mole, as a function of the carbon number of the* n*-alkyl ester group of the parabens.*

Table IV—Thermodynamic Values Obtained for the Parabens in Water

Solute	Enthalpy, cal/mole Fusion	Solution	Mixing	Entropy, cal/deg mole Fusion	Solution	Mixing	Free Energy, cal/mole Ideal	Actual	Excess
Methylparaben	4352	6516	1764	11.04	5.58	−5.46	985	4815	3830
Ethylparaben	4243	6906	2663	11.02	4.88	−6.14	882	5418	4536
Propylparaben	4026	8403	4387	10.90	7.72	−3.18	702	6093	5391
Butylparaben	3737	8778	5041	11.02	8.29	−2.73	376	6250	5874

Table V—Partial Excess Free Energy of the Solutes in Water

Solute	Partial Excess Free Energy of Solute, $\bar{F}_2^E$, cal/mole 25°	30°	35°	40°
Methylparaben	3915	3845	3783	3782
Ethylparaben	4637	4563	4512	4492
Propylparaben	5442	5404	5335	5225
Butylparaben	6040	5903	5828	5790

thermodynamic elements relative to carbon number or molecular size. However, these individual factors of enthalpy and entropy must be considered together to discern the overall effect, which would be the various free energy functions shown in Table IV. The ideal free energies follow a pattern of diminishing magnitude with carbon number; however, this pattern is not linear. The actual and excess free energies increase smoothly with the carbon number of the ester group in a nonlinear fashion.

The free energy function is a result of the combination of the appropriate values of enthalpy and entropy, where these factors act in opposition or in concert with one another depending on their signs and varying magnitudes.

In Fig. 4, the free energy functions calculated for these systems were plotted as a function of the carbon number of the ester group, and smooth nonlinear relationships were found. Specifically, the ideal free energy diminishes with the carbon number, whereas both the actual and excess

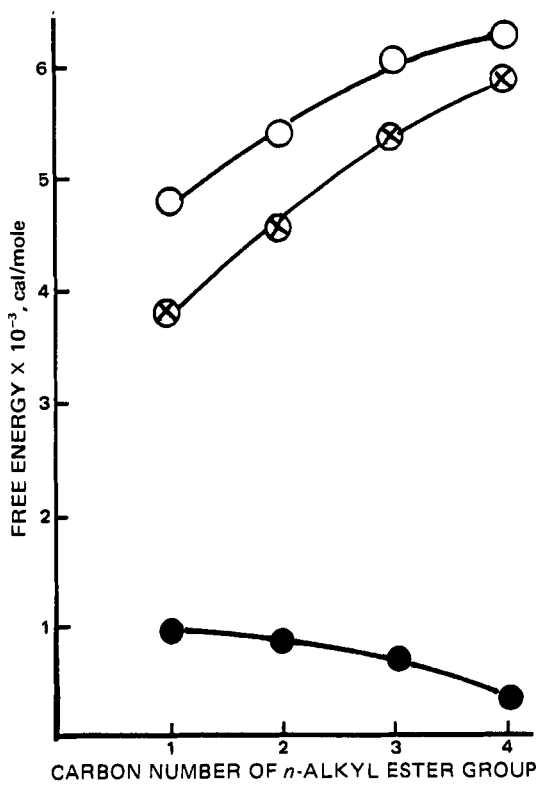

Figure 4—*Plot of the ideal (●), actual (○), and excess (⊗) free energies, in calories per mole, as a function of the carbon number of the* n*-alkyl ester group of the parabens.*

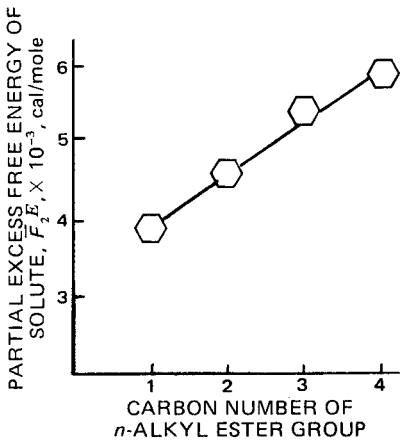

Figure 5—*Plot of the average partial excess free energy for the solute, in calories per mole, as a function of the carbon number of the* n*-alkyl ester group of the parabens.*

free energies increase with an increasing carbon number. The values obtained for these free energy functions, the actual and excess free energies, are substantial in magnitude and imply highly nonideal solution behavior for these parabens in water. This fact was also supported by the rather large activity coefficients determined for these solutes.

Thus, the free energy functions possess a nonlinear relationship with carbon number or molecular size.

An additional thermodynamic parameter, the partial excess free energy of the solute, was investigated. This parameter can be considered to be an overall property of the solution and can also be calculated from these results. The partial free energy, $\overline{F}_2^E$, can be written as:

$$\overline{F}_2^E = RT \ln \gamma_2 \qquad \text{(Eq. 1)}$$

where R is the gas constant, T is the absolute temperature, and γ_2 is the activity coefficient. Previously, it was stated that these activity coefficients were quite large in magnitude and implied highly nonideal behavior. However, when using these values and solving Eq. 1 for the partial free energy, entirely reasonable values of free energy were obtained (Table V). The calculated partial free energy values occurred in regular order with carbon number, and there were regular increases with increasing temperature. This result was expected as the solubility increased with temperature because the energy requirement of the overall system also increased.

Figure 5 shows plots of the partial excess free energy for these solutes as a function of the carbon number of the ester group. In this case, a linear relationship of partial excess free energy with carbon number is evidenced with an approximate slope of about 680 cal/mole/carbon atom.

The expression of the rate of change in partial excess free energy with carbon number allows for a definitive relationship of a thermodynamic parameter and molecular size.

Finally, a spectrum of thermodynamic elements can be easily obtained by studying solubilities at various temperatures once the heat of fusion values have been determined. Studies along these lines will be the subject of future reports.

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Electron-Capture GLC Determination of Ibuprofen in Serum

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Received April 25, 1977, from the *Research Laboratories, The Upjohn Company, Kalamazoo, MI 49001.* Accepted for publication August 22, 1977.

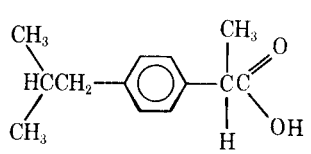
Abstract □ To evaluate drug–protein binding, a sensitive method for the determination of ibuprofen in submilliliter amounts of serum was required. A specific and highly sensitive procedure, based on benzene extraction of the acidified specimen, TLC of the benzene extract residue, formation of the pentafluorobenzyl esters of the materials eluted from the thin-layer chromatogram, and quantification of the pentafluorobenzyl esters by GLC, was developed. Utilizing electron-capture detection, the method is sensitive to 0.1 μg of ibuprofen/0.1 ml of serum. Statistical analyses indicated an average recovery of 97.7% with a standard deviation of ±7.3%. Mass spectrometric analysis, in conjunction with GLC, confirmed the specificity of the method for the intact drug. The procedure was applied successfully to drug absorption and drug–protein binding studies in humans.

Keyphrases □ Ibuprofen—electron-capture GLC analysis in serum, time course of protein binding in humans □ GLC, electron capture—analysis, ibuprofen in serum □ Protein binding—ibuprofen in humans, time course studied using electron-capture GLC analysis in serum □ Anti-inflammatory agents—ibuprofen, electron-capture GLC analysis in serum, time course of protein binding in humans

The pharmacology, toxicology, and biochemistry of ibuprofen[1] [(*RS*)-2-(4-isobutylphenyl)propionic acid] (I), a potent orally active anti-inflammatory agent in animals (1–6) and humans (7–9), have been reported.

In studies of the absorption, metabolism, and excretion of I, paper chromatographic (10) and GLC (5, 11–14) methods were utilized. All of these procedures required at least 1–5 ml of biological fluid for analysis of intact drug. This quantity constituted a serious limitation in studies where multiple blood specimens were needed for multiple analyses. The need for a sensitive analytical method to measure individual drugs in small amounts of biological fluids was apparent during drug interaction studies with I and aspirin (15).

To determine the time course of drug–protein binding in human volunteers participating in pharmacokinetic studies of I, a simple, specific, and highly sensitive method for the measurement of the intact drug in submilliliter amounts of serum was developed.



I

[1] Motrin, The Upjohn Co., and Brufen, Boots Co.