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Note

High-performance liquid chromatographic separation of amino sugars and peptides with metal ion-modified mobile phases

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The use of impregnated supports for the separation of closely related compounds has been reported for thin-layer chromatographic (TLC) systems[1,2]. In an application of these TLC techniques to high-performance liquid chromatography, Schomburg and Zegarski[3] have reported the use of an argentated mobile phase with a reversed-phase partition system for the separation of geometrical isomers of 2-alkenes, oleic and elaidic acid methyl esters. The separation of pharmaceutical compounds using a mobile phase containing silver nitrate has been reported by Tscherne and Capitano[4]. Other metal ions such as Ni(II), Cd(II) and Zn(II) have been used in two different manners in the reversed-phase mode. The metal ion can be added to the mobile phase directly[5] or in a chelated form[6].

In the present study we have used cadmium sulphate and zinc acetate in the mobile phase for separation of amino sugars and some peptides on an amino column, and explain the probable mechanism.

EXPERIMENTAL

The HPLC apparatus consisted of a Tracor 990 pump, a Rheodyne 7125 injector and a Schoeffel SF 770 variable-wavelength UV detector monitoring at 180–220 nm. 600-NH amino columns (Alltech) were used for all the studies. A precolumn of silica gel (Universal Scientific) was used to improve the life and stability of the column. The amino sugars and peptides were of reagent grade (Sigma, St. Louis, MO, U.S.A.). The $CdSO_4$, zinc acetate and acetonitrile (HPLC Grade) were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). 2,2,2-Trifluoroethanol (sequanol grade) was purchased from Pierce (Rockford, IL, U.S.A.).

All chromatograms were developed at room temperature. Amino sugars and peptides were dissolved in water. The columns were allowed to equilibrate in the appropriate mobile phase for about 120 ml prior to chromatography. The UV absorbance of the amino sugars was monitored at 190 nm and that of peptides at 202 nm.

RESULTS AND DISCUSSION

We first measured the effect of $CdSO_4$ on the retention of amino sugars. Although there is no retention of amino sugars with pure water as the mobile phase,

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TABLE I

EFFECT OF $CdSO_4$ CONCENTRATION IN THE MOBILE PHASE ON THE RETENTION TIMES (min) OF AMINO SUGARS

Amino sugars	*$CdSO_4$ concentration in water*			
	10^{-4} *M*	10^{-3} *M*	5×10^{-3} *M*	10^{-2} *M*
Glucosamine	1.8	2.5	3.0	3.2
Galactosamine	2.0	2.4	3.3	3.5
Mannosamine	3.6	4.3	7.15	7.3

the retention times increased with the amount of $CdSO_4$ in the mobile phase (Table I). Fig. 1 shows that the retention times increase in a sigmoidal fashion to a maximum at 10^{-2} *M*. The peak width also increased with increasing $CdSO_4$ concentration, an effect which could be reduced by addition of the organic modifiers acetonitrile and 2,2,2-trifluoroethanol to the mobile phase. It was found that 10^{-3} *M* cadmium sulphate–acetonitrile–trifluoroethanol (83:15:2) as mobile phase gave good separation of all amino sugars (see Fig. 2). We observed two peaks for each amino sugar with this mobile phase, one of the peaks having the same retention time for all the amino sugars. Since the samples are amino sugar hydrochlorides, one of the peaks is presumably due to HCl and the other is due to the amino sugar. In further support for this interpretation, we observed that injection of HCl gives a peak having the same retention time as the peak which is common in all the amino sugars. Hence this

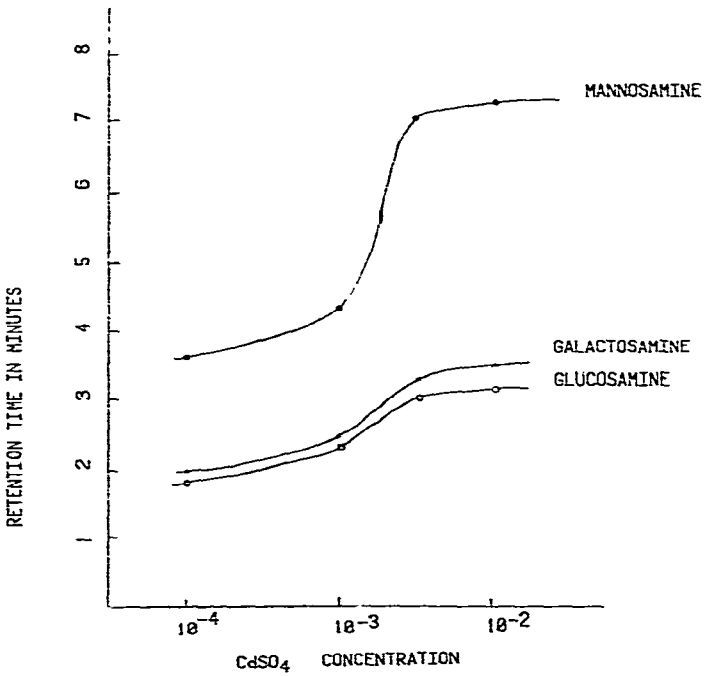


Fig. 1. Influence of $CdSO_4$ concentration on retention of amino sugars. Mobile phase: $CdSO_4$ in water.

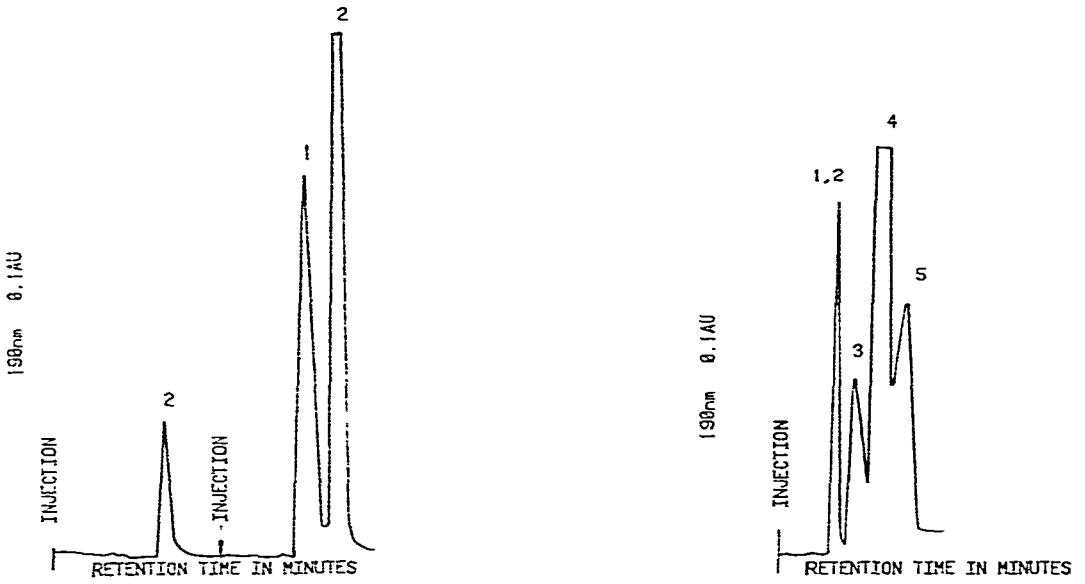


Fig. 2. Separation of glucosamine (1) and HCl (2). Conditions: eluent, 83 ml 10^{-3} *M* $CdSO_4$ + 15 ml acetonitrile + 2 ml trifluoroethanol; flow-rate, 1.0 ml/min.

Fig. 3. Separation of sugars. Eluent as in Fig. 2. Solutes: 1 = glucose; 2 = galactose; 3 = glucosamine; 4 = HCl; 5 = mannosamine.

system could be useful for the isolation of the free base from its hydrochloride. Fig. 2 shows the chromatogram of the HCl peak alone and that of glucosamine hydrochloride.

Fig. 3 shows that this mobile phase may be used to separate neutral sugars from amino sugars. The latter are all retained under these conditions, while the former migrate with the injection peak. We believe that the retention of the amino sugars results from the formation of weak metal ion complexes with Cd^{2+} forming bridges between the stationary phase amine and that of the amino sugar. The retention times of three different amino sugars are summarized in Table II.

Influence of $CdSO_4$ on the retention of peptides

Although the influence of $CdSO_4$ on the retention of peptides is similar to that

TABLE II

HPLC SEPARATION OF NEUTRAL SUGARS FROM AMINO SUGARS

Eluent: 83 ml 10^{-3} *M* $CdSO_4$ + 15 ml acetonitrile + 2 ml trifluoroethanol. Flow-rate: 1 ml/min.

Compounds	*Retention time (min)*
Glucose, galactose, mannose, maltose, lactose	1.4
HCl	2.6
Glucosamine	1.7
Galactosamine	2.0
Mannosamine	3.2

TABLE III

HPLC SEPARATION OF SOME PEPTIDES

Eluent: 89 ml $5 \cdot 10^{-4}$ *M* $CdSO_4$ + 10 ml acetonitrile + 1 ml trifluoroethanol. Flow-rate: 1.5 ml/min. Detection: 202 nm. pK_2 values are taken from ref. 7.

Peptide	*Retention time (min)*	pK_2 $(NH_3)^+$
Void volume	1.0	
Val-Val	3.2	
Ala-Val	4.1	
Ala-Leu	4.5	
Ala-Ala	5.2	8.14
Ala-Gly	5.4	8.18
Gly-Leu	9.0	8.29
Gly-Thr	4.0	8.40
Gly-Gly	11.1	8.17
Gly-Gly-Gly	13.7	7.91
Gly-Gly-Gly-Gly	15.2	7.75
Gly-Gly-Gly-Gly-Gly-Gly	15.8	7.60

on the amino sugars, *i.e.*, the retention increases with increasing $CdSO_4$ concentration in the mobile phase, the peptides are retained more strongly than amino sugars for the same concentration of $CdSO_4$. The most suitable mobile phase for the separation of peptides was found to be $5 \cdot 10^{-4}$ *M* cadmium sulphate–acetonitrile–tri-

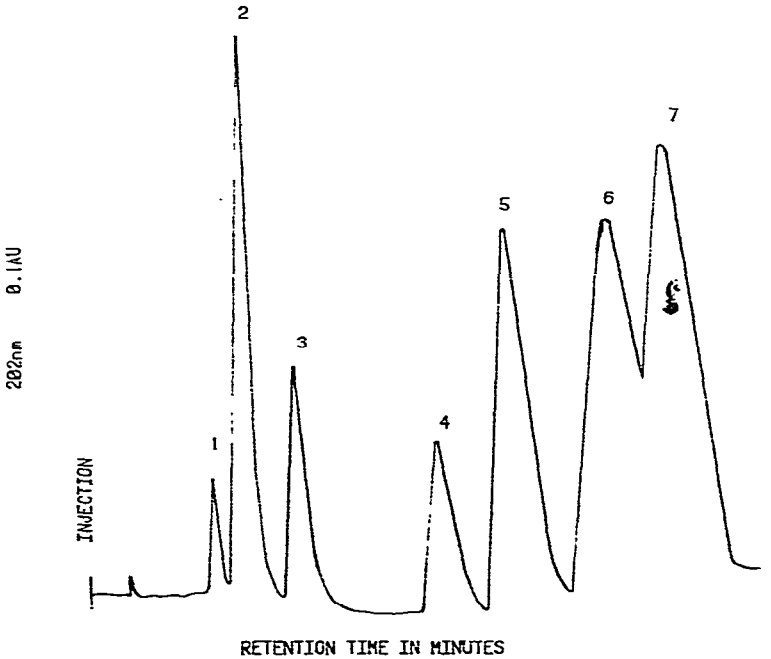


Fig. 4. Separation of peptides. Eluent: 89 ml $5 \cdot 10^{-4}$ *M* $CdSO_4$ + 10 ml acetonitrile + 1 ml trifluoroethanol. Solutes: 1 = Val-Val; 2 = Gly-Thr; 3 = Ala-Gly; 4 = Gly-Leu; 5 = Gly-Gly; 6 = Gly-Gly-Gly; 7 = Gly-Gly-Gly-Gly.

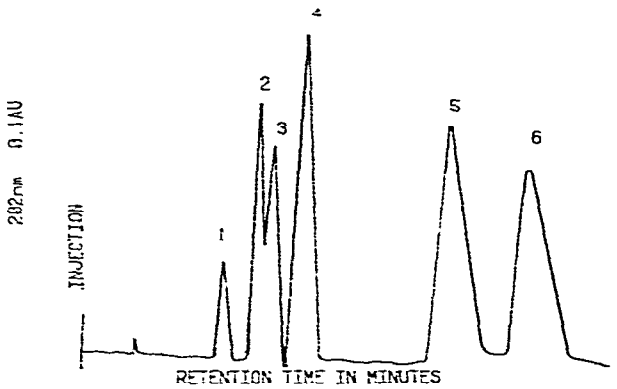


Fig. 5. Separation of peptides. Eluent as in Fig. 4. Solutes: 1 = Val-Val; 2 = Ala-Val; 3 = Ala-Leu; 4 = Ala-Ala; 5 = Gly-Leu; 6 = Gly-Gly.

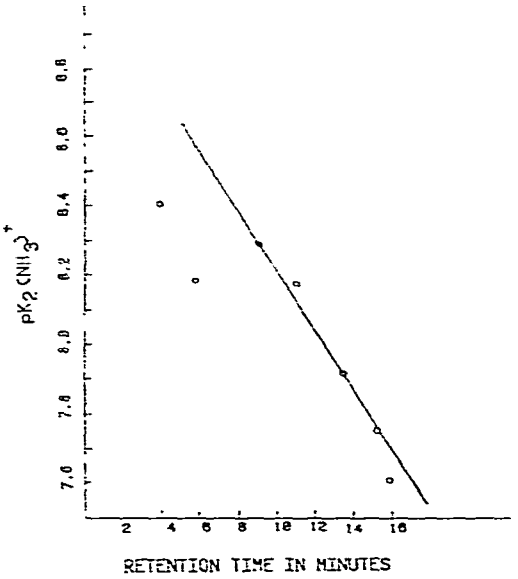
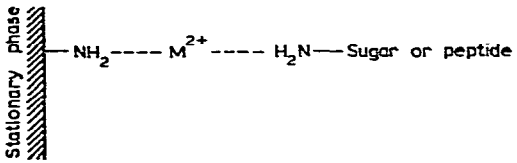


Fig. 6. Relationship between pK_2 $(NH_3)^+$ of peptides and their retention times.

fluoroethanol (89:10:1). The retention times are shown in Table III and the separation of some of the peptides is given in Figs. 4 and 5.

In order to establish whether there is any correlation between the retention times and basicities of the peptides, we plotted pK_2 value of peptide *vs.* retention time (Fig. 6). It can be seen that the retention decreases as the pK_2 value increases. Similar behavior was observed by Yasuda[1] in the TLC separation of aromatic amines on silica gel layers impregnated with cadmium sulphate. Hence the chromatographic behavior of amino sugars and peptides seems to be mainly due to the weak complex formation between the metal ion and the NH_2 group of the sugars or peptides as shown below:



We next attempted to separate amino sugars from peptides using several mobile phases containing $CdSO_4$. Since both the amino sugars and peptides were retained, we could not separate amino sugars from peptides. So we tried zinc acetate in the mobile phase which forms very weak complexes with amino sugars but stronger complexes with peptides. With 10^{-3} *M* zinc acetate as the mobile phase the amino sugars are not retained and elute with the solvent front while the peptides are retained. The results are shown in Table IV. Hence this system is most suitable for the separation of amino sugars from peptides.

TABLE IV

HPLC SEPARATION OF AMINO SUGARS FROM PEPTIDES

Eluent: 10^{-3} *M* zinc acetate. Flow-rate: 1.5 ml/min. Column: amino column.

Compound	*Retention time (min)*
Sugars	
Glucosamine	2.2
Galactosamine	2.2
Mannosamine	2.2
Glucose	2.2
Galactose	2.2
Peptides	
Ala-Ala	10.0
Ala-Leu	10.2
Ala-Val	8.4
Ala-Gly	9.3
Gly-Gly	11.0
Gly-Gly-Gly	13.6
Gly-Gly-Gly-Gly	14.2
Gly-Leu	12.3
Gly-Thr	10.2
Val-Val	8.9

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