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The Applicability of Chromatofocusing to the Concentration and Separation of Transition Metals with Tetraethylenepentamine-bonded Silica

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A new approach to the separation of metals is possible owing to a combination of the buffering and complexing properties of silica gel with immobilized tetraethylenepentamine molecules.

Chromatofocusing is now a well-established technique and was first described by Sluyterman and co-workers in 1979 as a novel chromatographic method for the separation of biological macromolecules.[1] The main features of chromatofocusing are: high resolution ability, column load ability and selectivity of separation. These advantages are achieved by formation of a linear pH gradient inside a chromatographic column packed with a weak base anion exchanger. Such a gradient is produced by initial equilibration of the column with the appropriate buffer at the higher limit of the desired pH range followed by flushing of the column with polybuffer ampholyte adjusted with acid to the lower limit of the pH range. Owing to the buffering effect of the column and polybuffer eluent, a linear pH gradient forms and moves at a much slower rate than the mobile phase.

As a rule weak anion-exchangers used in chromatofocusing have a set of amino functional groups possessing significant complexing ability towards transition metals. Such exchangers have been successfully used for the preconcentration of transition metals.[2,3] Thus, at neutral or weakly-acid pH ranges the preconcentration of transition metals on the top of the column can be performed. This can be combined with simultaneous equilibration of the column by a starting buffer with a higher pH limit of gradient. The consequent destruction of the surface complexes and the elution of metals can be performed by flushing the column with a polybuffer solution of lower pH value in accordance with the linear pH gradient formed in a column. Total separation of a mixture of Cu^{II}, Co^{II} and Ni^{II} was achieved on a column packed with a weak anion exchanger PBE-94 (Pharmacia, Uppsala, Sweden).[4] However, the softness of the polysaccharide PBE-94 matrix restricts its practical use in a system of higher pressure. Also, the ability of PBE-94 to preconcentrate metals from dilute solutions leaves something to be desired. One can propose that the use of a silica chemically modified by oligoethyleneamines as a polybuffer ion exchanger will eliminate the above drawbacks of PBE-94. The aim of the present paper is to demonstrate the possibility of formation of a linear pH gradient in a column packed with tetraethylenepentamine bonded silica (Tetren-SiO_2) and its applicability to the separation of transition metals.

The liquid chromatographic system, consisting of a peristaltic pump (Multiperpex 2115), a low pressure rotary injection valve equipped with 1 ml sample loop (Beckman, Berkeley, CA) and collector (RediRac 2112, LKB, Bromma) was used. A glass chromatographic column (30 cm × 1 cm internal diameter) packed with silica based polybuffer ion exchanger Tetren-SiO_2 was used. The potentiometric titration of Tetren-SiO_2 was performed with potentiometer pH-340 (Moscow, Russia) equipped with a glass electrode. The concentration of transition metals in the fractions was determined photometrically by reaction with PAR as described.[5]

The polybuffer ion-exchanger was prepared by modification of the silica surface with 3-glycidoxypropyltriethoxysilane followed by tetraethylenepentamine (Reakhim, Russia) treatment in a modified procedure.[6] Silochrom S-120 silica (specific surface area 120 $m^2 g^{-1}$, particle size 0.10–0.16 mm, Reakhim, Moscow) was used as a matrix for the synthesis of Tetren-SiO_2. Aqueous solutions of Tris (Serva, Heidelberg) and dilute solution of Polybuffer 74 (Pharmacia, Uppsala, Sweden) were used as starting buffer and a polyampholyte eluent, respectively. The required pH values of the eluents were adjusted with 0.1 M HCl. Stock solutions of transition metals containing 200 $mg\,l^{-1}$ were prepared from the corresponding nitrates and sulfate in the case of Fe^{III}.

Tetren-SiO_2 can be considered as a polybuffer ion-exchanger owing to the presence on its surface of several types of primary and secondary amino groups with different pK values. There are several reasons for this. Initially, the difference in basicity of the nitrogen atoms in oligoethyleneamine molecules is in accordance with the conjugation effect (pK_1 9.9; pK_2 9.1; pK_3 7.9; pK_4 4.3; pK_5 2.7[7]). The different possible reaction products can then be formed on treatment of the epoxy-activated surface of silica with tetraethylenepentamine. The tetraethylenepentamine molecule can therefore be attached to the sorbent surface for one or for several amino groups. Multiple acid-base interactions of residual silanol groups with amino groups should also be taken into consideration. As a result, Tetren-SiO_2 provides a set of amino groups of different basicity and a practically constant buffer capacity (70–120 μequiv g^{-1} pH $unit^{-1}$) over a wide pH range (Fig. 1). This is important for the formation of a really linear pH gradient in the corresponding pH range.

The important characteristic of the sorbent to be used in a proposed separation technique is the completeness of sorption of transition metals at the starting point of the pH gradient. As shown in Fig. 2, the sorption of Ni^{II}, Co^{II}, Cd^{II}, Zn^{II}, Cu^{II} and Mn^{II} is increased with growth of solution pH. The total sorption of all metals investigated can be achieved at a solution pH value equal to 7.5. In batch experiments total sorption of all metals was reached in 5–7 min. It was also found that a column packed with Tetren-SiO_2 allows total recovery of transition metals from the volume of sample injected in a flow of Tris-buffer with pH 7.5. The concentration factor for all metals exceeded 10^2. The complete elution of adsorbed Cd^{II}, Zn^{II}, Co^{II}, Mn^{II}, Ni^{II} and Fe^{III} from a column packed with Tetren-SiO_2 was achieved by flushing with eluent of pH < 3.†

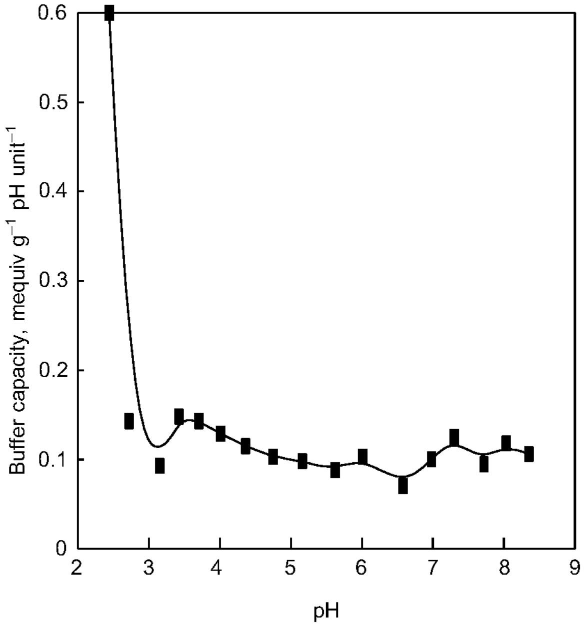

Fig. 1 Dependence of buffering capacity of Tetren-SiO_2 *versus* pH calculated from a titration curve with 0.1 M HCl.

Our preliminary results show the possibility of using Tetren-SiO_2 as a stationary phase in the chromatofocusing of transition metals. The separation of Mn^{II}, Cd^{II}, Zn^{II}, Ni^{II} and Cu^{II} was achieved in a low pressure liquid chromatographic system. The main features of the proposed method are: simplicity, high resolution ability and potential for metal preconcentration. The further development of the proposed method can be connected with the optimization both of the structure of the bonded layer of the polybuffer ion-exchanger and of the elution system. The use of microparticle Tetren-SiO_2 in combination with HPLC could improve both the efficiency and time of separation. Attention should be also paid to the development of reliable detection of separated metals in an eluent flow of very high buffering capacity.

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† *Separation of transition metals.* Tris solution (0.025 M) of pH 7.5 was chosen as a starting buffer due to the low complexing ability towards transition metals. A dilute 1:8 solution of Polybuffer-74 of pH 3.5 was used as a polyampholyte eluent for separation. As shown in Fig. 3, it was possible to separate Mn^{II}, Cd^{II}, Zn^{II}, Ni^{II} and Cu^{II}. The elution order of metals correlated well with the stability of the corresponding metal complexes with bonded amines. The peak widths for all metals are *ca.* 30–5 min. This is in accordance with the focusing effect usually observed for the gradient technique. The chromatographic behaviour of Fe^{III} was also studied under the same separation conditions. The chromatographic peak of Fe^{III} tailed due to the formation of hydroxy complexes in the neutral range of the pH gradient.

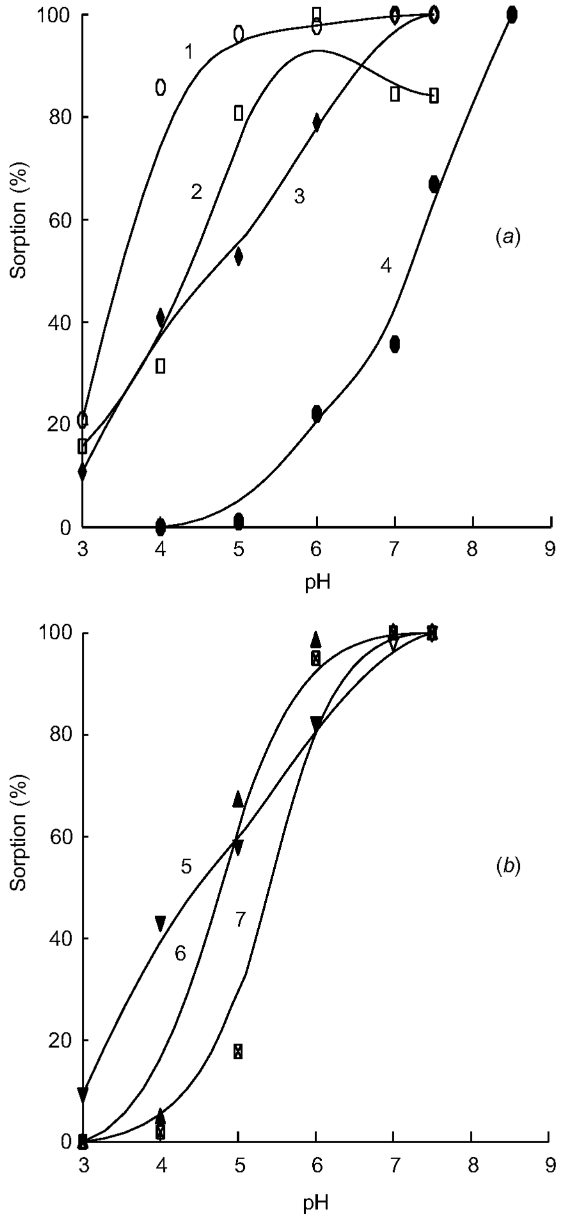

Fig. 2 Effect of pH on sorption of (*a*) Cu^{II} (1), Fe^{III} (2), Co^{II} (3), Mn^{II} (4); (*b*) Ni^{II} (5), Cd^{II} (6) and Zn^{II} (7) on Tetren-SiO$_2$; $c_{\mathrm{Me}} = 10^{-4}$ M, sorbent 0.2 g, solution volume 10 ml, duration of phase contact 15 min.

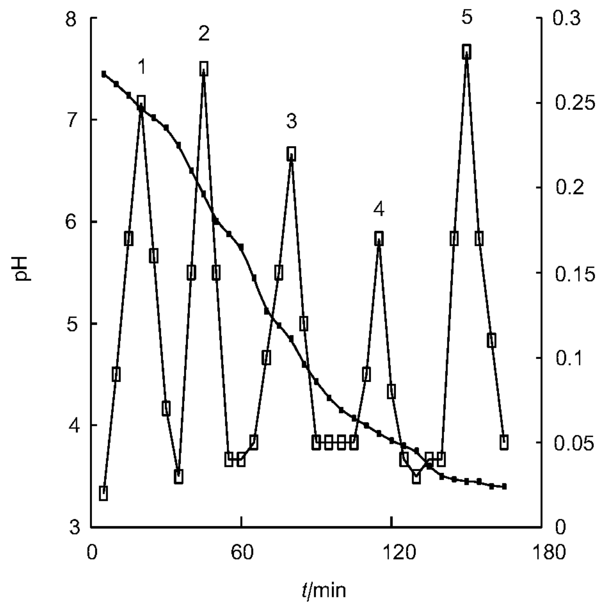

Fig. 3 Separation of standard mixture of metals. Column: 30 × 1 cm, Tetren-SiO$_2$. Starting buffer: 0.025 M Tris-HCl, pH 7.5. Polyampholite eluent: 1:8 Polybuffer 74, pH 3.4. Flow rate 1 ml min^{-1}. Sample: 1 ml of solution containing 0.1 mg of each metal. Peaks: 1, Mn^{II}; 2, Cd^{II}; 3, Zn^{II}; 4, Ni^{II} and 5, Cu^{II}.

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