Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Owens, George S. , Southard, Glen E. , Van Houten, Kelly A. and Murray, George M.(2005) 'Molecularly Imprinted Ion-Exchange Resin for Fe3', Separation Science and Technology, 40: 11, 2205 — 2211
To link to this Article: DOI: 10.1080/01496390500201177
URL: http://dx.doi.org/10.1080/01496390500201177

Separation Science and Technology, 40: 2205–2211, 2005
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ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390500201177

Molecularly Imprinted Ion-Exchange Resin for Fe^{3+}

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Abstract: Ion exchange resins selective for the sequestration of Fe^{3+} from aqueous solutions containing citrate were prepared by the molecular imprinting technique. Sorption characteristics of imprinted resins prepared with high (85 mole%) and low (3 mole%) amounts of covalent cross-linking were examined. Experiments to determine loading capacity and selectivity, relative to several metal ions of physiological significance, were performed. The Fe^{3+} capacity of the highly cross-linked resin was larger but the selectivity was lower.

Keywords: Molecularly imprinted polymer, ion exchange, ferric ion, hemochomatosis

INTRODUCTION

Iron overload, characterized by greater than normal deposition of iron within body tissues, is a problem that is both common and serious. Hemochromatosis is the disease resulting from significant iron overload (1). The typical manifestations are cirrhosis of the liver, diabetes, and cardiomyopathy. Pituitary failure is common and may be the cause of frequently observed testicular atrophy and loss of libido. Laboratory and clinical evidence even suggests that the excess iron in tissues which builds up during normal aging can

Received February 17, 2005, Accepted May 4, 2005

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promote coronary artery disease and foster the growth of latent cancers and infectious organisms (2). The current treatment is blood removal, otherwise known as therapeutic phlebotomy. During the treatment one unit (0.473 L) of whole blood, which contains about 250 mg of iron, is removed once or twice a week. The chelating agent deferoxamine is also used but is not specific for iron and so will leach other important metals as well. These treatments are inefficient and not clinically effective. A method to process blood through a membrane that complexes iron would obviously be of great benefit to hemochromatosis victims. This membrane could be incorporated into existing plasmaphereis machines that separate plasma from cellular blood elements. Treated plasma would be returned to the patient along with their cellular blood elements. Such a system would require a highly selective sequestering agent capable of removal of iron without significant amounts of electrolytes.

Vinyl benzoic acid is a commercially available polymerizable ligand. It has been used successfully to make uranyl ion selective resins (3). Uranyl ion is similar to Fe^{3+} in hardness, and Fe^{3+} behaves chemically in a manner similar to uranyl. The Pourbaix diagram for iron is similar to the one for uranium and shows that Fe^{3+} readily forms $Fe(OH)_3$ even at fairly low pH (4). Concentrated solutions of Fe^{3+} from $Fe(NO_3)_3$ were readily prepared by dissolving the salt in water, but allowing them to stand for several days resulted in solid matter accumulating on the bottom of the flasks in which they were prepared. Therefore, all the solutions used were maintained at a relatively low pH, ≤ 3.5.

EXPERIMENTAL

Reagents and Analyses

All reagents were purchased from Aldrich and used without further purification. Iron(III) vinylbenzoate was prepared by first dissolving 0.500 g (3.40 mM) of freshly prepared vinylbenzoic acid (VBA) in a solution of 50.0 mL of 0.058 M NaOH. The pH of the resulting solution was adjusted to 5.0 with drop-wise addition of 1.0 M HCl. Iron(III) nitrate nonahydrate (0.450 g) was dissolved in 50.0 mL of water and immediately added to the VBA solution. A yellow precipitate was formed. The precipitate was filtered, dried, and analyzed, but had only 8.9 ± 0.5% Fe. The product was washed with acidified demineralized water (pH ~ 6.0), acetone, and finally toluene, then dried in a vacuum dessicator. The product yield was 48%; IR(KBr): 3400 (br, H_2O), 3050 (br), 1600–1700, 1510, 1400, 850 cm^{-1}; elemental analysis (ICP-MS), 11.0 ± 0.5% Fe (calc. 11.2%). Nickel vinylbenzoate complex was prepared by the same procedure as used for the iron vinylbenzoate complex: yield, 86%.

A Hewlett-Packard Model 5400 ICP-MS (Yokogawa Analytical Systems, Tokyo, Japan) was used to verify metal concentrations in all sample solutions. NMR was performed using a Model EFT 90 MHz spectrometer (Anasazi Instruments, Indianapolis, IN). The purity of synthesized organics was established using a Model QP 5050A GC/MS (Shimadzu, Columbia, MD). Standard solutions of the metal ions were prepared by dilution of 1000 ppm standard solutions purchased from Fisher Scientific (Fisher Scientific, Pittsburgh, PA). The pH measurements were made on a model 350 Corning pH/ion Analyzer (Fisher Scientific, Pittsburgh, PA). Infrared spectra (IR) were recorded on a Bomem MB-122 FT-IR Spectrophotometer (Quebec, Canada). Samples for IR analysis were prepared as KBr pellets after grinding with a Wig-L-Bug Amalgamator (Crescent Dental Manufacturing Company, Elgin, IL).

Synthesis of Imprinted Polymers

Two types of polymers were synthesized, one with a low amount of covalent cross-linking and no porogen and one with a high amount of cross-linking and methanol as porogen. Polymer feed compositions are given in Table 1.

Iron (III) vinylbenzoate (4 mol% with respect to total monomer) dissolved in styrene was added to a screw cap vial containing divinyl benzene (DVB) and sonicated for 5 min. The solution was then purged with nitrogen for 5 min. Azobisisobutyronitrile, AIBN (1.0 mol% with respect to total monomer) were added to the mixture. The mixture was again purged with nitrogen. The polymerization reaction was carried out at 60°C for 24 h under a nitrogen atmosphere in an ultrasonic bath. Nickel (II) imprinted and blank (unimprinted) polymers were prepared in the same manner to serve as reference. The ground polymers were washed with acetone, then acetone and water with increasingly larger amounts of water until only water was used. This procedure removed unreacted monomers and served to swell the polymers. Once cleaned, the resins were allowed to dry overnight in ambient air. Subsequently, they were washed with 1 M HNO_3 until the wash solution was free of metal ions as verified by ICP-MS. The polymers were rinsed with deionized water until the wash water tested neutral to pH paper and were again allowed to dry overnight in ambient air. The imprinted resins were deemed ready for use in the loading and selectivity studies.

Table 1. Compositions of Fe^{3+} templated polymers

ID	Complex mol%	Styrene mol%	DVB mol%	AIBN mol%	Methanol porogen, mL
1	4	92	3	1	0
2	4	10	85	1	1.4

RESULTS AND DISCUSSION

Polymers imprinted with Fe^{3+} were tested by two methods. In the initial tests, a cleaned polymer was placed in a Fe^{3+} solution and the Fe^{3+} concentration was measured before and after stirring for 4 h with the polymer. A decrease in iron concentration indicated proper functioning of the polymer. For instance a 4.50 ppm solution of Fe^{3+} was found to have its concentration decrease to 0.412 ppm after stirring 4 mL of the solution with polymer **1**. Clearly the Fe^{3+} imprinted polymer was sequestering ferric ions. Stirring an equivalent amount of the Ni^{2+} imprinted resin showed no discernable decrease in the Fe^{3+} concentration.

The second type of test was designed to eliminate errors associated with nonspecific adsorption (5). In this method a polymer (200 mg) was loaded with Fe^{3+} by elution, washed with deionized water, then eluted with nitric acid. The loading solution of Fe^{3+} had a concentration of 15 ppm and a pH of 3. A 25 mL volume provided an excess of iron, and keeping the concentration of iron low mitigated problems with Fe^{3+} forming insoluble $Fe(OH)_3$ (6). The loading time was overnight, but elution time was only 4. The eluting solution was 1.0 M nitric acid. A run in which the imprinted polymers were stirred in 4.00 mL of 1.0 M nitric acid for 4 h and sampled for iron concentration was performed before loading the polymers with ferric ions. Results are presented in Table 2.

Concentrations from the preloading tests are compared to those obtained after a standard load/elute cycle for determination of iron capacity. Because the iron concentration after soaking polymers in nitric acid is significantly higher than the nitric acid soak solution, it is apparent that there is still some slight leaching of iron from nitric acid soaking. However, the amount of iron leached from the polymer is low in comparison to iron concentrations developed from an elution step after ferric ion loading (and, of course, water washing) (7). Therefore the iron loading capacity numbers for these resins reflect real iron sequestering. In determining iron loading capacity, the amount of iron appearing in the nitric acid soaking experiments prior to load and elute was subtracted from the amount of iron eluted after loading.

Table 2. Preliminary tests on templated polymers compared to subsequent load and elute tests

Sample ID	[Fe] after 4 hr stirring in 4.00 mL of 1 M HNO_3, ppm	[Fe] after standard load and elute test, ppm
Resin **1**	0.980	4.49
Resin **2**	1.00	13.7
1 M HNO_3	0.363	—
Distilled water	0.0765	—

For example, for polymer **2**, capacity = $\{(13.7\,\mu g/mL \times 4.00\,mL - 1.00\,\mu g/mL \times 4.00\,mL)/\text{polymer mass}\}/(55.86\,\mu g/\mu mol\ Fe)$ to give capacity of micromoles per gram polymer. This was a conservative calculation of loading capacity because correction for the amount of iron in the nitric acid elution solution was not made [that is subtract $(1.00-0.363)\mu g/mL \times 4.00\,mL = 0.637\,\mu g/mL \times 4.00\,mL$ from $13.7\,\mu g/mL \times 4.00\,mL$ instead of $1.00\,\mu g/mL \times 4.00\,mL$].

Polymer ion loading capacity tests were performed with a variety of interferent ions expected in blood plasma. Solutions of each of the metals were prepared by dilution of the appropriate metal nitrate to a concentration of 15.0 ppm at a pH of 3.5. The solutions were stirred overnight, the resin was rinsed with deionized water, and the amount of metal loaded was determined by elution with 1.0 M nitric acid. Results are presented in Table 3.

A more realistic test of iron loading capacity for a blood iron filter is with iron that is complexed by citrate at a physiological pH of 7.4. The citrate-complexed iron is representative of non-transferrin bound iron which occurs in hemochromatosis. Non-transferrin bound iron for patients with excessive iron loading has been reported at up to 9 μmol/L (8), or 0.5 μg/mL, so that a ferric ion loading solution concentration should be in the vicinity of this value. An iron-citrate solution was made from equimolar amount of ferric ion (from iron nitrate) and sodium citrate. Solution pH was adjusted to 7.4 and the solution was heated to boiling to solubilize the iron-citrate complex. The result was a dark-red solution free from solids. A loading solution of 0.81 ppm Fe was used: this is somewhat higher than a physiological value, but it is safely above background iron levels of 0.1 to 0.3 ppm. The higher concentration of ferric ion should not greatly affect results.

Results showed low capacities for both resins: 0.37 and 0.18 μmol/g for resin **1** and **2**, respectively. It is obvious that in competition with another complexing ligand (the log of the complexation constant of Fe^{3+} with citrate is 11.50 and with acetate is 3.2 (9)) imprinted resin capacity decreases. The question of iron loading in the presence of high concentrations of Ca^{2+} also

Table 3. Capacity values for Fe^{3+} imprinted polymers

	Resin **1** (no porogen)		Resin **2** (with porogen)	
Species	Polymer capacity, μmol/g	Capacity Fe^{3+}/capacity M^{n+}	Polymer capacity, μmol/g	Capacity Fe^{3+}/capacity M^{n+}
Fe^{3+}	0.740	1.00	1.54	1.00
Ca^{2+}	0.0120	61.6	0.168	9.16
Zn^{2+}	0.0196	37.8	0.0358	43.0
Mg^{2+}	0.00980	75.5	0.00277	556
Cu^{2+}	0.00325	228	0.0277	55.6

Table 4. Results for Fe^{3+} imprinted polymers and an unimprinted polymer in loading experiments with Fe^{3+}-citrate and 100 ppm Ca^{2+}

Polymer ID	Loading solution	Polymer Fe^{3+} capacity, μmol/g	Polymer Ca^{2+} capacity, μmol/g	α Fe/Ca
1	a	0.37	—	—
2	a	0.18	—	—
1	b	0.57	0.062	9.2
2	b	0.20	0.072	2.8
Control	b	0.18	0.11	1.6

a—Fe^{3+} citrate, b—Fe^{3+} citrate, and 100 ppm Ca^{2+}.

was addressed. A 0.8 ppm citrate-complexed Fe^{3+} loading solution with 100 ppm Ca^{2+} was made. This level of Ca^{2+} is typical (10) for serum. Results show iron capacities of 0.57 and 0.20 μmol/g for resin **1** and **2**, respectively. The apparent increase in capacity may be the result of a change in ionic strength of the solution affecting resin swelling (5). Calcium capacities are 0.062 and 0.072 μmol/g for resins **1** and **2**, respectively.

An untemplated polymer resin with 3 mol% vinylbenzoic acid when loaded with the citrate-complexed Fe^{3+}/100 ppm Ca^{2+} solution showed a Fe^{3+} capacity of 0.18 μmol/g and a Ca^{2+} capacity of 0.11 μmol/g. It is apparent that iron is selectively sequestered even in the presence of very high levels of Ca^{2+}. Imprinting offers some advantage in selectivity for Fe^{3+} ions. These results are summarized in Table 4. The selectivity for Ca^{2+} differs significantly from the test above due to citrate complexation, a different pH, and the much higher concentration of Ca^{2+} with respect to Fe^{3+}. Still, the resin remains selective to Fe^{3+}.

CONCLUSIONS

The method of molecular imprinting shows promise as an approach to make sequestering agents for metal ions of medical and environmental importance. The imprint process is shown to significantly enhance metal ion selectivity. The use of coordinators with better innate complexation constants should make resins with much better selectivity. We are currently preparing other vinyl substituted coordinators.

ACKNOWLEDGMENT

This work was supported by the U.S. Department of Energy through grant number DE-FG07-97ER14823.

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