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Note

Rôle of silanophilic retention in the reversed-phase chromatography of chloramphenicol intermediates

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Earlier[1–3], we reported the separation of chloramphenicol intermediates on chemically bonded polar phases[3]. Pellicular reversed-phase (RP) packings available at that time proved inadequate for this separation. The advent of efficient microparticulate reversed-phase packings and renewed interest in an improved separation of choramphenicol compounds led to the present study of their retention behaviour in an RP system.

Separations were carried out on a 250 × 4.6 mm I.D. thermostatted column packed with 10-μm LiChrosorb RP-18. The equipment and eluent preparation were as described[4]. The aqueous methanol eluents always contained 50 m*M* H_3PO_4 (the apparent pH of the eluent was always less than 3.1).

The compounds tested are listed in Table I. The logarithms of their capacity factors, k', are plotted against the methanol concentration in Fig. 1. The KI method[5] was used for the determination of the dead volume.

It is seen that *p*-nitro-α-aminoacetophenone (AK) which contains a terminal $-NH_2$ group does not follow the regular "hydrophobic" retention behaviour, exhibiting a minimum in log k'. The plots for the compounds OX and AC, which contain an –NH– and an –NH– and –OH group respectively, also deviate from linearity. These compounds have tailing peaks and k' decreases with increasing sample concentration.

Nahum and Horváth[6] and Bij *et al.*[7] proposed that a dual adsorption mechanism, consisting of a hydrophobic and a silanophilic part, accounts for this tupe of RP retention behaviour. They showed that the silanophilic contribution could be successfully suppressed by the addition of a long-chain quaternary amine, which effectively competed for the silanol groups of the alkylsilica surface. They proposed an equation which, in linearized form, permitted the determination of the binding constant, K_A, of the competing agent and the silanophilic retention coefficient, k'_2, of the solute

$$\frac{[A]}{k'_0 - k'} = \frac{1}{k'_2 K_A} + \frac{[A]}{k'_2}$$

where [A] is the concentration of the competing agent and k'_0 and k' are the capacity factors of the solute in the absence and presence of the competing agent, respectively.

To restore the "regular" hydrophobic behaviour of these compounds, to eliminate the tailing and the concentration dependence of k', long-chain *n*-alkylamines were added to the eluents in various concentrations. The rôle of the chain length in

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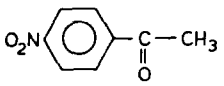
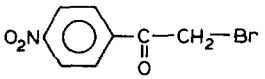
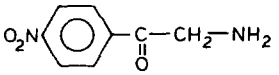
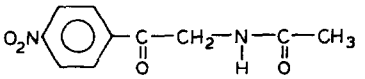
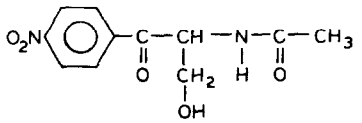
TABLE I

STRUCTURES AND ABBREVIATIONS OF THE CHLORAMPHENICOL INTERMEDIATES

Structure	*Symbol*
	PNAF
	BK
	AK
	AC
	OX

masking was investigated with *n*-hexylamine, *n*-dodecylamine and *n*-octadecylamine.

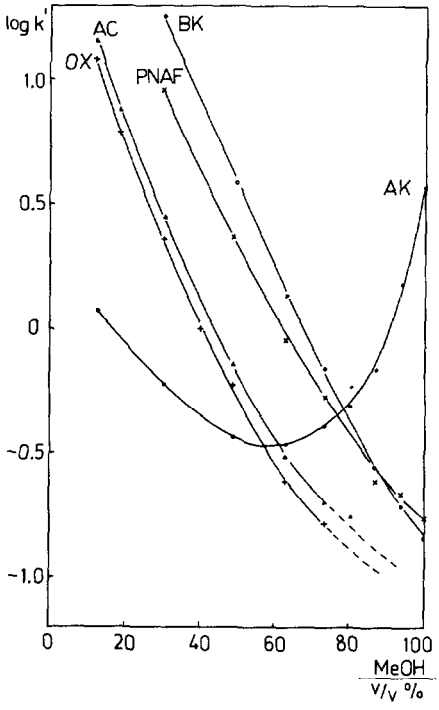
The log k' values of AK in pure methanol containing 50 m*M* H_3PO_4, where the silanophilic contribution to retention is strongest, are shown in Fig. 2 as a function of the alkylamine concentration. It is seen that the longer the chain length, the stronger is the silanol-masking effect of the competing amine.

The same alkylamines were also tested in 94% (v/v) methanol eluents. These and the previous results were analysed by the equation proposed in ref. 7. The correlation coefficients were better than 0.999. The results are shown in Table II, the last two columns of which show C_A^*, the concentration of masking agent which suppresses 90% of the silanophilic retention contribution.

The silanophilic retention contributions are characteristic of the solute, independent of the competing agent and their magnitude decreases with increasing water concentration of the eluent. Also, in pure methanol the observed k' is only slightly larger than k'_2, indicating that the hydrophobic contribution to retention is almost negligible.

The K_A values increase with the chain length of the amine reagent. Plots of log K_A against the carbon number of the alkyl chain (Fig. 3) are linear for both pure methanol and 94% (v/v) methanol eluents.

The cross over, *i.e.*, the reversal in order of the competing strength (binding strength) of the amines, indicates that the competing agent itself is retained by a dual



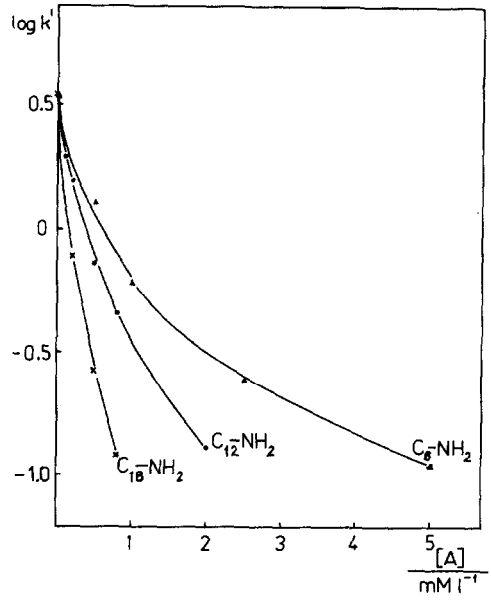


Fig. 1. Retention of the chloramphenicol intermediates as a function of the methanol (MeOH) concentration of the eluent. Column: LiChrosorb RP-18. Eluent: methanol–water containing 50 mM H_3PO_4. For abbreviations see Table I.

Fig. 2. The effect of *n*-alkylamines on the retention of AK in pure methanol containing 50 mM H_3PO_4.

retention mechanism. The relative magnitude of the hydrophobic and silanophilic contribution to this binding strength change with the water concentration of the eluent.

The K_A values of the *n*-alkylamines are of the same magnitude as those reported in ref. 7 for alkyltrimethylammonium ions of equal chain length.

Addition of alkylamines to the eluent restored the "regular" hydrophobic behaviour of the other compounds containing an –NH– group as well (OX and AC).

TABLE II

PARAMETERS OF THE SILANOPHILIC–HYDROPHOBIC RETENTION MODEL

Masking agent	k'_2		K_A (mM^{-1})		C^*_A (mM)	
	100% (v/v) methanol	*94% (v/v) methanol*	*100% (v/v) methanol*	*94% (v/v) methanol*	*100% (v/v) methanol*	*94% (v/v) methanol*
n-Hexylamine	3.5	1.7	3.6	1.7	2.8	6.0
n-Dodecylamine	3.5	1.7	6.2	6.7	1.6	1.5
n-Octadecylamine	3.6	1.7	14.0	28.3	0.7	0.4

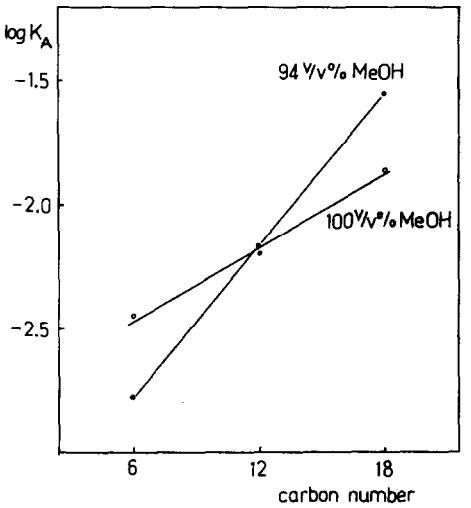


Fig. 3. Dependence of the binding constant, K_A, of various *n*-alkylamines on the carbon number and methanol (MeOH) content of the eluent.

However, the log k' values are so low that their analysis by the silanophilic model is impossible..

It can be concluded that the model proposed in ref. 7 can be used for the evaluation of the relative binding strength of the various alkylamines on alkylsilica, and that this binding strength is greatly dependent on the chain length of the masking agent.

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