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New Aspects Concerning Chelating Resins

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Object of the present paper is to provide an up-to-date overview the application of chelating resins, a functional polymer of a special kind. Attention has been in particular limited to the field of ligand exchange chromatography, sample handling and trace enrichment, metal-ion buffers and polymer catalysts with enzyme-like activities. The fundamental principles in the design and syntheses of chelating resins for usage in these respects are also described.

INTRODUCTION

The term "functional polymers", when used in the general sense, represents a broad range of polymers embodying various highly specific "chemical", "physicochemical", or "biophysical" functions compared with the mechanical functions of the more commonly used polymeric materials. In such a broad sense, functional polymers include diverse chemical structure, morphology, and functionality.[1]

More specifically, the term "functional polymers" is synonymous with "reactive polymers" and refers to insoluble (cross-linked) beaded resins carrying chemically reactive functional groups. Although classification of reactive polymers has not been well defined, for convenience, reactive polymers are classified in broad terms into two types; typical examples are shown in Table 1. In this sense,[2] functional polymers are employed as polymer supports and polymeric reagents for many and various chemical and biochemical applications, including chromatography,[3-5] peptide synthesis,[6-8] biochemical[9-11] and chemical[12,13] catalysis.

Functional polymers are produced either by chemical modification of preformed nonfunctional polymers, or by direct (co)polymerization of the desired functional monomers with suitably chosen structural and cross-linking monomers.[14] In many instances, either approach can be employed for the synthesis of a given functional polymer; the choice of the two possibilities is based on experience, availability of starting materials, convenience and economic considerations.

During the past 20 years, the synthesis of functional monomers, their copolymerization behavior and their use for the synthesis of new functional polymers have attracted considerable interest. Our own work in this area includes design, synthesis, characterization of new chelating resins — a functional polymer of a special kind,[15-23] and application of the synthesized polymer for the stationary phase of ion chromatography,[15,17-19,24-28] ligand-exchange liquid chromatography[22,25,29-32] and ligand-exchange gas chromatography,[31,33] for the packing material of on-line or off-line preconcentration,[15-17,19,27,28] for usage in the chelating ion-exchange colorimetry,[34] for the biochemical model of hydrolysis etc.[35-36] Although numerous general articles have treated the syntheses and applications in inorganic analysis,[25,37,38] there are practically no general articles relating the application of chelating resin to organic analysis. We have therefore limited our review to the use of chelating resins in ligand exchange chromatography, sample handling and trace enrichment, metal-ion buffer and polymer catalysts with enzyme-like activities.

Table 1. Classification of Reactive Polymers

Reactive intermediates	main-chain polymers for graft- and block-polymers, prepolymers for cross-linkage, reactive polymers for introducing functional groups into polymers etc.
End polymers	ion-exchange resins, chelating resins, photosensitive polymers, polymer reagents, polymer catalysts, reactive fibres, degradable polymers etc.

LIGAND EXCHANGE CHROMATOGRAPHY

In 1961, Helfferich[39] first introduced the term "ligand exchange" illustrating how metal ions, Cu(I), Cu(II), Ni(II), Zn(II), Cd(II), Ag(I) and Co(II) when loaded onto an ion exchanger could separate or isolate ammonia, organic amines, polyhydric alcohols, olefins and anions of organic

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acids and amino acids by forming complexes of varying strengths with these metals.

Ligand-exchange chromatography (LEC) is a process in which complex-forming compounds are separated through the formation and breaking of labile coordinate bonds to a central metal atom, coupled with partition between a mobile and a stationary phase. It separates ligands by causing them to change places around metal ions. The exchange can occur in either the stationary or mobile phase. The mobile phase may be either liquid or gaseous.[40] LEC is a useful technique to separate organic compounds having complex-forming ability, such as nitrogen-, sulfur-, oxygen-, or phosphorus-containing compounds. The most exciting application of ligand-exchange chromatography is the separation of enantiomers on chemically bonded chiral chelate phases. The principle of chiral LEC was introduced by Davankov, who bonded L-amino acids to chloromethylated polystyrene and applied these phases after complexation with transition metals to the separation of amino acid enantiomers.[41] A modification of this principle was described by Audebert, who used polyacrylamide as matrix to bind L-amino acids.[42] The first chiral LEC phase on the basis of chemically modified silica gel for the HPLC separation of enantiomers was developed by Gübitz.[43] L-amino acids were bonded to silica gel via 3-glycidoxypropyl trimethoxysilane. We have synthesized various chelating resin-metal complexes as stationary phases of LEC for the separation of dialkyl sulfides,[29,30,31] chlorophenols,[31] nitrophenols[31] and amino acids.[22,29]

Ligand-exchange liquid chromatography is a well established method to separate complex-forming organic compounds. It has, however, some disadvantage such as metal leakage from the stationary phase and the difficulty to adjust the metal concentration in the stationary phase. In gas chromatography, the former cannot occur and the latter can easily be overcome by impregnating or coating the support with metal salt in a suitable amount, instead of using a stationary phase bonded with chelating moiety in limited amount. In ligand-exchange gas chromatography the retention of the sample compounds depends on both their gas-phase basicity and their steric congestion: i.e., the smaller the gas-phase basicity and the more bulky the substituents around the donating element, the more brief is the retention period.[31,33,44,45] This retention behavior differs from that observed in conventional partition or adsorption gas chromatography. Furthermore, the selectivity in ligand-exchange gas chromatography depends on not only the temperature but also the concentration of the mobile-phase ligand.[31,33] These characteristics strongly indicate the suitability of the ligand-exchange gas-chromatographic method to separate homologues or isomers of organic compounds.

The establishment of ligand-exchange equilibrium between the resin and external solution depends on numerous factors, e.g. the nature of the support,[30] the composition of the eluent,[22,29-33] the complex-forming ability of sample compounds towards the cation present and the structure of the molecule,[22,29-33] i.e. the nature, number, position and properties of the functional groups they contain. The problem to separate optical isomers using LEC is related to the enantioselective effects in coordination compounds.[32] Although it was not difficult to propose a synthesis of a resin with chiral complex-forming fixed ligands starting from a chloromethylated polystyrene and an optically active substance, which seemed suitable to put to test the idea of LEC of racemic mixtures, many difficulties arose with this method.[32] For a resin to be convenient for practical use it has to meet several requirements: (i) the exchange capacity of the resin should be as large as possible; (ii) the structure of the resin elementary unit should be unique and exactly known; (iii) the racemization of the optically active starting component should be excluded during synthesis of the resin; (iv) the resin should be mechanically, chemically, and configurationally (with respect to asymmetric centers) stable; (v) the resin must be able to swell sufficiently to ensure rapid establishment of the interphase equilibria and constantly to prevent sealing off or channeling of the column on changing the eluent, and (vi) the final resin should be inexpensive.

Cyclodextrins (CD) are cyclic oligosaccharides, constructed from α-(1,4)-linked glucose units arranged in a torus, with the most common CD being α-, β-, and γ-CD, containing six, seven and eight glucose units respectively. The structures of these compounds have typically a central cavity which causes their remarkable ability to form inclusion complexes with various guest molecules. We prepared a more complex property of a chelating resin in which additional cyclodextrin groups are attached, so as to interact with both chelating behavior and molecular recognition; the product was suitable as stationary phase of LEC for the separation of phenolic compounds or dialkyl sulfides from hydrocarbons. The results were superior to that with either only chelating behavior or only molecular recognition (Fig. 1).[31]

SAMPLE HANDLING AND TRACE ENRICHMENT

An increasing need for sensitive and selective detection techniques to analyse organic trace constituents in

complex environmental matrices has been clearly recognized. Consequently sample-handling procedures have been developed which are more sophisticated than conventional liquid-liquid extraction, evaporation or steam distillation. A promising approach is to enrich trace compounds of interest on suitable sorbents, in order to isolate and preconcentrate them before their separation and detection by means of a suitable chromatographic technique. This approach has been discussed in several reviews.[46-50] Sorbents such as silica, porous polymers, alkylsilane-modified silica, alumina or carbon are generally contained in a cartridge or short stainless-steel or glass column; these are called pre-columns when operated on-line with a chromatographic column. Depending on their characteristics, the cartridge or column can be operated at ambient pressure-i.e. under gravity-flow condition or at elevated pressure. Sample volumes are generally between about 1 mL and 1 L. The samples themselves are diverse in nature; they range from aqueous samples, which include surface waters and biological fluids, to extracts from solid, liquid or even gaseous matrices, which are commonly encountered in, e.g. residue analysis.

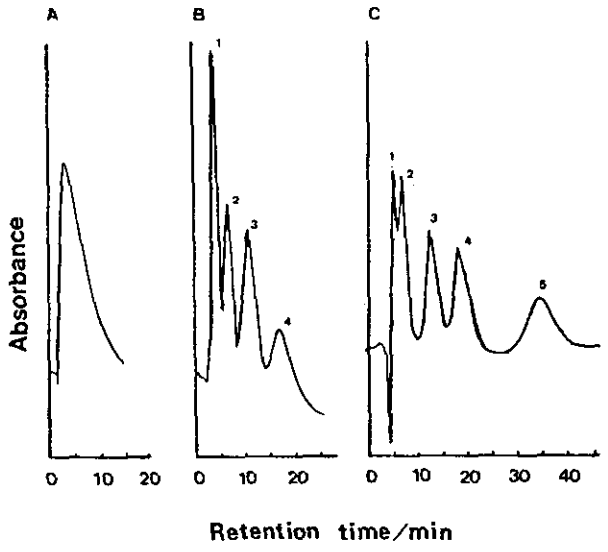

Fig. 1. Chromatogram for the separation of substituted phenols on various columns with various eluents. (A) S-CD column with methanol/water (35:65); (B) S-CDG-Zn column with methanol/water (45:55); (C) S-CDG-Cu column with acetonitrile/methanol/water (10:30:60). Column dimensions: 100 mm x 4.6 mm i.d. Particle size of resin: 325 - 400 mesh. Detection: UV (λ = 270 nm). Peak identification: (A) phenol; 2-chlorophenol; 2,4-dichlorophenol; 2-nitrophenol; 4-nitrophenol. (B) (1) phenol; 2-chlorophenol; (2) 2,4-dichlorophenol; (3) 2-nitrophenol; (4) 4-nitrophenol. (C) (1) phenol; (2) 2-chlorophenol; (3) 2,4-dichlorophenol; (4) 2-nitrophenol; (5) 4-nitrophenol. (Abbreviation: S: silica gel; CD: β-cyclodextrin; G:glycine).

Many sample preparation procedures include sample dissolution followed by liquid-liquid extraction. Traditional liquid-liquid extractions are performed in separatory funnels or automatically in dynamic systems. Liquid-liquid extractions are tedious, time-consuming and costly. These methods not only require several sample-handling steps but also cause many problems to the analyst such as phase emulsification, the evaporation of large solvent volumes, the disposal of toxic and inflammable solvents, impure and wet extracts, non-quantitative extraction and irreproducible results. An alternative approach is solid-phase extraction. When selecting a sorbent for solid-phase extraction in a particular application, one has to take into account some physico-chemical considerations, such as the nature of functional groups of compounds of interest, nature of solvated bonded phase, energetics of interactions between compounds of interest and bonded phases, secondary interactions between compounds of interest and bonded phase, interactions between components of sample matrix and bonded phase, and interaction between compounds of interest and sample matrix.[51] However one should bear in mind that sample isolation by solid-phase extraction is a combination processes of retention and elution; covalent retention is attractive but efficient elution is more difficult. Various interaction mechanisms and their energies appear in Fig. 2. The sorbents are used in solid-phase extraction as shown in Table 2.

The necessity and demand to develop automated sample-handling techniques requires that desorption and transfer of trace components to the analytical column be done on line. A typlcal scheme for an on-line procedure

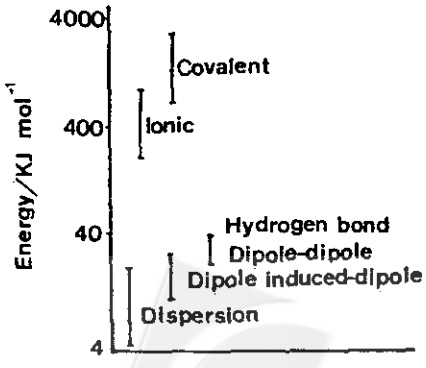

Fig. 2. Energetics of interactions in solid-phase extraction.

Table 2. Examples of Sorbents for Solid-Phase Extraction

Hydrophobic sorbents	C18, C8 or C2 bonded silicas Styrene-divinylbenzene copolymers, such as XAD-2 or PRPl sulfonic acid or carboxylic acid functional groups bound to silica or polymers Quarternary ammonium and secondary amine bonded phases
Ion pair	Sulfonates or quarternary ammonium salts, having non-polar carbon chains
Normal phase	Silica, alumina, or florisil sorbents, cyano-, diol- and amino-bonded silicas
Ligand exchange	Metal ion immobilized on a suitable support (silica or polymer-based material having functional groups such as ion exchange or ligand-exchange sites)
Size-exclusion	Sephadex columns
Miscellaneous	Wide-pore columns

appears in Fig. 3. On-line trace enrichment on a pre-column and desorption to the analytical column preferably take place with the aid of a switching valve. Selective on-line sample handling and trace enrichment are successfully performed using a small pre-column packed with materials shown in Table 2; the best way to enhance the selectivity in sample handling in volves the use of selective com-plexation on metal-loaded sorbents by the mechanism of ligand exchange. Nielen et al,[52] evaluated three commercially available stationary phases containing a thiol, 8-hydroxyquinoline (oxine), and 2-amino-1-cyclopentene-1-dithiocarboxylic acid (ACDA) functional group loaded with Hg(II), Pt(IV) and Ag(I) ions for their potential towards the selective on-line sample handling and trace enrichment. The thiol 2-mercaptobenzimidazole was used to study trace enrichment on Hg(II) loaded phases; the herbicide buturon, which contains an ethynic bond, was selected to study trace enrichment on Ag(I)-loaded phases. The Pt(IV)-loaded phases were investigated with chloroaniline as a model compound. The Hg(II)-ACDA phase was preferable for trace enrichment of thiols, a Pt(IV)-ACDA or a Pt(IV)-thiol phase for anilines and the Ag(I)-oxine phase for trace enrichment of ethynic compounds. Irth et al. studied adsorption-desorption of nitrogen heterocycles on metal-loaded thiol phases as a function of pH and metal ion.[53] Results indicated that the optimum pH for desorption could be predicted from the pK_1 of the *N*-heterocycle and the stability constant of the complex formed with the metal ion. Application of this material as a preconcentration phase was demonstrated with several barbiturates in plasma. Our investigations showed that both the copper-loaded *N*-(hydroxymethyl)thioamide concentrator column[22] and copper-loaded dithiocarbamate resin column[30] were suitable for trace enrichment and determination of 2-mercaptobenzimidazole or other sulfur containing compounds. The detection limit for the determination of 2-mercaptobenzimidazole using the metal chelate affinity precolumn connected on line to a reverse phase HPLC column were in the range 0.2-0.9 ppb.

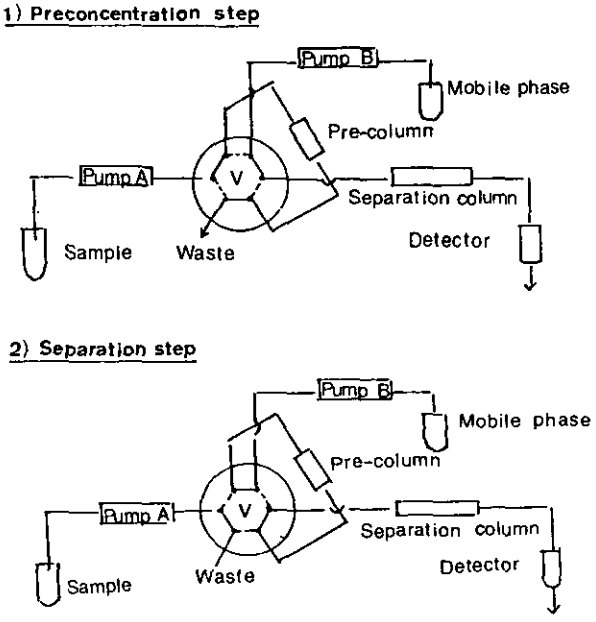

Fig. 3. On-line trace enrichment using a pre-column.

METAL ION BUFFER

By analogy with the definition of a pH buffer, which is a solution for which the pH value is little affected by dilution or addition of a relatively small proportion of a strong acid or strong base, a metal buffer solution can be defined as a solution for which the pM-value is only slightly affected by the addition of a metal ion or a ligand which complexes with the metal ion. Normally, the pM-value would also be almost independent of dilution. Buffer regions in acid-base titration curves are recognized as the regions of small slope along these curves. Similarly, pM buffer action occurs in the almost horizontal regions in the titration curves, that is, pM vs. fraction of metal ions titrated (f) with a ligand. When we consider a simple titration curve of a metal ion with a ligand forming 1:1 complexes with the metal ion, two parts in the titration curve are considered buffer regions: the first, immediately after the beginning of the titration (f

< 0.7) and the second after the equivalence point (f > 1.3).[54] These buffer regions are shown in Fig. 4.

Chemical and biological reactions generally depend critically on the presence of small concentrations of certain free metal ions. Concentrations less than 10^{-5} M are difficult to maintain in the presence of adventitious complexing agents, hydrolytic equilibria, adsorption and, possibly, contamination. Many problems are overcome by the use of metal-ion buffers which provide a controlled source of free metal ions in a manner similar to the regulation of hydrogen-ion concentration by pH buffers.

A major application of pM buffers is to maintain concentrations of necessary metal ions in biological nutrient media at essentially constant levels. As free metal ions are removed from the system, perhaps by hydrolysis or by incorporation into metalloenzymes, they are replenished by the reversible dissociation from a reservoir of metal complex. Among the first complexing agents used in this way were citrate and tartrate ions, but more recently aminopolycarboxylic acids such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA) and nitrilotriacetic acid (NTA) have become preferable chelating agents. Metal-ion buffers are useful to regulate enzyme synthesis and activity in vivo, in studies of enzyme activation and inhibition, to obtain information on the nature of metal-binding groups in enzymes and coenzymes, and to examine reaction rates and stabilities of many enzymes. This metal buffering is important when hydrolysis or insolubility limits the attainable free metal-ion concentration. Maintenance of constant pM levels may also be important to control the catalytic action of metal ions in industrial chemical processes. A promising application of metal-ion buffers is in the standardization of ion-selective electrode.[55]

Hendrickson, Turner and Corey[56] have described an approach to preparation of metal-ion buffers. A two-phase system consists of an aqueous solution and a chelating ion-exchange resin. In such a system several ion exchange equilibria are operative. In the simplest case, the solution contains only hydrogen ions and a metal ion. The resin is prepared by loading the protonated form partly with metal ions. When the resin is added to the solution, an equilibrium becomes formed between the two phases, such that

$$M^{z+} + z\,H^+R^- = M^{z+}R_z^- + z\,H^+ \quad (1)$$

in which R^- represents the resin matrix and H^+ is the counter ion. The equilibrium is described by the selectivity coefficient

$$K^M_{zH} = [M]_r[H]^z/([M][H]^z_r) \quad (2)$$

in which concentrations in the resin phase are denoted by a subscript r. After rearrangement, Eq. (2) is expressed in logarithmic form:

$$pM = z\,pH + \log K^M_{zH} - \log([M]_r/[H]^z_r) \quad (3)$$

The pM value of the aqueous solution is determined by the pH value in the aqueous phase, by the selectivity coefficient and by the relative loading of M^{z+} and H^+ on the resin. Provided that the loading is great enough for the last term in Eq. (3) to remain practically constant as a small proportion of M^{Z+} is displaced from the resin into solution, the pM value of the solution varies only with the pH value of the solution phase. This pH is controlled by a suitable buffer. Thus, at constant pH, the pM value of the solution also remains constant. Hence, a saturated chelating resin can be used as a solid-phase buffer to maintain pH and ionic metal activities for various chemical and biological studies. Our investigations showed that a chelating resin as metal-ion buffer for the calibration of ion-selective electrodes was superior to the conventional method, especially at a small metal-ion concentration (< 10^{-5} M).[57]

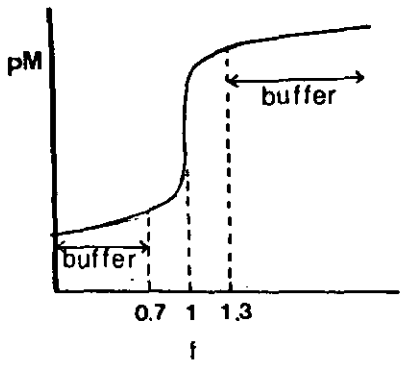

Fig. 4. Titration curve. f: fraction of metal ions titrated with a ligand.

POLYMER CATALYSTS WITH ENZYME-LIKE ACTIVITIES

Polymer catalysts with enzyme-like activities should:[58]

1. Have a large rate acceleration. A polymer catalyst may be used even under mild conditions such as ambient temperature, ambient pressure, and neutral pH in dilute aqueous solution.

2. Have efficient turnover or recycling. Even a small

proportion of polymer catalysts should be effective.

3. Be able to select the appropriate substrate from a complicated mixture of compounds. If this can be accomplished, crude raw material may be used in chemical industries.

4. Be able to select the appropriate reaction from many possible reactions.

5. Regulate or control the reaction.

A polymer catalyst should accelerate the reaction when a large proportion of the starting material is present, but retard it when the starting material is deficient and excess product has been formed. Although satisfying the fifth requirement requires further study, polymer catalysts having the other four requirements are prepared relatively easily by carrying out the following general procedures: (i) design a single small molecule which has the desired function, such as catalysis or metal binding; (ii) synthesize this molecule; (iii) attach this molecule to an appropriate "parent polymer" or a readily available polymer via chemical bonding or physical interaction (ion-pairing, adsorption, chelation). An alternative method is to design a molecular assembly (such as an inclusion complex, a metal cluster, a metal complex or a micelle) in which each particular molecule is designed to show only part of the total entire function and the proper synergetic combination of all parts leads to the desired total function. A sophisticated polymer having all necessary functions at appropriate sites is easy to imagine but difficult to prepare.

In order to prepare a polymer catalyst with enzyme-like activity, one must have a detailed understanding of the nature of enzymes. For practical applications, experimental procedures are generally limited to a narrow range in order to maintain the enzyme activity. For example, a polymer catalyst exhibiting only hydrolytically activity (without substrate specificity or regulation) can be easily prepared, but greater sophistication is not yet possible and remains a future challenge.

As the hydrolysis of carboxylic and phosphoric acid esters is of paramount importance in biological[59] and industrial processes, especially in the field of decontamination of areas exposed to pesticides or chemical weapons, we are interested to prepare polymer catalysts with enzyme-like activities for the hydrolysis reaction. Functional micelles[60] and vesicles[61] are investigated as effective catalytic systems as they provide the hydrophobic environment to bind the substrate and the function that may perform the catalytic process. Many hydrolytic processes in enzymes involve metal cations that are assumed to activate a water molecule or other nucleophilic group as well as the electrophilic center of the substrate. Working in this direction, our research group was stimulated to synthesize and investigate the properties of polymer-supported catalysts with a metal-chelating group, such as histidine, hydroxamate or combination of molecular recognition behavior for the hydrolysis of carboxylate[35,36] and phosphate[62] esters. The histidine-containing polymer was synthesized via azide coupling to the hydrolysis product of acrylonitrile-divinylbenzene copolymer. The observed kinetics of hydrolysis of *p*-nitrophenyl acetate (PNPA) in aqueous solution proceeded at 25 °C, below pH 7.8 in phosphate buffer, and obeyed Michaelis-Menten kinetics ($V_s = 4.02 \times 10^{-4}$ M/min, $k_3 = 5.90 \times 10^{-1}$ M/min cm^3 per gram resin and $K_s = 1.68 \times 10^{-2}$ M). The activation parameters, preexponential factor (A) and activation energy (E_a), have been calculated to be 6.64×10^{-4} min^{-1} and 37.6 KJ mol^{-1}, respectively. Nevertheless, above pH 7.8, the reaction rate remained almost constant ($K_{cat} = 0.024$ min^{-1}) and seemed to be controlled by the rate of diffusion of PNPA from bulk solution into the catalytically active site in the resin channel surface. For catalysed hydrolysis, the effect of ionic strength in solution demonstrated that bifunctional cooperation between adjacent histidine groups existed through nucleophilicity of nitrogen possessing unpaired electrons. The effects of nickel(II), copper(II) and zinc(II) ions on the hydrolysis of PNPA and the effect of adding aspartic acid and serine to the hydrolysis system catalyzed by the histidine-containing polymer to mimic the active site of α-chymotrypsin were also investigated.[35]

MISCELLANEOUS APPLICATIONS

An interesting use of outer-sphere coordination is the recovery of nonionic surfactants from polluted water and the separation of homologous surfactants from one another.[63] The stationary phase was an iminodiacetate chelating resin carrying the cations $[Co(NH_3)_6]^{3+}$ or, preferably, $[Co(NH_3)_5H_2O]^{3+}$. Aqueous solutions 5×10^{-5} M in the polyoxyethylene surfactants, $C_9H_{19} \cdot C_6H_4 \cdot O(CH_2CH_2O)_nH$, were passed through a small column of the chelating resin, which took up both these compounds and cationic surfactants, but not anionic surfactants. Stripping the column with ethanolic ammonia removed the nonionic surfactants, but not the cationic.

CONCLUDING REMARKS

Chelating resins have versatile applications distinguished by high selectivity. Experimental conditions are

flexible and selectivity orders can be manipulated almost at will. Although there are many practical difficulties, their solution is in progress.

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Key Words

Chelating resin; Ligand exchange; Chromatography; Sample handling; Trace enrichment; Metal-ion buffer; Polymer catalyst.

REFERENCES

1. Bergbeiter, D. E.; Martin C. R. *Functional Polymers;* Plenum: New York, **1989**.
2. Sherrington, D. C.; Hodge, P. *Syntheses and Separations Using Functional Polymers;* Wiley: Chichester, **1988**.
3. Allenmark, S. G. *Enantioselective Separations;* Ellis Harwood: Chichester, **1988**.
4. Fritz, J. S.; Gjerde, D. T.; Pohlandt, C. *Ion Chromatography;* Dr. Alfred Huthig Verlag: Heidelberg, **1982**.
5. Dean, P. D. G.; Johnson, W. S.; Middle, F. A. *Affinity Chromatography;* IRL Press: Oxford, **1985**.
6. Merrifield, R. B. *Makromol. Chem. Suppl.* **1988**, *19*, 31.
7. Fields, G. B.; Noble, R. L. *Int. J. Peptide Res.* **1990**, *35*, 161.
8. Smith, L. M. *Anal. Chem.* **1988**, *60*, 381A.
9. Chibata, I.; Tosa, T.; Sato, T. *J. Mol. Catal.* **1986**, *37*, 1.
10. Woodward, J. *Immobilized Enzymes and Cell's: A Practical Approach;* IRL Press: Oxford, **1985**.
11. Mosbach, K. *Methods in Enzymology;* Academic Press: New York, **1985**; Vol. 112.
12. Ford, W. T. *Polymeric Reagents and Catalysts;* American Chemical Society; Washington, D.C., **1986**.
13. *IUPAC Microsymposfum on Reactive Polymers,* Prague, Czechoslovakia, 1987, *Pure Appl. Chem.* **1988**, *60*(3).
14. Akelah, A.; Sherrington D. C. *Chem. Rev.* **1981**, *81*, 557.
15. Liu, C. Y.; Sun, P. J. *Anal. Chim. Acta* **1981**, *132*, 187.
16. Liu, C. Y.; Sun, P. *J. J. Chin. Chem. Soc.* **1981**, *28*, 75.
17. Liu, C. Y.; Fan, J. D.; Liu, C. B. *Proc. Natl. Sci. Counc., A. R.O.C.* **1986**, *10*, 352.
18. Liu, C. Y.;Sun, P. J. *Fresenius Z. Anal. Chem.* **1986**, *325*, 553.
19. Liu, C. Y. *Anal. Chim. Acta* **1987**, *192*, 85.
20. Liu, C. Y.; Lih, J. J. *Fresenius Z. Anal. Chem.* **1988**, *332*, 171.
21. Liu, C. Y.; Chang, H. T.; Hu, C. C. *Inorg. Chim. Acta* **1990**, *172*, 151.
22. Liu, C. Y.; Hu, C. C.; Yeh, K. Y.; Chen, M. *J. Fresenius Z. Anal. Chem.* **1991**, *339*, 877.
23. Liu, C. Y.; Chen, M. J.; Lee, N. M.; Hwang, H. C.; Jou, S. T.; Hsu, J. C. *Polyhedron* **1992**, *11*, 551.
24. Liu, C. Y.; Sun, P. J. *Talanta* **1984**, *31*, 353.
25. Liu, C. Y. *J. Chin. Chem. Soc.* **1989**, *36*, 389.
26. Lih, J. J.; Yeh, K. Y.; Liu, C. Y. *Fresenius Z. Anal. Chem.* **1990**, *336*, 12.
27. Liu, C. Y.; Chen, M. J.; Chai, T. J. J. *Chromatogr.* **1991**, *555*, 291.
28. Chen, M. J.; Fan, J. D.; Liu, C. Y. *J. Chin. Chem. Soc.* **1992**, *39*, 121.
29. Hu, C. C. Master's Thesis, National Taiwan University, June **1989**.
30. Chen, W. S. Master's Thesis, National Taiwan University, June **1990**.
31. Chang, C. H. Master's Thesis, National Taiwan University, June **1992**.
32. Chyueh, S. D.; Liu, C. Y. Abst. Annual Meeting Chin. Chem. Soc. **1991**; p 54.
33. Yeh, C. F.; Chyueh, S. D.; Chen, W. S.; Fan, C. D.; Liu, C. Y. *J. Chromatogr.* **1992**, in press.
34. Liu, C. Y.;Chang, H. T. *Fresenius Z. Anal. Chem.* **1987**, *328*, 61.
35. Hung, W. H. Master's Thesis, National Taiwan University, June **1984**.
36. Shieh, C. J. Master's Thesis, National Taiwan University, June **1987**.
37. Sahni, S. K.; Reedijk, *J. Coord. Chem. Rev.* **1984**, *59*, 1.
38. Myasoedova, G. V.; Savvin, S. B. *CRC Crit. Rev. in Anal. Chem.* **1987**, *17*, 1.
39. Helfferich, F. *Nature* **1961**, *189*, 1001.
40. Davankov, V. A.; Navratil, J. D.; Walton, H. F. *Ligand Exchange Chromatography;* CRC Press: Florida, **1988**.
41. Rogozhin, S. V.; Tsyurupa, M. P. *Chem. Commun.* **1971**, *490*.
42. Audebert, R. *J. Lig. Chromatogr.* **1979**, *2*, 1063.
43. Gübitz, G.; Jellenz, W.; Lofler, G.; Santi, W. *J. High Resolut. Chromatogr., Chromatogr. Commun.* **1979**, *2*, 145.
44. Fujimura, K., Ando, T. *J. Chromatogr.* **1975**, *114*, 15.

45. Fujimura, K.; Ando, T. *Anal. Chem.* **1977**, *49*, 1179.
46. Fishman, M. J.; Erdman, D. E.; Garbarino, J. R. *Anal. Chem.* **1983**, *55*, 102R.
47. Garbarino, J. R.; Steinheimer, T. R.; Taylor, H. E. *Anal. Chem.* **1985**, *57*, 46R.
48. MacCarthy, P.; Klusman, R. W.; Rice, J. A. *Anal. Chem.* **1987**, *59*, 308R.
49. MacCarthy, P.; Klusman, R. W.; Rice, J. A. *Anal. Chem.* **1989**, *61*, 269R.
50. Dorsey, J. G.; Forey, J. P.; Cooper, W. T.; Barford, R. A.; Barth, H. G. *Anal. Chem.* **1992**, *64*, 353R.
51. *Selective Sample Handling and Detection in High-performance Liquid Chromatography;* Frei, R. W.; Zech, K. Eds.; Elsevier: New York, **1988**.
52. Nielen, M. W. F.; van Ingen, H. E.; Valk, A. J.; Frei, R. W.; Brinkman, U. A. Th. *J. Chromatogr.* **1987**, *10*, 617.
53. Irth, H.; Tocklu, R.; Welten, K.; De Jong, G. J.; Frei, R. W.; Brinkman, U. A. Th. *J. Pharm. Biomed. Anal.* **1989**, *7*, 1679.
54. Wanninen, E. V.; Ingman, F. *Pure Appl. Chem.* **1987**, *59*, 1681
55. Azab, H. A. *Bull. Soc. Chim. Fr.* **1987**, 265.
56. Hendrickson, L. L., Turner, M. A.; Corey, R. B. *Anal. Chem.* **1982**, *54*, 1633.
57. Wu, C. S. Master's Thesis, National Taiwan University, June **1992**.
58. Ise, N.; Tabushi, I. *An Introduction to Speciality Polymers*; Cambridge University Press: Cambridge, **1983**.
59. Auld, D. S. In *Enzyme Mechanisms*; Page, M. I.; Williams, A. Eds. The Royal Society of Chemistry: London, **1987**.
60. Fendler, J. *Membrane Mimetic Chemistry;* Wiley: New York, **1982**.
61. Moss, R. A.; Scrimin, P.; Bhattacharya, S.; Swarup, S. *J. Am. Chem. Soc.* **1987**, *109*, 6209.
62. Hu, C. C.; Liu, C. Y. submitted to *J. Incl. Phenom. Mol. Recog. Chem.* for publication.
63. Carunchio, V.; Liberatori, A.; Messina, A.; Petronio, B. M. *Ann. Chim. (Rome)* **1979**, *69*, 165.