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THIN-LAYER CHROMATOGRAPHIC SEPARATION OF SOME TRIBUTYLPHENYL ETHYLENE OXIDE OLIGOMERS ACCORDING TO THE LENGTH OF THE ETHYLENE OXIDE CHAIN

TIBOR CSERHÁTI* and ARANKA SOMOGYI
Plant Protection Institute, Hungarian Academy of Science, Herman O.15, 1022 Budapest (Hungary)

SUMMARY

The separation of non-ionic surfactants such as tributylphenyl ethylene oxide oligomers according to the length of the ethylene oxide chain was carried out on precoated alumina thin-layer plates using various carbon tetrachloride–acetonitrile mixtures as the eluent. The optimum separation of surfactant oligomer pairs with longer ethylene oxide chains requires a higher eluent strength (higher acetonitrile concentration). The results suggest that at a maximum of thirteen fractions can be separated on a plate with a given eluent composition. Because of the possible folding of longer ethylene oxide chains in the eluent, oligomers with higher ethylene oxide numbers could not be separated under the conditions applied.

INTRODUCTION

Non-ionic surfactants have been increasingly applied in pesticide formulations, pharmaceutical products and cosmetics. They influence many biological and chemical processes, increasing the penetration of hydrocortisone through the skin[1], enhancing the fluorescence of metal chelates[2], preventing the adsorption of bacteria to hydrophobic surfaces[3,4], stimulating protein secretion in *Trichoderma resei*[5] and interacting with membrane phospholipids[6]. Their biological activity depends on the physicochemical character of the hydrophobic moiety and on the number of hydrophilic ethylene oxide groups[7,8].

The analytical application of non-ionic surfactants includes spectrophotometry[9,10] and chromatography. It was established that the presence of non-ionic surfactants in the eluent influences retention[11], and they are readily adsorbed on the surface of silica supports[12]. This effect depends on the type of surfactant and on the eluent composition[13].

According to the type of surfactant (anionic, cationic and non-ionic) separations have been carried out by traditional wet chemical procedures and by thin-layer chromatography (TLC)[14]. The separation of oligomeric series of polyethoxylated non-ionic surfactants containing identical hydrophobic moieties has been achieved by TLC[15] and by high-performance liquid chromatography (HPLC)[16,17].

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The objectives of our work were to separate some ethoxylated tributylphenyl derivatives according to the number of ethylene oxide groups by means of TLC and to optimize the chromatographic conditions[18].

EXPERIMENTAL

The non-ionic surfactants, ethoxylated tributylphenyl derivatives (Sapogenate T series) containing on average 4, 6, 8, 11, 13, 18, 30 and 50 ethylene oxide groups per molecule, were obtained from Hoechst (Frankfurt, F.R.G.). For the TLC separation, plates of DC-Alufolien Aluminiumoxid 60 (Merck, Darmstadt, F.R.G.) were applied without any pretreatment. The surfactants were dissolved in methanol at a concentration of 10 mg/ml; 2 μl of each solution were spotted on the plates. Acetonitrile–carbon tetrachloride mixtures were applied as mobile phases, the concentration of acetonitrile being changed from 0 to 80% (v/v) at 10% intervals. After development the plates were dried at room temperature and the spots were revealed by the modified Burger reagent according to ref. 14 (p. 517). The plates were evaluated with the Shimadzu dual-wavelength TLC scanner CS-930 at 500 nm. All experiments were performed in quadruplicate.

The R_M values were calculated separately for each individual spot in each eluent:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \tag{1}$$

The relative adsorption surfaces were calculated from the linear correlation between the decrease in R_M value and the increase in concentration of the eluent component of higher elution strength (acetonitrile)[19]. In our special case the relative adsorption surface increases linearly with the number of ethylene oxide groups per molecule, n_e, *e.g.*, the actual R_M value of a spot is a linear function of the number of ethylene oxide groups per molecule and of the acetonitrile concentration in the eluent, C:

$$R_M = a + bn_e + dC \tag{2}$$

To determine the numerical values of the parameters a, b and d, eqn. 2 was fitted to our experimental data.

On combination of eqns. 1 and 2, the R_F value being the dependent variable can be expressed as a function of the ethylene oxide number per molecule and that of the acetonitrile concentration, the independent variables:

$$R_F = \frac{1}{1 + 10^a \cdot 10^{bn_e} \cdot 10^{dC}} \tag{3}$$

The optimum eluent composition for the separation of two neighbouring fractions occurs when the ΔR_F value expressed by eqn. 4 is at a maximum:

$$\Delta R_F = \frac{1}{1 + 10^a \cdot 10^{bn_e} \cdot 10^{dC}} - \frac{1}{1 + 10^a \cdot 10^{b(n_e+1)} \cdot 10^{dC}} \tag{4}$$

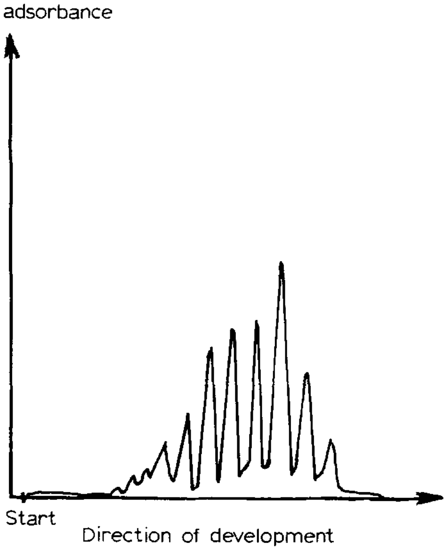

Fig. 1. Densitogram of tributylphenyl hexaethoxylate. Eluent composition: carbon tetrachloride–acetonitrile (2:3, v/v).

Eqn. 4 has a maximum when its first derivative equals zero. The numerical calculations were carried out on an Hewlett-Packard 9845B calculator. We calculated the optimum eluent composition for the separation of each pair of neighbouring compounds, the maximum ΔR_F values obtainable in these eluent systems and the ΔR_F values for a given eluent composition.

RESULTS AND DISCUSSION

The components of commercial non-ionic surfactants are well separated under the chromatographic conditions applied. As shown in Fig. 1 these surfactants contain a number of different fractions. Surfactants with lower ethylene oxide numbers are well separated, whereas the separation of higher homologues cannot be achieved even by increasing the acetonitrile concentration in the eluent. This result can be explained by the assumption that the shorter ethylene oxide chains are in a relatively elongated state in the eluent, thus the relative adsorption surface increases linearly with increasing number of ethylene oxide groups per molecule. This, in turn results in clear-cut differences in their adsorption behaviour and therefore in their retention on the alumina support. The longer ethylene oxide chains are presumbably in a folded state in the eluent, thus their relative adsorption surfaces hardly differ, resulting in inadequate separation.

As shown in Fig. 2 surfactants have a wide fraction distribution, some fractions being present in commensurable quantities. However, the data in Fig. 2 may be misleading. As we have already explained, the derivatives with longer ethylene oxide chains cannot be separated adequately. Fig. 2 therefore contains only the fractions with shorter ethylene oxide chains which were well separated under the TLC conditions applied.

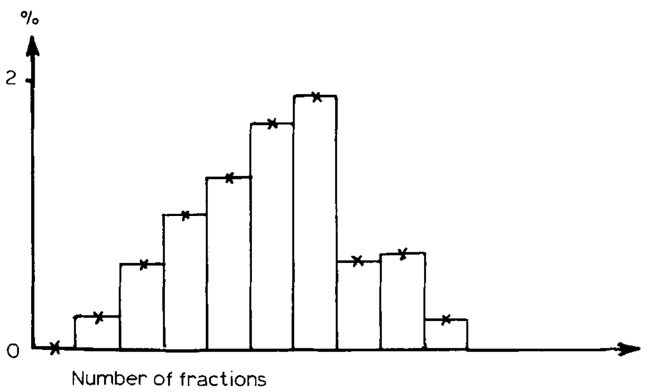

Fig. 2. Distribution of various ethoxylated fractions in tributylphenyl undecaethoxylate (only separated fractions).

The parameters of eqn. 2 are compiled in Table I. Eqn. 2 fits well the experimental data, the calculated F value is very high and the significance level of the fitting is over 99.9%. The coefficient of determination, r^2, shows that the change in the number of ethylene oxide groups per molecule and in the acetonitrile concentration of the eluent account for 96.59% of the change in R_M value. The slope values, b and d, indicate that the retention of tributylphenyl ethylene oxide oligomers increases with increasing number of ethylene oxide groups per molecule (positive b value for n_e). It decreases with increasing concentration of acetonitrile in the eluent (negative d value for C). The effect of both independent variables, n_e and C), on the retention is highly significant, the significance level being higher than 99.9% in both cases (high t values). The path coefficients indicate that the impact of each independent variable on the retention of surfactants is similar.

The optimum eluent compositions calculated from eqn. 4 for each neighbouring oligomer pair are compiled in Table II. The fractions indicated do not contain the same number of ethylene oxide groups as their numbering. Unfortunately we did not have pure surfactants with authentic numbers of ethylene oxide groups.

TABLE I

PARAMETERS OF THE LINEAR CORRELATION BETWEEN THE R_M VALUES OF SOME TRIBUTYLPHENYL ETHYLENE OXIDE OLIGOMERS, THE NUMBER OF ETHYLENE OXIDE GROUPS PER MOLECULE, n_e, AND THE ACETONITRILE CONCENTRATION, C, IN THE ELUENT

$R_M = a + b.n_e + dC$
$n = 140, a = 0.57, r^2 = 0.9659, s = 9.39 \cdot 10^{-2}, F = 1938.6$

	n_e	C
b and d	0.135	$-3.73 \cdot 10^{-2}$
s_b and s_d	$2.68 \cdot 10^{-3}$	$7.70 \cdot 10^{-4}$
Path coefficient	51.06	48.94
t	50.38	48.29

TABLE II

OPTIMUM ELUENT COMPOSITIONS FOR THE SEPARATION OF NEIGHBOURING OLIGOMER PAIRS OF TRIBUTYLPHENYL ETHOXYLATE DERIVATIVES

Fractions to be separated	*% (v/v) acetonitrile in the eluent*
1, 2	20.6
2, 3	24.2
3, 4	27.8
4, 5	31.4
5, 6	35.1
6, 7	38.7
7, 8	42.3
8, 9	45.9
9, 10	49.5
10, 11	53.1
11, 12	56.7
12, 13	60.3

Therefore our numbering indicates the order of fractions according to the increasing length of the ethylene oxide chain and not the exact number of ethylene oxide groups in the respective compounds. We are well aware of the fact that without identification of individual oligomers the value of our conclusions is limited.

According to the results in Table II, the amount of acetonitrile in the mobile phase has to be increased by 3.6% in order to separate two surfactants differing in one ethylene oxide group. In general, the higher the number of ethylene oxide groups in the oligomers to be separated, the higher the solvent strength has to be to achieve optimum separation. Eqn. 4 predicts that higher homologues can also be separated by increasing the eluent strength. On the other hand, our data and the considerations

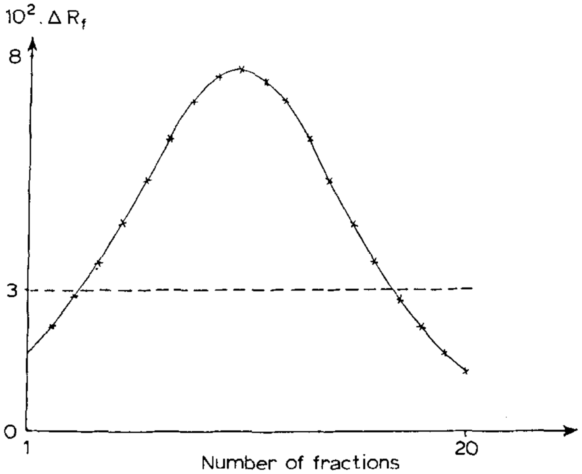

Fig. 3. ΔR_F values of neighbouring tributylphenyl ethylene oxide homologues. Eluent composition: carbon tetrachloride–acetonitrile (46.9:53.1, v/v).

above about the folding of longer ethylene oxide chains contradict this assumption. Practically, we could separate only the first thirteen fractions of the respective surfactants. The calculation showed that the ΔR_F values are the same (0.08) for each homologous surfactant pair at their optimum eluent composition, *e.g.*, these eluent mixtures are suitable for the adequate separation of tributylphenyl ethylene oxide oligomers with the limitations outlined above. The ΔR_F values of neighbouring oligomers calculated at 53.1% acetonitrile (optimum for the separation of fractions 10 and 11) are shown in Fig. 3. When we consider $\Delta R_F = 0.03$ as the separation limit, thirteen fractions can be separated under optimum conditions on one plate in a given eluent. In our case the best performance of a plate is expected with the eluent composition acetonitrile–carbon tetrachloride (45.9:54.1, v/v).

Summarizing our results, we found that tributylphenyl ethylene oxide oligomers can readily be separated according to the number of ethylene oxide groups on alumina plates in carbon tetrachloride–acetonitrile eluent mixtures. The longer the ethylene oxide chain, the higher the acetonitrile concentration has to be to achieve optimum separation. Our calculations suggest that, at maximum, thirteen fractions can be separated on a plate. Oligomers with longer ethylene oxide chains cannot be separated with this technique, probably due to the folding of ethylene oxide chains in the eluent.

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