Sorption of Monodisperse Alcohol Ethoxylates and Their Effects on the Mobility of 2,4-D in Isolated Plant Cuticles

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The sorption of homologous monodisperse alcohol ethoxylates in cuticles isolated from the leaves of bitter orange (*Citrus aurantium* L.) and thoroughly extracted with chloroform (yielding polymer matrix membranes, MX) has been investigated. Sorption isotherms exhibited a linear phase at low aqueous concentrations of surfactant, while above the critical micelle concentrations (cmc) the concentration sorbed in the MX was independent of external concentration. MX/water partition coefficients (25–148 000, depending on the homologue), maximum concentrations in the MX (91–318 mmol/kg), and cmc were derived from the isotherms. Quantitative structure–property relationships were established for estimating the MX/water partition coefficients, cmc, and maximum cuticular concentrations of alcohol ethoxylates from the number of carbon atoms and oxyethylene groups of their alcohol and poly(oxyethylene) moieties, respectively. These relationships were used for analyzing the effects of monodisperse alcohol ethoxylates and primary alcohols on the mobility of (2,4-dichlorophenoxy)acetic acid in isolated cuticular membranes from bitter orange leaves.

Keywords: *Plant cuticles; foliar penetration; surfactants; adjuvants; Citrus aurantium*

INTRODUCTION

Nonionic surfactants of the poly(oxyethylene) type are widely used in pesticidal formulations. Some enhance the activity of many foliage-applied pesticides by increasing the rates of uptake of active ingredients from droplets deposited on leaf surfaces (Stock and Holloway, 1993; Kirkwood, 1993; Stevens et al., 1991; Gaskin and Holloway, 1992; Stock et al., 1992; Knoche and Bukovac, 1992; Holloway and Edgerton, 1992). The cuticle, representing the main barrier to foliar uptake, has been the object of further studies performed under rigorously controlled experimental conditions with the objective of a mechanistic understanding of the processes involved during the enhancement of activity. Selected nonionic surfactants effectively increased the permeability of isolated cuticles for water (Geyer and Schönherr, 1989; Riederer and Schönherr, 1990; Schönherr and Bauer, 1992) and organic solutes (Schönherr and Bauer, 1992; Schönherr et al., 1991; Tan and Crabtree, 1992; Chamel et al., 1992; Knoche and Bukovac, 1993). Enhanced cuticular permeability in the presence of alcohol ethoxylates can be attributed in part to an increase in the mobility of the penetrant in the cuticle (Schönherr, 1993a,b). Adjuvants having this property have been termed accelerators (Schönherr, 1993b).

It is an essential prerequisite for accelerating the movement of the active ingredient across the plant surface that sufficient quantities of surfactant are present in the cuticle during permeation of the active ingredient. Supposing a linear dose–effect relationship, the efficacy of an accelerator should depend on its concentration in the cuticle, which in turn will directly depend on the accelerator's activity in the outside medium. Such data are needed to interpret the wide variation of efficiency between different classes of nonionic surfactants or among homologous series.

The sorption of nonionic surfactants from aqueous solutions in isolated plant cuticles has been studied repeatedly (Schönherr et al., 1991; Shafer et al., 1989; Bukovac and Petracek, 1993). These experiments have been performed using a technical polydisperse surfactant preparation containing a large number of individual homologues. Due to their complex and unknown composition, such polydisperse surfactants are not suitable for critical studies on the effect of chemical structure of a poly(oxyethylene) surfactant on its sorption by plant cuticles.

Therefore, the sorption of monodisperse alcohol ethoxylates has been investigated in this study. The objective of our work was to study sorption by standardized cuticular material as a function of concentration in the aqueous phase for a series of alcohol ethoxylates varying in chemical structure. Our findings will help elucidate the general principles governing the cuticular sorption of alcohol ethoxylates and thus surfactant concentrations in the cuticle during the permeation of surfactants and active ingredients. The results and the quantitative relationships derived will also be used to analyze the effects of monodisperse alcohol ethoxylates and primary alcohols on the mobility of 2,4-dichlorophenoxyacetic acid (2,4-D) in isolated cuticles (Schönherr, 1993a,b).

EXPERIMENTAL PROCEDURES

Cuticular Membranes. Adaxial cuticular membranes were enzymatically isolated (Schönherr and Riederer, 1986) from mature leaves of small bitter orange (*Citrus aurantium* L.) trees grown in growth chambers as described elsewhere (Geyer and Schönherr, 1990). The isolated cuticles were

Table 1. Molar Weights (MW) and Characteristic Molecular Volumes (V_x) of the Alcohol Ethoxylates Used in the Present Study

name	abbrev	MW	$V_x{}^a$ (cm³/mol)
diethylene glycol monobutyl ether	C_4E_2	162.23	141.19
triethylene glycol monohexyl ether	C_6E_3	234.34	203.42
diethylene glycol monooctyl ether	C_8E_2	218.34	197.55
triethylene glycol monooctyl ether	C_8E_3	262.39	231.60
tetraethylene glycol monooctyl ether	C_8E_4	306.45	265.65
pentaethylene glycol monooctyl ether	C_8E_5	350.50	299.70
triethylene glycol monodecyl ether	$C_{10}E_3$	290.45	259.78
tetraethylene glycol monodecyl ether	$C_{10}E_4$	334.50	293.83
pentaethylene glycol monodecyl ether	$C_{10}E_5$	378.56	327.88
hexaethylene glycol monodecyl ether	$C_{10}E_6$	422.61	361.93
heptaethylene glycol monodecyl ether	$C_{10}E_7$	466.66	395.98
octaethylene glycol monodecyl ether	$C_{10}E_8$	510.72	430.03
diethylene glycol monododecyl ether	$C_{12}E_2$	274.45	253.91
triethylene glycol monododecyl ether	$C_{12}E_3$	318.50	287.96
tetraethylene glycol monododecyl ether	$C_{12}E_4$	362.56	322.01
pentaethylene glycol monododecyl ether	$C_{12}E_5$	406.61	356.06
hexaethylene glycol monododecyl ether	$C_{12}E_6$	450.66	390.11
heptaethylene glycol monododecyl ether	$C_{12}E_7$	494.72	424.16
octaethylene glycol monododecyl ether	$C_{12}E_8$	538.77	458.21
triethylene glycol monotetradecyl ether	$C_{14}E_3$	346.56	316.14
pentaethylene glycol monotetradecyl ether	$C_{14}E_5$	434.66	384.24
heptaethylene glycol monotetradecyl ether	$C_{14}E_7$	522.77	452.34
octaethylene glycol monotetradecyl ether	$C_{14}E_8$	566.83	486.39
octaethylene glycol monohexadecyl ether	$C_{16}E_8$	594.88	514.57

^{a} Calculated according to the method of Abraham and McGowan (1987).

exhaustively extracted (16 h) with chloroform (Riedel-de Haën, Seelze, Germany) in a Soxhlet apparatus to remove cuticular waxes. The resulting cuticular material will be referred to as polymer matrix membranes (MX). This treatment was necessary to avoid problems during the gas chromatographic quantification of the amounts of alcohol ethoxylates sorbed in the cuticle since wax constituents and surfactants had similar retention times. MX consisting mainly of cutin are good models for the overall sorptive properties of plant cuticles for lipophilic compounds. It has been shown experimentally that the fundamental processes governing sorption are identical for extracted and nonextracted cuticles (Riederer and Schönherr, 1984, 1986; Kerler and Schönherr, 1988).

Monodisperse Alcohol Ethoxylates. Monodisperse alcohol ethoxylates were obtained from Fluka (Neu-Ulm, Germany) and their identity and purity confirmed by capillary gas chromatography/mass spectrometry. Twenty-four homologues were selected on the basis of purity (97—99%), structural diversity, and size (Table 1).

Sorption Isotherms. Stock solutions (80—150 mmol/L) of the different homologues were prepared in chloroform. Various volumes of these solutions were added to 100-mL glass vials, the solvent was evaporated under a gentle stream of N_2, and the surfactant was redissolved in 100 mL of water bidistilled over quartz. Sodium azide (1 mM) was added to the solutions to prevent growth of microorganisms. For each homologue, 12—14 different concentrations were prepared such that they covered a range of concentrations from 1 to 2 orders of magnitude below to about 1 order of magnitude above the critical micelle concentration (cmc).

The sorption experiments were started by adding polymer matrix membranes (ca. 6 mg) to the solution that had been brought to 25 °C before. The vials were closed with Teflon-lined septum caps and slightly agitated for 20 h in an incubator (Memmert, Schwabach, Germany) at a temperature of 25 ± 1 °C. Preliminary experiments had shown that after this period of time the concentrations of the alcohol ethoxylates in the MX were invariant with time. The variation of the amount of MX added to the surfactant solution had no influence on $K_{MX/W}$, indicating that, in the concentration range studied, surface adsorption was negligible with respect to absorption by the cutin.

Subsequently, the MX were carefully removed from the solution, gently blotted with soft tissue paper to remove adhering solution, and air-dried for at least 24 h. Internal standard (an alkane between hexadecane and nonacosane depending on the retention time of the respective alcohol

ethoxylate) was added to the dry MX in a 1-mL Reactivial (Wheaton, Millville, NJ). After extraction of the MX with 1 mL of chloroform at 60 °C (preliminary tests had shown that a 10-min treatment under these conditions was sufficient for complete extraction), the cuticular material was removed and the resulting solution was reduced to dryness under a stream of N_2. These samples were subsequently analyzed by gas chromatography, and the concentration of surfactant sorbed in the MX was calculated.

The surfactant concentration in the aqueous phase was determined by taking aliquots of various volumes and evaporating the water under a gentle stream of clean air from an air generator (Chrompack, Middelburg, The Netherlands) at 40 °C. Preliminary study established that volatility of the surfactants was not a problem. After an appropriate amount of internal standard was added, the samples were analyzed by gas chromatography.

Gas Chromatographic Analysis of Alcohol Ethoxylates. For gas chromatographic analysis, the terminal hydroxyl groups of the alcohol ethoxylates were transformed into the corresponding trimethylsiloxyl ethers by reaction with N,N-bis(trimethylsilyl)trifluoroacetamide (Macherey & Nagel, Düren, Germany) in dry pyridine (Merck, Darmstadt, Germany). Analyses were performed with capillary gas chromatographs (Fractovap 4200, Carlo Erba Strumentazione, Milano, Italy; GC-14A, Shimadzu, Kyoto, Japan; HP 5890 II, Hewlett-Packard, Millville, NJ) equipped with on-column injectors and flame ionization detectors and operated with H_2 as carrier gas. Separation was achieved on a 25 m × 0.32 mm fused silica WCOT capillary column ($d_f = 0.12$ μm; CP-Sil 5 CB, Chrompack) using temperature programs adjusted to the retention of the respective homologue.

For quantification, peak areas were multiplied with specific correction factors determined for each homologue separately by analyzing solutions of known concentrations of the alcohol ethoxylate and the internal standard. Correction factors were determined daily for each of the chromatographs and homologues used.

Tensiometry. The surface tension of aqueous solutions of monodisperse alcohol ethoxylates was measured at 25 °C as a function of concentration using an interfacial tensiometer (K8, Krüss, Hamburg, Germany) with a thermostated sample holder. The solutions containing 1 mM sodium azide were prepared by successive dilution of a stock solution. Care was taken that only the equilibrium values of surface tension were recorded (up to 60 min of equilibration time).

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Critical micelle concentrations (cmc) of monodisperse alcohol ethoxylates were determined in the conventional way by plotting the surface tensions *vs* the logarithm of the surfactant concentration in the solution. The cmc was obtained by solving the regression equation fitted to the descending branch of the graph for the constant value of surface tension measured at high surfactant concentrations.

The regression equations describing the change in surface tension with concentration were also used to measure partition coefficients of alcohol ethoxylates between the aqueous phase and MX. For this purpose, MX were added to a surfactant solution with a concentration below the cmc and exactly known surface tension. After incubation at 25 ± 1 °C for at least 20 h, the surface tension of the supernatant was measured again and the corresponding concentration estimated using the regression equation. The equilibrium concentration of surfactant sorbed in the MX was computed from the concentration difference, the volume of the aqueous phase, and the mass of the MX used in the experiment. The MX/water partition coefficient $K_{MX/W}$ was calculated according to

$$K_{MX/W} = C_{MX}/C_W \qquad (1)$$

with C_{MX} and C_W referring to the equilibrium concentration (mol/kg) of the surfactant in the MX and the aqueous supernatant, respectively.

To apply this method for determining MX/water partition coefficients to the maximum range of homologues, the mass ratio of MX to aqueous phase was adjusted for each homologue such that the concentration difference due to sorption was measurable and did not exceed the validity of the regression equations.

Effects on the Mobility of 2,4-D in Isolated Plant Cuticles. Data on the effects of monodisperse alcohol ethoxylates on the mobility of (2,4-dichlorophenoxy)acetic acid (2,4-D) in isolated cuticular membranes from leaves of bitter orange were taken from two previous publications (Schönherr, 1993a,b). Mobilities were deduced from the kinetics of unilateral desorption of 2,4-D from the outer surface of isolated cuticles. Effects were standardized to a common initial rate constant k_i of $1/k_i$ = 15×10^5 s. With compounds exhibiting time-dependent effects on 2,4-D mobility, only the standardized maximum values (i.e. maximum effect) were used. For details on the materials and methods see Schönherr (1993a,b).

Statistics. Data were statistically evaluated using SPSS for Windows (SPSS Inc., Chicago, IL). Unless stated otherwise, the coefficients of the regression equations (eqs 4–8) were significantly different from zero on a level of $p < 0.0005$. The coefficients are given together with their 95% confidence intervals.

RESULTS AND DISCUSSION

Sorption Isotherms. Monodisperse alcohol ethoxylates in aqueous solution were readily sorbed in cuticular polymer matrix materials isolated from bitter orange leaves. The equilibrium concentrations in the MX (C_{MX}) varied with those in the aqueous supernatant (C_W) (Figure 1A).

The isotherms can be fully characterized by three distinctive features: (1) linear initial slopes (Figure 1B), (2) relatively sharp points of inflection intermediate between the portions with linear slopes and (3) those with zero slopes due to constant values of C_{MX} (Figure 1A). This general shape has been found with all homologues studied even though the absolute values of initial slopes, the locations of the inflection, and the plateau values varied significantly and systematically among the different compounds studied.

Similar isotherms have been observed for the adsorption of nonionic surfactants on the surfaces of solids like silica, coal, or clay (Clunie and Ingram, 1983; Furlong and Aston, 1982; Celik and Yoon, 1991). Those adsorption isotherms could be treated according to the Lang-
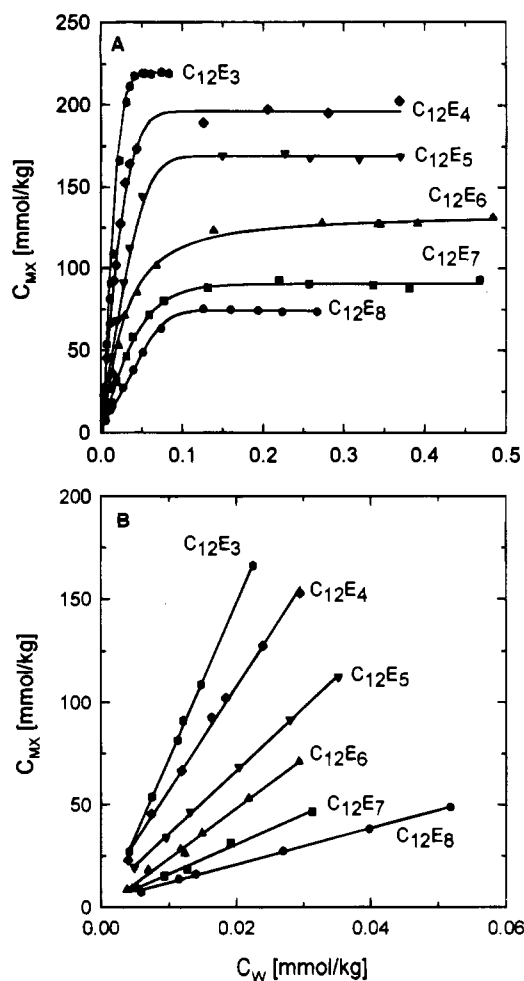
Figure 1. Isotherms (25 °C) for the sorption of a homologous series of alcohol ethoxylates ($C_{12}E_y$) in cuticular polymer matrix membranes from bitter orange leaves: (A) isotherms over the whole concentration range studied; (B) initial linear portions of these isotherms. Concentrations in the polymer matrix (C_{MX}) and the aqueous phase (C_W) were determined by the gas chromatographic method.

muir formalism (Adamson, 1982; Clunie and Ingram, 1983; Rosen, 1989). Langmuir adsorption isotherms approach a plateau which is due to the saturation of a limited number of sorption sites on the surface of the substrate. However, attempts to fit the Langmuir equation to the data describing the sorption of monodisperse alcohol ethoxylates in MX failed. Regression analyses showed that the initial portions of the isotherms were strictly linear (Figure 1B). Thus, the isotherms observed in this study consist of two linear components (the initial slope and the plateau) and a short transitional region in between (Figure 2).

The isotherms found in the present study (at aqueous concentrations 1–2 orders of magnitude below and 1 order of magnitude above cmc) can be classified according to Giles et al. as type C2 (Giles et al., 1974). The linear isotherms are indicative for conditions where the number of sorption sites remains constant throughout a wide range of solute concentrations. Such behavior is characteristic for amorphous solids which, at low concentrations, act as quasi-liquid phases (Giles et al., 1974). Thus, at low concentrations of surfactants in the aqueous solution, the distribution of alcohol ethoxylates between the cuticular matrix and the aqueous solution closely resembles partitioning between two liquid phases. Constant partitioning isotherms have also been described for monodisperse $C_{12}E_5$ in the system heptane/
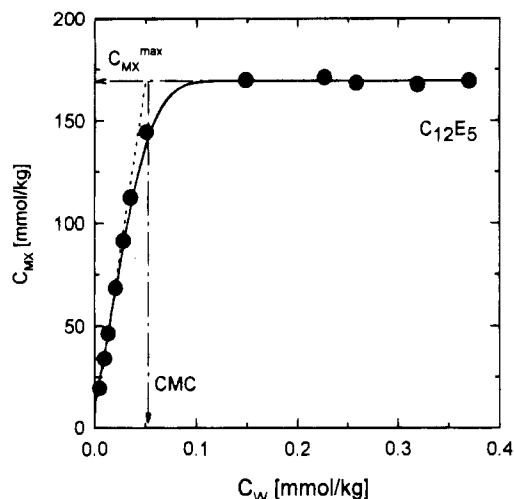
Figure 2. Isotherm (25 °C) for the sorption of $C_{12}E_5$ in polymer matrix membranes from bitter orange leaves. The arrows indicate how the critical micelle concentration (cmc) and the maximum concentration in the MX were deduced. The MX/water partition coefficient $K_{MX/W}$ was obtained from the slope of the initial linear portion of the isotherm.

water (Aveyard et al., 1990). Linear sorption isotherms have already been described for the sorption of several lipophilic organic compounds in the cuticles isolated from various plant species (Riederer and Schönherr, 1984, 1986).

The intercepts of the initial linear portions of the sorption isotherms measured in this study are not significantly different from the origin. This fact underlines that a constant partitioning phenomenon (obeying Henry's law) is taking place at low concentrations of alcohol ethoxylates in the aqueous phase. The linear portions can therefore be described by

$$C_{MX} = K_{MX/W}C_W \qquad (2)$$

where the slope $K_{MX/W}$ is the MX/water partition coefficient.

The plateaus of the sorption isotherms (Figure 1) found in this study could, theoretically, be attributed to two different causes: (1) the filling of the sorptive capacity of the MX or (2) a dramatic concentration-dependent change of the activity of the solute in the aqueous phase. The validity of the first hypothesis can be assessed by estimating the volume fraction occupied by the alcohol ethoxylates in the MX in the plateau region. The characteristic molecular volumes of the different homologues were computed according to the method of Abraham and McGowan (1987), and a specific mass of 1100 kg/m³ was assumed for the MX (Schreiber and Schönherr, 1990). The resulting volume fractions vary from 4.7 vol % for $C_{16}E_8$ to 9.7 vol % for $C_{12}E_2$. Such a large variation (by almost a factor of 2) is not compatible with the hypotheses that the plateaus are due to attainment of a maximum sorption capacity because this capacity should be equal for all homologues. In addition, the volume fractions occupied by the alcohol ethoxylates are more than a factor of 2 lower than the maximum sorption capacity of approximately 21 vol % determined for 4-nitrophenol (Riederer and Schönherr, 1986).

For these reasons, the alternative explanation of the plateaus of the sorption isotherms is favored. Independent of the treatment of micellization in aqueous solutions of nonionic surfactants (Moroi, 1992; Nagarajan

and Ruckenstein, 1977, 1991; Puvvada and Blankschtein, 1990; Ruckenstein and Nagarajan, 1975, 1981; Tanford, 1973) it has been stated that, above the critical micelle concentration (cmc), any added surfactant molecules will not increase the concentration of the free monomers in solution. Instead, they will form micellar aggregates. Therefore, the activity of free monomers will increase linearly with the bulk concentration in the aqueous phase up to a concentration close to the cmc. Above the cmc, the activity of free monomers of monodisperse surfactants will remain nearly constant even though the bulk concentration still rises (Levitz, 1991).

The sorption of monodisperse alcohol ethoxylates in plant cuticular matrix closely follows the concentration dependence of the activity of the free monomer in the aqueous phase as predicted by a theoretical model (Levitz, 1991). This shows that the species taking part in the partitioning between the aqueous solution and the MX is only the free monomer and not those aggregated in micelles. The micelles act as a reservoir, keeping the concentration of the free monomers at a constant level even when sorption by the MX removes part of them. Consequently, the plateau portions of the isotherms represent the maximum concentrations (C_{MX}^{max}) that can be reached by sorption from aqueous solution at a given temperature. The maximum concentration is in equilibrium with a constant concentration of the free monomers in the aqueous phase which is practically equal to the cmc. According to this model, it should be possible to estimate cmc from sorption isotherms by solving eq 2 for $C_{MX} = C_{MX}^{max}$ using $K_{MX/W}$ (Figure 2). Thus, complete sorption isotherms as determined by the gas chromatographic method provide the three basic parameters ($K_{MX/W}$, cmc, C_{MX}^{max}) necessary for a description of the concentration-dependent sorption of alcohol ethoxylates in plant MX.

This interpretation of the sorption isotherms of monodisperse alcohol ethoxylates can be tested by measuring MX/water partition coefficients and cmc by an independent method and by estimating C_{MX}^{max} according to

$$C_{MX}^{max} = K_{MX/W}(cmc) \qquad (3)$$

In this study, tensiometry has been used as an independent method for measuring cmc and $K_{MX/W}$. The results obtained by both methods will be compared and discussed in the following sections.

MX/Water Partition Coefficients. The partition coefficients of a series of monodisperse alcohol ethoxylates determined in the system *C. aurantium* leaf MX/water (Table 2) ranged from 25 (C_8E_5) to 148 000 ($C_{16}E_8$). The gas chromatographic and tensiometric methods produced results identical on the 95% confidence level (Table 2).

For monodisperse alcohol ethoxylates with various alkyl chain lengths and degrees of ethoxylation, the MX/water partition coefficients depended systematically on chemical structure. Within a homologous series having an equal number of oxyethylene groups but differing in the length of the alkyl chain, the values of $K_{MX/W}$ increased markedly with the numbers of methylene groups in the alcohol moiety. For instance, partition coefficients measured for the series C_xE_3 increased from 69 (C_8E_3) to 69 200 ($C_{14}E_3$) with $C_{10}E_3$ and $C_{12}E_3$ having intermediate values of 740 and 7590, respectively. Homologous series with a constant alkyl chain length but a varying degree of ethoxylation exhibited a comparable, even though much less pronounced, dependence

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Table 2. MX/Water Partition Coefficients ($K_{MX/W}$), Critical Micelle Concentrations (cmc), and Maximum Cuticular Concentrations (C_{MX}^{max}) for Monodisperse Alcohol Ethoxylates

	log $K_{MX/W}$		cmc (mmol/kg)		C_{MX}^{max}	
	TM[a]	GC[b]	TM[a]	GC[b]	mmol/kg	g/kg
C_4E_2			537.0			
C_6E_3			81.0			
C_8E_2		2.02 (1.97–2.06)[c]				
C_8E_3		1.84 (1.81–1.86)				
C_8E_4		1.60 (1.58–1.63)	6.58			
C_8E_5		1.40 (1.39–1.42)				
$C_{10}E_3$		2.87 (2.83–2.90)		0.42	318	92
$C_{10}E_4$		2.68 (2.67–2.70)				
$C_{10}E_5$	2.58	2.50 (2.45–2.55)	0.69	0.68	217	82
$C_{10}E_6$		2.39 (2.31–2.46)		0.62	148	63
$C_{10}E_7$		2.22 (2.16–2.27)		0.79	133	62
$C_{10}E_8$	2.00	1.95 (1.90–2.00)	1.1	1.1	107	55
$C_{12}E_2$	4.06	4.21 (4.15–4.26)	0.031			
$C_{12}E_3$	3.88	3.88 (3.86–3.90)	0.031	0.029	259	82
$C_{12}E_4$	3.74	3.72 (3.68–3.75)	0.042	0.037	231	84
$C_{12}E_5$	3.56	3.49 (3.47–3.50)	0.053	0.053	169	67
$C_{12}E_6$	3.39	3.39 (3.34–3.43)	0.065	0.063	140	63
$C_{12}E_7$	3.19	3.16 (3.12–3.20)	0.085	0.079	109	54
$C_{12}E_8$	3.08	2.95 (2.91–2.98)	0.089	0.089	97	52
$C_{14}E_3$		4.84 (4.78–4.89)		0.0027	234	81
$C_{14}E_5$		4.44 (4.39–4.49)		0.0040	182	79
$C_{14}E_7$	4.22		0.0070			
$C_{14}E_8$	4.06	4.08 (4.01–4.15)	0.0088	0.0090	91	52
$C_{16}E_8$	5.05	5.17 (5.13–5.20)	0.00073			

[a] Determined by tensiometry. MX/water partition coefficients determined by this method are exact to approximately ±0.1 log unit. [b] Determined by capillary gas chromatography. [c] Upper and lower limits of the 95% confidence intervals.

of MX/water partition coefficients on chemical structure. Partition coefficients in the series from $C_{12}E_2$ to $C_{12}E_8$ steadily decreased from 16 200 to 890, respectively (Table 2).

The dependence of the MX/water partition coefficients on the chemical structure of the monodisperse alcohol ethoxylates can be expressed by a simple multiple-regression equation using the number of carbon atoms (C) and the number of oxyethylene groups (E) as descriptors for molecular structure:

$$\log K_{MX/W} = -1.78(\pm 0.11) + 0.52(\pm 0.01)C - 0.17(\pm 0.01)E \quad (4)$$

$$N = 33, \text{ adjusted } r^2 = 0.997, F = 5357$$

The data obtained using both methods for measuring partition coefficients were combined for establishing this quantitative structure–property relationship (QSPR). Values of $K_{MX/W}$ estimated from this equation are in good agreement with the experimental results (Figure 3). Equations with coefficients not significantly different from those in eq 4 were obtained when the results from both methods were treated separately.

Equation 4 can be used to derive general rules for the dependence of MX/water partition coefficients of alcohol ethoxylates on chemical structure. This QSPR predicts that increasing the alkyl chain length by two methylene groups will increase the MX/water partition coefficient by almost exactly 1 order of magnitude. A decrease of the same extent would result from an elongation of the poly(oxyethylene) part of the molecule by six units.

Critical Micelle Concentrations. Critical micelle concentrations give the upper limit of the concentration range where partition coefficients can serve as valid descriptors for the sorption of alcohol ethoxylates in
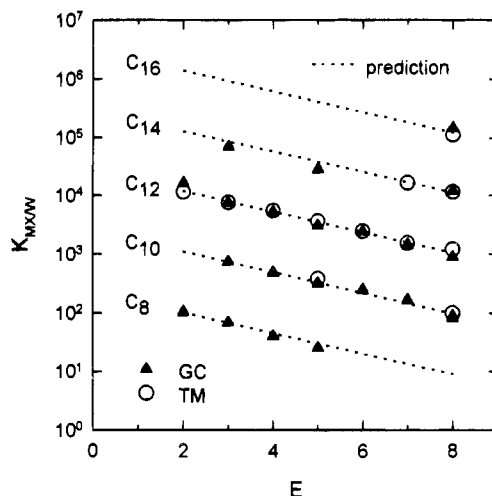
Figure 3. Plots of the MX/water partition coefficients ($K_{MX/W}$) of a homologous series of alcohol ethoxylates *vs* the number of ethylene oxide units (E) in the poly(oxyethylene) parts of the molecules. Data obtained by the gas chromatographic (GC) and the tensiometric methods (TM) are shown together with the prediction calculated from eq 4.

plant cuticular material (Figures 1 and 2). The cmc of the monodisperse alcohol ethoxylates used in this study varied from 0.00073 ($C_{16}E_8$) to 537 (C_4E_2) mmol/kg (Table 2). Both the tensiometric and the gas chromatographic methods used for determining cmc produced results identical within the limits of error. This supports the interpretation of the shape of the sorption isotherms outlined above. Reaching the cmc in the aqueous phase causes the inflection between the initial linear portion with a constant slope to the plateau of the sorption isotherms (Figures 1A and 2).
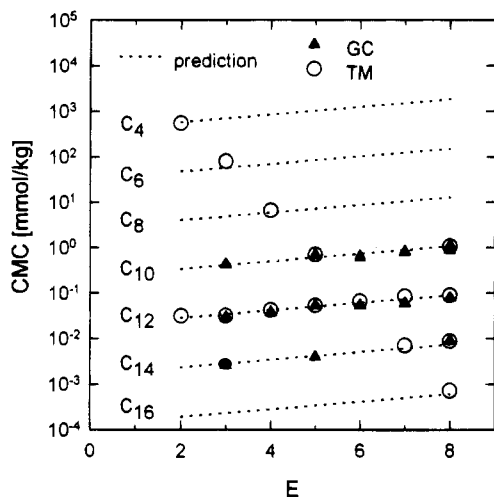
Critical micelle concentrations of homologues varying in alkyl chain length and ethoxylation also depended systematically on chemical structures. Within the homologous series of C_xE_3, the cmc decreased from 0.68 ($C_{10}E_3$) to 0.0027 mmol/kg ($C_{14}E_3$) while it increased for the series $C_{12}E_y$ from 0.029 ($C_{12}E_3$) to 0.089 mmol/kg ($C_{12}E_8$). The values of cmc measured in this study are in good agreement with data in the literature (Meguro et al., 1987). Using the results obtained from both methods, the following multiple regression equation was established

$$\log \text{cmc} = 4.75(\pm 0.09) - 0.54(\pm 0.01)C + 0.09(\pm 0.01)E \quad (5)$$

$$N = 29, \text{ adjusted } r^2 = 0.998, F = 9114$$

describing the dependence of the critical micelle concentration (in mmol/kg) on the number of the carbon atoms in the alkyl (C) and those of the oxyethylene groups (E) in the poly(oxyethylene) chains, respectively. This equation successfully predicts the cmc values determined experimentally (Figure 4). The regression equations obtained for the results from the two independent methods were not significantly different from eq 5.

This QSPR shows that the cmc of the homologous monodisperse alcohol ethoxylates used in this study again depend markedly on the chain lengths of the alcohol moieties. Increasing the length of the alkyl chain by two methylene groups decreases the cmc by a factor of approximately 10. The same decrease of cmc is predicted by eq 5 for a shortening of the poly(oxyethylene) chain by 11 ethylene oxide units. The

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Figure 4. Plots of the critical micelle concentrations (cmc) of a homologous series of alcohol ethoxylates *vs* the number of the ethylene oxide units (E) in the poly(oxyethylene) parts of the molecules. Data obtained by the gas chromatographic (GC) and the tensiometric methods (TM) are shown together with the prediction calculated from eq 5.

Figure 5. Plot of the experimentally determined maximum concentrations of various homologous alcohol ethoxylates in bitter orange MX (C_{MX}^{max}) *vs* values predicted from eq 6. The slope of the graph is not significantly different from 1.0 (adjusted $r^2 = 0.969$).

coefficients for C and E in eqs 4 and 5 have opposite signs, respectively, showing that, for instance, a structural change leading to a lower cmc will simultaneously increase the MX/water partition coefficient. As far as variations of the alkyl chain length are concerned, they will even result in equal absolute changes of cmc and $K_{MX/W}$ but in opposite directions.

Maximum Concentrations in the MX. When the concentration of a monodisperse alcohol ethoxylate in aqueous solution exceeds the cmc, its equilibrium concentration in the MX phase remains constant (Figures 1 and 2). As the activity of the free monomer in an alcohol ethoxylate solution remains practically constant at concentrations above the cmc, the corresponding equilibrium concentration in the MX is the maximum concentration (C_{MX}^{max}) attainable in an MX/aqueous solution system. Using the gas chromatographic method, values of C_{MX}^{max} were determined for some homologues (Table 2). The experimental values of C_{MX}^{max} ranged from 91 ($C_{14}E_8$) to 318 mmol/kg ($C_{10}E_3$). Generally, variation of C_{MX}^{max} as a consequence of chemical structure was small (at the maximum, by a factor of 3.5) compared to that observed with $K_{MX/W}$ and cmc, which varied over several orders of magnitude. Within homologous series, C_{MX}^{max} decreased with increasing numbers of both C and E (Table 2).

This dependence of the experimentally determined values of C_{MX}^{max} (mmol/kg) on C and E can be expressed by the multiple regression equation

$$\log C_{MX}^{max} = 2.96(\pm 0.16) - 0.02(\pm 0.01)C -$$
$$0.09(\pm 0.01)E \quad (6)$$

$$N = 14, \text{ adjusted } r^2 = 0.969, F = 206.3$$

In contrast to the QSPRs for $K_{MX/W}$ and cmc (eqs 4 and 5, respectively), the coefficients for the independent variables have both negative signs and the dependence of C_{MX}^{max} on C is fairly small but, nevertheless, significantly different from zero ($p = 0.0026$). Here, only a variation of the alkyl chain length by 50 methylene groups would produce a change of C_{MX}^{max} by a factor of 10, while in the cases of $K_{MX/W}$ and cmc a variation by
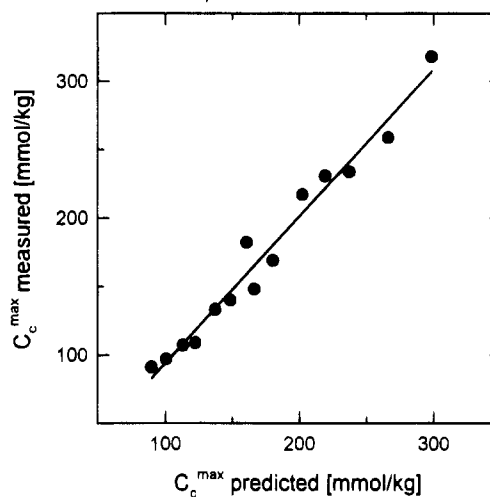
two carbon atoms would have the same effect. Also in contrast to $K_{MX/W}$ and cmc, C_{MX}^{max} is affected to a much higher extent by a variation of the number of the ethylene oxide units than by the carbon number of the alcohol moiety. Elongating the poly(oxyethylene) chain by 11 units would decrease C_{MX}^{max} by 1 order of magnitude.

A different approach leads to an equivalent quantitative relationship between C_{MX}^{max} and C and E, respectively. Based on eq 3, eqs 4 and 5 can be combined to give (C_{MX}^{max} again in mmol/kg)

$$\log C_{MX}^{max} = 2.97 - 0.02C - 0.08E \quad (7)$$

This combination of the QSPRs for $K_{MX/W}$ and cmc illustrates how the drastic effect of C on both single parameters is reduced such that it exerts a fairly small effect on C_{MX}^{max}. This is due to almost identical absolute values of the coefficient for the C variable and to its opposite signs in eqs 4 and 5. On the other hand, the coefficients of the E variable in eqs 4 and 5 do not counteract completely, resulting in a slight negative dependence of C_{MX}^{max} on the degree of ethoxylation. This dependence is dominated by that of $K_{MX/W}$ on the length of the poly(oxyethylene) chain.

The multiple regression equation derived from experimental data (eq 6) and that obtained by combining eqs 4 and 5 (eq 7) are not significantly different. This fact supports the interpretation of the sorption isotherms of monodisperse alcohol ethoxylates in plant cuticular matrix as outlined above. Both equations can be used to predict maximum concentrations in the MX because the estimates agree well with the experimental data (Figure 5).

It can be expected that similar relationships between the numbers of C and E and $K_{MX/W}$, the cmc, and C_{MX}^{max} exist for other groups of ethoxylated surfactants. The dependence on the number of oxyethylene groups may even be identical, while the quantitative structure–property relationships will have intercepts varying with the nature of the hydrophobic part of the surfactant molecule.

Analysis of the Effects of Alcohol Ethoxylates on the Mobility of 2,4-D in Isolated Cuticles. Most of

Table 3. MX/Water Partition Coefficients ($K_{MX/W}$), Critical Micelle Concentrations (cmc), Equilibrium Concentrations (C_{MX}), and Volume Fractions in the MX as well as the Maximum Effects of the Compounds on the Mobility of 2,4-D in Isolated Bitter Orange Leaf Cuticles

| compd[a]	$K_{MX/W}$[b]	cmc[c] (mmol/kg)	C_{MX}[d] (mmol/kg)	vol fraction[c]	max effect[f]
C_7E_0[g]	72		1066	0.135	30.8
C_8E_0[g]	240		1024	0.146	24.9
C_8E_1	162	3.3	510	0.092	25.4
C_8E_2	110	4.1	420	0.091	21.0
C_8E_3	74.1	5.0	340	0.086	17.6
C_8E_4	50.1	6.2	280	0.081	15.6
C_8E_5	33.9	7.6	220	0.074	13.4
C_9E_0[g]	794		776	0.123	29.8
$C_{10}E_0$[g]	2630		660	0.115	22.8
$C_{10}E_1$	1780	0.28	470	0.099	16.9
$C_{10}E_2$	1200	0.34	380	0.094	16.1
$C_{10}E_3$	813	0.42	310	0.088	14.9
$C_{10}E_4$	550	0.51	250	0.081	18.2
$C_{10}E_5$	372	0.63	200	0.074	17.5
$C_{10}E_6$	251	0.78	170	0.066	12.0
$C_{10}E_7$	170	0.95	130	0.059	8.4
$C_{10}E_8$	115	1.17	110	0.052	5.4
$C_{12}E_0$[g]	28840		456	0.093	15.5
$C_{12}E_2$	13180	0.028	350	0.097	16.8
$C_{12}E_3$	8910	0.035	280	0.089	15.4
$C_{12}E_5$	4070	0.052	190	0.073	14.1
$C_{12}E_6$	2750	0.065	150	0.065	12.3
$C_{12}E_7$	1860	0.079	120	0.057	8.4
$C_{12}E_8$	1260	0.098	100	0.050	8.67
$C_{14}E_7$	20410	0.0066	110	0.056	9.12
$C_{16}E_8$	151400	0.00068	83.2	0.047	5.3

[a] C_xE_y, monodisperse alcohol ethoxylates; C_xE_0, 1-alkanols. [b] Estimated according to eq 4. [c] Estimated according to eq 5. [d] Estimated according to eq 6 except for compounds marked by [g]. [e] Equilibrium volume fractions occupied by the compounds sorbed in the MX were calculated from the corresponding C_{MX}, the characteristic molecular volumes (V_x) (Abraham and McGowan, 1987), and a specific mass of 1100 kg/m^3 of the MX (Schreiber and Schönherr, 1990). [f] Data from Schönherr (1993a,b). Effects are standardized to an initial rate constant of desorption (k_i) of $1/k_i = 15 \times 10^{-5}$ s. [g] Equilibrium concentrations of 1-alkanols in the MXs were estimated from their aqueous solubilities S_W according to $C_{MX} = K_{MX/W}S_W$. Aqueous solubilities used: (Yalkowsky and Valvani, 1980) 1-heptanol, 15 mmol/kg; 1-octanol, 4.3 mmol/kg; 1-nonanol, 0.98 mmol/kg; 1-decanol, 0.25 mmol/kg; 1-dodecanol, 0.016 mmol/kg.

the monodisperse alcohol ethoxylates used in this study have also been tested for their effects on the mobility of 2,4-D in isolated cuticular membranes from leaves of *C. aurantium* (Table 3) (Schönherr, 1993a,b). In addition, the effects of some primary alcohols have been investigated. The method used for measuring adjuvant effects on 2,4-D mobility was unilateral desorption from the outer surface (Schönherr, 1993a,b). This method is especially suitable for this kind of study as it allows for the equilibration of the adjuvant between the desorption medium and the cuticle. Thus, the equilibrium approach used in the present study for describing the partitioning of alcohol ethoxylates between an aqueous phase and the cuticular matrix is applicable. Therefore, data and concepts from the present study can be used to analyze quantitatively the adjuvant effects on 2,4-D mobility by relating them to the concentrations effective within the cuticle during the experiments. A further prerequisite for this approach is a linear relationship between the partition coefficients of the surfactants in the systems MX/water and wax/water. Only under this condition is C_{MX}^{max} a valid relative measure for the surfactant concentration in the cuticular wax representing the actual transport barrier. The validity of this
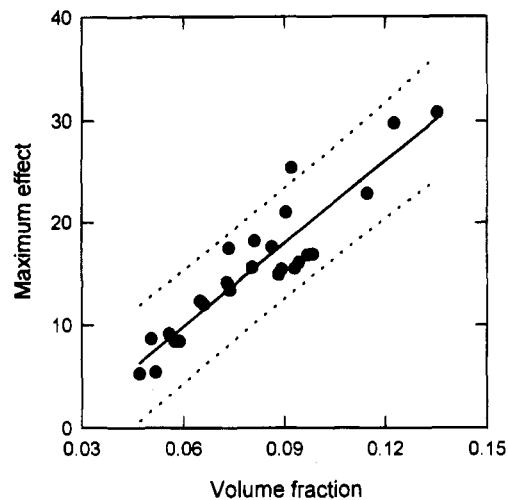
Figure 6. Dose–effect curve for alcohol ethoxylates and primary alcohols on the mobility of 2,4-D in isolated cuticular membranes from bitter orange (data from Table 3).

assumption has recently been shown experimentally (Riederer and Schreiber, 1995; Burghardt and Riederer, unpublished results, 1995).

For the different alcohol ethoxylates and alcohols used in the mobility studies, MX/water partition coefficients, critical micelle concentrations (where appropriate), and equilibrium concentrations in the cuticle can be estimated using the QSPRs developed in the present work (Table 3). Only those homologues of alcohol ethoxylates were included that had been used in the desorption experiments at concentrations significantly exceeding their cmc as estimated from eq 5.

A linear dose–effect relationship was obtained when the maximum effects on the mobility of 2,4-D in isolated cuticles were plotted *vs* the volume fractions of the alcohol ethoxylates or alcohols in the cuticle (Figure 6). The relationship can be described by

$$\text{maximum effect} = -6.5(\pm 4.1) + 272(\pm 48.5)\text{volume fraction} \quad (8)$$

$$N = 25, \text{ adjusted } r^2 = 0.847, F = 134.1$$

Equilibrium volume fractions occupied by the compounds in the cuticle were calculated from the corresponding equilibrium concentrations in the cuticle, the characteristic molecular volumes (Abraham and McGowan, 1987), and the specific mass of the cuticle (Schreiber and Schönherr, 1990). Concentrations in the cuticle were estimated either according to eq 7 (alcohol ethoxylates) or from the product of the cuticle/water partition coefficient (Table 3) and the aqueous solubilities (Yalkowsky and Valvani, 1980) (alcohols). In the latter case concentrations in the aqueous solutions used as desorption medium always exceeded aqueous solubilities. A somewhat smaller r^2 was obtained when the equilibrium concentration in the cuticle was used instead of the volume fraction.

The type of dose–effect relationship found here (eq 8) may help to advance the understanding of the mode of action of aliphatic alcohols and their poly(oxyethylene) derivatives on the cuticular transport properties. It suggests that their effects can be expected to linearly depend on the volume fraction or concentration of surfactants in the transport limiting barrier of the cuticle. The fact that all homologues of alcohol ethoxylates studied and even the free alcohols share one

common linear dose—effect relationship further suggests that the individual dose—effect curves of each of these compounds are also linear. The slopes of these (hypothetical) individual plots must be equal because only then do effects measured at widely different volume fractions combine to form a common dose—effect curve (Figure 6). This further implies that all compounds included in this study have a common intrinsic activity (i.e. slope of the individual dose—effect relationship) and that, within the range of validity of eq 8, differences in the chemical and physical properties of the compounds included in this study do not affect the acceleration of 2,4-D diffusion in isolated cuticular membranes.

The concepts and hypotheses put forward here must be tested and expanded to be useful from a practical point of view. First, the partitioning of technical polydisperse surfactants between cuticular material and aqueous solutions should be investigated to assess the validity of the proposed QSPRs for real-world situations. Second, the sorption of surfactants in cuticular waxes and their effects on the mobility of active ingredients in the wax barrier of the cuticle have to be studied to evaluate the assumptions underlying some of the conclusions drawn in this paper. Both subjects are currently under investigation in the authors' laboratories.

ACKNOWLEDGMENT

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