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Mechanistic study on the derivatization of aliphatic carboxylic acids in aqueous non-ionic micellar systems

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SUMMARY

A model is proposed for the derivatization mechanism of carboxylic acids with a fluorescent label, 4-bromomethyl-7-methoxycoumarin, in an aqueous non-ionic micellar system with the use of a tetraalkylammonium ion-pairing cation. Using experimentally obtained extraction constants and partition coefficients for aliphatic carboxylic acids in the micellar solution, the model was compared with the observed derivatization rate constants of these acids; this gave a satisfactory correlation. Owing to its similarity with phase-transfer catalysis, the present micelle-mediated derivatization is termed micellar phase-transfer catalysis.

INTRODUCTION

Recently, aqueous micellar systems have been used for the derivatization of carboxylic acids [1,2]. By using micelles, carboxylic acids can be derivatized directly in an aqueous (physiological) matrix with minimum sample pretreatment, *i.e.*, tedious extraction procedures can be circumvented. In a previous paper we reported the influence of micelles on the derivatization of 10-undecenoic acid with the fluorophore 4-bromomethyl-7-methoxycoumarin (BrMMC)[1]. Optimum derivatization rates were obtained in non-ionic micelles in the presence of an ion-pair agent, *e.g.*, tetrahexylammonium bromide. We suggested that the reaction mechanism in the aqueous micellar systems is related to the mechanism known as phase-transfer catalysis (PTC).

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$$\begin{array}{ccccccccc}
 & & K_a & & & & & & \\
HA_w & \rightleftharpoons & H^+_w & + & A^-_w & + & Q^+_w & + & Br^-_w \quad\text{aqueous phase} \\
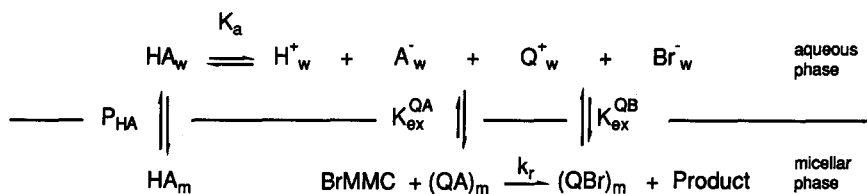\end{array}$$

Fig. 1. Model for the MPTC mechanism in a non-ionic micellar system. The symbols are explained in the text.

PTC is based on the principle that an analyte is derivatized in an organic phase directly after it has been extracted from an aqueous phase using an ion-pair agent[3,4]. Because of the observed similarity, the micelle-mediated derivatization reaction is termed micellar phase-transfer catalysis (MPTC). The distinct advantage of using MPTC systems is that the reaction mixture, unlike in traditional PTC systems, can be injected directly into reversed-phase high-performance liquid chromatographic (RP-HPLC) systems without encountering the problem of peak deterioration.

The aim of this study was to investigate further the MPTC mechanism, for which a micellar system was selected composed of an aqueous solution containing Arkopal N-130 as a non-ionic surfactant, tetrahexylammonium bromide as ion-pair agent, several even-numbered saturated aliphatic carboxylic acids as analytes and BrMMC as derivatization reagent. A quantitative model for the MPTC mechanism is proposed which proved to be in satisfactory agreement with the experimental results.

THEORY

Micellar phase-transfer catalysis

The model for the derivatization mechanism of carboxylic acids in a non-ionic micellar system is based on the supposition that the reaction of the acid with the reagent occurs in the hydrocarbonaceous core of the micelle (Fig. 1)[1]. With excess of BrMMC reagent present in the micelles, the derivatization rate, v, can be expressed by

$$v = k_r[(QA)_m] \tag{1}$$

where k_r is the pseudo-first-order rate constant and $[(QA)_m]$ is the concentration of the ion-pair complex of the acid in the micelle. The value of $[(QA)_m]$ is given by[3,4]

$$[(QA)_m] = K^{QA}_{ex}[Q^+_w][A^-_w] \tag{2}$$

where K^{QA}_{ex} is the extraction constant of the acid and $[Q^+_w]$ and $[A^-_w]$ are the concentrations of the tetraalkylammonium cation and of the conjugate base in the aqueous bulk phase, respectively. The extraction of the counter ion of the ion-pair agent, Br^-, into the micelle is expressed by

$$K^{QB}_{ex} = [(QBr)_m]/[Q^+_w][Br^-_w] \tag{3}$$

where K^{QB}_{ex} is the extraction constant of the counter ion. The acidity constant of the acid in the aqueous phase, K_a, is given by

$$K_a = [H^+_w][A^-_w]/[HA_w] \tag{4}$$

The non-dissociated acid HA partitions between the aqueous and micellar phases. The partition coefficient P_{HA} is given by the ratio of the concentrations of HA in the micellar and aqueous phases:

$$P_{HA} = [HA_m]/[HA_w] \tag{5}$$

The partitioning of the conjugate base is not considered, because such a transfer will be restricted electrostatically[5,6]. The partitioning of salts of A$^-$ is also not taken into account, because the partition coefficients are a few decades smaller than those of the non-dissociated acid[7,8]. For reasons of simplification, in the present model the formation of ion pairs in the aqueous phase[9,10] and the dissociation of the ion-pair complex[9,11] or of the acid[12] in the micellar phase and the formation of oligomers of the ion pair[9] and the formation of dimers of the acid[13] in the micellar phase are not taken into account.

The analytical concentration of the acid, $[HA]_t$, is then given by

$$[HA]_t = \varphi_m([HA_m] + [(QA)_m]) + \varphi_w([HA_w] + [A_w^-]) \tag{6}$$

where φ_m and φ_w are the volume fractions of the micelles and the aqueous bulk phase, respectively. φ_m, which is 0.036 at 50 mM Arkopal N-130, is equal to CV, where C is the concentration of the surfactant exceeding the critical micelle concentration (CMC = 90 μM, ref. 14), and V (0.9 ml/g, ref. 15) is the specific volume of the surfactant[16]. After substitution of eqns. 2, 4 and 5 into eqn. 6 and rearranging, $[(QA)_m]$ can be expressed as

$$[(QA)_m] = \frac{K_{ex}^{QA}[Q_w^+][HA]_t}{(\varphi_w + P_{HA}\varphi_m)[H^+]/K_a + \varphi_w + K_{ex}^{QA}\varphi_m[Q_w^+]} \tag{7}$$

$[Q_w^+]$ is calculated from the known ion-pair agent analytical concentration, $[Q]_t$. If the analytical concentration of the acid is much smaller than that of the ion-pair agent, then $[(QA)_m]$ is small compared with the other Q-containing species and can be neglected in the calculation of $[Q_w^+]$. In this case, $[Q]_t$ is given by

$$[Q]_t = \varphi_w[Q_w^+] + \varphi_m[(QBr)_m] \tag{8}$$

Provided that $[Q_w^+] = [Br_w^-]$, substitution of eqn. 8 into eqn. 3 results in

$$K_{ex}^{QB}\varphi_m[Q_w^+]^2 + \varphi_w[Q_w^+] - [Q]_t = 0 \tag{9}$$

The physically relevant solution for this equation is

$$[Q_w^+] = -\varphi_w + \frac{\{\varphi_w^2 + 4\varphi_m K_{ex}^{QB}[Q]_t\}^{\frac{1}{2}}}{2K_{ex}^{QB}\varphi_m} \tag{10}$$

In other words, if K_{ex}^{QA}, K_{ex}^{QB} and P_{HA} can be determined, $[(QA)_m]$ can be calculated using eqns. 7 and 10.

Determination of K_{ex}^{QA}, K_{ex}^{QB} and P_{HA}

Data in the literature on extraction constants[3,4,11] and partition coefficients[11,17] could not be used in this study, because they are reported only for traditional two-phase systems and depend on the properties of the two-phase system[3,4,11,17]. The determination of extraction constants or partition coefficients in micellar solutions by simply measuring the concentrations of the species in the two pseudo-phases will be very difficult because of the macro-homogeneity of micellar solutions.

However, previous studies have demonstrated that it is possible to calculate the partition coefficient of an analyte in a micellar solution from the relationship between the observed acidity constant of an acid in the micellar solution, termed K_a^{obs}, and φ_m[5,6,14,18–21]. This approach is based on the supposition that all acid species present in either the micellar or the aqueous bulk phase contribute to K_a^{obs}:

$$K_a^{obs} = \frac{[H^+]([A_w^-]\varphi_w + [A_m^-]\varphi_m)}{[HA_w]\varphi_w + [HA_m]\varphi_m} \tag{11}$$

In this study, the above expression was modified by neglecting the $[A_m^-]\varphi_m$ term (see above) and introducing $[(QA)_m]\varphi_m$ as a species which in this instance contributes to K_a^{obs}:

$$K_a^{obs} = \frac{[H^+]([A_w^-]\varphi_w + [(QA)_m]\varphi_m)}{[HA_w]\varphi_w + [HA_m]\varphi_m} \tag{12}$$

Substitution of eqns. 2, 4 and 5 into eqn. 12 yields

$$K_a^{obs} = \frac{K_a(\varphi_w + K_{ex}^{QA}\varphi_m[Q_w^+])}{(\varphi_w + P_{HA}\varphi_m)} \tag{13}$$

or, in logarithmic form,

$$pK_a^{obs} = pK_a + \log(\varphi_w + P_{HA}\varphi_m) - \log(\varphi_w + K_{ex}^{QA}\varphi_m[Q_w^+]) \tag{14}$$

This equation allows the calculation of the partition coefficient, P_{HA}, and the extraction constant, K_{ex}^{QA}, from the relationship between pK_a^{obs} and $[Q_w^+]$. First, in the absence of the ion-pair agent the value of P_{HA} can be calculated from the difference between pK_a^{obs} in the pure micellar solution and in water. Next, after addition of the ion-pair agent the value of K_{ex}^{QA} can be calculated from the slope of the relationship between K_a^{obs} and $[Q]_t$, which is almost linear. From the deviation of linearity of this relationship, because $[Q_w^+]$ is not directly proportional to $[Q]_t$ (*cf.*, eqn. 10), the value of K_{ex}^{QB} can be deduced. In practice, the above approach was carried out by using a three-parameter (*i.e.*, P_{HA}, K_{ex}^{QA} and K_{ex}^{QB}) non-linear optimization procedure, as will be explained under Experimental.

EXPERIMENTAL

Materials

Milli-Q water (Millipore, Milford, MA, U.S.A.) was used throughout. Except

for the Arkopal N-130 surfactant [a polyoxyethylene(13)nonylphenyl ether], the chemicals were of analytical-reagent grade. The chemicals were used as received. The even-numbered aliphatic carboxylic acids were purchased from Merck (Darmstadt, F.R.G.). Arkopal N-130 was a gift from Hoechst Holland (Amsterdam, The Netherlands). Tetrahexylammonium bromide (THxABr) and tetrabutylammonium bromide (TBuABr) were obtained from Fluka (Buchs, Switzerland) and 4-bromo-methyl-7-methoxycoumarin (BrMMC) from Fluka or Sigma (St. Louis, MO, U.S.A.).

The aliphatic carboxylic acids were dissolved in acetone at a concentration of 100 mM (titrimetric experiments) or 5 mM (derivatization experiments) and stored at 4°C. A saturated solution of the reagent, made by adding 8 mg of BrMMC per millilitre of acetone, was stored at 4°C; it was prepared fresh every week. Prior to derivatization the BrMMC was completely dissolved by heating to *ca.* 40°C.

Derivatization procedure

A summary of the derivatization procedure[1] is as follows. To 965 μl of a 10 mM phosphate buffer (pH 7.0) containing 50 mM Arkopal N-130 and 36 mM THxABr, 10 μl of the carboxylic acid stock solution were added, if not stated otherwise. The incubation was started by the addition of 25 μl of BrMMC stock solution. All incubations were carried out protected from light, in a laboratory-built swerve-water-
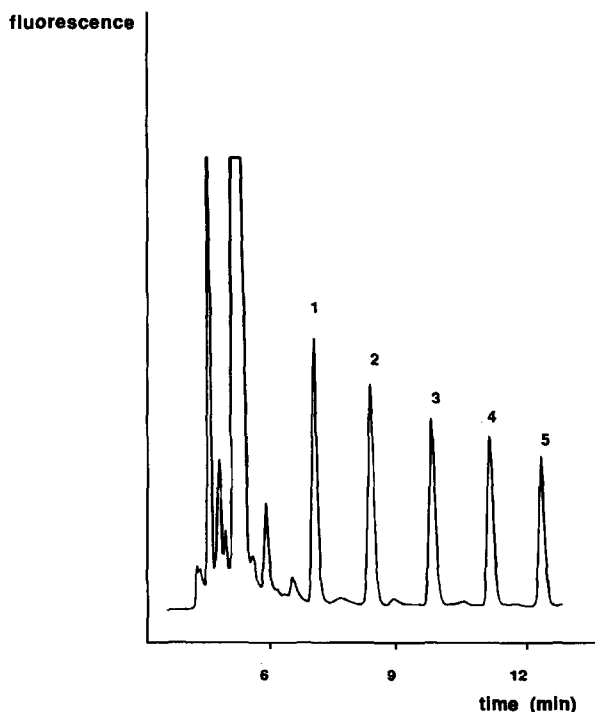

Fig. 2. Reversed-phase HPLC of MMC derivatives of five fatty acids (50 μM each) obtained from an aqueous non-ionic micellar system containing 50 mM Arkopal N-130 and 36 mM THxABr at 70°C and pH 7.0. Peaks: 1 = decanoyl-MMC; 2 = dodecanoyl-MMC; 3 = tetradecanoyl-MMC; 4 = hexadecanoyl-MMC; 5 = octadecanoyl-MMC.

bath at 70°C. At given times, 75-μl samples were taken and added to 75 μl of acetonitrile.

Chromatographic system

As described previously[1], 20 μl of the diluted sample were analysed using an automated high-performance liquid chromatographic (HPLC) system. A 100 × 3.0 mm I.D. analytical column (5-μm Chromspher C_{18}; Chrompack, Middelburg, The Netherlands) was used. A linear gradient was run in 12 min from methanol–water (60:40, v/v) to 100% methanol. A Model 650 fluorescence detector (Hitachi–Perkin-Elmer, Tokyo, Japan) was used with optimized excitation and emission wavelengths of 330 and 395 nm, respectively. Retention times and peak areas were measured with an SP 4270 integrator (Spectra-Physics, Santa Clara, CA, U.S.A.). The performance of the chromatographic system is illustrated by Fig. 2, which shows that the MMC derivatives of five saturated fatty acids (50 μM) are well separated.

Identification of the derivatives

The product of dodecanoic acid with BrMMC was prepared in acetone and in the micellar system. The derivatization in acetone, using potassium carbonate and 18-crown-6 ether to accelerate the reaction, has been described previously[1,22]. After reaction for 30 min at 60°C, the supernatant was transferred to a clean vial, in which the acetone was evaporated under a stream of nitrogen.

After derivatization in the micellar system for 1 h at 70°C, the derivative was extracted into 1,2-dichloroethane (DCE). Next, the DCE layer was transferred to a clean vial and evaporated under a stream of nitrogen. In both instances the dodecanoyl-MMC derivative was isolated using thin-layer chromatography on silica with toluene–ethyl acetate (75:15, v/v) as the eluent[23]. The HPLC retention times of the purified compounds were the same as before the isolation procedure. The mass spectra, obtained using electron impact ionization with an MS 80 spectrometer (Kratos, Ramsey, NJ, U.S.A.), of the derivatives obtained from acetone and the micellar system were identical; three main peaks occurred, at m/z 388, corresponding to the molecular weight, and at m/z 206 and 190, from the chromophoric label.

Determination of pK_a^{obs}

In general, 250 μl of the carboxylic acid stock solution were added to 20 ml of carbon dioxide-free Milli-Q water in which the surfactant, ion-pair agent and other additives had been dissolved. After acidification with 1 M hydrochloric acid, the pK_a^{obs} values of the acids were determined in duplicate at 25.0 ± 0.2°C, using a glass–calomel pH electrode (Metrohm, Herisau, Switzerland), by titration with 0.1 M sodium hydroxide solution with a 636 Titroprocessor (Metrohm). For the long-chain acids ($\geqslant$ C_{10}), the pK_a values could not be determined experimentally because of the poor solubility of the acids. For these acids the pK_a values were obtained from ref. 24.

Calculation of K_{ex}^{QA}, K_{ex}^{QB} and P_{HA}

For the calculation of the extraction constants and the partition coefficients, eqn, 10 was substituted into eqn. 13 and a three-parameter non-linear optimization procedure (Marquardt algorithm[25]) of this equation was performed through the experimental pK_a^{obs} vs. [Q]$_t$ data points. To ensure a satisfactory statistical significance

of the fitting procedure, the number of degrees of freedom, *i.e.*, the number of data points minus the number of parameters, P_{HA}, K_{ex}^{QA} and K_{ex}^{QB}, was at least seven in all instances.

For several acids the value of P_{HA} was also determined from the relationship between pK_a^{obs} and φ_m[5,6,18]:

$$(\varphi_w - K_a^{obs}/K_a)\varphi_m^{-1} = P_{HA}(K_a^{obs}/K_a) - P_{A^-} \qquad (15)$$

which is a different expression for eqn. 11.

RESULTS AND DISCUSSION

First in this section, the effects of additives on the pK_a values of acids in the micellar system are discussed. From these experimental data, the partition coefficients and extraction constants for the acids were calculated, and were subsequently fitted into the proposed model for the derivatization rate in the micellar system. Finally, by comparing the model with experimental reaction rate data, its validity was evaluated.

Determination of acidity constants

Fig. 3 shows the pK_a^{obs} values of the acids in various solutions as a function of their chain length. The pK_a values of the shorter chain homologues ($\leqslant C_8$) are in good agreement with those reported in the literature[24,26]. In aqueous 50 mM Arkopal N-130 solution, no significant pK_a^{obs} shift was observed with the short-chain acids ($\leqslant C_4$), indicating that the partition coefficients of these acids are small (*cf.*, eqn. 14). In contrast, in the micellar solution pK_a^{obs} increases for the long-chain acids. This marked
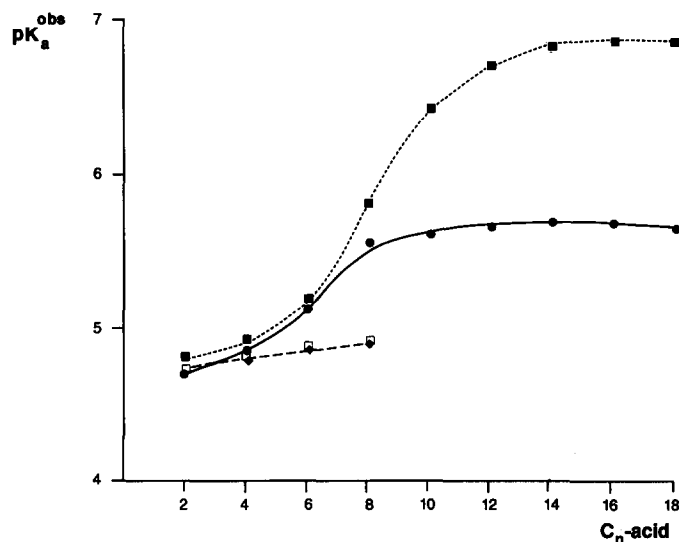

Fig. 3. Dependence of pK_a^{obs} (at 25°C) of aliphatic carboxylic acids with alkyl chain length C_n on the composition of the aqueous micellar system. ◆ = Water; □ = 18 mM THxABr; ■ = 50 mM Arkopal N-130; ● = 50 mM Arkopal N-130 with 36 mM THxABr.

shift cannot be attributed to the influence of the micelles on the pH electrode[27,28]. Shifts in the pK_a^{obs} values of ionizable analytes in the presence of micelles are often observed[12,29] and are related to the influence of the low dielectric constant, ε, in the micelle on the acidity constant of the analyte[12,30]; that is, a low ε restricts the dissociation of the non-charged acid. Therefore, the partitioning of the acid from the aqueous bulk phase, with $\varepsilon = 80$[30], to the micelle, with ε(interface) $= 25$ and ε(core) $= 3$[30], probably causes the shift in the pK_a^{obs} found in this present study. From the value of pK_a^{obs} of ca. 6.85 for the long-chain acids in the pure micellar system (Fig. 3), it can be concluded that the carboxylic moiety mainly resides in an environment with medium polarity, probably the hydrated polyoxyethylene interface of the micelle.

The addition of 36 mM THxABr to the micellar solution causes a decrease in the pK_a^{obs} values relative to those observed in pure micellar systems (Fig. 3), whereas with the addition of 18 mM THxABr (solubility limit) to an aqueous solution no marked effect on the pK_a^{obs} is found. As is also true for surfactants, it is unlikely that the shift in pK_a^{obs} in the micellar solution originates from an influence of the ion-pair agent on the pH electrode. Probably the anionic species, A^-, is extracted by the ion-pair agent into the micellar phase. This decreases the ratio HA/A^- (Fig. 1) and hence causes a decrease of pK_a^{obs}.

The MPTC mechanism predicts that $[(QA)_m]$ will decrease in the presence of other anions that compete for extraction into the micelle. This obviously depends on both the extraction constant and the concentration of the competitive ions[3,4]. The decrease in $[(QA)_m]$ in the micellar system results in an increase in pK_a^{obs} of the acid and a decrease in the derivatization rate[1]. Fig. 4 shows the influence of perchlorate, bromide and chloride anions on the pK_a^{obs} of dodecanoic acid in an aqueous solution of 50 mM Arkopal N-130 and 36 mM THxABr. The effect is strongest for the perchlorate
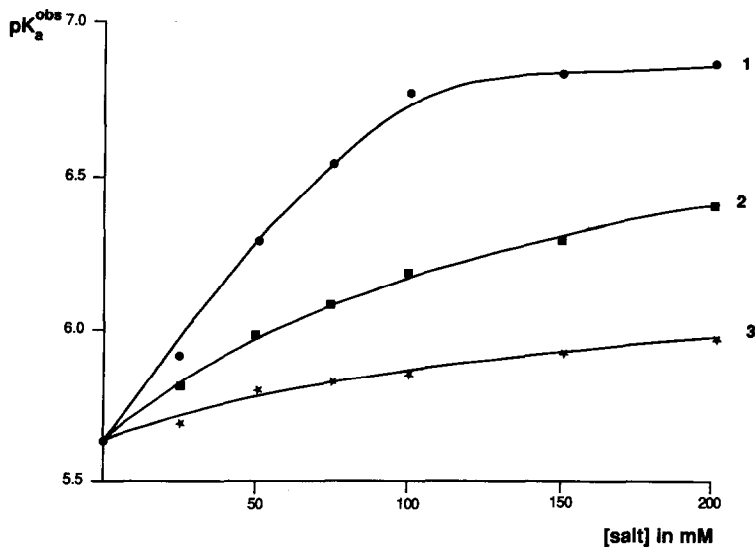

Fig. 4. Influence of 0–200 mM (1) NaClO$_4$, (2) KBr or (3) NaCl on the pK_a^{obs} of dodecanoic acid in an aqueous solution of 50 mM Arkopal N-130 and 36 mM THxABr at 25°C.

TABLE I

COMPILATION OF CALCULATED EQUILIBRIUM CONSTANTS

Acid	$Log\ K_{ex}$ using				$Log\ P_{HA}$ using			
	$THxA^+$		$TBuA^+$		$Eqn.\ 14$		$Eqn.\ 15$	$Ref.\ 11^a$
	A^-	Br^-	A^-	Br^-	$THxABr$	$TBuABr$		
C_6	2.7	1.6	1.9	1.2	1.4	1.3	1.5	0.9
C_8	3.4	1.5	2.3	1.1	2.2	2.2	2.3	2.1
C_{10}	4.0	1.5	2.9	1.0	3.0	3.0	ND^b	3.3
C_{12}	4.2	1.4	3.5	0.9	3.1	3.2	ND	4.5
C_{14}	4.2	1.3	3.8	0.7	3.2	3.1	ND	5.7
C_{16}	4.2	1.3	4.2	0.8	3.2	3.2	ND	6.7
C_{18}	4.2	1.3	4.2	0.5	3.2	3.2	ND	8.1

^{a} P_{HA} determined in a chloroform–water two-phase system.
^{b} ND = Not determined.

ion and is in good agreement with the order of the extraction constants of these ions $(ClO_4^- \gg Br^- > Cl^{-3,4})$.

In the absence of the ion-pair agent, the influence of 100 mM sodium chloride on the pK_a^{obs} of dodecanoic acid in an aqueous 50 mM Arkopal N-130 micellar system is
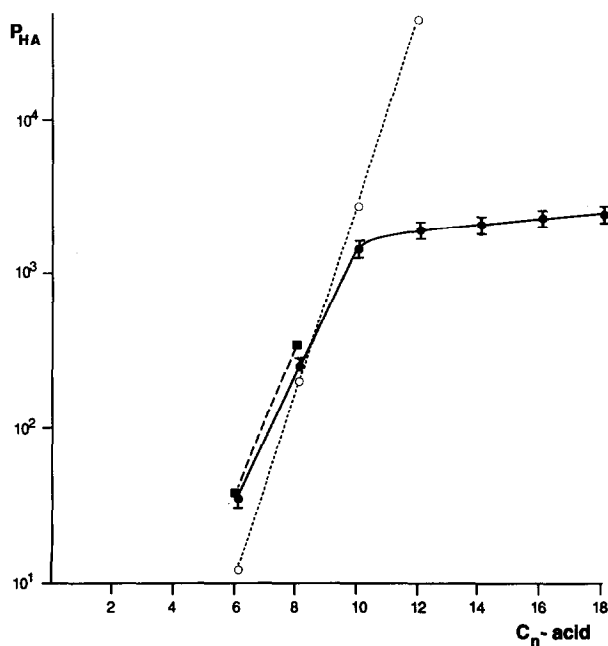

Fig. 5. Partition coefficient, P_{HA}, calculated for fatty acids with chain length C_n. ● = P_{HA} calculated from eqn. 14; ■ = P_{HA} calculated from eqn. 15; ○ = P_{HA} with dichloromethane as the organic phase[11]. The vertical bars indicate the standard deviation of the determined values.

small, *viz.*, *ca.* 0.1 pH unit. Obviously, the partitioning of the carboxylate salts is small; this was an additional argument for neglecting it in the present model.

Determination of P_{HA}

Table I and Fig. 5 show the P_{HA} values calculated for the acids in the micellar solutions using eqn. 14. For acids up to decanoic acid, log P_{HA} increases linearly with increasing carbon number (Fig. 5). A similar relationship has been observed in other two-phase systems[11,17], and is to be expected on the basis of thermodynamic considerations. For the long-chain acids the calculated P_{HA} values abruptly reach a plateau at a value of 1600, which was explained as follows. If the first derivative of eqn. 14 is taken, *i.e.*, dpK_a^{obs}/dP_{HA}, it is evident that the sensitivity of this expression decreases with increasing P_{HA}. Eventually, the sensitivity becomes less then the experimental error of 0.02 pH unit, that is, large P_{HA} values cannot be discriminated by the optimization algorithm, which results in a plateau being reached.

The partition coefficients were determined using the relationship between pK_a^{obs} and φ_m (*cf.*, eqn. 15), as shown in Fig. 6 and Table I. The P_{HA} values for decanoic acid and larger homologues could not be determined because these acids form mixed micelles with the non-ionic surfactant[31]. Even at low surfactant concentrations, this yielded a high and constant pK_a^{obs} of 6.85. For butanoic acid and smaller homologues, the P_{HA} values could not be determined either, because their pK_a^{obs} values turned out to be insensitive to even high surfactant concentrations. The P_{HA} values calculated for hexanoic and octanoic acid using eqn. 15 are in satisfactory agreement with those obtained from eqn. 14. The P_{HA} values determined for the acids in the present micellar and in conventional two-phase systems[11,17] are similar (Fig. 5). This suggests that it may by possible to use the P_{HA} values, which have been extensively reported for common two-phase systems[17], also for micellar systems.

From the influence of the number of carbon atoms in the acid on the partition coefficients of hexanoic acid to decanoic acid, it is possible to calculate the incremental free energy of transfer from water to the micelle per methylene group, n, in the acid[21,32]:

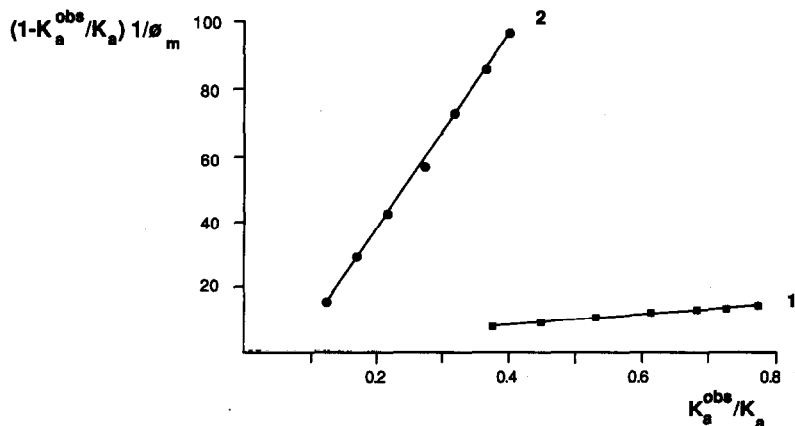$$d\mu_t^0 = d\mu_x^0 + nd\mu_c^0 = -RT\ln(55.5\,K) \tag{16}$$

Fig. 6. Plots of $(1 - K_a^{obs}/K_a)/\varphi_m$ *vs.* K_a^{obs}/K_a according to eqn. 13 for (1) hexanoic acid and (2) octanoic acid.
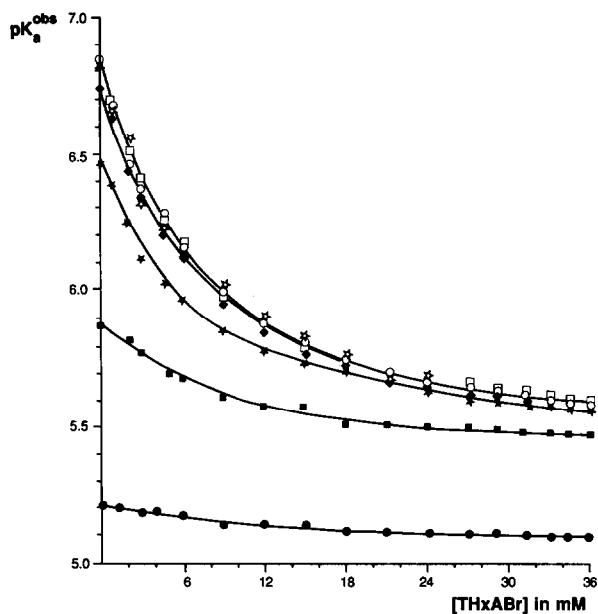
Fig. 7. Dependence of pK_a^{obs} for acids with chain length C_n on the THxABr concentration in an aqueous solution of 50 mM Arkopal N-130. ● = Hexanoic acid; ■ = octanoic acid; ★ = decanoic acid; ◆ = dodecanoic acid; ☆ = tetradecanoic acid; □ = hexadecanoic acid; ○ = octadecanoic acid.

where $d\mu_t^0$ is the standard free energy of transfer, $d\mu_c^0$ and $d\mu_x^0$ are the contributions of the methylene group and the residual groups and K is the absolute temperature in Kelvin, respectively. Using eqn. 16, a value for $d\mu_c^0$ of ca. -2.3 kJ mol^{-1} is calculated, which is in good agreement which those reported for other micellar systems (-2.4 to -3 kJ mol^{-1})[21,32].
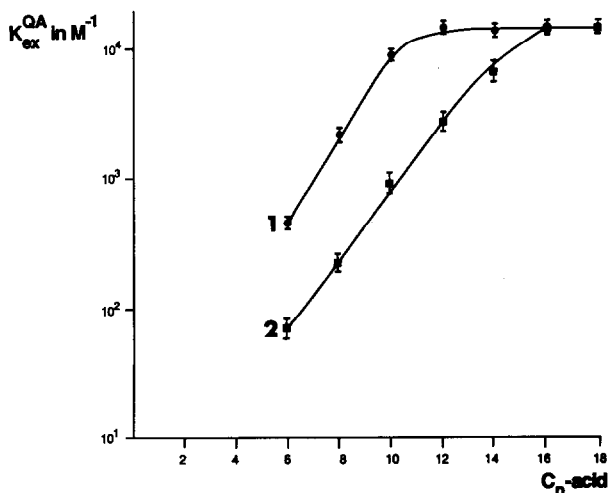

Fig. 8. Extraction constants, K_{ex}^{QA}, for C_n carboxylic acids calculated using eqn. 13, with (1) THxABr and (2) TBuABr. The vertical bars indicate the standard deviation of the determined values.

In conclusion, the partition coefficients determined from hexanoic acid through to decanoic acid in the micellar solution seem to be reliable for use in further calculations.

Determination of K_{ex}^{QA}

Fig. 7 shows the influence of THxABr concentration on the pK_a^{obs} values of several acids in aqueous 50 mM Arkopal N-130 solution. From these curves, the K_{ex}^{QA} values for the acids were calculated using eqn. 14; they are shown in Fig. 8 and Table I. Log K_{ex}^{QA} increases linearity with increasing number of carbon atoms in the carboxylic acid from hexanoic acid through to decanoic acid. A similar behaviour in two-phase systems is known from the literature[3,4,11], and is to be expected on the basis of thermodynamic considerations. For dodecanoic acid and larger homologues, log K_{ex}^{QA} reaches a plateau, for reasons mentioned in the discussion on the determination of P_{HA}. The extraction constants for the acids with TBuABr were also determined in the micellar solution. Up to hexadecanoic acid no plateau is reached (Fig. 8). This also suggests that the plateau observed for K_{ex}^{QA} is caused by the inadequacy of eqn. 14 to discriminate between large extraction constants, rather than by factors such as non-ideal behaviour of the extraction equilibrium in the micellar system. The variation in the calculated values of K_{ex}^{QB} for THxABr and for TBuABr is small (Table I), as is to be expected.

The combined results emphasize the reliability of eqn. 14 for calculating extraction constants in micellar solutions if they are not too large.

Effect of pH on the derivatization rate

The influence of the pH on k_{obs} in the micellar system was investigated for the reaction between decanoic acid and BrMMC. Fig. 9 shows that the observed derivatization rate constant is sigmoidally related to the pH with an inflection point at pH 5.5 $\pm$ 0.1, while a conventional sigmoidal titration curve was obtained with a pK_a^{obs}

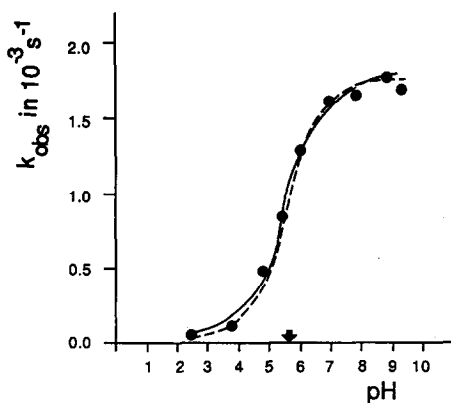Fig. 9. Dependence of k_{obs} of the reaction of decanoic acid (50 μM) with BrMMC, on the pH of an aqueous solution containing 50 mM Arkopal N-130 and 36 mM THxABr at 70°C. The arrow indicates the pK_a^{obs} as determined titrimetrically for decanoic acid in the micellar system at 25°C. The dashed line indicates the influence of the pH on [(QA)$_m$] obtained from eqn. 7.
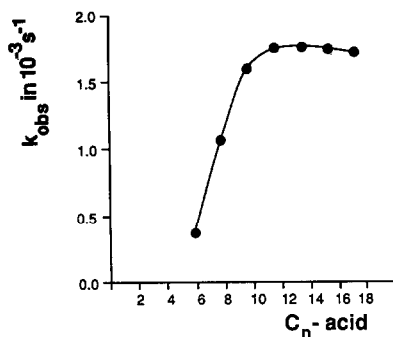
Fig. 10. Dependence of k_{obs} on the number of carbon atoms in the carboxylic acids, C_n. Conditions: aqueous solution containing 50 mM Arkopal N-130 and 36 mM THxABr at 70°C and pH 7.0.

of 5.64 ± 0.02 (data not shown). The direct proportionality of the derivatization rate and [A⁻] is to be expected, as can easily be verified by combining eqns. 1 and 2.

In addition, Fig. 9 includes data on the influence of pH on [(QA)$_m$] calculated using eqn. 7 and the parameters given in Table I. A good correlation is observed, which supports the validity of the present model.

From the above results, it can be inferred that, as in other micelle-mediated reactions[29], the pH of the micellar solution is an important parameter. From Figs. 3 and 9 it can be deduced that the derivatization of aliphatic carboxylic acids has to be performed at neutral pH, which is ca. 1.5 units above pK_a^{obs} in the micellar solution, to ensure optimum reaction rates for these acids.

Effect of the acid chain length on the derivatization rate

Fig. 10 shows the relationship between k_{obs} and the chain length of the aliphatic carboxylic acids. The k_{obs} values of the short-chain acids ($\leqslant$ C$_4$) could not be determined owing to high reagent blanks. Up to decanoic acid, k_{obs} increases, which reflects the expected influence of the increasing extractability of the acids on the derivatization rate. For the long-chain acids the derivatization rate reaches a plateau, because of the complete extraction of the conjugate bases having a large K_{ex}^{QA}. The overall reaction rate is then likely to be limited by the rate constant in the micelle, k_r, which is probably independent of the alkyl chain length of the acid.

For two acids, hexanoic and decanoic acid, the validity of the model for the prediction of their derivatization rate in the micellar solution (eqn. 7) was investigated further. Eqn. 1 shows that, if k_r is constant, the derivatization rate is linearly related to [(QA)$_m$]. The latter parameter was, therefore, used as a measure of the derivatization rate in the micellar solution. Fig. 11 shows the value of [(QA)$_m$], expressed as a percentage of [HA]$_t$, for hexanoic acid and decanoic acid as a function of the ion-pair agent concentration in aqueous 50 mM Arkopal N-130 solution, using the data given in Table I. Fig. 11 shows that the general shape of the calculated extraction curves is in line with expectation[3,4]: first, the curves reflect the influence of the extractibility of the acid on [(QA)$_m$] and, second, a plateau is reached in [(QA)$_m$] for decanoic acid owing to its large extraction constant. The model also predicts such a plateau for hexanoic acid; however, this should occur at a much higher ion-pair agent concentration, which is not attainable experimentally, because of the occurrence of phase separation above 36 mM THxABr[1].
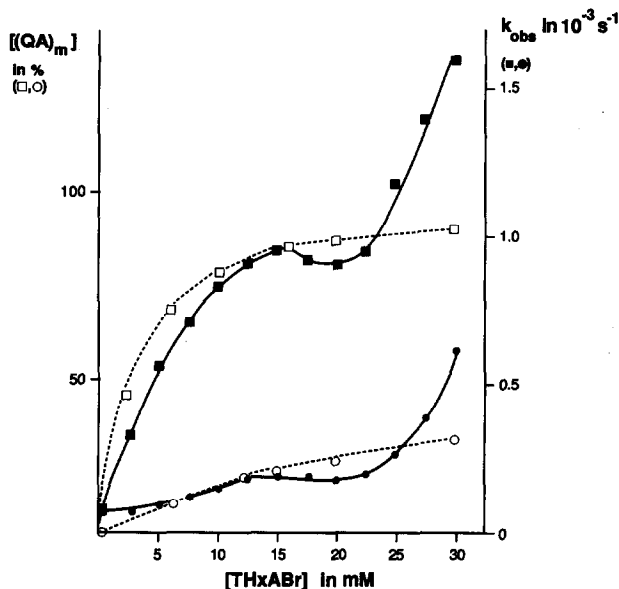
Fig. 11. Effect of THxABr concentration on the calculated concentration of $[(QA)_m]$, expressed as a percentage of $[HA]_t$, using eqn. 7, and on the rate constant, k_{obs} (at 70°C), for hexanoic and decanoic acid in aqueous 50 mM Arkopal N-130 solution. $\bigcirc$ = $[(QA)_m]$ calculated for hexanoic acid; $\square$ = $[(QA)_m]$ calculated for decanoic acid; $\bullet$ = observed rate constant for hexanoic acid; $\blacksquare$ = observed rate constant for decanoic acid.

The observed derivatization rate constants for hexanoic and decanoic acid in aqueous 50 mM Arkopal N-130 solution at pH 7.0 and 70°C are also shown in Fig. 11. The calculated $[(QA)_m]$ values are seen to match the actual derivatization profiles fairly well up to ca. 20 mM THxABr. The similarity indicates that below this THxABr concentration, the observed derivatization rate is likely to be limited by $[(QA)_m]$ (eqn. 1). Above 20 mM THxABr the derivatization profiles deviate from the calculated curve, which will be discussed below.

It has been shown that above 20 mM thxABr the micellar size increases markedly, which finally results in opaque solutions at the so-called cloud temperature, T_c, at 36 mM THxABr[1,33,34]. An increase in the size of micelles is not likely to reduce φ_m significantly and is, therefore, considered not to be the main cause of the deviating derivatization rates observed. The conclusion that changes in the micellar system as such have a small effect on the derivatization rate is further supported by the observation that for the fluorescence reagent 9-bromomethylacridine (BrMAC) the calculated extraction curve is very similar to the actual derivatization profile[35], that is, a plateau in the rate constant is observed above 20 mM THxABr.

A likely explanation for the observed derivatization profile is as follows. Next to the micellar size, the physico-chemical properties of the micellar solution also change on approaching T_c, e.g., water is expelled from the micelles[30,36]. Because it has been demonstrated that reactions involving BrMMC are very sensitive to the presence of water[37,38], the latter effect may well be responsible for the optimum in the reaction rate at T_c. These combined results indicate that below ca. 20 mM THxABr, the increasing

derivatization rate is related to an increase in $[(QA)_m]$, whereas for 20–36 mM THxABr the increase is caused by an increase in the reaction rate constant in the micelle.

The decrease in the observed derivatization rate above 36 mM THxABr, which occurs for both BrMMC[1] and BrMAC[35], can be attributed to the occurrence of phase separation[1], which probably disturbs the MPTC mechanism.

CONCLUSIONS

The MPTC model presented here is supported well by the experimental results. For example, the influence of the pH on the derivatization rate (Fig. 9) and the derivatization profiles is adequately described (Fig. 11). In addition, the discrepancies can be satisfactorily explained. It is obvious however, that despite the good results, the present model may require refinement.

The major goal of this paper and ref. 1 was to elucidate the derivatization mechanism of carboxylic acids in the micellar system. The main conclusions from these studies and their practical implications are discussed below.

The derivatization in the micellar system exhibits most of the properties that are characteristic for phase-transfer catalysis (PTC) systems, such as the following. In the absence of an ion-pair agent, only low derivatization rates are observed in both ionic and non-ionic micellar systems. In non-ionic micellar systems, the derivatization rate increases with increasing hydrophobicity of the analyte and of the ion-pair agent. This means that lipophilic ion-pair agents such as THxABr should be used to obtain high derivatization rates. The derivatization reaction is inhibited by the presence of anions that can compete for extraction into the micelle. The degree of inhibition is related to the extractability of the anions. This implies that the use of MPTC is not easily compatible with deproteination agents such as perchloric acid.

The pH of the micellar system is of primary importance for the derivatization rate. It should be *ca.* 1.5 pH units above the pK_a^{obs} of the acid in the micellar system to ensure its complete dissociation. With common carboxylic acids the derivatization reaction should therefore be performed at neutral pH.

Factors that affect the properties of the micellar system can also influence the derivatization reaction. The derivatization rate increases with the size of the non-ionic micelles. With BrMMC, unexpectedly high derivatization rates are observed if the micellar system is turbid at the derivatization temperature. An obvious reason for this marked increase in the observed reaction rate is that in these turbid micellar solutions water is expelled from the micelles, which is of significant importance, because reactions that involve BrMMC are very sensitive to the presence of water. The turbid solutions can be obtained with several combinations of non-ionic surfactants and ion-pair agents, *i.e.*, the actual composition of the micellar system is of secondary importance. We selected a micellar system that contains 50 mM Arkopal N-130 and 36 mM THxABr, because this system performs well with respect to the derivatization rate and with respect to the fact that the maximum derivatization rate is not critically related to the precise composition of the micellar system. The addition of large amounts of organic solvents, *e.g.*, above 10% (v/v) acetone, results in a decrease in the derivatization rate in the Arkopal N-130–THxABr micellar solutions, because micellization is then inhibited. Therefore, when using the Arkopal N-130–THxABr

system, organic solvents cannot be used for the precipitation of proteins. However, it is not obligatory to remove plasma proteins prior to the incubation procedure. This has been demonstrated for the anti-epileptic drug valproic acid (2-propylpentanoic acid), which can be derivatized directly in diluted plasma using MPTC[2].

Finally, one can safely conclude that MPTC will offer a convenient and versatile technique for the derivatization of carboxylic acids in aqueous matrices. As an illustration, in a subsequent paper we shall demonstrate that free fatty acids in plasma can be determined using a fully automated HPLC system with an on-line MPTC unit[39].

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