# Thermodynamics of the Lipase-Catalyzed Esterification of 1-Dodecanoic Acid and 1-Dodecanol in Organic Solvents

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Lipase immobilized on controlled-pore glass beads was used to catalyze the esterification of 1-dodecanol and 1-dodecanoic acid in organic solvents. Equilibrium measurements were performed in hexane, heptane, cyclohexane, 2,2,4-trimethylpentane, and toluene at the temperature $T = 298.15$ K. The equilibrium constants correlate well with the (octanol + water) partition coefficients and the dielectric constants of the solvents. The saturation molalities and the (2,2,4-trimethylpentane + water) partition coefficients of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate have been determined at $T = 298.15$ K. The equilibrium constant of the esterification reaction in water has also been calculated.

## Introduction

For the past 20 years biocatalysis in organic solvents (Laane et al., 1987; Klibanov, 1990) has become a potentially important method for the synthesis of stereoselective compounds, with lipase (EC 3.1.1.3) being one of the most commonly used biocatalysts in these solvents (Chang and Rhee, 1990; Kamiya et al., 1995; Janssen et al., 1993b). Lipase-catalyzed reactions have been used for the acylation of the primary position OH group in glycols (Cesti et al., 1985), the interesterification of fats (Goto et al., 1995), the stereoselective esterification of menthol (Kamiya et al., 1995), and transesterification reactions (Klibanov, 1990; Goto et al., 1995) in organic solvents. Biocatalysis in organic solvents has several advantages. It overcomes the difficulty of dissolving hydrophobic substances. It shifts the thermodynamic equilibrium of many enzymatic reactions to the formation of desired products. It eliminates bacterial contamination of fermentors, which is a major problem in aqueous solutions.

There are only a few reports dealing with the thermodynamics of reactions in nonaqueous solvents (Janssen et al., 1993a; Valivety et al., 1991; Tewari et al., 1995, 1996). This is unfortunate since these results are needed to gain a better understanding of these reactions. In this study, the esterification of 1-dodecanoic acid and 1-dodecanol has been investigated. The equilibrium constants for the reaction have been measured in hexane, heptane, cyclohexane, toluene, and 2,2,4-trimethylpentane at $T = 298.15$ K.

The equilibrium constants have been correlated with the (octanol + water) partition coefficients $K_{o/w}$ and the relative permittivities of the solvents. The saturation molalities $m_{sat}$ and (2,2,4-trimethylpentane + water) partition coefficients $K_{iso/w}$ of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate have also been measured at $T = 298.15$ K. The equilibrium constant for the hydrolysis reaction of dodecyldodecanoate in water has also been calculated.

## Experimental Section

The substances used in this study, their Chemical Abstract Service (CAS) numbers, empirical formulas, molar masses, vendors (see footnote *c* in Table 1), and purities determined by gas chromatography (GC) are given in Table 1. The GC analysis of 1-decanol, 1-dodecanol, 1-dodecanoic acid, dodecyl dodecanoate, and dodecyl tetradecanoate confirmed the purities reported by the vendors. These substances were used as received without further purification. The lipase (EC 3.1.1.3) used in this study was type II crude powder obtained from porcine pancreas.

The analysis of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate was carried out with a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a flame ionization detector. The column was a fused silica HP 5 column (5% cross-linked phenylmethylsilane, 30 m long × 0.53 mm i.d. with a film thickness of 0.80 $\mu$m). The head pressure of the helium carrier gas was 2.8 bar. The injector and detector temperature were set at 270 °C. The initial column temperature of 100 °C was held for 1 min and then raised to 225 °C at a rate of 20 K per min and then held at 225 °C for 20 min. An internal standard method was used for the analysis of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate. 1-Decanol was used as the internal standard for 1-dodecanol and 1-dodecanoic acid; dodecyl tetradecanoate was used for dodecyl dodecanoate. The retention times of 1-decanol, 1-dodecanol, 1-dodecanoic acid, dodecyl dodecanoate, and dodecyl tetradecanoate were 3.0, 4.35, 4.88, 13.0, and 18.7 min, respectively.

The enzyme was immobilized on silanized controlled-pore glass (CPG) beads. The method for the immobilization of the lipase on glutaraldehyde-treated CPG beads was similar to that described previously (Tewari et al., 1995). Approximately 4 g of lipase (type II from porcine pancreas) was dissolved in $K_2HPO_4$ buffer (0.1 mol dm$^{-3}$, pH = 7.2). The resulting solution was centrifuged at 2000 rpm for 10 min to remove any insoluble substances. The CPG beads were then suspended in this solution and the resultant solution was stored overnight at 4 °C. The next day, the solution was shaken at 25 rpm for an hour in a bath set at $T = 298.15$ K. The suspension in solution was then filtered and washed with $K_2HPO_4$ buffer (0.1 mol dm$^{-3}$, pH = 7.2). The immobilized enzyme on the CPG beads was stored at 4 °C in this phosphate buffer.

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**Table 1. Principal Substances Used in This Study with Their Chemical Abstracts Service (CAS) Registry Numbers,[a] Empirical Formulas, Molecular Weights $M_r$, Vendor (B = Baker, S = Sigma, M = Mallinckrodt), and Mole Fraction Purity[b] $x$ as Stated by Vendor[c]**

| substance | CAS no. | formula | $M_r$ | supplier | $x$ |
|---|---|---|---|---|---|
| 1-dodecanol | 112-53-8 | $C_{12}H_{26}O$ | 186.34 | S | 0.988 |
| 1-dodecanoic acid | 143-07-7 | $C_{12}H_{24}O_2$ | 200.32 | S | 0.998 |
| dodecyl dodecanoate | 13954-76-1 | $C_{24}H_{48}O_2$ | 368.64 | S | 0.987 |
| 1-decanol | 112-30-1 | $C_{10}H_{22}O$ | 158.28 | S | 0.99 |
| dodecyl tetradecanoate | 22412-97-1 | $C_{26}H_{52}O_2$ | 396.70 | S | 0.99 |
| toluene | 108-88-5 | $C_7H_8$ | 92.14 | M | 0.99 |
| hexane | 110-54-3 | $C_6H_{14}$ | 86.14 | B | 0.99 |
| heptane | 142-82-5 | $C_7H_{16}$ | 100.20 | M | 0.997 |
| cyclohexane | 110-82-7 | $C_6H_{12}$ | 84.16 | M | 0.99 |
| 2,2,4-trimethylpentane | 540-84-1 | $C_8H_{18}$ | 114.23 | B | 1.00 |
| dipotassium phosphate | 7778-77-0 | $K_2HPO_4$ | 174.18 | S | |
| phosphoric acid | 7664-38-2 | $H_3PO_4$ | 98.00 | M | |
| lipase[d] | 9001-62-1 | | $5.0 \times 10^4$ | S | |

[a] Supplied by authors. [b] Gas chromatographic method was used by the vendor(s) to determine the purities of these substances. The GC analyses of 1-decanol, 1-dodecanol, 1-dodecanoic acid, dodecyl dodecanoate, and dodecyl tetradecanoate that were done in this study confirmed the purities reported by the vendors. [c] Certain commercial equipment, instruments, or materials are identified in this paper to specify the experimental procedures adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose. [d] Type ll crude powder obtained from porcine pancreas.

Equilibrium measurements were carried out by approaching equilibrium from both directions of reaction. 1-Dodecanol and 1-dodecanoic acid were used for the forward direction; dodecyl dodecanoate was used for the reverse direction. Following dissolution of these substances in the organic solvents saturated with water, ≈3 g of immobilized enzyme was added to each solution. Water is essential for carrying out this reaction from the reverse direction. Therefore, by using organic solvents saturated with water, we achieve this aim and also keep the molality of water essentially the same in the reaction started from both directions. The solutions of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate in organic solvent were clear and homogeneous. This phase was separate from the immobilized enzyme, which, in the solvents used in this study, was on the bottom of the flask. The Teflon stoppered glass bottles containing these solutions were then placed in a shaker bath (25 rpm) set at $T = 298.15$ K and allowed to equilibrate. The temperature of the shaker bath used in this study was measured with a NIST calibrated thermometer. The temperature of the bath was held constant to within ±0.1 K. The solutions were periodically analyzed to determine the extent of reaction and to see if the reaction quotients (i.e., the ratio of molalities of products to reactants) obtained from both directions of the reaction were equal and if equilibrium had been achieved. After 15 days equilibration, it was deemed necessary to add an additional 2 to 3 g of immobilized enzyme to each bottle. A total equilibration time of ≈30 days was required for the reaction in each solvent in order to reach equilibrium as evidenced by the agreement (or near agreement) of the reaction quotients determined from both directions of the reaction.

A standard solution of 1-decanol, 1-dodecanol, 1-dodecanoic acid, dodecyl dodecanoate, and tetradecyl dodecanoate was prepared in hexane. The response factor ratios (concentration/area) for 1-dodecanol and 1-dodecanoic acid with respect to 1-decanol and dodecyl dodecanoate to dodecyl tetradecanoate were determined. For the analysis of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate, 2.0 $cm^3$ of equilibrated organic phase and 100 $\mu$L of an internal standard solution containing 1-decanol and dodecyl tetradecanoate in hexane were gravimetrically added to a vial and then capped tightly. Approximately 0.6 $\mu$L of the solution was injected into the GC and analyzed. The molalities of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate were determined from their respective chromatographic areas, the response factor ratio, and the chromatographic areas and the known molalities of the internal standards. The molality of the internal standard 1-decanol was kept close to the molalities of 1-dodecanol and 1-dodecanoic acid in the reaction mixture and the molality of the internal standard dodecyl tetradecanoate to that of dodecyl dodecanoate.

The molalities of water in the organic solvent reaction mixtures were measured with a Metrohm model 633 Karl Fischer titration apparatus and a model 665 Dosimat. The Karl Fischer titration apparatus, enclosed in a Plexiglass box, was continuously purged with dry nitrogen. The instrument was calibrated with 1-octanol saturated with water. Approximately 0.03 g of 1-octanol saturated with water was withdrawn with a 50 $\mu$L syringe and injected into the (methanol + Hydranal Composite 2). Reaction mixture samples (0.1 g to 0.4 g) were withdrawn with an airtight 500 $\mu$L syringe and were injected into the (methanol + Hydranal Composite 2) solution to determine the molality of water in the organic solvents. The molalities of water in the reaction mixtures are based on the solubility of water in 1-octanol at $T = 298.15$ K reported by Leo and Hansch (1971).

The pH measurement was carried out with an Orion model 811 pH meter and a Radiometer combination glass microelectrode. The pH meter was calibrated with Radiometer standard buffers. The pH measurements are judged to be accurate within ±0.02.

The saturation molalities $m_{sat}$ and (2,2,4-trimethylpentane + water) partition coefficients $K_{iso/w}$ of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate were also measured. The saturation molalities were determined by approaching the position of equilibrium from two different temperatures. For each substance, approximately 0.5 g of substance and 45 g of water were added to two 50-$cm^3$ Erlenmeyer flasks, which were sealed with ground glass stoppers. One of these flasks was then placed in a constant temperature shaker bath (25 rpm) set at $T = 288.15$ K; the other flask was placed in a similar bath set at $T = 308.15$ K. After 24 h all flasks were placed in a single shaker bath at $T = 298.15$ K and allowed to equilibrate for an additional 3 days. This procedure was adopted to gain confidence that equilibrium had been reached.

Approximately 30 g of the equilibrated aqueous layer was then carefully transferred into a Teflon bottle with a

**Table 2. Results of Equilibrium Measurements at $T = 298.15$ K for the Esterification Reaction 1 in Hexane, Heptane, Cyclohexane, 2,2,4-Trimethylpentane, and Toluene[a]**

| direction | $m(C_{12}H_{24}O_2) \times 10^3$/mol (kg soln)$^{-1}$ | $m(C_{12}H_{26}O) \times 10^3$/mol (kg soln)$^{-1}$ | $m(C_{24}H_{48}O_2) \times 10^3$/mol (kg soln)$^{-1}$ | $m(H_2O) \times 10^3$/mol (kg soln)$^{-1}$ | $K$ | $K$(combined) |
|---|---|---|---|---|---|---|
| | | | Hexane | | | |
| forward | 1.09 ± 0.06 | 3.57 ± 0.06 | 14.5 ± 0.6 | 10.7 ± 0.6 | 39.9 ± 6.8 | 39.6 ± 7.0 |
| reverse | 1.30 ± 0.08 | 3.12 ± 0.09 | 14.8 ± 0.6 | 10.7 ± 0.6 | 39.0 ± 7.3 | |
| | | | Heptane | | | |
| forward | 1.07 ± 0.06 | 3.61 ± 0.13 | 4.89 ± 0.26 | 28.02 ± 1.16 | 35.5 ± 6.6 | 35.3 ± 8.6 |
| reverse | 1.13 ± 0.09 | 4.01 ± 0.10 | 5.70 ± 0.08 | 28.03 ± 1.16 | 35.3 ± 6.5 | |
| | | | Cyclohexane | | | |
| forward | 2.86 ± 0.15 | 2.17 ± 0.05 | 6.11 ± 0.51 | 24.55 ± 1.74 | 24.2 ± 5.7 | 23.3 ± 7.4 |
| reverse | 2.53 ± 0.09 | 2.81 ± 0.10 | 6.57 ± 0.49 | 24.56 ± 1.74 | 22.7 ± 4.9 | |
| | | | 2,2,4-Trimethylpentane | | | |
| forward | 2.81 ± 0.14 | 1.43 ± 0.01 | 4.07 ± 0.09 | 24.17 ± 1.08 | 24.5 ± 3.0 | 27.2 ± 5.3 |
| reverse | 1.45 ± 0.04 | 3.20 ± 0.03 | 6.74 ± 0.44 | 24.19 ± 1.08 | 35.1 ± 5.2 | |
| | | | Toluene | | | |
| forward | 6.14 ± 0.24 | 5.61 ± 0.05 | 5.10 ± 0.27 | 103.0 ± 17.6 | 15.3 ± 4.2 | 17.9 ± 6.8 |
| reverse | 5.08 ± 0.15 | 5.12 ± 0.03 | 5.84 ± 0.29 | 103.0 ± 17.6 | 23.1 ± 5.9 | |

[a] The equilibrium constants $K$ were calculated with eq 2. The initial molalities of 1-dodecanol and 1-dodecanoic acid in the forward reaction mixtures were ≈0.013 mol kg$^{-1}$ and of *n*-dodecyl dodecanoate for the reverse solutions were ≈0.015 mol kg$^{-1}$. The molalities $m$ for the solutes in solution at equilibrium are given in columns 3 to 6. $C_{12}H_{26}O$ is 1-dodecanol, $C_{12}H_{24}O_2$ is 1-dodecanoic acid, and $C_{24}H_{48}O_2$ is dodecyl dodecanoate. $K$(combined) was calculated as the weighted average of the values of the equilibrium constants, which were measured from both directions of the reaction. These uncertainties are based on two estimated standard deviations of the mean. Final uncertainties are given in the text (see Results and Discussion).

10-cm$^3$ disposable plastic syringe. Then 2.0 cm$^3$ of hexane and 100 $\mu$L of the internal standard solution in hexane were quantitatively added to the bottle. The contents of the bottles were shaken and then centrifuged at 2000 rpm for 15 min. The hexane layer was analyzed for the molality of the substance as described above. Control experiments showed that a second extraction of the aqueous phase with hexane did not give any measurable peaks corresponding to the compounds whose molalities were being determined.

For the measurement of $K_{iso/w}$, approximately 0.5 g of substance was quantitatively dissolved in 2.0 cm$^3$ of 2,2,4-trimethylpentane in a 50-cm$^3$ Erlenmeyer flask and 40 g of water was added to the flask. The flasks were then placed in a shaker bath (25 rpm) at $T = 298.15$ K and allowed to equilibrate for 3 to 5 days. These solutions were also briefly shaken vigorously by hand on a daily basis. The molality of the solute in the aqueous phase was then determined in the same way as was done for the determination of the saturation molalities. The molality of the solute in the organic phase is well-known from its preparation by gravimetric methods. In the calculation of $K_{iso/w}$, the molality of this substance in 2,2,4-trimethylpentane was corrected for the amount of this substance in the aqueous phase.

## Results and Discussion

The reaction that occurs in the organic solvents is

$$\text{1-dodecanol(sln)} + \text{1-dodecanoic acid(sln)} = \text{dodecyl dodecanoate(sln)} + H_2O\text{(sln)} \quad (1)$$

where "sln" denotes the various organic solvents that were used in this study. The equilibrium constant $K$ for reaction 1 in each organic solvent is

$$K = m(\text{dodecyl dodecanoate}) \cdot m(H_2O) / \{m(1-\text{dodecanol}) \cdot m(1-\text{dodecanoic acid})\} \quad (2)$$

Here $m$ is the molality. It is important to note that the molality of the water is also included in eq 2 and that the equilibrium constant is symmetrical and therefore automatically dimensionless. Since the molalities of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate in the organic solvents are ≤0.016 mol kg$^{-1}$ and also since they are almost certainly un-ionized in these solvents, it is reasonable to assume that the activity coefficients of 1-dodecanol, dodecanoic acid, and dodecyl dodecanoate in these solvents are close to unity. Therefore, the equilibrium constants reported in this study can also be identified with the thermodynamic equilibrium constants defined in terms of activities.

The equilibrium molalities of the reactants and products are given in Table 2. The molalities of water in the organic solvents in this table are the averages of three to five measurements. The molalities of the other reactants are the averages of five or six measurements except in the case of the results for 2,2,4-trimethylpentane, where the molalities are an average of two measurements for the forward and three measurements for the reverse direction of reaction. The reported equilibrium constants $K$(combined) are the weighted averages of the results obtained from the forward and the reverse directions of reaction. In all cases the reported uncertainties in the molalities and equilibrium constants are equal to two estimated standard deviations of the mean.

The saturation molalities $m_{sat}$ of 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate in water are given in Table 3. Since the values of the saturation molalities are very low, a direct measurement of the equilibrium constant for the esterification reaction in aqueous solutions could not be carried out. However, it was possible to calculate a value of the equilibrium constant for this reaction in aqueous solutions by using a thermochemical cycle (see below). This cycle requires a knowledge of the partition coefficients of the 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate between one of the organic solvents and water. The solvent 2,2,4-trimethylpentane was selected for this purpose.

The measured values of the (2,2,4-trimethylpentane + water) partition coefficients $K_{iso/w}$ for these three substances are given in Table 3. The 1-dodecanol and dodecyl dodecanoate are assumed to exist only as the neutral species both in the organic solvents and in the aqueous phase. However, the molality of 1-dodecanoic acid as measured

**Table 3. Saturation Molalities $m_{Sat}$ in Water and (2,2,4-Trimethylpentane + Water) Partition Coefficients $K_{Iso/w}$ of 1-Dodecanol, 1-Dodecanoic Acid, and Dodecyl Dodecanoate at $T = 298.15$ K[a]**

| substance | $m_{sat}$/mol kg$^{-1}$ | $K_{iso/w}$[b] |
|---|---|---|
| 1-dodecanol | $(3.91 \pm 0.26) \times 10^{-5}$ | $(7.61 \pm 0.15) \times 10^{4}$ |
| 1-dodecanoic acid | $(3.11 \pm 0.26) \times 10^{-5}$ | $(3.44 \pm 0.21) \times 10^{4}$ [c] |
| dodecyl dodecanoate | $(3.897 \pm 0.083) \times 10^{-6}$ | $(2.03 \pm 0.15) \times 10^{6}$ |

[a] These uncertainties are based on two estimated standard deviations of the mean. Final uncertainties are given in the text (see Results and Discussion). [b] The molalities of the solutes in the aqueous phases were 1-dodecanol, $(1.87 \pm 0.04) \times 10^{-5}$ mol kg$^{-1}$; 1-dodecanoic acid, $(2.64 \pm 0.07) \times 10^{-5}$ mol kg$^{-1}$; dodecyl dodecanoate, $(2.94 \pm 0.20) \times 10^{-6}$ mol kg$^{-1}$. [c] The value given in this table was obtained from the molality of aqueous 1-dodecanoic acid at pH = 4.9. This measured molality is the sum of the molalities of both the ionized and nonionized forms of 1-dodecanoic acid. The value $K_{iso/w} = (6.88 \pm 0.42) \times 10^{4}$ pertains to the transfer of the nonionized form of 1-dodecanoic acid to 2,2,4-trimethylpentane (see Results and Discussion).

by GC is equal to the total molality of both the ionized and nonionized forms of this acid. Thus, the measured value $K_{iso/w} = (3.44 \pm 0.21) \times 10^{4}$ pertains to this mixture of species; formally it is an apparent equilibrium constant. However, it is desired to obtain a value of $K_{iso/w}$ that pertains only to the nonionized form of 1-dodecanoic acid. To do this it is necessary to know the p$K$ of 1-dodecanoic acid. The value p$K = 4.9$ for 1-dodecanoic acid from the known p$K$'s of 1-hexanoic, 1-heptanoic, and 1-octanoic acids (Martell et al., 1993). Thus, at the pH = 4.9 at which the experiments were performed, the fractions of the ionized and nonionized forms of this acid are equal. This information is used to calculate a value of $K_{iso/w} = (6.88 \pm 0.42) \times 10^{4}$, which pertains to the transfer of the nonionized form of 1-dodecanoic acid to 2,2,4-trimethylpentane.

The uncertainties assigned thus far are based on the random errors in the measurements expressed as two estimated standard deviations of the mean. Since these uncertainties allow for only random errors, it is desirable to consider possible systematic errors in the measurements. It is judged that the determinations of the molalities $m$ of water are reliable to within $0.03 \cdot m$. The use of internal standards also helps to minimize errors, and it is judged that the systematic errors in the measured molalities of 1-dodecanol, dodecyl dodecanoate, and 1-dodecanoic acid are $< 0.01 \cdot m$. The final values of the equilibrium constants for reaction 1 are based on results in which the position of equilibrium was approached from two different directions. Thus, systematic errors due to lack of equilibrium are already included in the present uncertainty intervals. A similar situation holds for the values of the saturation molalities $m_{sat}$. Here, however, the tactic used was to approach the position of equilibrium from two different temperatures. While the method of approaching equilibrium from two different temperatures was not used in the measurement of the values of $K_{iso/w}$, it had already been established from the solubility measurements that 3 days equilibration time was adequate for the attainment of equilibrium. Thus, total systematic errors in the reported quantities are judged to be, respectively, $< 0.035 \cdot K(1)$, $< 0.01 \cdot m_{sat}$, and $< 0.014 \cdot K_{iso/w}$.

These estimates of possible systematic error are combined in quadrature together with the statistical uncertainties in the measured values of these quantities, expressed as one estimated standard deviation of the mean, to obtain combined standard uncertainties. These combined standard uncertainties are then multiplied by 2 to arrive at a final set of results with somewhat larger estimates of total error. Thus, the values of the equilibrium constant $K(1)$ are $(39.6 \pm 7.5)$ for hexane, $(35.3 \pm 9.0)$ for heptane, $(23.3 \pm 7.6)$ for cyclohexane, $(27.2 \pm 5.6)$ for 2,2,4-trimethylpentane, and $(17.9 \pm 6.9)$ for toluene. The values of the saturation molalities $m_{sat}$ in water are $(3.91 \pm 0.27) \times 10^{-5}$ mol kg$^{-1}$ for 1-dodecanol, $(3.11 \pm 0.27) \times 10^{-5}$ mol kg$^{-1}$ for 1-dodecanoic acid, and $(3.90 \pm 0.12) \times 10^{-6}$ mol kg$^{-1}$ for dodecyl dodecanoate. The values of the partition coefficient $K_{iso/w}$ are $(7.61 \pm 0.27) \times 10^{4}$ for 1-dodecanol, $(6.88 \pm 0.47) \times 10^{4}$ for 1-dodecanoic acid, and $(2.03 \pm 0.16) \times 10^{6}$ for dodecyl dodecanoate.

The aforementioned thermochemical cycle is now described. The chemical reference reaction in water that will be used is

$$\text{1-dodecanol(aq)} + \text{1-dodecanoic acid(aq)} = \text{dodecyl dodecanoate(aq)} + \text{H}_2\text{O(l)} \quad (3)$$

The charges on all of the substances in reaction 3 are zero and therefore are omitted. The equilibrium constant $K_m$ for this reaction is

$$K_m = \{m(\text{dodecyldodecanoate}) \cdot m^\circ / \{m(1-\text{dodecanol}) \cdot m(1-\text{dodecanoic acid})\} \quad (4)$$

The standard molality ($m^\circ = 1$ mol kg$^{-1}$) has been used in the above equation to keep the equilibrium constant dimensionless. The partition coefficients $K_{iso/w}$ pertain to the following reactions:

$$1-\text{dodecanol(aq)} = 1-\text{dodecanol(iso)} \quad (5)$$

$$1-\text{dodecanoic acid(aq)} = 1-\text{dodecanoic acid(iso)} \quad (6)$$

$$\text{dodecyl dodecanoate(aq)} = \text{dodecyl dodecanoate(iso)} \quad (7)$$

In this case, "iso" denotes 2,2,4-trimethylpentane. Also, needed in the thermochemical cycle is the standard molar Gibbs free energy change $\Delta_r G_m^\circ$ for reaction 1 in 2,2,4-trimethylpentane and the solubility of water in 2,2,4-trimethylpentane:

$$\text{H}_2\text{O(l)} = \text{H}_2\text{O(iso)} \quad (8)$$

$\Delta_r G_m^\circ$ for reaction 3 can then be calculated with

$$\Delta_r G_m^\circ(3) = \Delta_r G_m^\circ(1) + \Delta_r G_m^\circ(5) + \Delta_r G_m^\circ(6) - \Delta_r G_m^\circ(7) - \Delta_r G_m^\circ(8) \quad (9)$$

The values of $K_{iso/w}$ (see Table 3) were used to calculate values of $\Delta_r G_m^\circ$ for reactions 5, 6, and 7. The value of the equilibrium constant for reaction 1 with 2,2,4-trimethylpentane as the organic solvent (see Table 2) give $\Delta_r G_m^\circ$ for reaction 1. The solubility of water as determined in the equilibrium measurements with the solvent 2,2,4-trimethylpentane (see Table 2) was used to calculate $\Delta_r G_m^\circ$ for reaction 8. This seems reasonable since the aqueous and the organic phases had equilibrated for 30 days. Also, it seems unlikely that the solubility of water in the 2,2,4-trimethylpentane would be significantly affected by the dilute solutes that were also present in the organic phase. The thermochemical cycle gives $\Delta_r G_m^\circ = -(36.9 \pm 2.9)$ kJ mol$^{-1}$ for reaction 3; the corresponding equilibrium constant $K_m = (2.9^{+6.5}_{-2.0}) \times 10^{6}$. Therefore, in aqueous solution, the formation of dodecyl dodecanoate is favored over

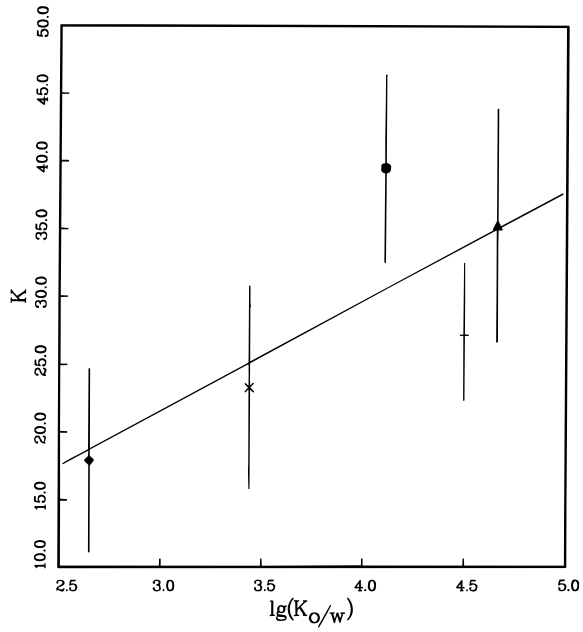


**Figure 1.** Equilibrium constants for reaction 1 vs the (octanol + water) partition coefficients $K_{o/w}$ of the five solvents used in this study. The solvents and their respective symbols are hexane, ○; heptane, ▲; cyclohexane, ×; 2,2,4-trimethylpentane, +; toluene, ◆.

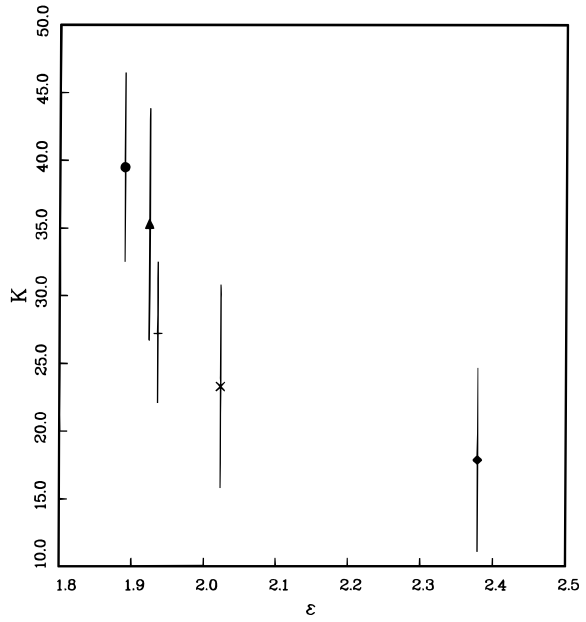


**Figure 2.** Equilibrium constants for reaction 1 vs the relative permittivities $\epsilon$ of the solvents. The solvents and their respective symbols are hexane, ○; heptane, ▲; cyclohexane, ×; 2,2,4-trimethylpentane, +; toluene, ◆.

hydrolysis. Also, the equilibrium constant for reaction 3 is significantly larger than the equilibrium constants for reaction 1 in the organic solvents. Both findings are contrary to what has been previously found (Tewari et al., 1995, 1996). However, in this reaction, the saturation molalities of the reactants are much lower than in the earlier studies. Perhaps, very hydrophobic compounds such as 1-dodecanol, 1-dodecanoic acid, and dodecyl dodecanoate act differently in aqueous solutions.

Valivety et al. (1991) studied the lipase-catalyzed esterification reaction

$$\text{1-dodecanol(sln)} + \text{1-decanoic acid(sln)} = \text{dodecyl decanoate(sln)} + H_2O\text{(sln)} \quad (10)$$

Several organic solvents were used in their investigation. However, in reporting their results, they assumed that the activity of water in the reaction was unity and they did not include the concentration of the water in the calculation of the equilibrium constant for reaction 10. Thus, they reported the ratio [dodecyldecanoate(sln)]/{[1 − dodecanol-(sln)]·[1 − decanoic acid(sln)]}, where the square brackets denote concentration. The reported (Valivety et al., 1991) ratios for this reaction in toluene, hexane, and 2,2,4-trimethylpentane are 610, 700, and 1600 $mol^{-1}\ dm^3$, respectively. By using the molalities of water in these organic phase determined in the present study and the densities of the respective solvents (Dreisbach, 1959), the equilibrium constants for reaction 10 in toluene, hexane, and 2,2,4-trimethylpentane are calculated to be 72.4, 11.5, and 56.1, respectively. Although the reaction they studied was not identical to the reaction studied herein, the values of the equilibrium constants obtained for reaction 10 are comparable to the values obtained in this study for reaction 1.

Previous thermodynamic studies of reactions in nonaqueous media (Janssen et al., 1993a; Valivety et al., 1991) have attempted to correlate the results with the partition coefficients and the relative permittivities. Shown in Figure 1 is a plot of the equilibrium constants for reaction 1 vs the decadic logarithm of the octanol water partition coefficients $K_{o/w}$ for the pertinent solvents. The values of $K_{o/w}$ for hexane, heptane, and toluene are from Tewari et al. (1982), and the values for cyclohexane and 2,2,4-trimethylpentane are from Hansch and Leo (1979). There is a good correlation between the equilibrium constants and the octanol water partition coefficients $K_{o/w}$ of the solvents. Since the solvents have similar hydrophobicity and (octanol + water) partition coefficients, this is not surprising. Figure 2 is a plot of the equilibrium constants for reaction 1 against the relative permittivities of the pertinent solvents, which were taken from Dreisbach (1959). Here again the equilibrium constants correlate well with relative permittivities of the solvents. However, since the relative permittivities of the solvents used in this study had a relatively narrow range, it does not seem wise to make any generalization in regard to this matter.

The results given in Table 2 also show that the molality of the product, dodecyl dodecanoate, in hexane is twice as high as in any other solvent. This suggests that, among these solvents and barring other complications, hexane is the preferred solvent for the esterification of 1-dodecanol and 1-dodecanoic acid.

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