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Retention of some ethylenediamine oligomers in reversed-phase chromatography

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ABSTRACT

The retention behaviour of four ethylenediamine oligomers containing two, three, four and five monomer units was studied in reversed-phase thin-layer chromatography using various alcohols, salts, acetic acid and ammonia as eluent additives. It was established that with salt-free eluents the oligomers showed very high retention. The retention decreased with increasing salt concentration in the eluent. This phenomenon may be due to the dissociation-suppressing effect of the salts and/or to the competition for the free silanol groups on the silica surface uncovered by the impregnating agent. The salts with higher activity coefficients had a greater effect on the retention whereas lower dielectric constants of the alcohols decreased their influence on the retention. The results suggest that to obtain the minimum retention of ethylenediamine oligomers (and probably other polyamines), the use of a salt with a high activity coefficient and organic mobile phases with high dielectric constants can be recommended.

INTRODUCTION

The chromatographic separation of amines is complicated by the strong silanophilic interaction between the amino groups and the silanol groups of the stationary phase. Nevertheless, several chromatographic techniques have been developed for the analysis of amines. Adsorption and reversed-phase thin-layer chromatography (RP-TLC) [1] and soap chromatography [2,3] have also been applied to separate amine mixtures. Cation [4,5] and anion [6] exchangers could also produce good separations. In adsorption chromatography, the silanophilic interactions have been modified by treating the stationary phase, *e.g.*, with phenols [7] or transition metal complexes [8].

Various eluent additives have a considerable effect on the retention of polar compounds in (RP-TLC). When the solute contains one or more dissociable polar substituents, the pH of the eluent strongly modifies the retention [9,10]. Salts may exert a similar effect on the retention. A salt adsorbed on the silica surface changes the retention characteristics of the sorbent [11], and salts added to the eluent markedly

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change the reversed-phase mobility of polar solutes [12]. The retention of some methylated amino acids [13] and crown ether derivatives [14] decreased non-linearly with increasing salt concentration in the eluent. The type of cations also influenced the retention of some aniline derivatives in RPTLC [15]. With the antimicrobial agent chlorhexidine {1,1-hexamethylene-bis[5-(4-chlorophenyl)biguanidine]} it was established that the ionic strength has a greater effect than the pH value on the retention [16]. As the apparent dissociation constant of salts depends strongly on the dielectric constant of the solvent [17] and most reversed-phase eluents contain an organic mobile phase, which decreases the dielectric constant, it is reasonable to suppose that the effect of salts also depends on the composition of the eluent.

The objectives of this work were to study the RP-TLC behaviour of some ethylenediamine oligomers and to elucidate the relationship between the role of salts and organic mobile phases on the retention. Ethylenediamine oligomers are used as epoxy resin hardeners, but only a few methods have been developed for their separation [18–21].

EXPERIMENTAL

Diethylenetriamine (compound I), triethylenetetramine (II), tetraethylenepentamine (III) and pentaethylenehexamine (IV) were synthesized at the EGIS Pharmaceutical Works (Budapest, Hungary). The compounds were chromatographically pure. All other reagents were of analytical-reagent grade. Silufol plates (Kavalier, Sklárny, Czechoslovakia) were impregnated by overnight predevelopment in the eluent paraffin oil-*n*-hexane (5:95, v/v). Silufol plates are prepared from large-pore silica with a starch binder having a fairly low developing time. The compounds were separately dissolved in methanol at a concentration of 5 mg/ml and 2-μl volumes of these solutions were spotted on the plates. Water–methanol (11:9, 9:11 and 7:13, v/v), water–ethanol (12:8, 11:9 and 10:10, v/v) and water–1-propanol (14:6, 13:7 and 12:8, v/v) were used as eluents. The salt concentration in the eluent was 0–4.5 *M* in steps of 0.5 *M* for lithium chloride and ammonium acetate and 0–2.25 *M* in steps of 0.25 *M* for lithium acetate and ammonium chloride. To study the effect of pH on retention, acetic acid and ammonia were added to the eluents in the concentration range 0–4.37 *M* in steps of 0.88 *M*. After development the plates were dried at 105°C and the ethylenediamine oligomers were detected with ninhydrin. To increase the sensitivity of detection, the plates developed in alkaline eluents were first sprayed with 2 *M* acetic acid. Each experiment was run in quadruplicate.

The R_M values of the ethylenediamine oligomers were calculated according to the equation [9]

$$R_M = \log(1/R_F - 1) \tag{1}$$

To elucidate the individual effects of eluent additives on the R_M values of ethylenediamine oligomers, stepwise regression analysis [22] was applied to select the independent variables that influence the R_M value significantly. The R_M value in eqn. 1 was taken as dependent variable. The total salt (independently of the type of salt), acetic acid and ammonia concentrations (three variables) and as combined variables (each salt concentration multiplied by each alcohol concentration = 12 variables) served as independent variables. The inclusion of the combined variables in the calculation was motivated by the theoretical considerations outlined in the Introduction and the facts that the ions account mainly for the modification of the retention of polar compounds and that the dissociation of salts is influenced by the dielectric constant of the eluent, which depends on the type and concentration of the alcohols. The stepwise regression analysis was carried out separately for each ethylenediamine oligomer, the significance level of the acceptance of the individual independent variables was set at 95% and the number of accepted variables was not limited.

To find the relationship between the effect of significant variables on the retention and physico-chemical parameters, correlations were calculated between the regression coefficient values of the independent variables selected by the stepwise regression analysis and the activity coefficient of salt, the number of ethylenediamine units and the dielectric constant of the alcohols. The dielectric constants and the activity coefficients of lithium salts and ammonium chloride were taken from refs. 23, 24 and 25, respectively. The regression coefficients in Tables I–IV served as dependent variables. The independent variables were the number of ethylenediamine units in the oligomer (x_1), the activity coefficient of the corresponding salts (x_2), the dielectric constant of the alcohols (x_3) and two combined variables, $x_4 = x_2/x_4$ and $x_5 = x_1x_4$. The inclusion of the last two variables was motivated by the assumptions outlined above.

The calculation was carried out with the same stepwise regression analysis programme under the same conditions. Data for which the relative standard deviation between parallel determinations was higher than 6% were omitted from the calculations.

RESULTS AND DISCUSSION

The ethylenediamine oligomers remained at the start or very near the start with each salt-free eluent, even with the most acidic and alkaline eluents. This indicates that these highly polar compounds bind very strongly to the free silanol groups of the silica surface in both dissociated and undissociated forms. The retention of the ethylenediamine oligomers decreased linearly with increasing salt concentration in the eluent (Fig. 1) and the effect was higher for compound IV than for compound I. This observation can be explained by the assumption that the longer oligomers occupy more adsorption sites than the smaller oligomers on the silica surface and therefore their retention changes more rapidly when the ions saturate the adsorption sites. The phenomenon seems to be of competitive character. The retention order does not follow the general rule of reversed-phase chromatographic separations in neutral and alkaline eluent systems: the most polar compound IV is the most strongly retained followed by compounds III, II and I. This anomalous retention behaviour can also be explained by the suppositions outlined above.

The concentration of the organic mobile phase has a relatively small effect on the retention (Fig. 2). This observation again proves the strong adsorption capacity of ethylenediamine oligomers. Acidification of the eluent also decreased the retention of each oligomer (Fig. 3). At higher acetic acid concentrations the retention order changes and at the highest salt and acid concentrations it is totally reversed (that is, it follows the general rule): the longest oligomer shows the lowest retention and the

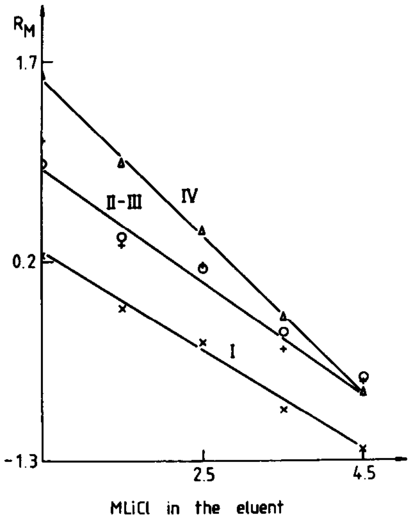
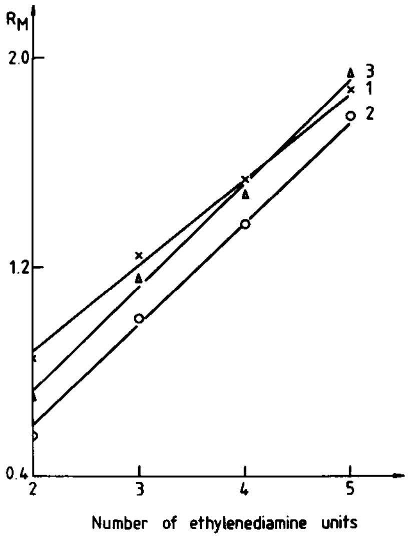


Fig. 1. Effect of LiCl concentration on the R_M value of ethylenediamine oligomers (40 vol.% ethanol). I = diethylenetriamine; II = triethylenetetramine; III = tetraethylenepentamine; IV = pentaethylenehexamine.



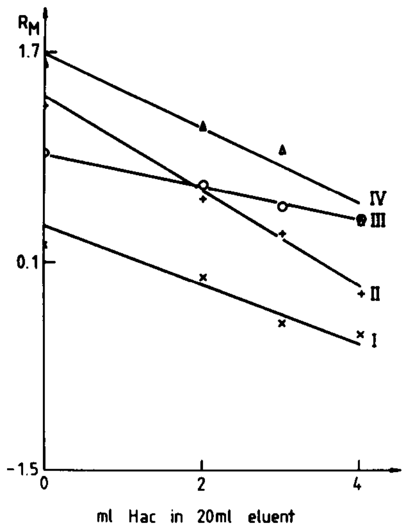


Fig. 2. Effect of the number of ethylenediamine monomer units on the R_M value at a 0.375 *M* lithium acetate concentration. Methanol concentration: 1, 45 vol.%; 2, 55 vol.%; 3, 65 vol.%.

Fig. 3. Effect of acetic acid (Hac) concentration on the R_M value of ethylenediamine oligomers at 40 vol.% ethanol and 0.5 *M* LiCl concentration. I = diethylenetriamine; II = triethylentetramine; III = tetraethylenepentamine; IV = pentaethylenehexamine.

TABLE I

EFFECT OF ELUENT COMPOSITION ON THE R_M VALUE OF DIETHYLENETRIAMINE: RESULTS OF STEPWISE REGRESSION ANALYSIS

$R_M = a + b_1C_1C_2 + b_2C_1C_3 + b_3C_1C_4 + b_4C_5C_2 + b_5C_5C_3 + b_6C_5C_4$

where C_1 = vol.% methanol in the eluent, C_2 = NH_4Cl concentration in the eluent (*M*), C_3 = LiCl concentration in the eluent (*M*), C_4 = CH_3COOLi concentration in the eluent (*M*) and C_5 = vol.% ethanol in the eluent.

$n = 31$; $a = -8.40$; $F = 23.52$; $r^2 = 0.8547$.

Parameter	Number of independent variable					
	1	2	3	4	5	6
b	−0.87	−0.29	0.94	−1.36	−0.53	1.07
s_b	0.18	0.08	0.23	0.22	0.09	0.36
Path coefficient (%)	17.01	12.12	14.40	21.15	20.77	10.27

smallest oligomer is the most strongly retained. This result is probably due to two effects, *viz.*, the compounds are in a highly dissociated state and the adsorption centres on the silica surface are totally occupied by the ions.

The results of stepwise regression analysis are compiled in Tables I–IV. The equations selected by the stepwise regression analysis fit the experimental data well, the significance level is in each instance being over 99.9% (see F values). The equations account for about 85–95% of the total variance (see r^2 values), that is, the change in the independent variables (chromatographic conditions) explains most of the change in the dependent variable (retention of the ethylenediamine oligomers). The results support our previous suppositions; the combined variables explain most of the total variance, that is, the effects of salt and organic modifier are not independent of each other. This result lend support to the assumption that only the dis-

TABLE II

EFFECT OF ELUENT COMPOSITION ON THE R_M VALUE OF TRIETHYLENETETRAMINE: RESULTS OF STEPWISE REGRESSION ANALYSIS

$R_M = a + b_1C_1C_2 + b_2C_1C_3 + b_3C_1C_4 + b_4C_5C_2 + b_5C_5C_3 + b_7C_6C_3 + b_8C_7 + b_9C_8$

where C_6 = vol. % 1-propanol in the eluent, C_7 = acetic acid concentration in the eluent (*M*), C_8 = salt concentration in the eluent (*M*) and other symbols are as in Table I.

$n = 32$; $a = 80.68$; $F = 37.08$; $r^2 = 0.9280$.

Parameter	Number of independent variable							
	1	2	3	4	5	7	8	9
b	−1.30	−0.34	0.71	−1.65	−0.63	−0.58	−29.6	−9.67
s_b	0.16	0.08	0.21	0.20	0.09	0.20	4.29	3.79
Path coefficient (%)	17.64	9.70	7.61	17.96	17.73	6.51	15.62	7.22

TABLE III

EFFECT OF ELUENT COMPOSITION ON THE R_M VALUE OF TETRAETHYLENEPENTAMINE: RESULTS OF STEPWISE REGRESSION ANALYSIS

$R_M = a + b_1C_1C_2 + b_2C_1C_3 + b_4C_5C_2 + b_5C_5C_3 + b_7C_6C_3 + b_8C_7 + b_{10}C_6C_2$

Symbols as in Tables I and II.

$n = 32$; $a = 86.00$; $F = 18.33$; $r^2 = 0.8424$.

Parameter	Number of independent variable						
	1	2	4	5	7	8	10
b	−1.25	−0.57	−1.53	−0.88	−0.82	−31.24	−2.36
s_b	0.30	0.12	0.30	0.12	0.29	6.31	0.61
Path coefficient (%)	12.49	13.94	15.36	22.80	8.49	15.21	11.71

sociated ions of the salts influence the retention, and the extent of dissociation depends on the composition of the eluent.

Ammonium acetate, having the lowest activity coefficient [25], has no significant effect on the retention. The acetic acid concentation significantly decreases the retention of the longer oligomers but not of the smallest oligomer. This result can be explained by the supposition that the increasing number of polar groups makes the compound more sensitive to the acidity of the environment.

The effects of the various combined variables on the retention of ethylenediamine oligomers are commensurable (see path coefficients), but the absolute value of the regression coefficient differs considerably. The path coefficients (normalized slope values) are dimensionless numbers reflecting the relative impact of the independent variables on the dependent variable. As they are normalized values they are independent of the orginal dimension of the independent variables.

The activity coefficients of the salts and the dielectric constants of the alcohols explain well their effect on the retention of the ethylenediamine oligomers (Table V).

TABLE IV

EFFECT OF ELUENT COMPOSITION ON THE R_M VALUE OF PENTAETHYLENEHEXAMINE: RESULTS OF STEPWISE REGRESSION ANALYSIS

$R_M = a + b_1C_1C_2 + b_2C_1C_3 + b_3C_1C_4 + b_4C_5C_2 + b_5C_5C_3 + b_7C_6C_3 + b_8C_7 + b_{10}C_6C_3 + b_{11}C_9$

where C_9 = ammonia concentration in the eluent (M) and other symbols are as in Tables I and II.

$n = 31$; $a = 112.91$; $F = 48.72$; $r^2 = 0.9543$.

Parameter	Number of independent variable								
	1	2	3	4	5	7	8	10	11
b	−1.47	−0.64	1.80	−1.75	−0.96	−0.84	−31.07	−2.60	−34.33
s_b	0.22	0.08	0.31	0.21	0.09	0.20	4.42	0.49	6.38
Path coefficient (%)	10.62	12.95	9.41	13.30	18.54	6.58	11.47	8.53	8.59

TABLE V

EFFECT OF ACTIVITY COEFFICIENTS OF SALTS (*AC*) AND DIELECTRIC CONSTANTS OF THE ALCOHOLS (*DC*) ON THEIR IMPACT ON THE RETENTION (*b*) OF ETHYLENEDIAMINE OLIGOMERS: RESULTS OF STEPWISE REGRESSION ANALYSIS

$b = a + b_1 AC + b_2 AC/DC$

$n = 25;\ a = 14.92;\ F = 48.69;\ r^2 = 0.8157.$

Parameter	Number of independent variable	
	1	2
b	−18.68	−60.24
s_b	2.60	16.76
Path coefficient (%)	66.66	33.34

The equation fits the experimental data well, the significance level being over 99.9% (see F value). The changes in the independent variables account for about 82% of the retention changes of the ethylenediamine oligomers (see r^2 value). The data prove that the salts with higher activity coefficients decrease the retention more strongly (higher amount of dissociated ions) and the alcohols with lower dielectric constants decrease the effect of salts more strongly.

Our data are in good agreement with the solvophobic theory in ref. 26, according to which three types of interactions may occur between the solutes and the stationary phases, namely coulombic, hydrogen bonding and hydrophobic (solvophobic). As the ethylenediamine oligomers contain both polar (amino groups) and apolar (ethylene groups) substructures and the results of stepwise regression analysis indicate significant effect of salts, organic modifiers and pH on the retention, we assume the each type of interaction may have some influence on the retention behaviour of the ethylenediamine oligomers.

In conclusion, the calculations suggest that to decrease the retention of polar compounds in reversed-phase chromatography, salts with high activity coefficients and organic modifiers with high dielectric constants are strongly recommended.

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