LAUR-01-5415

Final Report

U. S. Department of Energy

SYNTHESIS OF NEW WATER-SOLUBLE METAL-BINDING POLYMERS: COMBINATORIAL CHEMISTRY APPROACH

Principle Investigator:
Barbara F. Smith
Los Alamos National Laboratory
Los Alamos, NM 87544
Phone: 505-667-2391
e-mail: bfsmith@lanl.gov

Co-Investigators:
Name: Drs. R. Bryan Miller* and Mark Kurth
Institution: University of California,
Davis, CA;
Phone: 916-752-0963,
e-mail: mjkurth@ucdavis.edu
*deceased

Drs. Samuel Sawan and Sonny Ekhorutomwen
University of Massachusetts
Lowell, MA
978-934-3680
Samuel_Sawan@uml.edu

Drs. George Havrilla and Lisa Colletti
Los Alamos National Laboratory
Los Alamos, NM 87544
Phone: 505-667-9627
e-mail:havrilla@lanl.gov

Students: Chris Halm (UCD), Darin Kizer (UCD), Rob Sammelson (UCD), Wenjing Zhou (UCD), Christopher Lubeck (LANL), Everett Neal (LANL), Cecilia Olivares (LANL), Yen Truong (LANL)

Project Number: [54724]

Project Duration: 1997 to 2000

September 2001

TABLE OF CONTENTS

EXECUTIVE SUMMARY 3
Publications 5

BACKGROUND 7
Enabling Science 7
Combinatorial Chemistry 8

TECHNICAL DESCRIPTION OF WORK 11
Synthesis Methods Development 11
1,2-Amino Alcohols Synthesis Development 11
Polyisoxazole Synthesis Development 13
Pyrazolones and Acylpyrazolones Synthesis Development 17
Pendant-Heteroatoms Synthetic Development 22

Ultrafiltration Synthesis Reactor 26

Rapid Polymer Assaying Techniques 30
Micro x-ray fluorescence spectroscopy 30
Radiochemical Survey 32

Polymer Characterization 35

ACKNOWLEDGEMENT 49

REFERENCES 49

EXECUTIVE SUMMARY

The overall objectives of the project were: (1) to develop rapid generation, assay, and characterization approaches to new water-soluble metal-binding polymers for use in Polymer Filtration (PF) systems and (2) to evaluate the concept of using water and organic soluble polymers as new "solid" supports for combinatorial synthesis. The ultimate goal was to rapidly develop new polymers for Polymer Filtration technology to address DOE separations' needs in such areas as ground-water treatment, advanced processing, waste water treatment, decontamination and decommissioning activities, and pollution prevention.

Polymer Filtration (PF), which uses water-soluble metal-binding polymers to sequester metal ions in dilute solutions followed by ultrafiltration to separate and concentrate the polymer-metal complexes, is an emerging technology to selectively remove and/or recover dilute hazardous and valuable metal ions from a variety of aqueous process and waste streams. Future directions in Polymer Filtration must include the rapid development, testing, and characterization of new water-soluble metal-binding polymers. Thus, we adapted and built upon the combinatorial chemistry approach developed for rapid molecule generation used in the drug industry for the rapid development of new chelating polymers. Commensurate with the development of new water-soluble metal-binding polymers is the necessity for the development of rapid metal-binding assay techniques and soluble polymer characterization capabilities.

We focused on four areas of development: (1) synthetic procedures, (2) small ultrafiltration equipment compatible with organic-and aqueous-based combinatorial synthesis, (3) rapid metal-assay techniques, and (4) polymer characterization techniques.

(1) Synthetic Methods Development: Several classes of materials were pursued and developed, which included: acylpyrazolones, polyisoxazoles, 1,2-aminoalcohols, and pendant-heteroatoms. We focused our efforts on obtaining both polymers and small molecules that could be incorporated into the polymers. The polyisoxazole syntheses utilized the 1,3-dipolar cycloaddition of nitrile oxides with alkynes as the dipolarophile. The hope was that these isoxazoles, along with other electron donating atoms, would have favorable metal binding properties. Our goal was to synthesize a 3,3'-diisoxazole and then tether it with other heterocyles to form tetradentate ligands. Our studies focused on the construction of pyrazoloisoxazol-(in)e-based heterocycle, utilizing the intramolecular cycloaddition of 1,3-dipole, which in turn generated dipolarophile "tethered" pyrazolo aldehydes. In the acylpyrazolones, we prepared over 40 derivatives having variation mainly in the side chain at C-4. A variety of heterocycles having multidentate ligands, such as thiophene, furan, and pyridine, were prepared through polymerization. Not all the materials were tested for a variety of reasons including poor water solubility or insufficient material. The series of polymers that were tested for metal binding mostly included a series of functional groups attached to polyethylenimine as they had superior solubility under a variety of extraction conditions.

(2) Small Ultrafiltration Equipment: One of the goals of the project was to demonstrate that water-soluble polymers could be used as soluble "solid" supports for the synthesis of the metal-binding polymers in both organic and aqueous solvents. We assembled a synthesis unit

based on reverse dead-end ultrafiltration. This ultrafiltration mode was faster than dead-end ultrafiltration, as a gel layer from concentration polarization was not produced on the membrane surface. Centrifugal force was used as the pressure source to drive the liquid through the membrane. The ultrafiltration unit was constructed of a polypropylene housing with a polysulfone membrane. The synthesis was performed in separate glass tubes using a parallel synthesis approach. When the reaction was complete ultrafiltration units were placed in the reaction tubes and the solutions ultrafiltered simultaneously using centrifugation to force permeate through the UF membrane. Excess reagents and byproducts were readily washed from the soluble polymeric product using a diafiltration process, thus successfully demonstrating the approach of using soluble "solid" supports in the combinatorial synthesis.

(3) Rapid Polymer-Metal Complex Assaying Techniques: In the process of rapidly generating large numbers of polymeric materials, it was necessary to develop rapid metal-binding assaying procedures for the developed materials. We focused on two approaches described below.

Micro-X-ray fluorescence (MXRMF) Survey: X-ray fluorescence (XRF) is considered a robust and mature analytical technology usually regarded as a bulk analytical method with limited sensitivity and requiring several grams of sample for analysis. In the past, this had typically restricted application of XRF to large sample masses and to concentrations well above the parts-per-million level. That sub-ppm detection limits could be achieved on macro-instrumentation using a dried spot methodology was shown in 1991. Dried spot methodology allows the direct analysis of radioactive samples using energy dispersive micro-X-ray fluorescence. Matching the excitation area of the X-ray spot to the dried sample spot minimizes much of the scatter background and increases sensitivity. Detection limits demonstrated were sub-ppm in the liquid and approached less than 1 ng of material in the dried spot. We optimized the spot drying technique in the presence of the water-soluble polymer, addressed background noise issues, optimized thickness and spot-size, evaluated Mylar-based materials, improved sensitivity, and performed multi-element analysis. Better sensitivity was realized with lower polymer concentration. We successfully detected 10 ppb concentrations of metal ions in an aqueous test solution containing five metal ions. Further improvements with apertures on both the X-ray tube and the detector can increase the method sensitivity if necessary. Analysis of metal ions was, thus far, qualitative, but could be semiquantified by peak height/area analysis, but this was sufficient for rapid survey of metal binding by the developed water-soluble polymers.

Radiochemical Survey: A rapid survey technique using direct radiochemical counting techniques of the ultrafiltration units was developed. We adapted commercially available, centrifugation driven, ultrafiltration units developed for protein purification to a rapid survey technique of the water-soluble metal-binding polymers under various metal-binding conditions. A dead-end ultrafiltration unit having sample volumes from 0.5 to 2 mL were used. The membranes typically had a molecular weight cut off (MWCO) of 10,000 to 100,000 Daltons and were composed of a variety of materials such as cellulose acetate, polysulfone, and fluoropolymers. The UF units were separated into their two halves and each half was directly counted after placing them in scintillation vials with the appropriate scintillation solution.

Permeate samples could also be directly analyzed using other analytical instrumentation such as ion chromatography, inductively coupled plasma spectroscopy, and atomic absorption. Attempts at using autoradiography as an alternative approach proved to be too slow and insensitive.

(4) Polymer Characterization: The weight average molecular weights, as well as other characteristics such as the second virial coefficients and root-mean-square (RMS) radii of gyration of polyamines and various derivatives, have been determined in solution light scattering studies on selected polymers. The solution dynamics of polyamines and carboxylated and phosphorylated derivatives were studied at pH 3.3, 7.0 and 10.0. Measurements were made in freshly distilled and de-ionized water as well as in 0.1 M, 1 M and 5-M solutions of sodium chloride in water. Molecular weights were calculated from Berry plots. The concentration of sodium chloride leads to an increase in the root mean square radius of gyration, as the polymer chains become more extended and relaxed in a more concentrated salt medium. The apparent molecular weights were observed to decrease in proceeding from acidic to alkaline medium, especially with the phosphorylated polymer. However, the RMS radius of gyration of the carboxyl modified polymer was high at both ends of the pH scale, i.e., the polymer chains were extended at both the acidic and alkaline pH ends. The increase in RMS radius of gyration was indicative of increasing solvent-polymer interaction.

In summary, we have developed a number of synthetic modes to new water-soluble metal-binding polymers and prepared a number of new polymers using combinatorial chemistry approach. These new polymers have potential application to metal-ion separations needs of DOE. Two new approaches to rapid assay of the metal-binding capability of the new polymers were developed, along with developing polymer characterization techniques. Information was transmitted to the public to aid in technology transfer through a number of reports, patent disclosures, and by published papers as summarized below. Further publications are in progress along with testing of new materials for specified applications.

Publications

B. F. Smith, T. W. Robison and G. D. Jarvinen, "Water-Soluble Metal-Binding Polymers with Ultrafiltration: A Technology for the Removal, Concentration, and Recovery of Metal Ions from Aqueous Streams", in the ACS Symposium Series 716, Metal-Ion Separation and Preconcentration, R. Rogers, A. Bond and M. Dietz, Eds, Chapt. 20, pp.294-330, **1999**.

Lisa Colletti, George Havrilla, "Trace Element Detection with Micro-x-Ray Fluorescence", Advances in X-Ray Analysis, Vol 44, **1999**.

Darin E. Kizer, R. Bryan Miller, and Mark J. Kurth. "Fused Pyrazolo Heterocycles: Intramolecular [3+2]-Nitrile Oxide Cycloadditions Applied to Syntheses of Pyrazolo[3,4-g][2,1]dihydrobenzoisoxazol(in)es." Tetrahedron Letters, 40: 3535-38, **1999**.

Robert E. Sammelson, R. Bryan Miller, and Mark J. Kurth. "The Synthesis of Tetraheterocyclic Polyisoxazole Chelating Ligands Using the Intermolecular 1,3-Dipolar Cycladdition Reaction." Journal of Organic Chemistry, 65, 2225-8, **2000**.

Sammelson RE, Miller RB, Kurth MJ, "The synthesis of tetraheterocyclic, polyisoxazole, chelating ligands using the intermolecular 1,3-dipolar cycloaddition", ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY, v. 217(pt.2) pp. 415-ORGN MAR 21, **1999**

In-Prep: Robert E. Sammelson, R. Bryan Miller, and Mark J. Kurth. The Synthesis of Tetraheterocyclic Polyisoxazole Chelating Ligands Using the Intermolecular 1,3-Dipolar Cycladdition Reaction. Journal of Organic Chemistry.

Sonny A. EKHORUTOMWEN, Gurusamy MANIVANNAN, Samuel P. SAWAN, Barbara F. SMITH, Thomas W. ROBISON, Kennard V. WILSON, "Water-soluble Metal Binding Polymers: Light Scattering and ^{113}Cd NMR spectroscopic Investigation"

BACKGROUND

Enabling Science

A difficult problem is the development of an efficient, inexpensive, and selective process that can reach the very low aqueous metal-ion levels required by the U.S. Department of Energy (DOE), Environmental Protection Agency (EPA) and other state and federal agencies. The diversity of target metals, the large variation in waste stream types (acidic, basic, salt, etc.) and the high potential for interfering complexing ions --both organic and inorganic-- requires that a tool chest of new, inexpensive, highly selective, high-binding, reversible metal-binding materials and processes be developed for metal-ion concentration and removal from aqueous solutions.

The interdisciplinary, interdivisional Metal Separations Research and Development team at Los Alamos National Laboratory has been developing metal separations technologies for a variety of applications important to both the DOE and the U. S. industrial complex. One of the technologies under development is Polymer Filtration, a technology to selectively remove or recover hazardous and valuable metal ions and radionuclides from various dilute aqueous streams. Not only can this technology be used to remediate contaminated soils, solid surfaces and treat aqueous wastes, it can also be incorporated into facilities as a pollution prevention and waste minimization technology.[1-3] From the past successes of Polymer Filtration in application to the electroplating industry, it was felt that with the appropriate water-soluble chelating polymers many dilute-solution separations could be affected both efficiently and inexpensively using Polymer Filtration technology.[4]

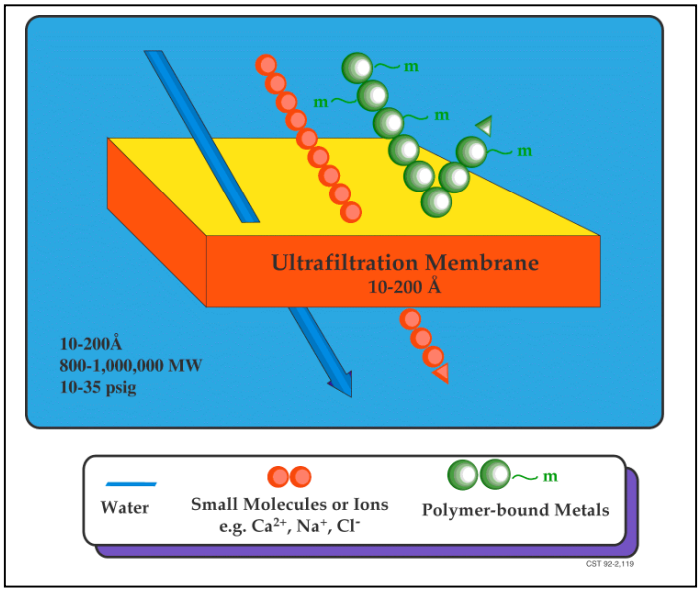


Figure 1. Cartoon of how Polymer Filtration functions.

Polymer Filtration uses water-soluble metal-binding polymers to sequester metal ions in dilute solution.[5,6] The water-soluble polymers have a sufficiently large molecular size that they can be separated and concentrated using commercially available ultrafiltration technology. Water, small organic molecules, and unbound metals pass freely through the ultrafiltration membrane while concentrating the metal-binding polymer as shown in Figure 1. The polymers

are regenerated by changing the solution conditions to release the metal ions. The metal-ions are recovered in concentrated form for recycle or disposal using a diafiltration process. The water-soluble polymer can be recycled for further aqueous-stream processing. Some of the advantages of this approach over other treatment technologies such as ion exchange, reverse osmosis, precipitation, liquid-liquid extraction, and/or evaporation include: (1) high metal-ion selectivity and capacity (3-8 times greater than ion exchange resins); (2) rapid metal-binding/release kinetics; (3) room temperature and low-pressure operation; (4) all aqueous-based processes; (5) no sludge formation/reduced reagent usage; and (6) small footprint. These advantages and others translate into rapid, low-energy processing, low waste volume, and reduced capital and process costs.

Though the concept of using water-soluble metal-binding polymers for metal ion recovery and concentration from aqueous solutions has been in the literature for about 30 years, it has never before been applied on an industrial scale or utilized as an engineered unit, even though ultrafiltration alone is commonly used in industrial operations.[6,7] The LANL Separations Team was able to develop Polymer Filtration technology (4 US patents and a number of foreign patents) for real-world applications.[1,2,4,5,8-10] The team received a 1995 R&D 100 award for developing and demonstrating a pilot-scale system for application to the electroplating industry. An international review panel selected Polymer Filtration as one of the top 100 technologies that became commercially available in 1995. From this experience we have developed a good understanding of the fundamental research needs to further this technology. The understanding of Polymer Filtration Technology is at the level of development that resin-based ion exchange technology was over 30 years ago.

To advance Polymer Filtration technology to the selectivity levels required for DOE needs, future directions in Polymer Filtration must include rapid development, testing, and characterization of new metal-binding polymers. The development of new chelating molecules can be equated to the process of new drugs or new materials discovery. Thus, we have built upon and adapted the combinatorial chemistry approaches developed for rapid molecule generation for the drug industry to the rapid development of new water-soluble metal-binding polymers.

Combinatorial Chemistry

The chelating polymers are key to the success of Polymer Filtration technology. The traditional approach to chelator development involves the laborious one-at-a-time process of repetitive synthesis, modification, and testing. This same approach had been used in the drug industry for years. A new strategy called combinatorial chemistry has been shown to enhance the rate of the discovery process. The medicinal chemistry community has been captivated by the revolutionizing potential of combinatorial chemistry for drug discovery.[11,12] In the same manner that diversity can be built into small molecules for pharmaceuticals [13-19], molecules with selective metal binding can also be discovered.[20]

Combinatorial chemistry allows the simultaneous evaluation of a large number of chemical variants, tests them for binding with target metal ions, and then isolates and identifies the most promising compounds for further development. The combinatorial chemistry approach does not change the necessity for screening large numbers of materials for metal-ion specificity, but allows a greater range of molecular diversity to be tested. The molecular diversity represents

not only the ability to address the large number of different metals ions that exist, but also the ability to optimize the selectivity, kinetics, and thermodynamics of metal-ion binding.

The basic strategy is to assemble many possible combinations of a given set of chemical building blocks (BB) that make up the main features of the chelator, while simultaneously recording the order of use. The resulting molecules are then assayed all at once, and reference to the record allows determination of the identity of the compounds that look most promising. Since the introduction of the concept of combinatorial synthesis, there has been a multitude of variations on the approach. The earliest and most successful so far has been the peptides that are based on sequential attachment of amino acids, as a considerable amount of chemistry had been previously developed for solid-phase peptide synthesis.[21-27] Since 1991, there has been a big thrust in small, stable molecule synthesis in the 500 molecular weight range of the natural product type.[13,28-33]

Libraries of new molecules are built by varying the reagent substituents. If three building blocks or reagents are required to make a molecular structure and each building block has three variants that will give a library of nine compounds. If there are five variants then 15 molecular structures are produced. It is easy to see that the number of diverse molecules can grow exponentially and why amino acid chemistry was its first success. With the large number of commercially available amino acids the number of combinations appears almost limitless.

There are two basic approaches to building libraries of compounds: the parallel approach and the split synthesis approach as shown in Figure 2.[34] In the split synthesis approach, compounds are assembled on resin beads. In each step, beads from previous steps are separated into several groups and a new building block is added. The different groups of beads are recombined and separated again to form new groups. This step is repeated until the combinatorial library is assembled. In Parallel synthesis approach, compounds are synthesized in separate vessels without mixing the beads. There are advantages and disadvantages to each approach. Larger libraries of small amounts of material can result from split synthesis. Larger amounts of pure materials, but of smaller libraries, can be prepared in the parallel approach.

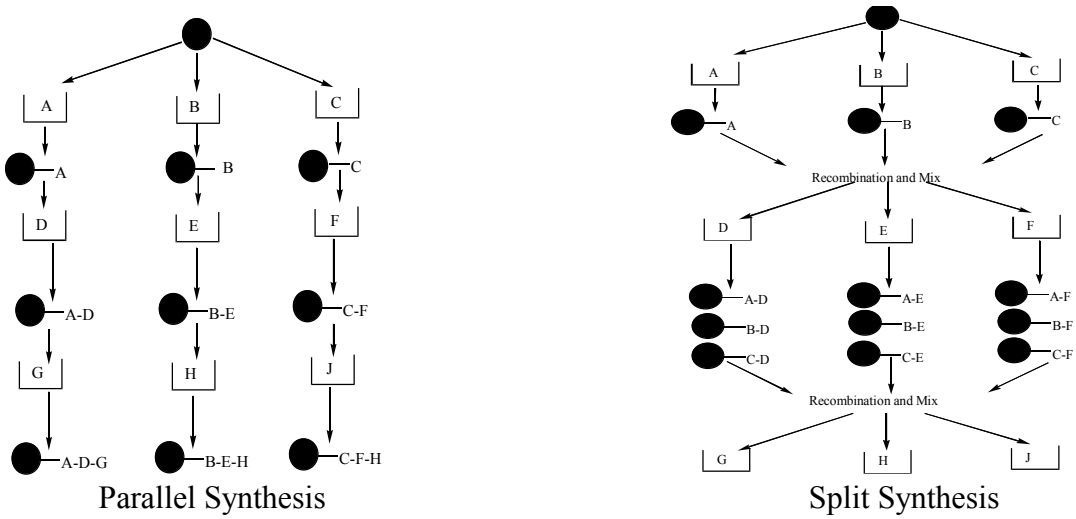


Figure 2: Schematic of Slit and Parallel synthesis method.[12,35]

Solid phase synthesis is a mainstay of both the approaches. The solid phases can be resin beads such as Merrifield's resin, solid polymeric films, ceramics, or silica-based materials. Solid-phase synthesis is necessary for rapid and complete purification of the intermediates and for the ability to use excess reagents to force the reactions to completion. The drawback of the solid-phase synthesis is that of the thousands of organic synthetic reactions developed over the years, only approximately 150 have been adapted to work under solid-phase conditions.[12] Two-phase reactions are difficult if not impossible with resin-based syntheses. If solids are formed as reaction byproducts, they too are difficult to separate from resins. A considerable amount of research effort is currently being spent in the direction of adapting reactions to solid supports.

Researchers have been attaching chelators to resins for years with the purpose of using them for chelating ion exchange resins.[36] Thus, the concept of attaching chelators with stable linkers is quite common. Likewise, the concept of having chelators attached to water-soluble polymers has been reported in the literature.[6] We have been able to show that there are some advantages to using water-soluble chelating polymers for metal binding relative to chelating ion exchange resins.[4,5] Some of the same advantages that exist for metal binding can also be advantages in using water-soluble polymers as "solid" supports for combinatorial synthesis. These advantages include rapid reaction kinetics, higher capacity, and many solution-based reactions. Solid reagents and resulting byproducts are not a problem when soluble polymers are used in synthesis.

Janda has demonstrated the concept of liquid-phase combinatorial synthesis (LPCS) using a linear homogenous polymer, polyethylene glycol monomethyl ether (MeO-PEG), as a terminal protecting group support.[37] All reactions were solution-phase reactions. Excess reagent could be used to force the reactions to completion, because at the end of the reaction the polymer was precipitated using diethylether, allowing the solid to be washed free of any unreacted reagents. The polymer was redissolved for the second and sequential steps of the combinatorial synthesis. The one drawback of using MeO-PEG is that only one terminal attachment point for the synthesis exists, thus giving low capacity. As different reaction products were produced they had different solubilities and the ether precipitation was less dependable giving variable yields. Soluble polymers with more functionality for attachment can have considerably higher capacity. Several researchers have stated that this new soluble polymer approach can readily lend itself to automated techniques, as sample movement of solutions is much easier and more quantitative than with solids.[35] The main drawback is the compatibility of synthetic reagents with the linking sites.

TECHNICAL DESCRIPTION OF WORK

We focused on four areas of development: (1) synthetic procedures, (2) small ultrafiltration equipment compatible with organic-and aqueous-based combinatorial synthesis, (3) rapid metal assay techniques, and (4) polymer characterization techniques.

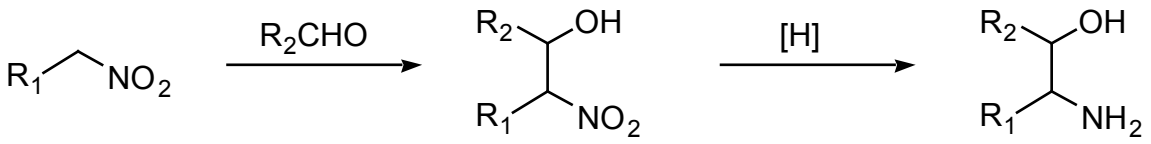
(1) Synthetic Methods Development

Four general classes of ligands were addressed: (1) acylpyrazolones, (2) polyisoxazoles, (3) 1,2-aminoalcohols and (4) pendant-heteroatoms. We synthesized representative examples of these four classes of ligands by solution-phase chemistry in order to master the chemistry of these compounds. In the acylpyrazolones, we prepared over 40 derivatives having variation mainly in the side chain at C-4. One significant finding was that Mitsunobu chemistry allowed good regiochemical control for synthesis of O-alkylated systems. Two types of polyisoxazoles were prepared; one ready for attachment to a water-soluble polymer and another as a monomer for polymerization into a polyacrylamide system. 1,2-aminoalcohols were prepared, but this chemistry did not promise to be adaptable to solid phase organic synthesis (SPOS) and therefore we tried incorporating this motif as a monomer structural feature into other polymer systems. However, the acylpyrazolones and polyisoxazoles were studied for use in SPOS and eventual combinatorial application.

1,2-Amino alcohols Synthesis Development

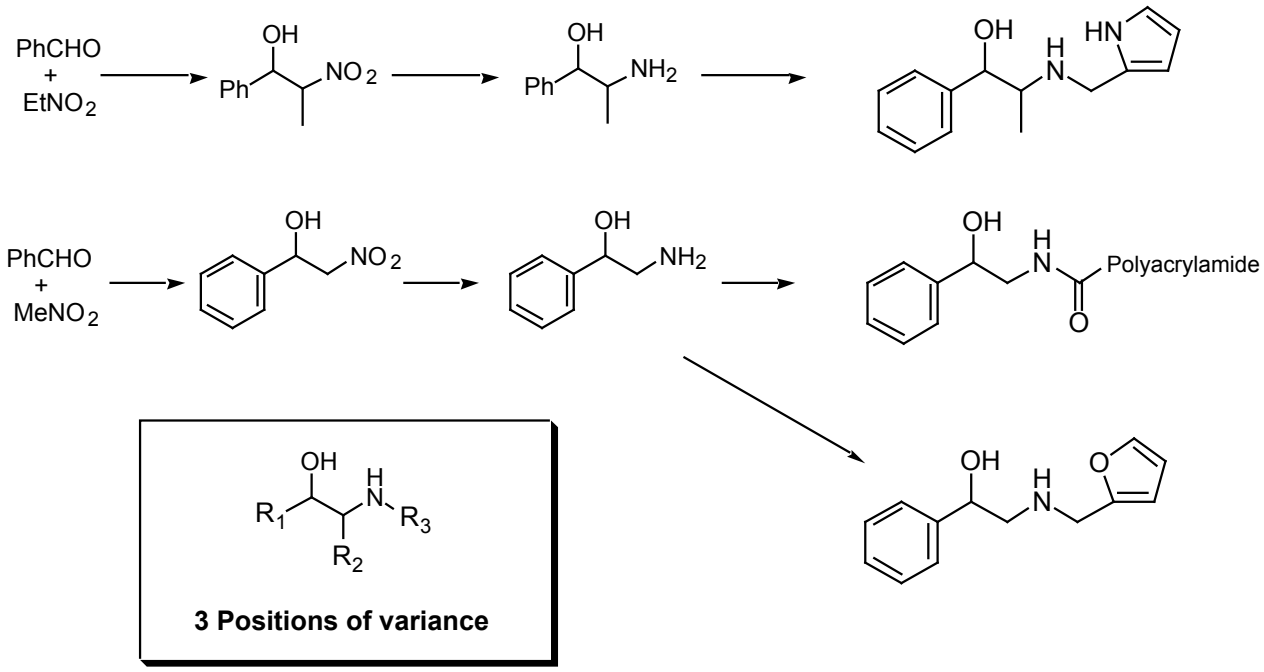
A well-established route to 1,2-amino-alcohols is the nucleophilic opening of epoxides with amines. This key transformation was exploited using a Lewis acid amination of epoxides (Scheme A-1) as well as a variation that gave the opposite regioisomer (see R^1 and R^2).

Two routes were envisioned for the synthesis of 1,2-aminoalcohols. The first utilized a nitro-aldol (Henry) reaction followed by reduction to an amine:



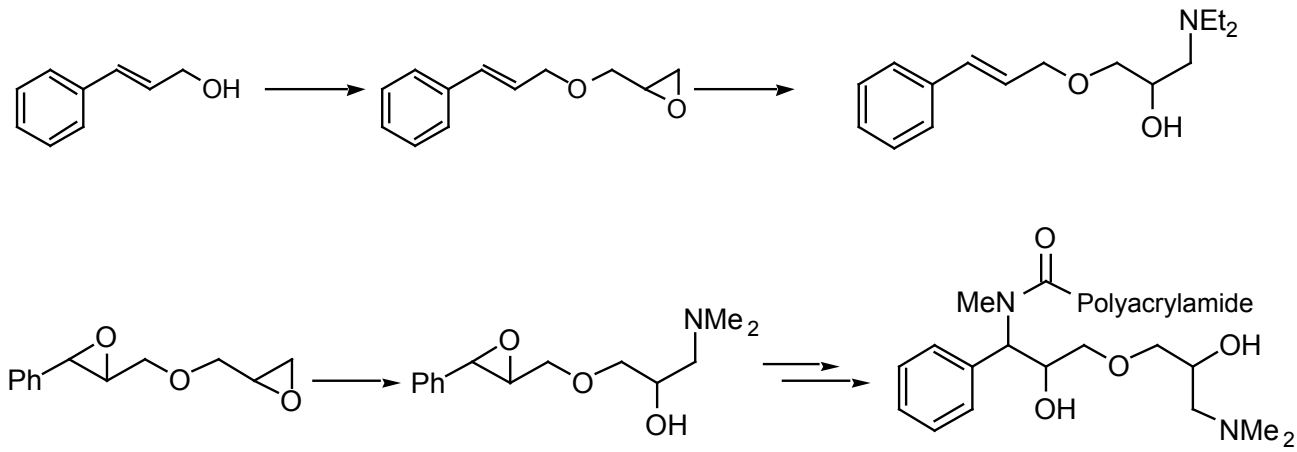
Scheme A-1

Both R_1 and R_2 positions were varied to give a library of amino alcohols using a combinatorial synthetic approach. Three examples of molecules synthesized using this approach are shown below in Scheme A-2. Reductive amination or acrylation at nitrogen gave a third position of diversification.

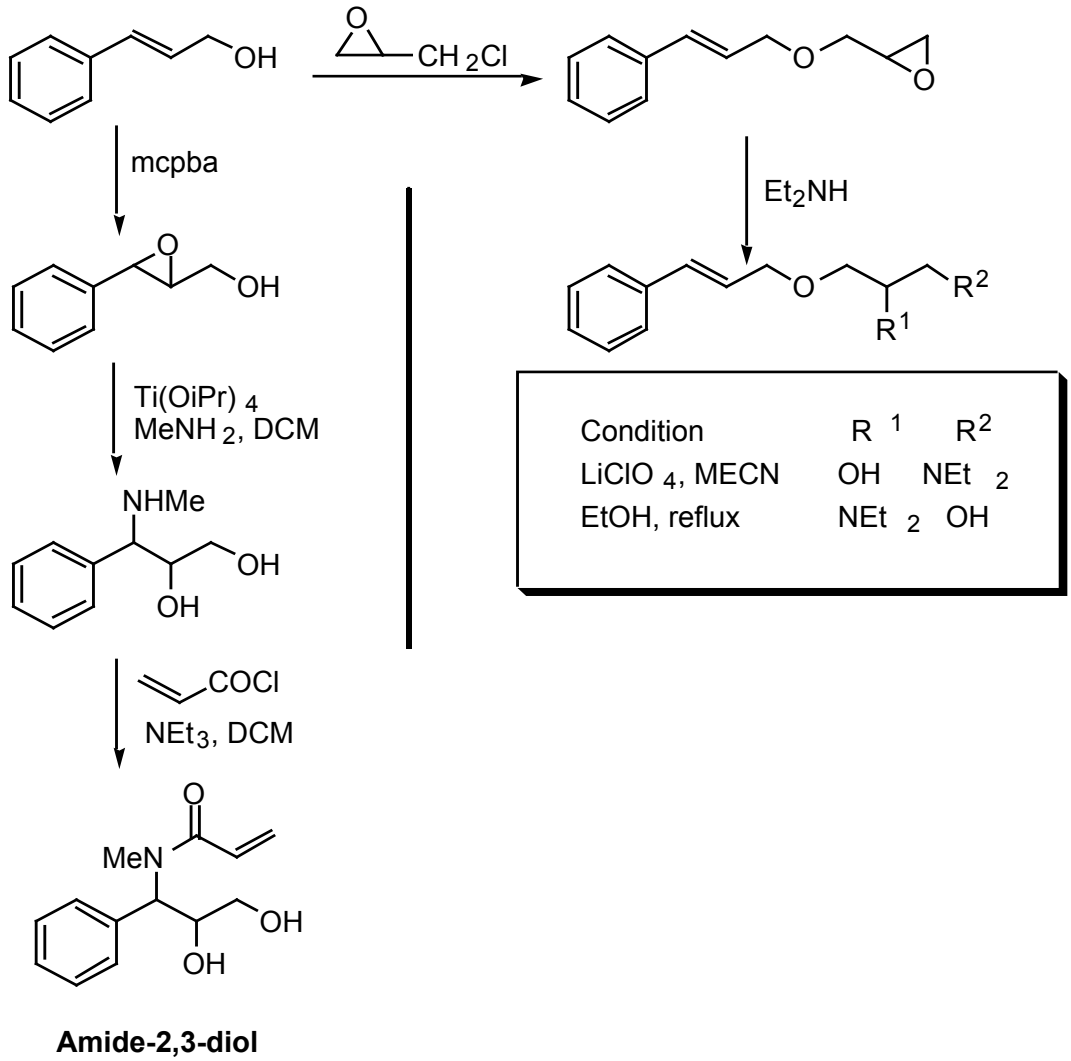


Scheme A-2

The second approach shown in Scheme A-3 utilized a regioselective attack of an epoxide by an amine. Depending on conditions used, either regioisomer can be obtained. Using lithium perchlorate as a Lewis acid, primary and secondary amines have been shown to attack an epoxide with almost complete regioselectivity. In refluxing ethanol without Lewis acid assistance, the opposite regioisomer was the major product in very good yield. Regioselectivity depended on epoxide substitution and the conditions employed.[38] Solution-phase 1,2-amino alcohols can now be synthesized easily from key intermediate epoxides. In this way, different amines can be reacted with one key polymer intermediate to generate many different amino alcohol ligands for a combinatorial approach to ligand design as shown in Scheme A-4.



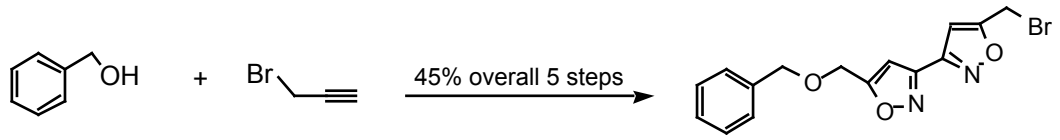
Scheme A-3



Scheme A-4

Polyisoxazole Synthesis Development

This task utilized the 1,3-dipolar cycloaddition of nitrile oxides with alkynes as the dipolarophile to make polyisoxazoles. These isoxazoles along with other electron donating atoms should have favorable metal binding properties. Our goal was to synthesize a 3,3'-diisoxazole and then tether it with other heterocyles to form tetradentate ligands. The first step in this task was the synthesis of the 3,3'-diisoxazole (Scheme B-1), which would allow for a 5-member ring metalocycle.

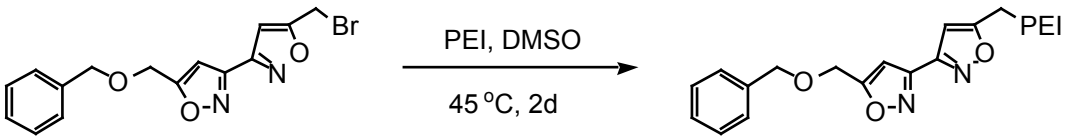


1. KH, THF; 2. $O_2NCH_2CO_2CH_3$, p-$OCNC_6H_4NCO$, Et_3N; 3. Dibal-H, CH_2Cl_2, -78°C; 4. NH_3OHCl, NaOAc, EtOH, H_2o; 5. Propargyl bromide, NaOCl, Et_3N

Scheme B-1

The first 1,3-dipolar cycloaddition used a modification of Mukaiyama's procedure where 1,4-phenylene diisocyanate and triethylamine form the nitrile oxide in situ from methylnitroacetate. The second 1,3-dipolar cycloaddition used bleach and triethylamine to form the nitrile oxide through in situ preparation of the conjugate base of the corresponding hydroxamoyl chloride, which was then dehalogenated. It was these two cycloaddition reactions where the overall yield was compromised. Both of these reactions' yields were in the upper 70's.

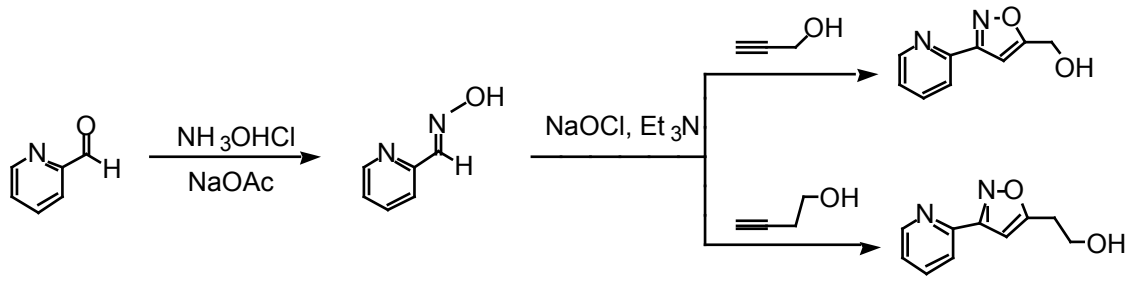
Next, we attached our diisoxazole, using the primary halide functionality, to the base polymer. This polyethylenimine or PEI is a water-soluble polymer and had a cutoff MW 30,000. The attachment was performed by incubation of the diisoxazole bromide and the polymer in DMSO at 45 ^{o}C for 2 days.



Scheme B-2

After incubation the mixture was purified by dialysis using a cutoff molecular weight of 3,000. The dialysis was against 30% (v/v) ethanol-water (3 times), 10% ethanol-water (2 times), and finally water (3 times). The water was then removed by rotary evaporation and the polymer was then dried in a vacuum oven.

We then synthesized several different nucleophiles, which could displace the diisoxazole bromide. Our nucleophile prospects were primary aliphatic alcohols attached to the 5 position of another isoxazole and different aromatic heterocycle containing oxygen, nitrogen, or sulfur at the 3 position. In each case we started with the commercially available 2-heterocyclic aldehyde, which was converted to the aldoxime using hydroxylamine hydrochloride and sodium acetate. A bleach solution was used to convert the oxime to the corresponding nitrile oxide, which then underwent 1,3-dipolar cycloaddition with either propargyl alcohol or 3-butyn-1-ol (Scheme B-3). The first that we examined was the pyridine heterocycle.

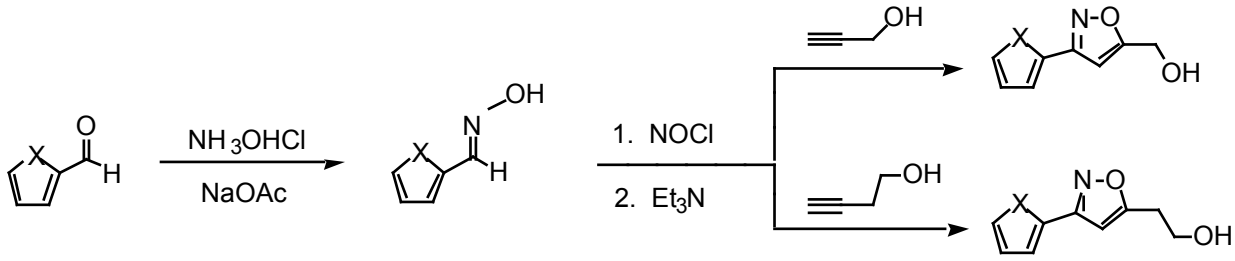


Scheme B-3

We also looked at these structures using the molecular modeling program called Spartan. This program can give us information on how much strain would be present in the geometry of our ligands. Even though it would be ideal if an eight coordinate metal that could bind to two of

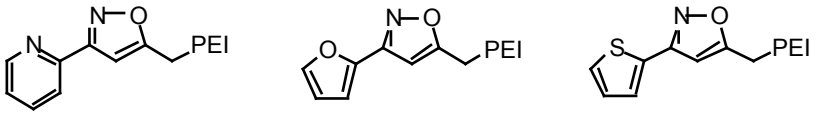
our tetradentate ligands, we could not do calculations for this since the highest coordination number available to us was six. We choose the octahedral structure and used only the ligand along with two water molecules at the remaining sites. The strain was great when our beginning alcohol had one methylene group. Adding a second methylene group brought down the strain considerably. On experimenting with longer chains we found that three methylene groups were still somewhat better than two.

Next we went on to examine the furan and thiophene heterocycles using the same methods. All products were isolated and yields for the cycloaddition step were good for pyridine derivatives but low (10-25%) for the furan and thiophene derivatives. One possibility was that the bleach was giving us chlorination on the furan or thiophene instead of converting the oxime to the conjugate base of the hydroxamoyl chloride. This side product has been found when NCS was used instead of bleach. Another possibility was that the bleach caused polymerization of the nitrile oxide. So we next tested other synthetic methods for oxime to nitrile oxide conversion. Finally, we chose nitrosyl chloride to form the hydroxamoyl chloride followed by slow addition of base to produce nitrile oxide (Scheme B-4).



Scheme R-4

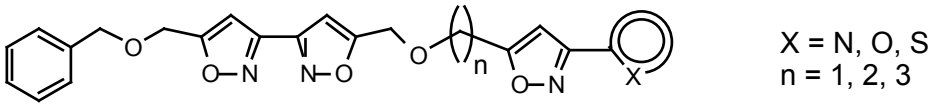
Next we made triisoxazoles tetradentate ligands, but first we made the same heterocyclic isoxazoles but used propargyl bromide in the place of the alkynols. This functionality then allowed for the attachment of the ligands to the water-soluble polymer (Scheme B-5). The same procedure was followed as before.



Scheme R-5

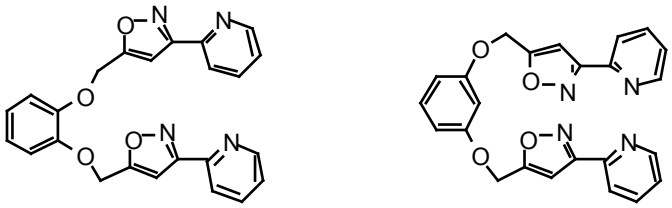
After attachment of these to the PEI, any impurities not attached were removed by dialysis (CMW 3,000). Removal of water gave the polymer bound ligand. We also examined the solubilities of the ligands attached to the PEI polymer. The pyridine-isoxazole, and furan-isoxazole were slightly soluble in neutral and acidic aqueous solution. The thiophene-isoxazole was sparingly soluble as a turbid mixture at neutral pH. Allowing the turbid solution to sit for two weeks did not allow the solution to become clear and did not allow any of the solid to settle out to the bottom. The diisoxazole did not show any significant solubility at neutral or acidic conditions. This is probably due to the benzyl ether attached rather than the 3,3'-diisoxazole moiety. All of the solubilities tested were 1 mmol per gram loading.

We attached our diisoxazole to the heteroisoxazoles using the Williamson ether synthesis (Scheme B-6). This lead to our 7 target ligands, that could be tested for metal binding by solvent-solvent extraction.



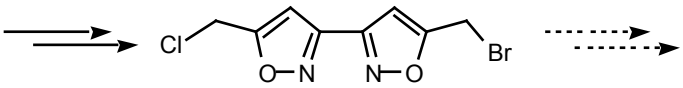
Scheme B-6

The alkylation of resorcinol and catechol was also examined. Two equivalents of 5-methylbromide with 3-(2'pyridyl)isoxazole were used to form a tetradentate chelating ligand for various metals. In these reactions sodium hydride was used as the base and the dianion was formed before addition of the alkyl bromide (Scheme B-7).



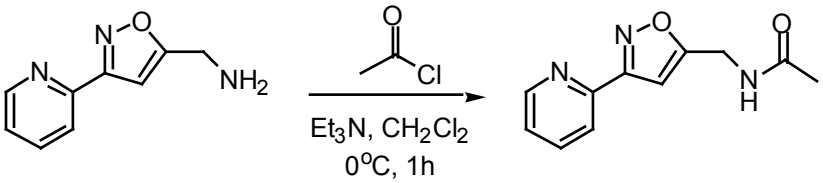
Scheme B-7

We have prepared a diisoxazole that contains two different nucleophilic sites. These two sites contain a primary bromide and a primary chloride. Selective nucleophilic displacement could then follow. Below in Scheme B-8 the synthesis is shown. The future strategy is to first displace the bromide with the heteroisoxazole alcohols, then attach to PEI using the chloride functionality. This could also be adapted to other combinatorial approaches.



Scheme B-8

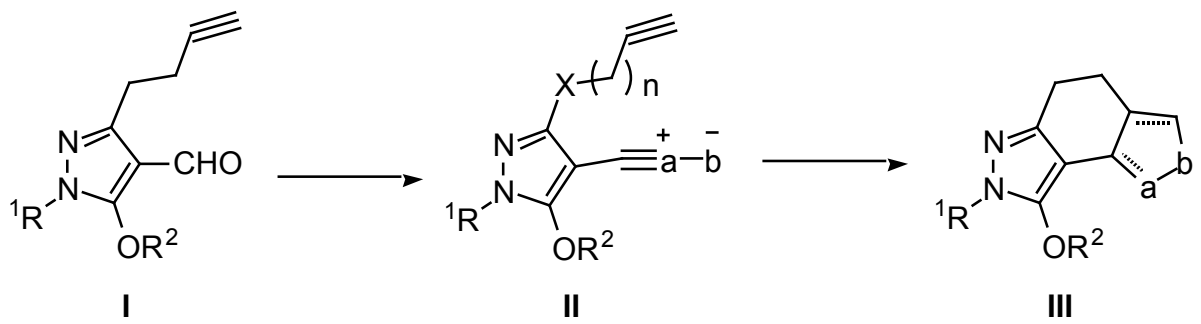
To evaluate the metal-binding effectiveness of these ligands, the following monomeric ligand was prepared as shown in Scheme B-9.



Scheme B-9

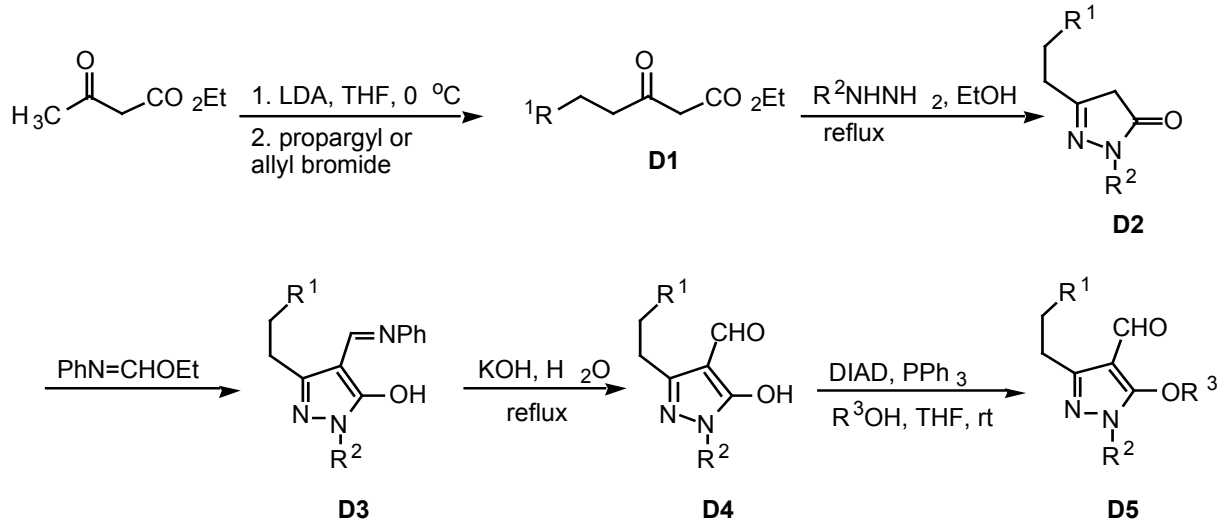
Pyrazolones and Acylpyrazolones Synthesis Development

Our initial studies were on the construction of pyrazoloisoxazol(in)e-based heterocycle III utilizing the intramolecular cycloaddition of 1,3-dipole II, in turn generated from dipolarophile "tethered" pyrazolo aldehyde I (Scheme C-1).



Scheme C-1

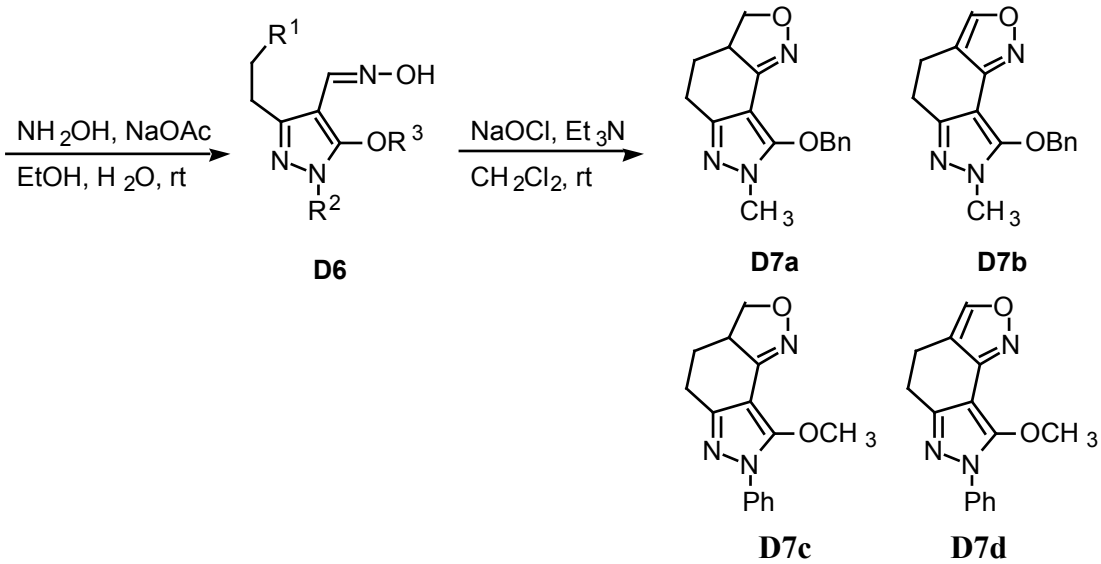
Synthesis of the targeted heterocycles (III) began as depicted in Scheme C-2. Formation of the dianion of ethyl acetoacetate with 2 eq. of LDA at 0°C in THF followed by alkylation (propargyl or allyl bromide) provided β-keto ester D1 (R^1 = -C≡CH or -CH=CH_2) in 62% and 75% yield, respectively. Condensation of D1 with phenyl or methyl hydrazine in refluxing anhydrous ethanol gave the corresponding pyrazolone D (R^1 = -C≡CH or -CH=CH_2; R^2 = -C_6H_5 or -CH_3). Attempts to install a formyl group at C-4 using classic Vilsmeir conditions (DMF, $POCl_3$, 0°to 100°C, 2h) were not successful. Thus, introduction of the formyl group at C-4 was accomplished using a two step procedure involving formation of Schiff base D3 by condensing ethyl N-phenylformimidate with D2 in refluxing toluene. Subsequent hydrolysis of D3 with aqueous potassium hydroxide and neutralization with concentrated HCl provided 4-formyl derivative D4.



Scheme C-2

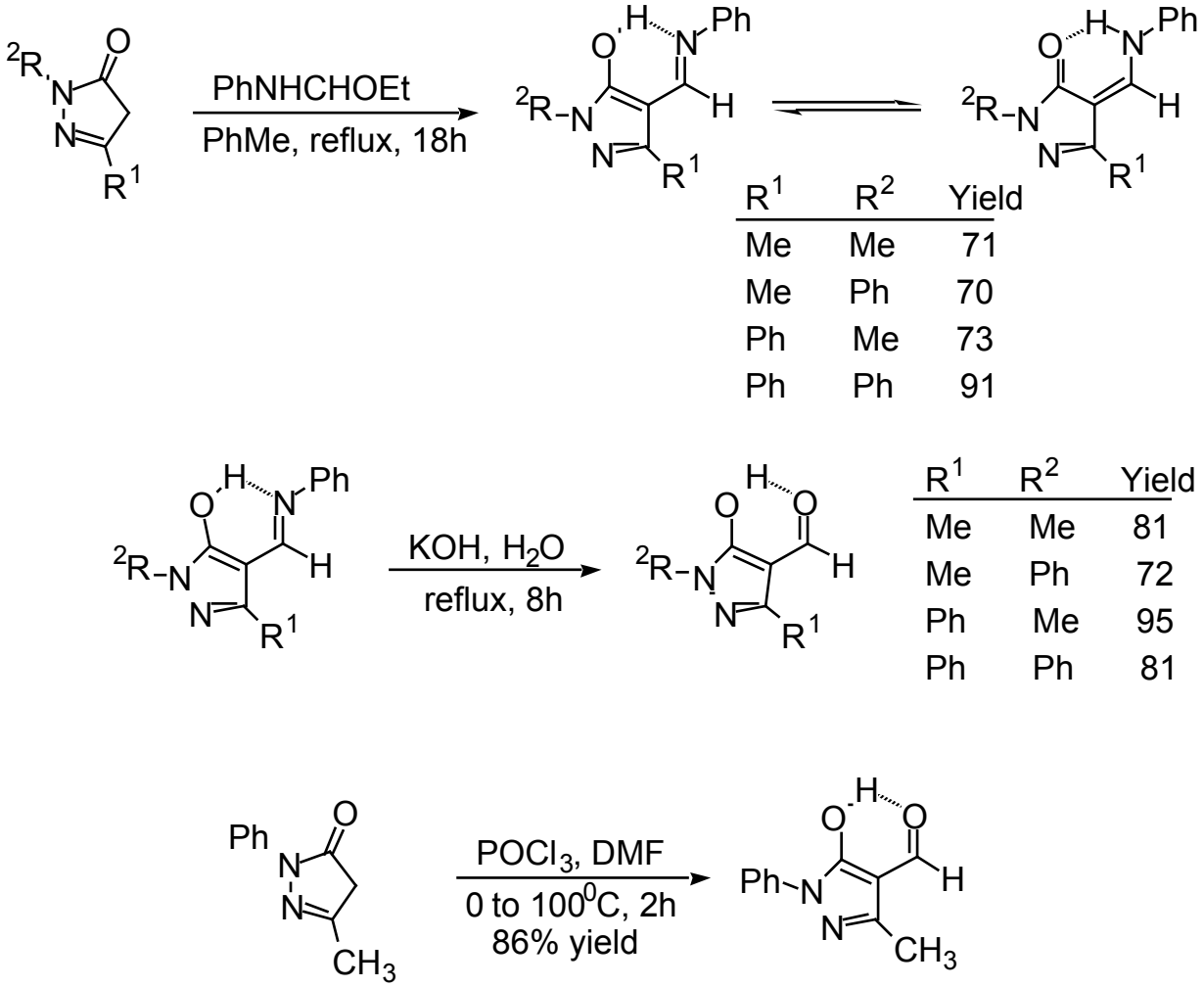
Model experiments revealed that it was necessary to mask the C-5 hydroxyl moiety prior to intramolecular nitrile oxide cycloaddition. Accordingly, D4 was alkylated under Mitsunobu conditions with methyl or benzyl alcohol to give pyrazole D5 (R^1 = -C≡CH or -CH=CH_2; R^2 = -C_6H_5 or -CH_3; R^3 = -CH_3 or -$CH_2C_6H_5$; in 32-40% yield from D4).

Room temperature condensation of aldehyde D5 with hydroxylamine hydrochloride in 95% EtOH containing NaOAc (2.5 eq.) gave oxime D6. Subsequent dropwise addition of aqueous sodium hypochlorite (5.25%) to a solution of this oxime and triethylamine in CH_2Cl_2 at 0^oC generated the nitrile oxide intermediate which underwent concomitant cycloaddition to D7a-d in 65-80% yield (Scheme C-3).



Scheme C-3

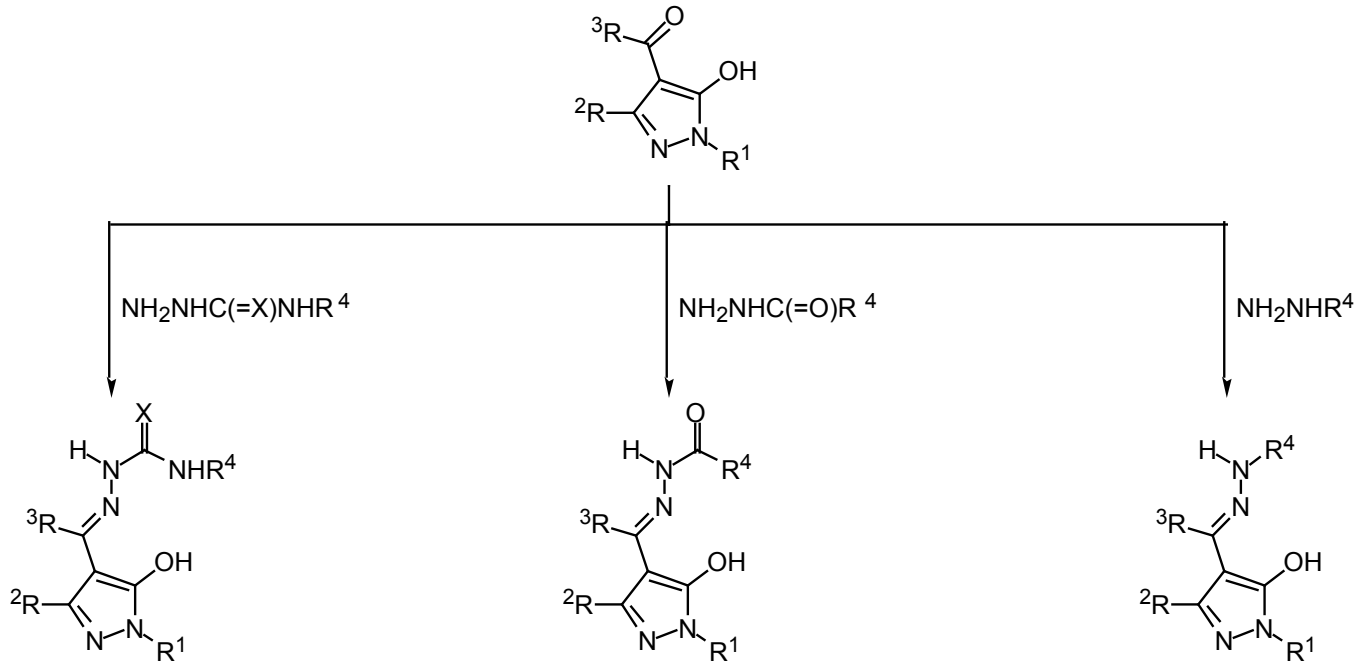
Further efforts in the synthesis of new metal chelating pyrazolones and acylpyrazolones are summarized below in Schemes C-4 and C-5.



R^1	R^2	Yield
Me	Me	71
Me	Ph	70
Ph	Me	73
Ph	Ph	91

R^1	R^2	Yield
Me	Me	81
Me	Ph	72
Ph	Me	95
Ph	Ph	81

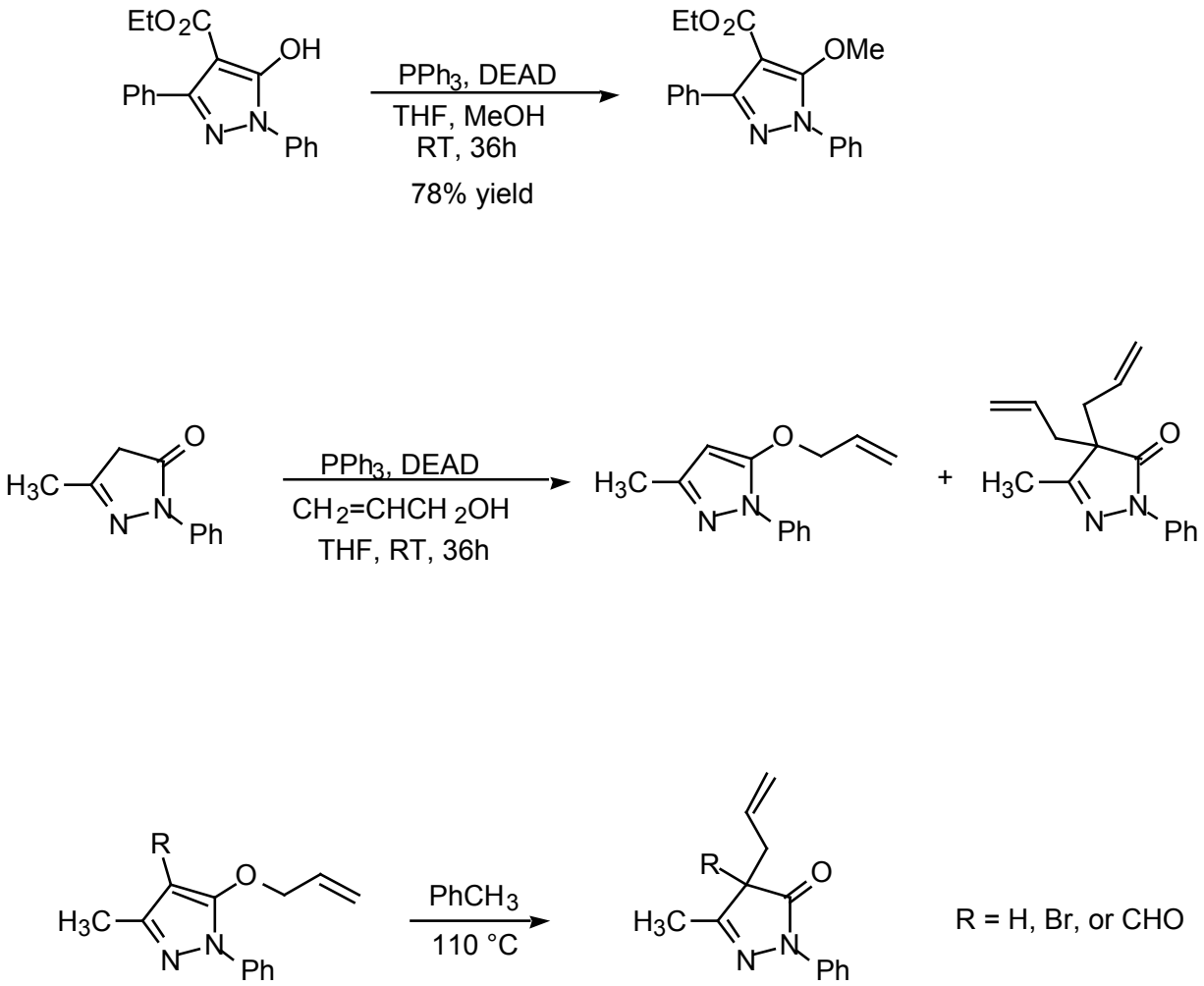
Scheme C-4



"semicarbazides"						"hydrazides"					"hydrazines"				
R^1	R^2	R^3	R^4	X	yield	R^1	R^2	R^3	R^4	yield	R^1	R^2	R^3	R^4	yield
Ph	Me	Me	H	O	76	Ph	Me	Me	Ph	38	Ph	Me	Me	Ph	58
Ph	Me	Me	H	S	77										
Ph	Me	H	H	O	74										
Ph	Me	H	H	S	46										
Me	PhH	H	H	S	45										
Ph	PhH	H	H	S	82										
Ph	PhH	H	H	O	68										
Me	Me	H	H	O	49										
Me	Me	H	H	S	50										

Scheme C-5

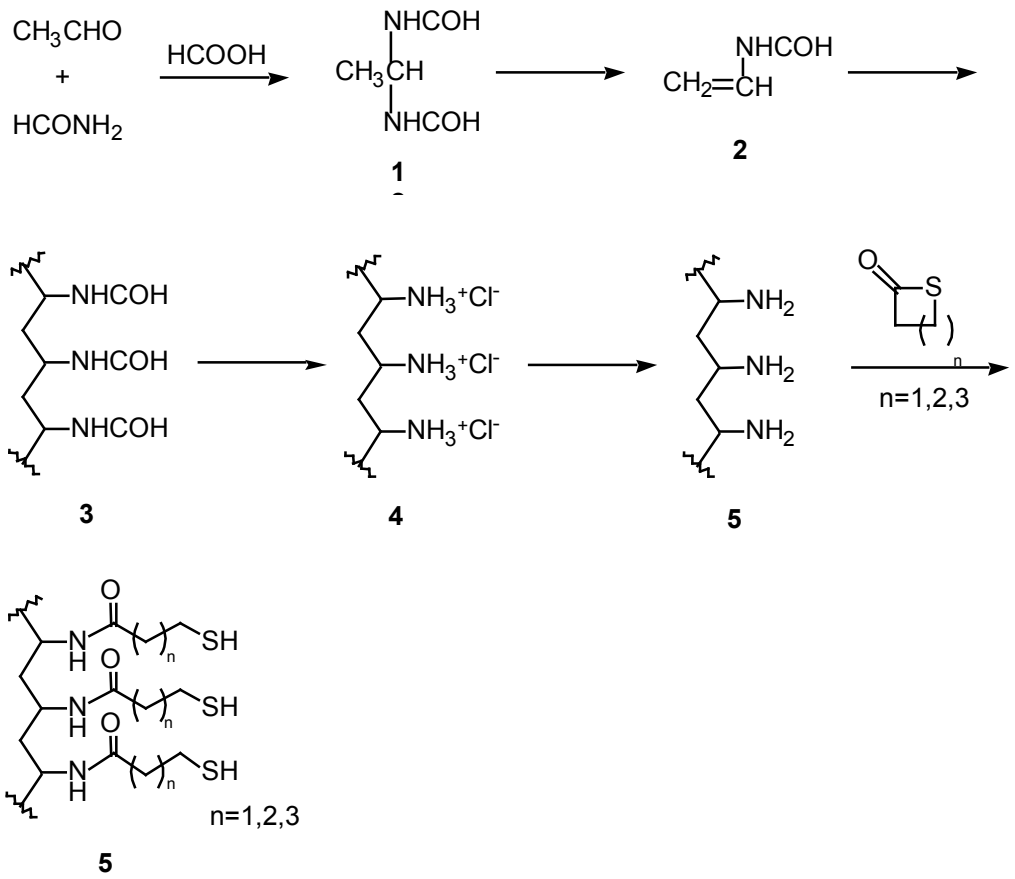
Mitsunobu and Claisen reactions of pyrazolones gave the materials shown in Scheme C-6.



Scheme C-6

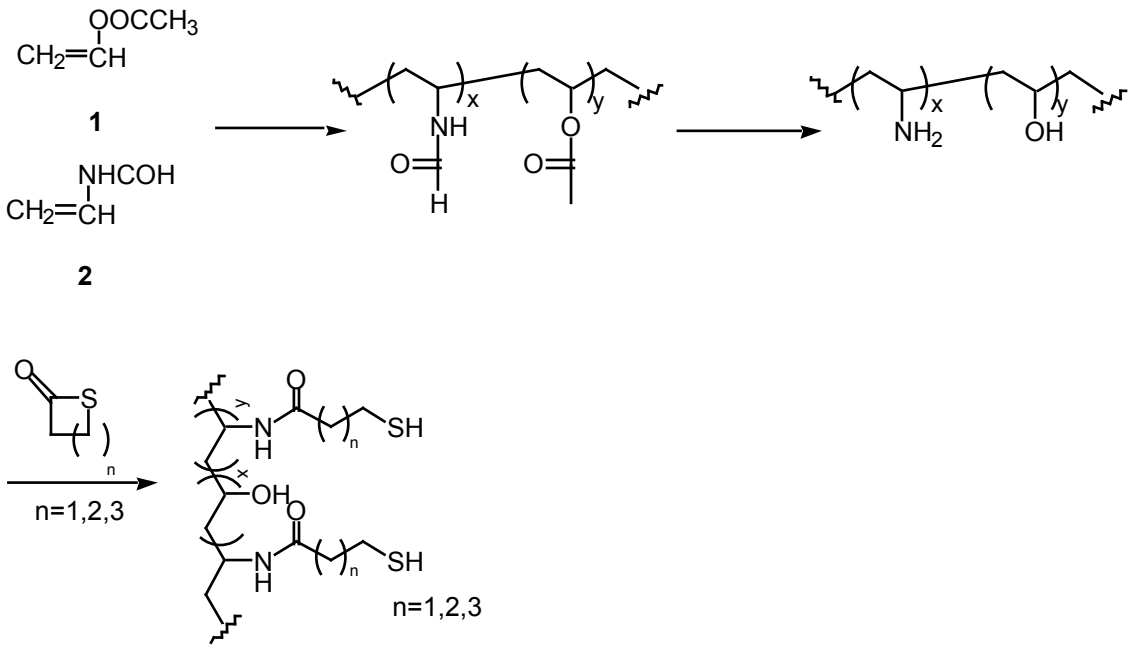
Pendant-Heteroatoms Synthetic Development

Polymer with Pendant Thiols: Since thiols are good nucleophiles, synthesis of water-soluble polymers with pendant thiols would offer polymers, which could be further functionalized to other polymers, was under taken as shown in Scheme D-1. We started from formamide and acetaldehyde to give ethylidenebis(formamide) in 65% yield. Pyrolyzation of ethylidenebis(formamide) gave the desired monomer N-vinylformamide in 89% yield. Polymerization of the monomer (2) yielded homopolymer poly(N-vinylformamide) in good yield. Hydrolysis and purification of the polymer (4) by a strong anionic exchange column gave the polyvinylamine in ca. 58%. The reaction of polyvinylamine with thiolactone in methanol or DMSO did not give the water-soluble polymer with pendant thiol because of the crosslinking of the thiols to make (S-S) linkage. We observed that the isolated polymer, which was initially soluble in DMSO, was no longer soluble in any tested solvents.



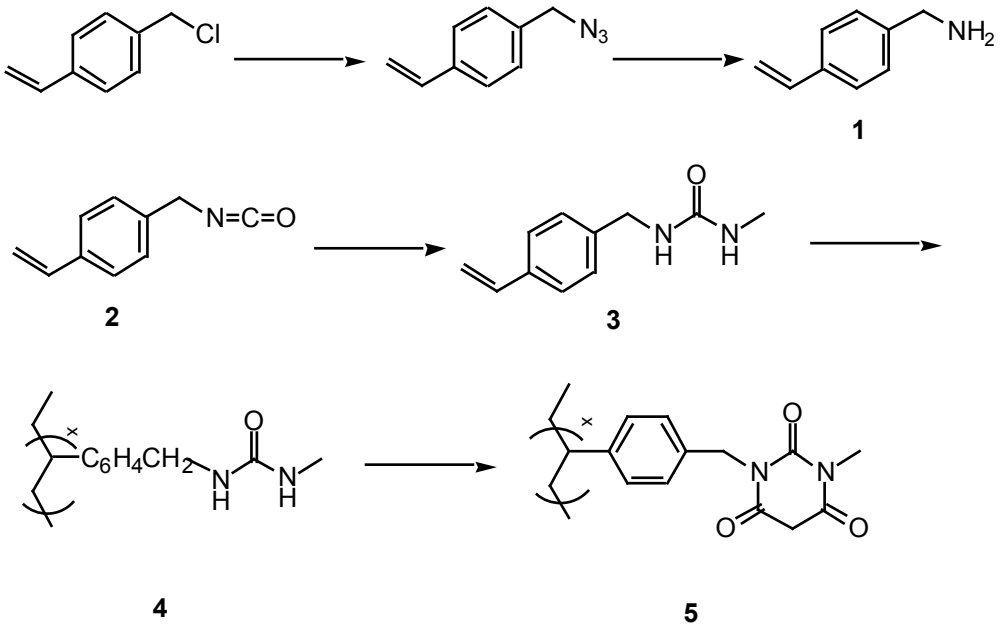
Scheme D-1

In an attempt to overcome the crosslinking problems, the thiol content in the polymer was decreased. Copolymerization of N-vinylformamide with vinylacetate gave a copolymer poly(N-vinylformamide-co-vinylacetate). Hydrolysis of the copolymer gave a polyvinyl alcohol with desired amine functionality. Subsequent reaction of the copolymer gave polyvinyl alcohol with different thiol groups (Scheme D-2). The synthesis of polyvinyl alcohols with decreased thiol groups indicated that the copolymer had not crosslinked in DMSO under a nitrogen atmosphere.



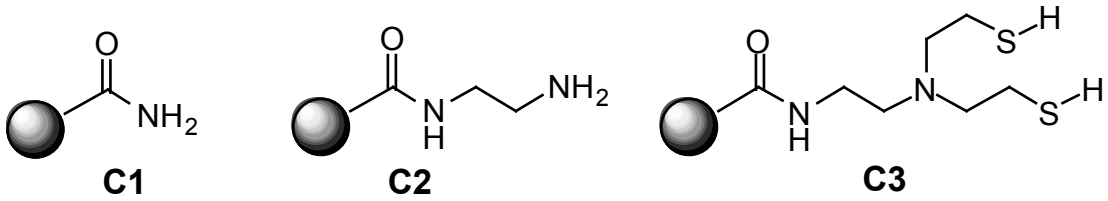
Scheme D-2

Polymers with Barbituric Moieties: We synthesized polymers with barbituric moieties.(Scheme D-3) Barbituric acid has a pKa~4, so under pH=7, polymer 5 will be deprotonated and exists as a polyelectrolyte with the barbituric moiety as the chelating ligand. Our initial results indicated that polymer 5 was not readily soluble in water, but at pH~7 adjusted by Na_2CO_3, the polyelectrolyte 5 was soluble.



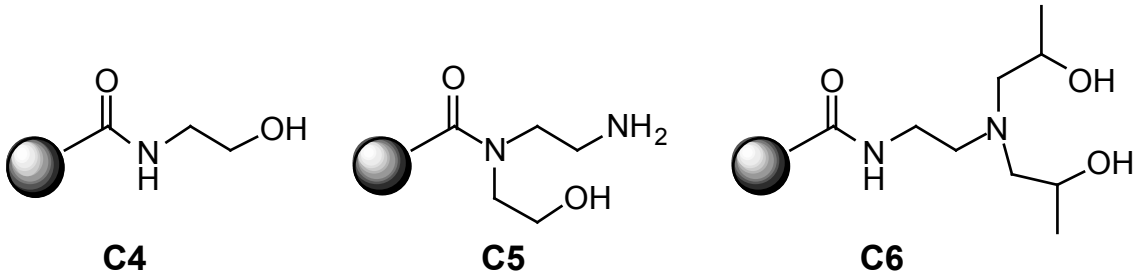
Scheme D-3

Acrylamide Polymer Synthesis: A wide variety of simple polyacrylamide polymers were synthesized. Polymers C2, and C3 were derived from a common polyacrylamide homopolymer C1 and were submitted to an atomic absorbance (AA spectroscopy) pre-screening. The polymers used in the AA screening were crosslinked. The structures of the polymers are shown in Scheme E-1. Polymers C1 and C2 were mildly effective at adsorbing mercury ions from an aqueous solution, but failed at high [Hg^{2+}]. Polymer C3 was found to be an excellent material for mercury sequestration, due to mercury's relatively irreversible binding to sulfur (thiophillicity). The synthesis of Polymer C2 required a transamidation with ethylenediamine, while the synthesis of Polymer C3 utilized a ring-opening amination of episulfide (polymer contains polythioethyl ether repeating units). Polymer C3 was isolated as a white solid after prolonged heating.



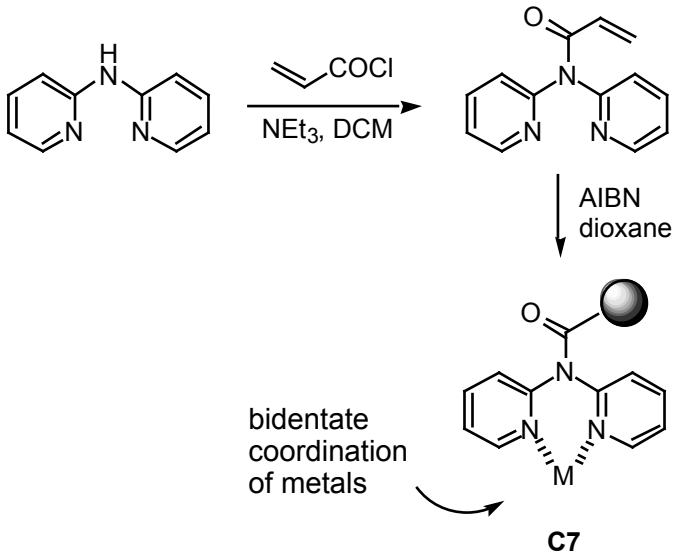
Scheme E-1

Other polymers synthesized are shown in Scheme E-2. We chose amides as a ligand class because amides have proven to be an effective chelating functional group due to its less Lewis basic amine. The amide shows selectivity for Hg over other more Lewis acidic metals such as iron, copper, and nickel. This is important when dealing with trace metals in an aqueous environment with high concentrations of relatively non-toxic metals.



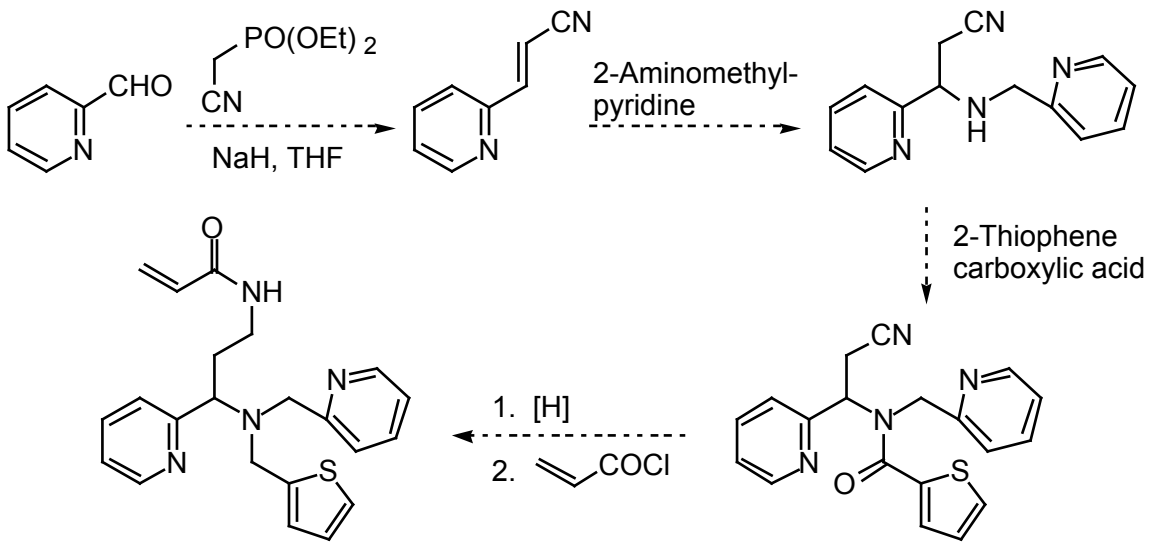
Scheme E-3

Dipyridyl ligands are known to strongly bind metals through a bidentate orientation of the pyridyl rings as shown in Scheme E-4. We synthesized a homopolymer of C7 in order to test this binding when the secondary amine was acylated with acryloyl chloride and then polymerized.

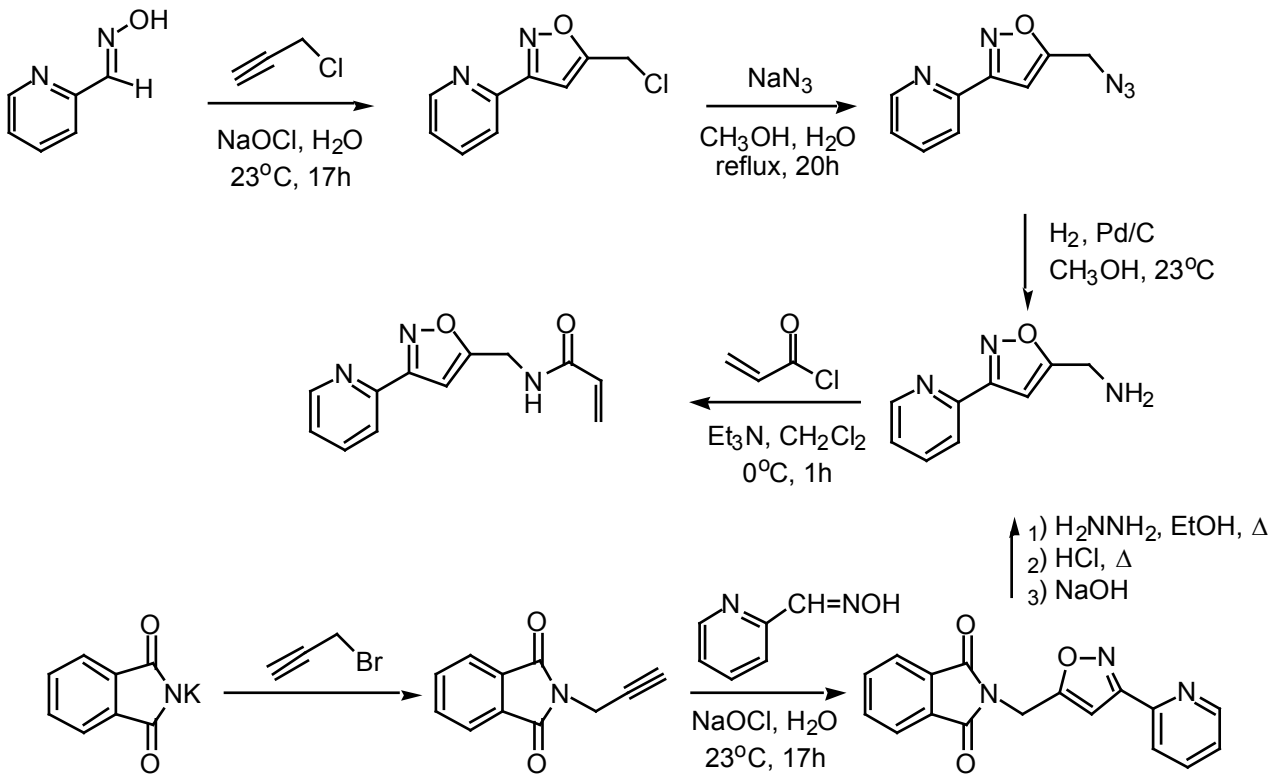


Scheme E-4

Acrylamide monomers with pendant heterocycles were synthesized. We hope to independently synthesize 10 monomers, copolymerize each of them (or a mixture of two different monomers for cooperative binding between ligands), and develop a combinatorial approach to monomer synthesis using different aldehydes, carboxylic acids, and amines as shown in Schemes E-5 and E6.



Scheme E-5


NaOCl, H2O
23°C, 17h
NaN3
CH3OH, H2O
reflux, 20h
H2, Pd/C
CH3OH, 23°C
Et3N, CH2Cl2
0°C, 1h
1) H2NNH2, EtOH, Δ
2) HCl, Δ
3) NaOH
NaOCl, H2O
23°C, 17h

Scheme E-6

(2) Ultrafiltration Synthesis Reactors: One important concept we developed for this project was the use of soluble polymers as the "solid" supports for the synthesis of the ligands. Soluble "solid" supports are desirable because the target metal-binding ligands were directly obtained ready for assay, and would have the advantage of homogenous synthesis conditions in comparison to the heterogeneous resin synthesis. The ability to accomplish this "homogenous synthesis" could revolutionize the potential automatability of combinatorial synthesis because it is much easier to accurately add and transfer liquids than solid resins. Water and organic soluble polyamines were used where the heteroatoms imparted excellent aqueous solubility to the polymeric supports. The polymeric supports were also soluble in a number of organic solvents useful for organic synthesis. The advantages of the multifunctional polymers was that they have greater capacity than the MeO-polyethyleneglycols used previously, as polyamines have multiple attachment points as opposed to only one per strand and the separation is accomplished with ultrafiltration instead of precipitation.

In order to accomplish our goal of using soluble polymers in a homogenous combinatorial system it was necessary to develop an apparatus in which to perform parallel syntheses. The unit must be compatible with acids, bases, and organic solvents; it must be able to allow reactions to be performed under an inert, heating, or cooling atmospheres, and it has to allow for ultrafiltration for ready recovery of the soluble polymer.

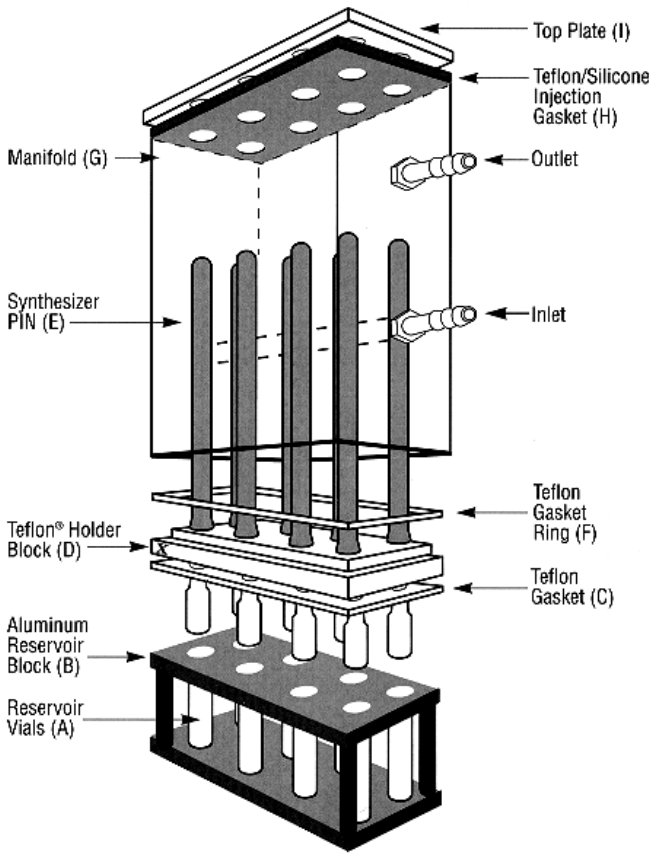


Figure 3. Schematic of Diversomer™ unit used for resin-based synthesis.

A commercially available unit produced by Diversomer™ that accomplishes this for resin synthesis is shown in Figure 3. The unit is an assembly of reaction vials held in an aluminum rack that can be heated or cooled if necessary. Into these vials are inserted glass-fritted tubes into which the resin is placed. A cover is sealed with gaskets over the bottom assembly that allows for the addition of nitrogen for an inert atmosphere, and a top plate with an injection gasket through which the reagents can be added. At the completion of the reaction the fritted tubes, called pins, can be lifted from the reaction vials and the excess reagent is drained and rinsed from the resin. The full vials are replaced with empty vials and a second set of reactions can be performed on the same resin and the cycle repeated as necessary.

We adapted this concept for ultrafiltration with soluble polymers. In our approach we reacted the polymer solution and reagents in glass tubes similar to the Diversomer system, but a reverse dead-end ultrafiltration unit was placed into the reaction solution after completion of the homogenous reaction, replacing the fritted pins. The whole unit was centrifuged to remove the excess reagents and soluble byproducts from the soluble polymeric product. After the first concentration by ultrafiltration, wash solution was added to the polymeric solution to complete the washing diafiltration process and ready the soluble polymer for the next step in the synthesis. The advantage of the reverse dead-end ultrafiltration approach was that filtration was rapid, minimizing the gel layer from concentration polarization on the membrane surface as the centrifugal force is in the opposite direction of the membrane driving the solution up into the filtration unit from the under side. The ultrafiltration membrane unit was placed into the reaction tubes after the reaction was completed to minimize membrane contact time. Multiple reactions could be performed in special holders that were also placed directly into the centrifuge (Ependorf, Model 5810R) for multiple sample processing. The challenge was of ensuring materials compatibility of the ultrafiltration modules with the chemical reagents. Many of the available membrane materials and holders were not compatible with the organic solvents and reagents used in the syntheses. We evaluated ceramic systems, all polysulfones systems, and disc inserts and decided on the use of Centraprep-10 (Amicon) for the ultrafiltration units. Though the materials were not optimized at this time, new materials are regularly appearing on the market that offer better chemical compatibility, such as units that have all polypropylene housing. Thus, the equivalent of a Diversomer™ unit could be used for all the synthesis except the fritted pins, which were replaced with the Centriprep units shown below in Figure 4.

Thus, the sequence of the synthetic procedure consists of reagent and polymer addition to the glass tubes along with the reaction solvent. The reaction proceeded under the desired conditions of mixing, temperature and atmosphere. An ultrafiltration module was added to each of the glass reaction tubes and the unit placed in a centrifuge and spun to force the solvent and excess reagent through the UF module. The liquid was removed from inside the ultrafiltration module and wash solution was added to the outside of the ultrafiltration module and the ultrafiltration process was repeated. The washed soluble polymer was then ready for the next synthetic step. To recover dry water-soluble polymer, the aqueous product was frozen in liquid nitrogen and the tubes were placed on a freeze dryer unit (Labconco, Freezone 4.5) at ca. 10^{-5} torr. The resulting dry polymeric materials, because of their parallel combinatorial synthesis approach, were available in amounts for characterization and testing without further purification.

Figure 4. Picture of unit for multiple syntheses using soluble polymers with ultrafiltration.

This apparatus and combinatorial approach were tested on the synthesis of a number of chelating polymers using Michael addition reactions to vinyl compounds by polyethylenimine. The reagents are given in Table 1 below. All product polymers were obtained in greater than 80% yield and were analyzed by NMR and IR spectroscopy.

Table 1. Summary of reagents and product polymers prepared using the ultrafiltration apparatus.

Reagents	Polymer	Products
CH_2=CH-CO-OCH_2CH_3	PEI	PEI-CH_2-CH_2-CO-OCH_2CH_3
CH_2=CH-CO-NH_2	PEI	PEI-CH_2-CH_2-CO-NH_2
CH_2=CH-PO-$(OCH_2CH_3)_2$	PEI	PEI-CH_2-CH_2-PO-$(OCH_2CH_3)_2$
CH_2=C-PO-CO-$(OCH_2CH_3)_3$	PEI	PEI-CH_2-CH-PO-CO-$(OCH_2CH_3)_3$
CH_2=C-$(PO)_2$-$(OCH_2CH_3)_4$	PEI	PEI-CH_2-CH-$(PO)_2$-$(OCH_2CH_3)_4$
CH_2=CH-CO-$N(CH_3)_2$	PEI	PEI-CH_2-CH_2-CO-$N(CH_3)_2$
CH_2=CH-CO-NH-$CH(CH_3)_2$	PEI	PEI-CH_2-CH_2-CO-NH-$CH(CH_3)_2$
CH_2=C(CH_3)-CO-NH-$CH(CH_3)_2$	PEI	PEI-CH_2-CH(CH_3)-CO-NH-$CH(CH_3)_2$

Conditions: Mixed for 2 hours under atmospheric conditions in 1:1 ethanol and water.

(3) Rapid Polymer Assaying Techniques: The resulting metal-binding polymers developed from the combinatorial synthesis (see Table 1) were assayed for metal-ion binding strength and selectivity. Since in any combinatorial approach there is the potential for generating a large number of compounds, assay techniques are needed that are near quantitative, sensitive, rapid, multi-element, nearly concurrent and require a small amount of sample. These techniques often require quantification of metal ions in the presence of polymers.

In this project we chose two techniques to develop for the metal-binding studies: micro-X-ray fluorescence spectroscopy (MXRF) and radiochemistry techniques.

Micro-X-ray fluorescence (MXRMF): X-ray fluorescence (XRF) is considered a mature and robust analytical technology usually regarded as a bulk analytical method with limited sensitivity and requiring several grams of sample for analysis. In the past, this high concentration need had typically restricted application of XRF to large sample masses and to concentrations well above the parts-per-million level. New techniques have changed the need for micro-X-ray fluorescence instrumentation. Meltzer demonstrated that sub-ppm detection limits could be achieved on macro-instrumentation using a dried spot methodology.[39] Dried spot methodology allows the direct analysis of radioactive samples using energy dispersive micro-X-ray fluorescence. Matching the excitation area of the X-ray spot to the dried sample spot minimizes much of the scatter background and increases sensitivity. Sensitivity was increased when the liquid matrix was removed and by focusing on the dried residue components. Detection limits demonstrated were sub-ppm in the liquid and approached less than 1 ng of material in the dried spot.

We have demonstrated the use of MXRF for trace metals analysis, using the dried spot methodology, for application to analysis of both RCRA and radioactive samples. The radioactive background was minimized by physically reducing the specimen size and permitting direct analysis of radioactive samples. We demonstrated that metal ions could be measured in the presence of water-soluble chelating polymers.

The procedure involved preparation of a polymer thin film as a sample support. Typical films were polycarbonate, polyester, and polypropylene that were 2-4 micrometers thick. The films were stretched and glued over a 35 mm slide mount. Normally 5-50 microliters of a liquid sample was applied to the surface of the film, and allowed to dry. This analytical specimen was analyzed directly. For radioactive samples, the liquid was placed on the well side of the slide, allowed to dry, and another layer of film was placed on top to seal the dried spot. This captured the radioactive specimen and provided a contained sample, which could be handled in a cold environment. Recent advances in thin film technology have led to the development of a commercially available sample system that can hold up to nine individual specimens on one slide, permitting multiple sample analysis and reducing both sample preparation time and cost per sample. An additional advantage of the commercial film was that it was 0.15 micrometers thick, which lowers the background and increased sensitivity. Our analyses were done using a Kevex (San Carlos, CA) Omicron micro-X-ray fluorescence spectrometer equipped with a rhodium X-ray tube, operated at 50 kV and 1 mA. The AAPS proprietary software allowed an almost universal calibration based on several known standards. Typical acquisition times were around 300 live seconds. Spectrum processing included peak identification, background modeling and subtraction and collection of both gross and net intensities. Figure 5 shows the various MXRF operations.

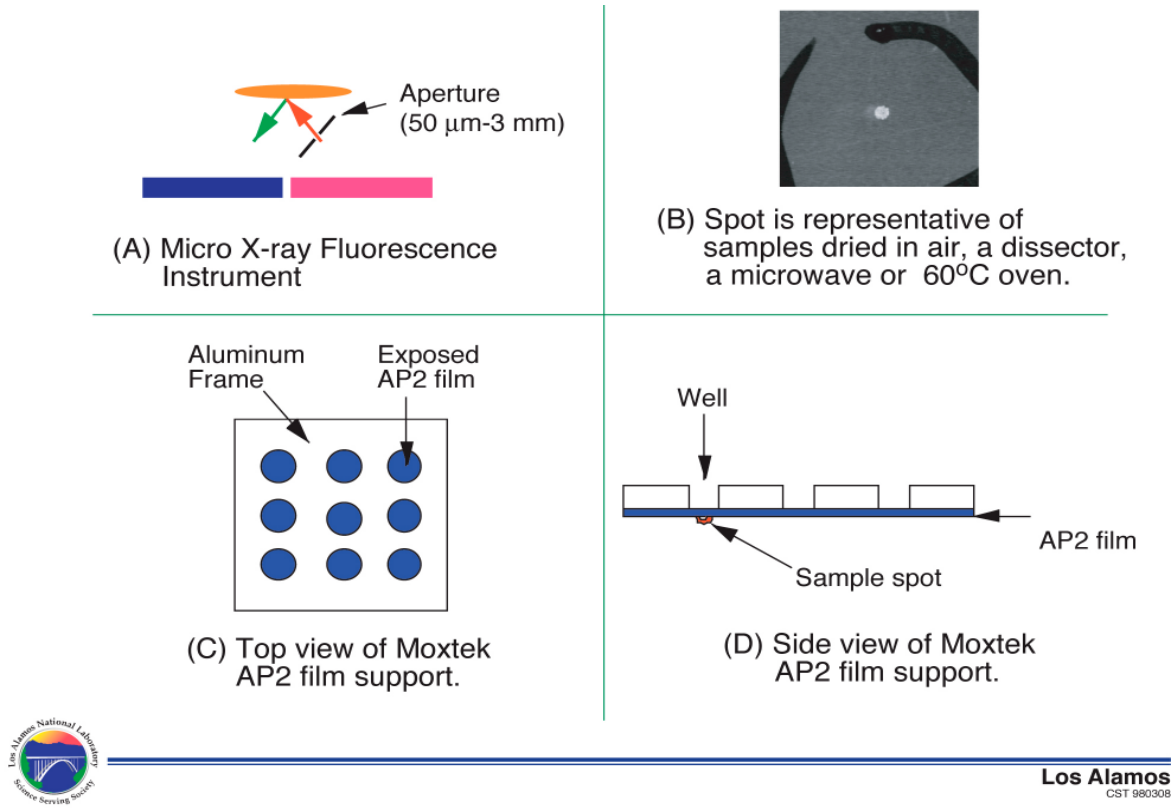


Figure 5. Diagrams of various operations in preparing a film with sample.

Spotting was on a variety of substrates including polypropylene, Moxtek's AP1 and AP2 films. The AP2 films, which could be heated, exhibited the best spot formation, while a silicone film (Rain-X) helped with spot formation on the polypropylene. Experiments with four surrogate water-soluble polymers at a concentration of 0.5% and 0.1 M $NaNO_3$ resulted in large backgrounds, which obscured analyte signal for the 100 ppb test solution. Further experiments with polymer at 0.1% and no $NaNO_3$ resulted in improved sensitivity. All elements in the test solution were observed in the starting solution, retentate and permeate solutions. Qualitatively, the retentate showed greater elemental intensities than the starting solution or the permeate, indicating a concentration effect by the polymer. The observation of the metals in the permeate, demonstrated sensitivity approaching 10's of ppb. Figure 6 gives the spectrum of the samples tested in a multiple element analysis, using the micro X-ray fluorescence analysis technique. Increasing counting time increased analyte peak intensities as well as the background. These results indicate the feasibility of using MXRF as a means for screening water-soluble polymer for metal ion binding effectiveness.

The next step was to develop a calibration to quantify the metal distribution among the different solutions. Lower polymer concentrations were studied to further reduce the background and increase sensitivity. Preliminary design plans indicated a good potential for matching the combinatorial reaction vessel with the Moxtek sample holder to maximize sample throughput and minimize confusion during sampling.

We have been able to optimize the spot drying technique, deal with background noise, optimize thickness and size of the spot, evaluate Mylar base materials, improve sensitivity, and

do multi-element analysis. We can see from the insert in Figure 7 that better sensitivity was realized with lower polymer concentration. We have been successful in detecting 10 ppb concentrations of metal ions in an aqueous test solution. Further improvements with apertures on both the X-ray tube and the detector may increase the method sensitivity.

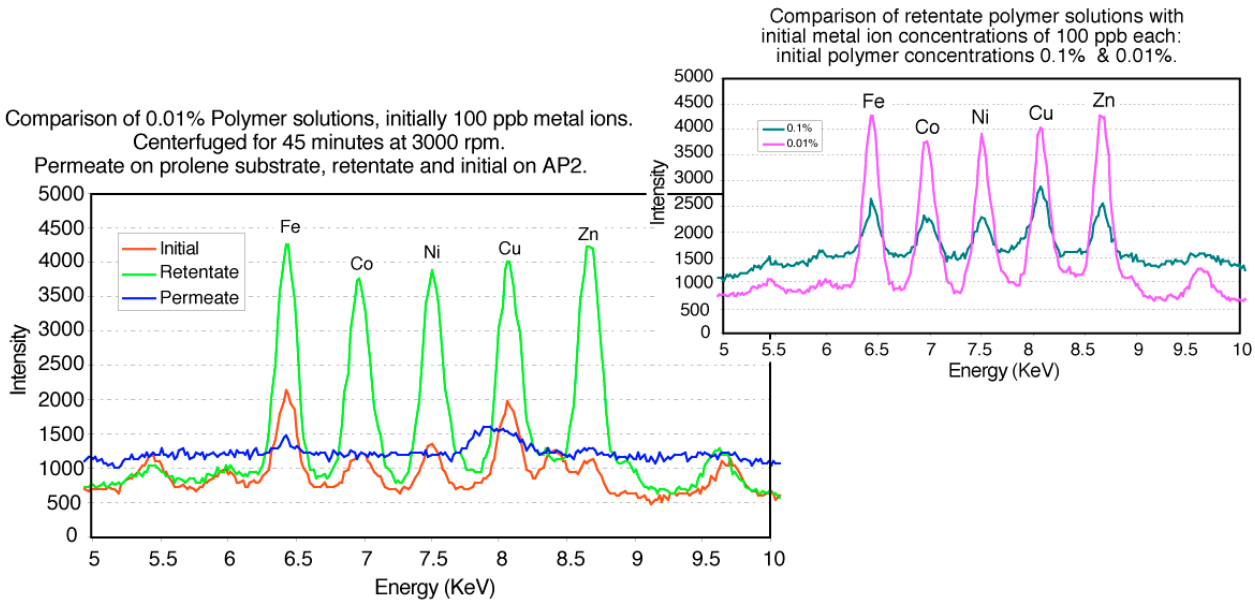


Figure 6. Spectrum of the samples tested in a multiple element analysis, using the micro X-ray fluorescence analysis technique.

Quantification of dried spot residues continues to show promise based on further development work with microwave drying. This approach to drying has potential to make the sample preparation more rapid, more reproducible and simpler. The microwave work demonstrated the following: 1) rapid drying of aqueous samples, 2) quantitative recovery of dried residue, 3) reduction in loss of volatile chloride species due to thermal drying. Microwave drying in combination with gentle thermal drying is necessary due to the reduction in water drop size below the effective interaction wavelength of the microwaves. This approach holds much promise in making the dried spot a viable quantitative method for trace elemental analysis. For now we are looking at the relative peak areas of the samples in the starting material and in retentate.

Radiochemical Survey: Since many of the targeted metal ions are radionuclides, radiochemistry techniques can be used for rapid assaying of metal binding. The metal ions of particular interest are plutonium (Pu) and americium (Am). Tests using autoradiography proved to be to slow as a rapid survey approach. Thus, we developed a survey technique using direct radiochemical counting techniques of small ultrafiltration modules (Amicon Centricon-10). We adapted centrifugation driven ultrafiltration units developed for protein purification to a rapid survey technique of our polymers under various conditions. These units are commercially available through companies such as Amicon, Fisher, Millipore, etc. They use dead-end filtration as in the Centricon-10 units (Amicon) and have sample volumes from 0.5 to 2 mL. The membranes used

had a molecular weight cut off (MWCO) of 10,000 to 100,000 Daltons and were composed of a variety of materials such as cellulose acetate, polysulfone, and fluoropolymers. A picture of the Centricon-10 unit is shown in Figure 7.

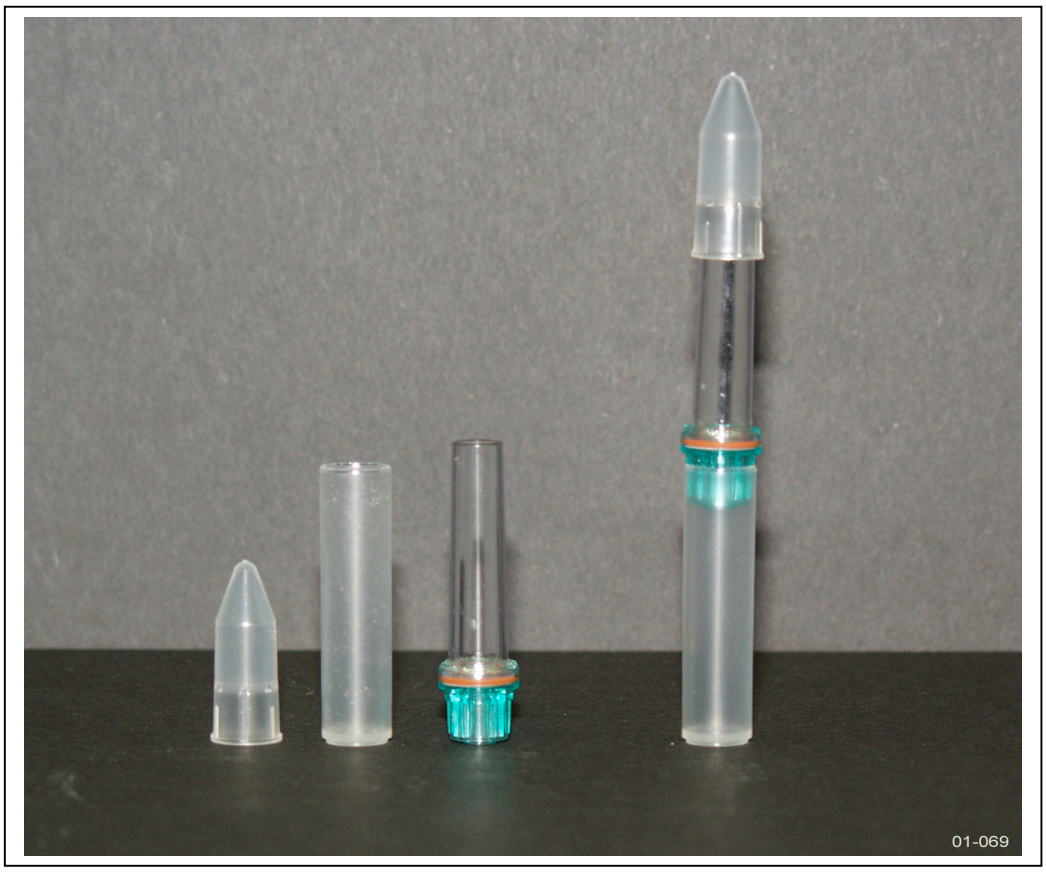


Figure 7. Photograph of Centracon-10 units (Amicon) both an assembled unit and parts consisting of the top and bottom half and a cap, that was used for the rapid survey of new water-soluble polymers.

These small centrifugal units were particularly useful for waste minimization purposes when evaluating polymers for actinides and other radionuclide separations because of the small sample required for testing and there were no transfer losses. Both the top and the bottom compartments of the unit were placed in separate scintillation vials and the whole unit was counted. The data can be reported as ppm metal or pCi/L remaining in the permeate as a function of the particular parameter being tested or as percent metal extracted or remaining. Data for actinide removal is often reported as a distribution coefficient (D) as a function of the parameter under study. The D value calculation was adapted from the D determinations for ion exchange resins where D = (Total Bound Metal/Total Unbound Metal) X Phase Ratio, where the Phase Ratio is (Initial Solution Volume in mL/Initial Polymer Weight in grams). Since this equation includes a phase ratio and very small amounts of soluble polymer can have large effects,

we can realize some very large D values. For example, a 0.1% w/v polymer solution that retains 99% of a metal-ion gives a log D value of 5.0.

Figure 8. Centracon-10 Ultrifiltration units and holder for multiple sample preparation for analysis.

The polymers prepared from Table 1 were tested using Centricon-10 ultrafiltration units using ultracentrifugation with the holders shown in Figure 8. To test the concept of rapid testing, in this study we used ICP analysis instead of counting techniques. Six metals (50 ppm) in groupings of three each were tested at four different pH values under conditions of constant ionic strength (0.1 with $NaNO_3$) and constant polymer concentration (0.1%) for the nine polymers that were prepared. This represented 24 different test conditions that were completed in just a few days using this rapid survey technique. The plot of the data for europium (Eu), often a surrogate for americium (Am), is given in Figure 9. Analysis of the results indicate that polymer PEI-E should be evaluated further as a possible candidate for study as it gave some binding at lower pH values compared to the other polymers.

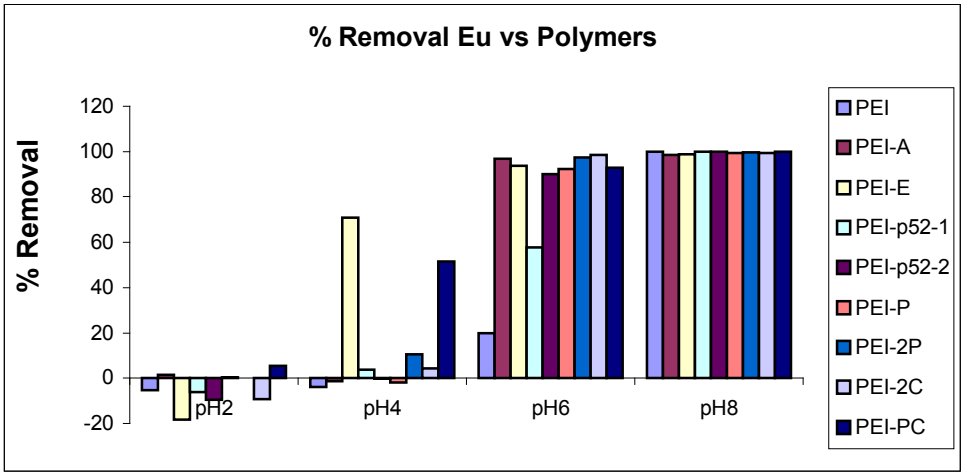


Figure 9. Plot of europium binding studies as a function of pH for 9 polymers prepared by the combinatorial approach.

(4) Polymer Characterization:

Characterization of the polymeric materials pose an especially unique and, oftentimes, complex problem due to the fact that these materials are high in molecular weight and are polydisperse, that is, not all of the molecules comprising a sample are either chemically or physically equivalent. Chemical derivatization and/or modification of polymers generally introduce further significant changes in the properties of the materials. The structure of polymers for specific functions and properties is obviously part of the molecular architecture of those molecules. To completely understand the properties of these materials it is highly desirable to correlate metal chelation with the molecular structure of the polymer.

Aqueous polymer behavior is, in many respects, much more difficult to characterize, predict, and evaluate than are organic soluble materials. This is in large part due to the complex nature of water itself, where ionic strength, added ions, pH and temperature all combine to form a very complex environment. Coupling this with the nature of polymeric chelating agents, which can be ionic, the chemical and physical behavior of such systems can span a wide range. Understanding the behavior of polymers under such conditions will allow for the optimization of performance of these materials for their intended application in Polymer Filtration.

The present characterization study focuses on water-soluble modified and unmodified poly(ethylenimine) (PEI). Principally, due to their polyelectrolyte character as well as their large macromolecular sizes, they are very useful in sequestering metal ions from dilute solutions. Light scattering spectroscopic studies were employed to obtain base characterization of these polymers.

Despite extensive studies in the last two decades, the dynamic light scattering behavior of flexible or branched polyelectrolytes in aqueous solutions is not yet fully understood.[40] Most of the studies that have been carried out in the past couple of decades have been done on typical model polymers such as poly(styrene)[41], poly(ethylene) [42] and poly(vinyl acetate)[43]. Not much information had been available in the literature about the dilute solution properties of branched or linear poly(ethylenimine) (PEI) [44] in comparison to these model polymers. A recent paper by Park and Choi characterizes the solution properties of branched poly(ethylenimine).[45] Part of the reason for the lack of studies on PEI, has been due to its weak polyelectrolyte character.[46] Development of such experimental approaches will have advantages in the in situ investigation of these macromolecules in solution in terms of conformational changes induced by external factors such as polymer-solvent interactions, ligand binding, pH, and temperature.

<u>Light Scattering Spectroscopic Study:</u>

Since its early development by Einstein,[47] Raman,[48] Debye[49] Zimm[50] and others,[51] the theory of light scattering from macromolecular solutions and suspensions has represented one of the major successes of chemical physics. Light scattering, indeed, is one of the few absolute methods available for the determination of molecular mass and structure and certainly is applicable over the broadest range of molecular weights of any method. Until samples were able to be separated by high performance size exclusion chromatography (HPSEC), or gel permeation chromatography,[52] however, these measurements produced only weight average molecular weights, Mw, and the corresponding z-average square radii (r_g^2) z together with the second virial coefficient, A_2. The use of HPSEC has resulted in the ability to analyze the distribution of broadly disperse samples as well as to obtain details of branching and molecular conformations.

Thus, weight, number, and z-average values for both mass and size may be obtained for most samples by combining light scattering and HPSEC.

We employed laser light scattering photometry to evaluate the change in hydrodynamic volume, association of polymers, weight-average molecular weight, and radius of gyration under conditions of varying pH, temperature, ion strength, and added ions to understand the complex conformational aspects of these systems. We evaluated the effect of different levels of functionalization, counter ion effects, and metal-binding effects. Figure 10 gives a diagram of the principle of the light scattering instrument.

In this work, we present results of a study of the solution behavior of PEI and PEI modified polymers in aqueous salt solutions. Light scattering measurements in solution of varying ionic strengths, pH, and electrolyte concentrations have been made to attempt to determine how the polymers exist in solution and the effects of added electrolyte.

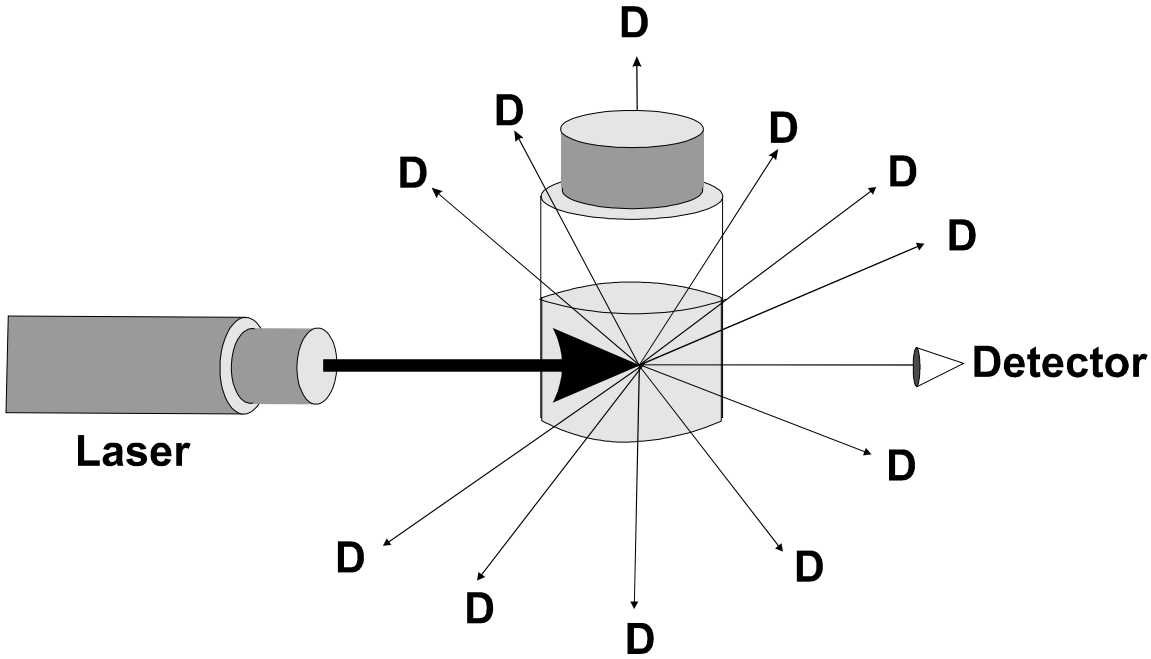


Figure 10. Diagram of the principle of the light scattering instrument.

Materials: Poly(acrylic acid) (PAA, MW = 450.000), poly(ethylenimine) (PEI, MW = 10,000), poly(methyacrylic acid), (PMAA), poly(methyacrylamide) (PMA) were purchased from Polysciences, Inc. potassium hydrogen phthalate, picolinic acid, NaOH pellets, poly(ethyleneimine) was obtained as a commercial product from BASF. The polymers consisted of purified PEI, unpurified PEI (with about 50% water), and carboxylated (PEIC) and phosphorylated (PEIP) poly(ethylenimines). All of the chemicals were best available commercial grade and were used as such without any further purification. The phosphorylated and carboxylated materials were prepared by previously published methods.[10] The number indicates the percent functionalization (e.g., PEIP0.5 indicates 50% functionalized).

Preparation of samples: Freshly distilled and de-ionized water was used in this investigation after filtering with 2-μm membrane filters. The pH values of the water were taken immediately

and after 3 days with an Orion Research pH meter. The pH values of 3 different concentrations (0.1, 1 and 5 M) of buffer solutions were also measured. The molecular weights (MW) of PEI were determined in purified water as well as buffer solutions.

Measurement of dn./dc: Wyatt Technology Optilab Differential refractometer 903 was used with a series of solutions of the polymer with different concentrations to measure the differential refractive index changes with concentrations.

The light scattering measurements: were done on a DAWN F Laser photometer (Wyatt Technology, Inc., CA), using a series of concentrations of the polymer solution. The final polymer solutions were filtered again with 0.2-μm membrane filters to avoid light scattering from extraneous dust and other particles. The MW was calculated from Berry plots.

Basic Theory and light scattering equation

Light scattering investigations are based on the following basic principles: 1) the amount of light scattered is directly proportional to the product of molecular weight and solute concentration[46], and 2) the angular variation of scattered light is directly related to the size of the molecule.[45]

$$K^*C/R(\theta) = 1/M\, P(\theta) + 2A_2C \qquad (1)$$

Where
K* = $4\pi^2(dn./dc)2n2/(N_A\lambda^4)$, and n is the refractive index of the solvent;
C is the concentration of the solute molecules (g/ml);
R (θ) is the fraction of light scattered relative to the incident beam;
$\mathbf{N_A}$ is Avogadro's number;
λ is the wavelength of light;
Dn./dc is the refractive index increment, dependant on solute concentration;
M is the weight-average molecular weight of the solute;
A is the second virial coefficient, which is a measure of solvent-solute interaction;
P (θ) is the "scattering function" which tells how the scattered light varies with angle. This variation is determined by $< r^2 >$, the mean square radius or radius of gyration;
$\mathbf{< r^2 >}$ is the mean square radius, describing the distribution of mass within the molecule, and it is given by the equation:[18]

$$< r^2 > = \Sigma\, r_i^2 m_i/M; \qquad (2)$$

The symbol R (θ), the Rayleigh ratio describes the angle-dependent light scattering, and it is described as

$$R(\theta) = I_\theta r^2/(I_0 V); \qquad (3)$$

I_θ is the scattered intensity, I_0 is the intensity of the incident beam, V is the volume of the scattered medium, and r is the distance between the scattering volume and the detector. Equation

3 implies that the dimensions of R (θ) are length^{-1}, and the DAWN instruments are provided with vertically polarized light sources. When studying a solution of solvent plus solute, it is convenient to use R (θ) to represent the *excess* scattering of the solution compared to that of solvent alone as:

$$R(\theta) = (I_\theta - I_{0,\ solvent})\ r^2/(I_0 V) \qquad (4)$$

where $I_{0,\ solvent}$ is the scattered intensity of the solvent. Equations 3 and 4 are used for pure solvent and for a solution respectively. Upon describing the scattering after subtracting the 'baseline' of pure solvent, R (θ) is called the excess Rayleigh ratio of the solute. The knowledge of Rayleigh ratio at a number of different angles leads directly to the weight-average molecular weight and mean square size of the solute molecules, making R (θ) the most important measured quantity in light scattering experiments.

The determination of molecular parameters of solute particles in solution is carried out by using the equation derived by Zimm,[50] as:

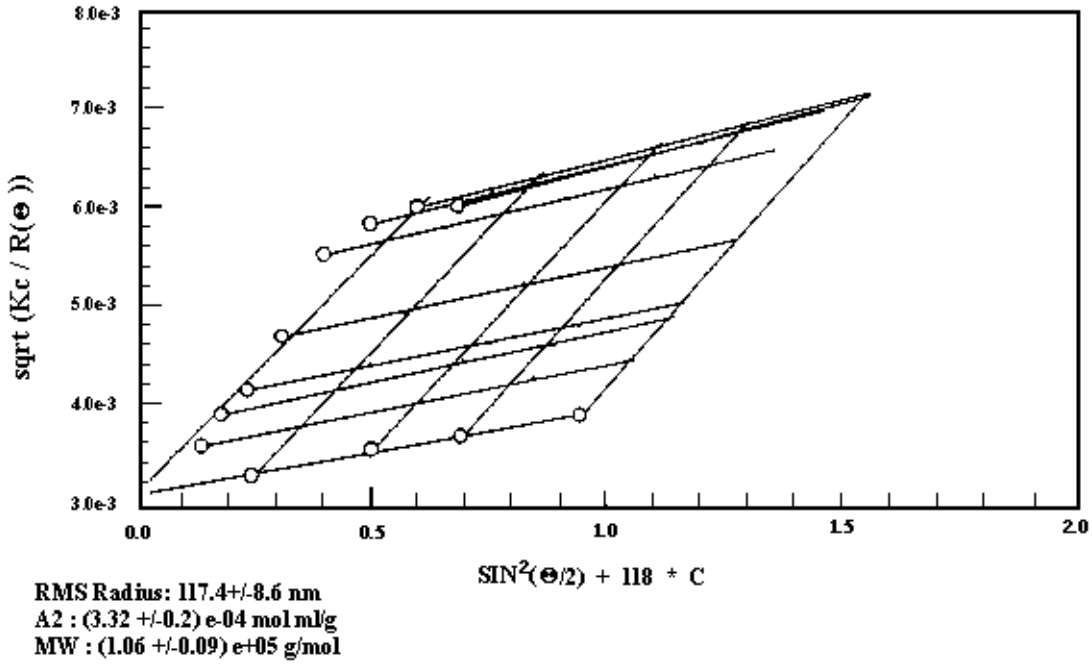
$$R(\theta)/K^*C = Mw\ P(\theta) - 2A_2CM_w^2P^2(\theta) \qquad (5)$$

where Mw is the weight-average molecular weight, and other parameters defined previously. P (θ) is related to $< r^2 >$ by the following equation:

$$P(\theta) = 1 - 2\mu^2 < r^2 >/3 + \qquad (6)$$

where $\mu = (4\pi/\lambda) \sin(\theta/2)$ and gives us the relation between P (θ) and mean square radius for any molecule regardless of molecular conformation in the limit of $\mu^2 < r^2 > << 1$. For a polydisperse sample the mean square radius obtained is z-average, usually written $<r^2>z$.

The determination of molecular weights and mean square radii are done through the use of Zimm, Debye or Berry plot.[53] (see Figure 11) The Zimm and Debye plots are similar. To construct a Debye plot, Y and X axes are R (θ)/K*C and $\mathrm{Sin}^2(\theta/2) + KC$ respectively, where K is a "stretch factor" which scales the contributions from c to be roughly equal to the contributions from $\sin^2(\theta/2)$. It is chosen to be the reciprocal of the maximum concentration. In the case of the Zimm plot, Y = K*C/R (θ) and X-axis = $\mathrm{Sin}^2(\theta/2) + KC$, and the y-axis is the reciprocal of that used for Debye plot.



DN/DC = 0.174, for polyethylenimine (PEI).

Figure 11. A representative Berry plot used to calculate the molecular weight, second virial coefficient and the root mean square radius of gyration of PEI.

Unfunctionalized and functionalized poly(ethylenimine)s: The effect of pH on polyelectrolytes in aqueous solutions is extremely important in the determination of their molecular dimensions. In this report, the pH of all the solutions including distilled water and the aqueous solutions were strictly controlled. The standard pH values of the starting solutions are shown in Table 2. The Molecular Weight characteristics for two batches of samples are provided in Table 3. It contains measured quantities such as RMS radius of gyration, $\langle r^2 \rangle$, second virial coefficient, A_2, and weight-average molecular weight, M_w. The RMS radius of gyration describes the distribution of mass within the molecule. This defines the radius of the volume within which a single polymer molecule gyrates.[54] The Second virial coefficient is derived from osmotic pressure. The free energy of mixing of a polymer solution with normal heat of mixing is given as:

$$\Delta G = KT\,[N_1 \ln V_1 + N_2 \ln V_2 + \chi_1 N_1 V_2]; \qquad (7)$$

the partial molar free energy of mixing is given as:

$$\Delta G_1 = KT\,[\ln (1-V_2) + (1-1/X)\, V_2 + \chi_1 V_2^2]; \qquad (8)$$

from which osmotic pressure, π is derived as:

$$\pi = KT/V_1\,[\ln (1-V_2) + (1-1/X)\, V_2 + \chi_1 V_2^2]; \qquad (9)$$

and finally gives rise to osmotic pressure as:

$$\pi = KT/V_1 \, [V_2/X + (1/2 - \chi_1) \, V_2^2 + \ldots\ldots], \qquad (10)$$

where V_1 = the molecular volume of the solvent and the coefficient of V_2^2 is known as the second virial coefficient, A_2, which is given as:

$$A_2 = V_2^2/N_O V_1 \, (1/2 - \chi_1) \qquad (11)$$

where V_2 is the specific volume of the polymer, and A_2 is directly proportional toV_2^2. MW is molecular weight, and M_w is the weight-average molecular of the polymers.

These materials were dissolved in 1 M NaCl solution and adjusted to pH 7.0. PEI-1 has a weight-average molecular weight of 39,600 g/mol while PEI-2 has a weight-average molecular weight of 43,100 g/mol. This should be expected because a purified material (almost always slightly fractionated) has a higher molecular weight than the unpurified one. The process of purification, as in fractional precipitation, separates the useful high molecular weight fractions from the low molecular weight fractions, which are usually washed out. This explains the sharp difference in the molecular weights of PEI-1 and PEI-2. Similarly, PEI-6, PEI-8 and PEI-11 also showed high molecular weights (Table 3). This might be due to the functionalization and eventual purification of the materials. Some of them have very large crystal sizes (e.g. PEI-8). The large molecular dimensions measured for some of the functionalized polymers could be due to the increased polyelectrolyte character due to derivatization and the increased tendency of larger macromolecular aggregations resulting from polar (ionic) attractions.

Table 2. Standard pH values of the experimental solutions.

Serial #	**Solution**	**pH at 23 ^oC**
1	Fresh distilled water	7.79
2	Distilled water after 3 days	7.78
3	NaCl, 0.1 M	6.82
4	NaCl, 1 M	6.17
5	NaCl, 5M	5.61

Table 3. Properties of functionalized poly(ethylenimine)s.

ID #	Remark	RMS (nm)	A_2 x 10^4 (mol ml/g)	MW x 10^4 (g/mol)
PEI-1	PEI Purified	39.8 ± 6.7	1.30 ± 1.0	3.96 ± 0.1
PEI-2	PEI Unpurified	38.8 ± 6.9	0.57 ± 2.0	4.31 ± 0.2
PEI-3	PEI Unpurified	42.3 ± 6.0	5.09 ± 0.4	5.19 ± 0.2
PEI-4	PEIP 0.5	39.3 ± 4.3	4.96 ± 0.7	3.16 ± 0.1
PEI-5	PEIC 0.5	47.1 ± 7.0	7.49 ± 3.0	1.60 ± 0.07
PEI-6	PEIP	45.6 ± 5.6	3.88 ± 0.2	0.41 ± 0.4
PEI-7	PEIP	40.2 ± 5.3	67.7 ± 0.5	59.7 ± 0.3
PEI-8	PEIC 0.5	49.6 ± 6.5	5.40 ± 0.7	5.69 ± 0.3
PEI-9	PEIP	46.1 ± 5.6	6.61 ± 7.0	96.1 ± 0.7
PEI-10	PEIP	44.5 ± 6.9	18.9 ± 0.4	1.89 ± 0.1
PEI-11	PEIP 0.5	39.8 ± 5.7	1.41 ± 0.2	0.11 ± 0.04
PEI-12	PEIP 0.5	38.1 ± 4.9	10.9 ± 0.4	1.29 ± 0.06
1	PEIC0.25	38.4 ± 5.4	0.62 ± 2.0	5.86 ± 0.3
2	PEIC0.5	37.6 ± 6.6	3.40 ± 1.0	1.11 ± 0.05
3	PEIC0.5	40.5 ± 6.7	0.77 ± 0.5	0.12 ± 0.05
4	PEIC0.5	36.6 ± 4.9	4.22 ± 0.8	7.26 ± 0.3
5	PEIC0.5	36.4 ± 7.0	0.29 ± 0.6	3.43 ± 0.2
6	PEIC0.75	37.8 ±5.6	0.65 ± 0.9	6.14 ± 0.4
7	PEIC1.0	34.5± 6.0	3.43 ± 0.4	4.76 ± 0.3
8	PEIC1.0	36.8 ± 5.0	2.64 ± 0.05	3.62 ± 0.1

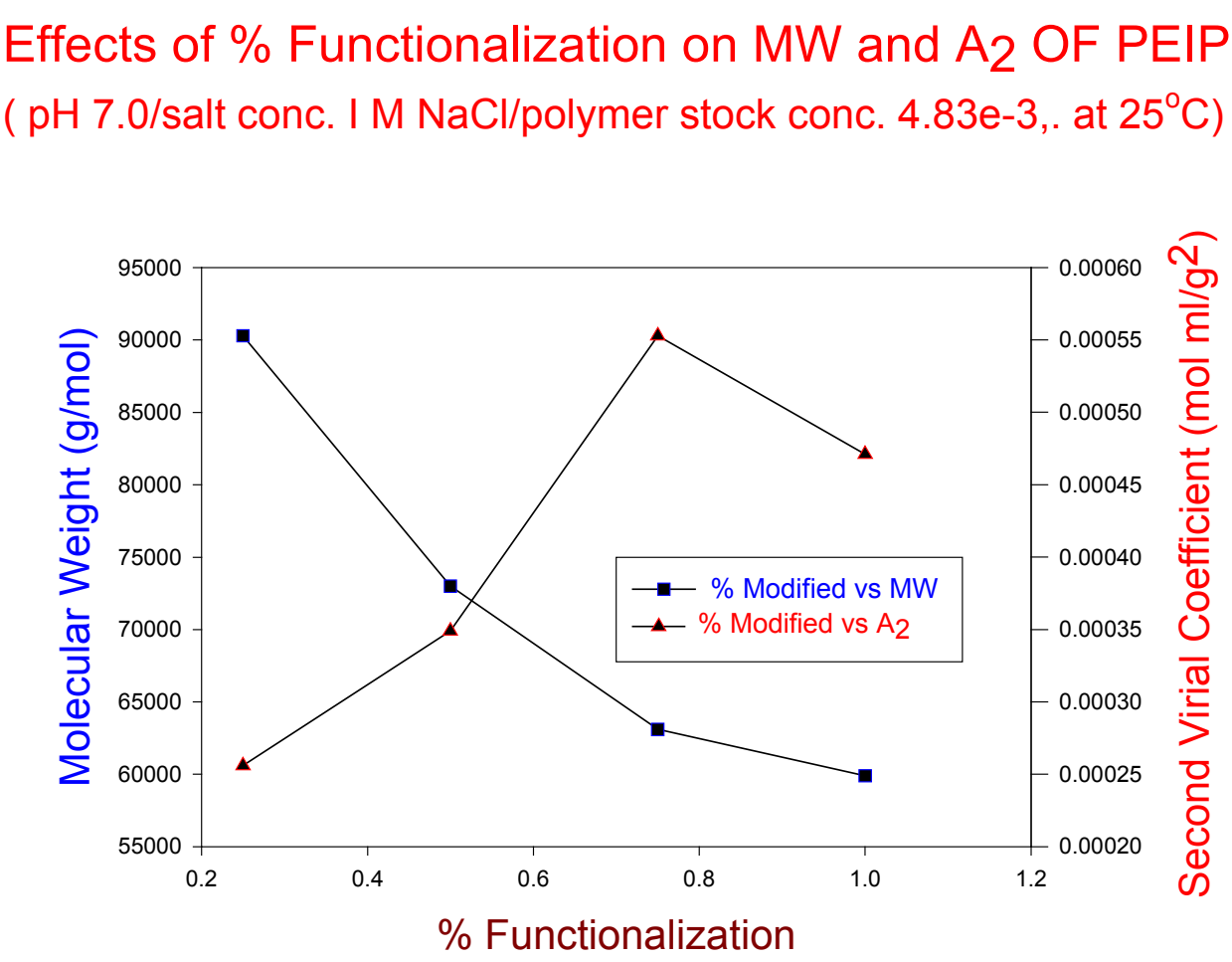


Figure 12. Plot of effects of functionalization percentage on the molecular weight and second virial coefficient of PEIP.

Effects of Salt Concentration, pH and Polymer concentration on the molecular weight of Phosphorylated PEI (PEIP): Figure 12 summarizes the effects of functionalization percentage on the molecular weight and second virial coefficient of PEIP. As the functionalization percentage increases from 25% to 100%, the molecular weight has been found to decrease, since polar and ionic interactions become stronger. On the contrary, the second virial coefficient increases with the increase in the functionalization percentage, as the possible effect of the solvent, NaCl solution.

For the PEIP 1.0 polymer, we have investigated the effect of pH on the molecular weight, second virial coefficient and RMS radius of gyration. The molecular size at the investigated pH range of 2.5 - 10.12, shows that the molecular weight is the highest at the neutral pH range of about 7.15. At both ends of the pH scale, the molecular weights are relatively smaller. The acid and alkaline ranges exhibit strong polar and ionic interactions that effectively coil the polymer

molecules. A similar trend is also observed for the second virial coefficients and the root mean squared radius of gyration. All the observed results for the investigated pH range are summarized in Table 4.

Table 4. Effect of pH on MW, Second virial coefficient and RMS radius of gyration of PEIP 1.0, [NaCl] = 1M; Polymer = 3.91 x 10^{-3}; T = 25 ^{o}C

pH	MW (g/mol) x 10^5	A_2 (mol ml/g^2) x 10^5	RMS radius (nm)
2.5	1.61	4.60	32.2
3.3	1.62	5.67	34.2
7.15	1.87	2.40	41.7
10.12	1.60	7.65	24.8

For an effective determination of the dimensions of polymer chains, it was very important that no aggregations were formed in solution. To this end, a small quantity of NaCl was dissolved in distilled and deionized water to prevent polymer aggregations. The NaCl concentrations used were 0.1 M, 1 M, 3 M and 5 M with the pH adjusted to 7.0. The effects of salt concentrations are summarized in Table 5. At low salt (0.1 M NaCl) concentration, both molecular weight and root mean radius of gyration were found to be very high, as the low salt concentration was not sufficient enough in buffering the polymer molecules. Increasing the salt concentration to 1 M leads to drastic reduction of both RMS radius and MW and further stabilizes the polymers. From the observed data (Table 5) describing the effect of salt concentration on the second virial coefficient, A_2, it is interesting to note that as the ionic concentration increases to 1 M, it increases and stabilizes as the ionic concentration increases (i.e., higher salt concentration makes a better solvent).

Table 5. Effect of NaCl on MW, Second virial coefficient and RMS radius of PEIP 0.75 Polymer = 3.88 x 10^{-3}; pH = 7.0; T = 25°C

Salt Conc. (moles)	RMS Radius (nm)	MW (g/mol) x 10^5	A_2 (mol ml/g^2) x 10^5
0.1	39.3	2.32	22.90
1.0	34.5	1.81	9.40
3.0	35.6	1.75	18.90
5.0	36.4	1.64	10.8

Similarly, the effects of polymer concentration on MW and A_2 of PEIP 0.5 are shown in Table 6. At low polymer concentration, the molecular chains are more relaxed and stretched out, leading to a rather high RMS radius of gyration. As the polymer concentration increases the RMS radius of gyration decreases and eventually reaches equilibrium, as the polymer chains have no more space to extend.

Table 6. Effect of [Polymer] on Second virial coefficient and Molecular weight of PEIP 0.5. pH = 7.0, [NaCl] = 1 M, T = 25 ^{o}C

[Polymer] X 10^3 (g/ml)	**A_2 (mol ml/g^2) x 10^5**	**MW (g/mol) x 10^5**
1.98	22.8	2.31
3.94	8.57	1.97
4.99	7.86	1.96
10.0	6.86	1.95

Effects of salt and polymer concentration and pH on the molecular weight characteristics of PEIC, Carboxyl modified PEI. The effect of pH on the carboxyl modified PEI seems to be dependent on whether medium is acid or alkaline. Both molecular weight and the root mean square radius of gyration are relaxed at the acid pH region as seen in Figure 13 for PEIC 0.75 at 25°C for a polymer concentration of 2.98 x 10^3. PEI is more basic, but when functionalized by carboxylation, the polymer chains exhibit more acidic than basic nature. As the pH of the medium progressively becomes alkaline, both the molecular weight and root mean square radius of gyration was found to decrease because the strong acid environment is progressively neutralized. It seems that the controlling factor here is the imbalance of ionic forces at both ends of the pH scale that leads to repulsion and extension of the molecules, or vice versa. The behavior of the second virial coefficient is also similar to those of MW and A_2.

The effects of salt concentration on PEIC was investigated choosing PEIC 0.25 as a model compound at pH 7.0 for a polymer concentration of 2.86 x 10^3 at 25°C. Observed results are compiled in Table 7. At low ionic concentration (e.g., 0.1 M NaCl) both molecular weight and root mean square radius of gyration have small values. At a higher salt concentration (1 M NaCl), MW and RMS have their highest values. At both acid and alkaline regions, polar and ionic interactions are strongest, which leads to smaller molecular dimensions at these regions.

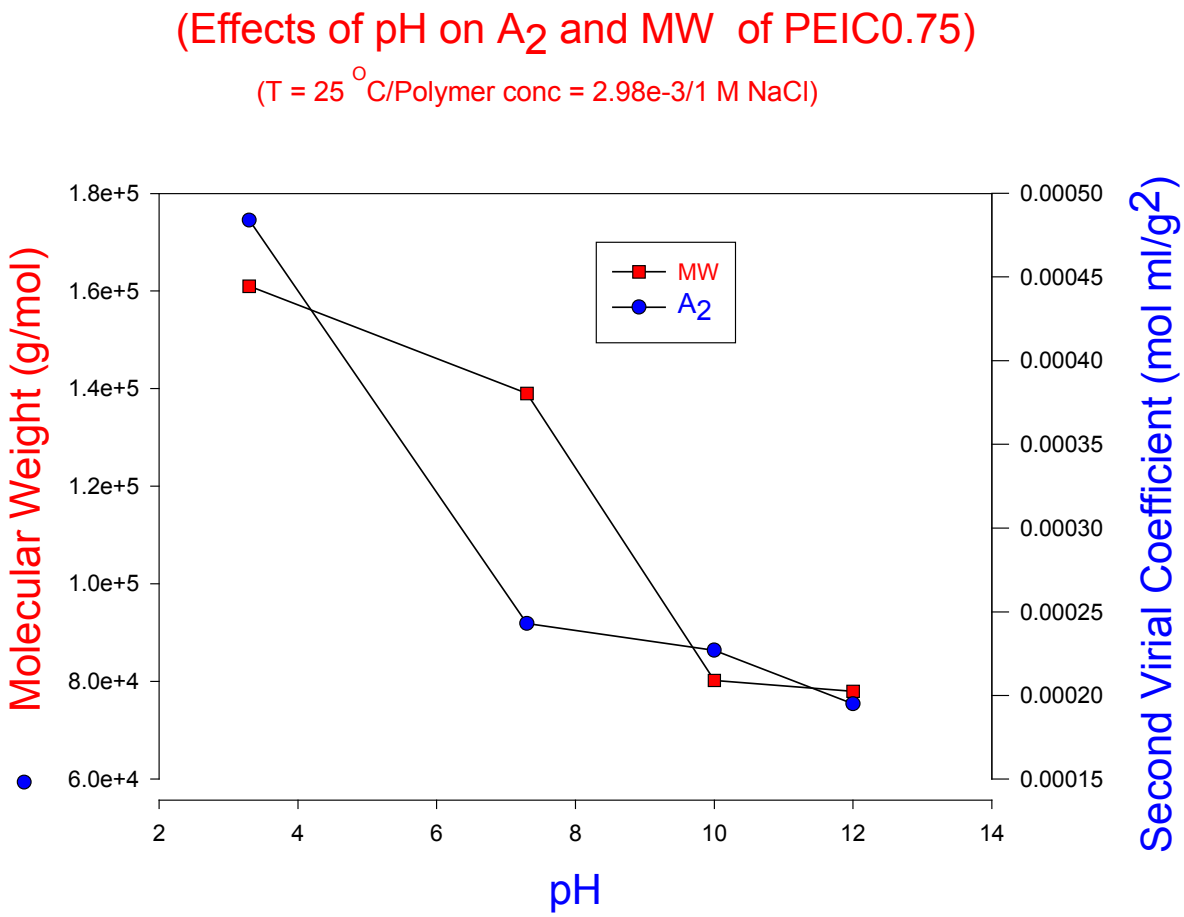


Figure 13. Plot of effects of pH on A_2 and MW of PEIP-.75.

Table 7. Effect of [NaCl] on Molecular weight, RMS radius of gyration and second virial coefficient of PEIC 0.25. pH = 7.0, [Polymer] = 2.86 x 10^{-3}, T = 25 °C.

Salt Conc. (Moles)	MW x 10^5 (g/mol)	RMS Radius (nm)	A_2 x 10^4 (mol ml/g^2)
0.1	1.41	7.1	1.07
1.0	1.47	14.5	1.28
3.0	1.35	13.8	1.48
5.0	1.07	13.2	1.82

Concentration of polymer has also demonstrated a significant effect on the molecular weight of PEIC 1.0 as shown in Figures 14 and 15. At low polymer concentrations, the molecular weight and root mean radii of gyration are lower values. As the concentration increases, MW and RMS were found to increase. At a polymer concentration of 4.00 x 10^{-3} g/ml, MW and RMS also increase rapidly. These rapid increases in these molecular dimensions can be explained from the concept of aggregation of polymer chains, above the critical concentration of

4.00 x 10^{-3} g/ml. It is however better to run the experiments at lower polymer concentrations for polymers with very high molecular weight. This will avoid saturation of the detectors leading to faulty results due to excessive scattering of light rays from large particles.

Critical Micelle Concentration [CMC] and aggregation: Some polymers form different agglomerations or aggregations in solution due to either polar/ ionic interactions or hydrogen bond formations. In either case it is extremely difficult to determine the exact molecular weights

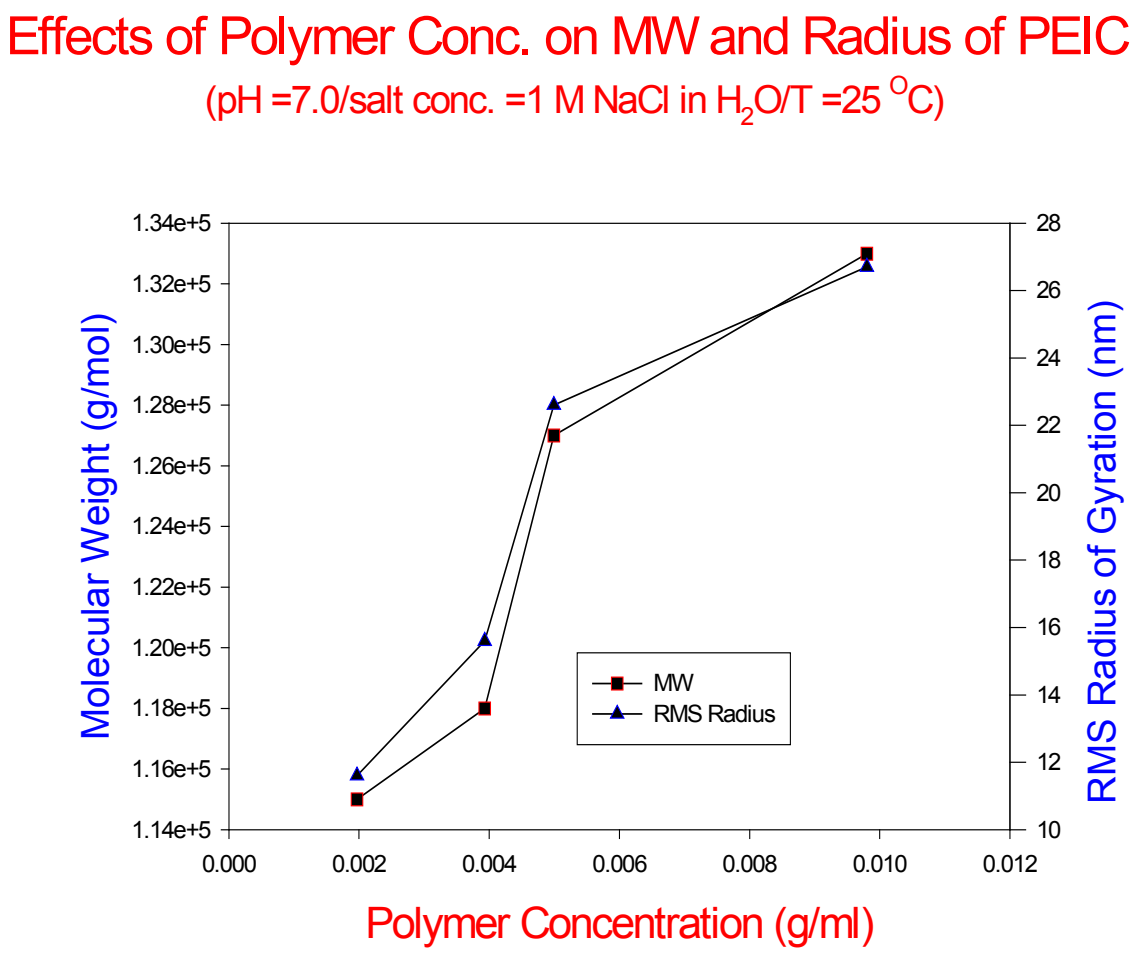


Figure 14. Plot of the effects of polymer concentration on MW and radius of PEIC-1.

of the individual molecules. The critical micelle concentration (CMC) is the concentration above which polymer molecules aggregate together due to polar or other interactions. Due to the large molecular weight values measured for some of the polymers, it is extremely important to ascertain if the polymer molecules aggregated at the certain concentrations of interest for PEIC 0.5 and PEIP 0.5. We therefore measured the molecular weights for an industrial use concentration range of 0.416 to 0.020 Wt % and at a low concentration range of 1.06 x 10^{-2} to

.0707 x 10^{-2} Wt %. For both functionalized polymers there were no significant differences observed between the molecular weights calculated from low concentrations and those calculated from industrially used concentrations.

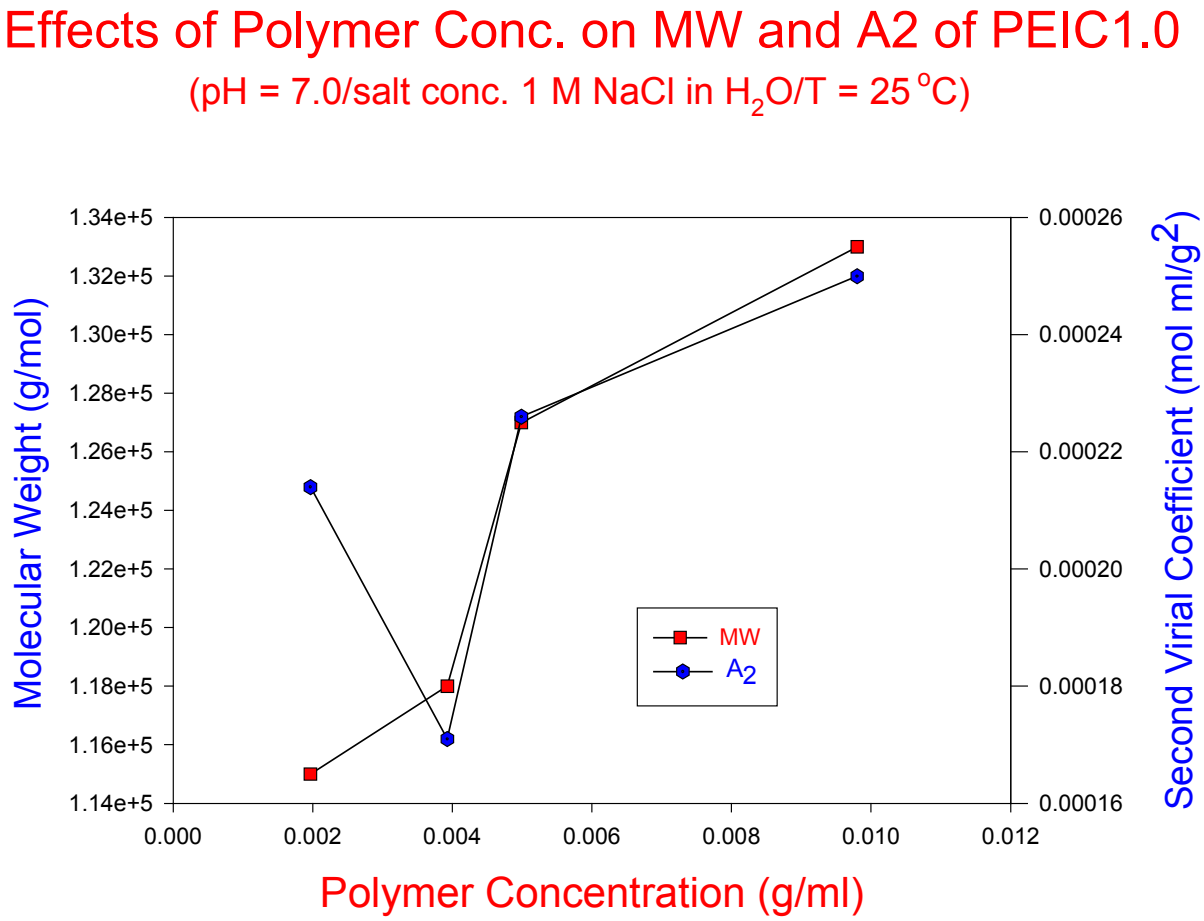


Figure 15. Plot of the effect of polymer concentration on the MW and A2 of PEIC-1.

In summary, various parameters of functionalized PEIs have been investigated by light scattering method. Generally, it was observed that the MW of the phosphorus modified PEI, as well as the second virial coefficient and root mean square radius of gyration decrease as a function of increasing polymer concentration initially, then stabilize above a concentration of 4.00 x 10^{-3} g/ml. For PEIP polymer, the polymer concentration range should be between 4.00 x 10^{-3} to 1.00 x 10^{-2} g/ml. For PEIP polymers, there are strong ionic and polar interaction at both ends of the pH scale, whereas for PEIC, opposite charges attract more strongly as pH increases into the alkaline range leading to the decrease in MW, RMS radius of gyration and second virial coefficient. All the observed results suggest that, the metal binding characteristics of

unfunctionalized and functionalized polymers can be tuned to target the specific metal ions of choice as well as the chemical nature of the solution containing the metal ion to be removed.

Increasing the concentration of sodium chloride leads to an increase in the root mean square radius of gyration, as the polymer chains become more extended and relaxed in a more concentrated salt medium. The apparent molecular weights were observed to decrease in proceeding from acidic to alkaline medium, especially with the phosphorylated polymer. However, the RMS radius of gyration of the carboxyl modified polymer was high at both ends of the pH scale, i.e., the polymer chains were extended at both the acidic and alkaline pH ends. The increase in RMS radius of gyration is indicative of increasing solvent-polymer interaction.

ACKNOWLEDGEMENT

The author would like to acknowledge the DOE, Environmental Management Science Program (EMSP) for supporting this work. The work was performed under DOE contract #W-7405-ENG-36. I would like to thank Thomas W. Robison for reviewing this report. Generally, the work was divided between the University of Massachusetts, Lowell collaborators performing the polymer characterization studies, the University of California, Davis collaborators performing much of the synthetic development work, the Los Alamos National Laboratory team developing the rapid assay techniques, some of the synthesis, testing, and development of the ultrafiltration apparatus and concepts, and coordinating the project. Our sincere regret is expressed at the death of Professor Bryan Miller (UCD) that occurred while participating in this project. He will be in our thoughts and hearts.

REFERENCES

1. Smith, B. F., Robison, T. W., Sauer, N. N. & Ehler, D. S. (No. 5,891,956, The Regents of the University of California, USA, 1999).
2. Smith, B. F. & Robison, T. W. (No. 5,766,478, The Regents of the University of California, USA, 1998).
3. Smith, B. F., Robison, T. W., Rogers, Y. C. & Lubeck, C. in *Waste Management-99* (W. M. Symposia Inc., Tucson, AZ, 1999).
4. Smith, B. F. *et al.* in *International Technical Proceedings from SURFIN '95* 607 (1995).
5. Smith, B. F., Robison, T. W. & Jarvinen, G. D. in *ACS Symposium Series 716, Metal-Ion Separations and Preconcentration: Progress and Opportunities* (eds. Bond, A. H., Dietz, M. L. & Rogers, R. D.) 294-330 (American Chemical Society, Washington, DC, 1999).
6. Geckeler, K. E. & Volchek, K. Removal of Hazardous Substances from Water Using Ultrafiltration in Conjunction with Soluble Polymers. *Environmental Science and Technology* **30**, 725-734 (1996).
7. Michaels, A. S. in *Progress in Separations and Purification* (ed. Perry, E. S.) 297-334. (Interscience, New York, 1968).
8. Smith, B. F., Gibson, R. R., Jarvinen, J. D., Robison, T. W. & Schroeder, N. C. Preconcentration of Ultra-Low Levels of Americium and Plutonium from Waste Waters by Water-Soluble Metal-Binding Polymers with Ultrafiltration. *Journal of Radioanalytical and Nuclear Chemistry* **234**, 225-229 (1998).
9. Smith, B. F. *et al.* Evaluation of Synthetic Water-Soluble Metal Binding Polymers with Ultrafiltration for Selective Concentration of Americium and Plutonium. *Journal of Radioanalytical and Nuclear Chemistry* **234**, 219-223 (1998).

10. Smith, B. F., Robison, T. W. & Gohdes, J. W. (No. 5,891,956 The Regents of the University of California, USA, 1999).
11. Alper, J. Drug Discovery On the Assembly-Line. *Science* **264**, 1399-1401 (1994).
12. Borman, S. Combinatorial Chemists Focus on Small Molecules, molecular Recognition, and Automation. *Chem. and Eng. News* **74**, 29 (1996).
13. Thompson, L. A. & Ellman, J. A. Synthesis and applications of small molecule libraries. *Chemical Reviews* **96**, 555-600 (1996).
14. DeWitt, S. H. & Czarnik, A. W. Combinatorial organic synthesis using Parke-Davis's DIVERSOMER method. *Accounts of Chemical Research* **29**, 114-122 (1996).
15. Armstrong, R. W., Combs, A. P., Tempest, P. A., Brown, S. D. & Keating, T. A. Multiple-component condensation strategies for combinatorial library synthesis. *Accounts of Chemical Research* **29**, 123-131 (1996).
16. Ellman, J. A. Design; synthesis; and evaluation of small-molecule libraries. *Accounts of Chemical Research* **29**, 132-143 (1996).
17. Gordon, E. M., Gallop, M. A. & Paatel, D. V. Strategy and Tactics in Combinatorial Organic Synthesis. Application to Drug Discovery. *Acc. Chem. Res.* **29**, 144-154 (1996).
18. Still, W. C. Discovery of sequence-selective peptide binding by synthetic receptors using encoded combinatorial libraries. *Accounts of Chemical Research* **29**, 155-163 (1996).
19. HsiehWilson, L. C., Xiang, X. D. & Schultz, P. G. Lessons from the immune system: From catalysis to materials science. *Accounts of Chemical Research* **29**, 164-170 (1996).
20. Xiang, X. D. *et al.* A Combinatorial Approach to Materials Discovery. *Science* **268**, 1738-1740 (1995).
21. Furka, A., Sebestyen, F., Asgedom, M. & Dibo, G. General method for rapid synthesis of multicomponent peptide mixtures. *Int. J. Pept. Protein Res.* **37**, 487-493 (**1991**).
22. Geysen, H. M., Rodda, S. J., Mason, T. J., Tribbick, G. & Schoofs, P. G. Strategies for epitope analysis using peptide synthesis. *J. Immunol. Methods* **102**, 259-274 (**1987**).
23. Houghten, R. A. Peptide libraries: Criteria and trends. *Trends Genet.* **9**, 235-239 (1993).
24. Jung, G. & Becksickinger, A. G. Multiple Peptide-Synthesis Methods and Their Applications. *Angewandte Chemie-International Edition in English* **31**, 367-383 (1992).
25. Sebestyen, F., Dibo, G., Kovacs, A. & Furka, A. Chemical synthesis of peptide libraries. *Bioorg. Med. Chem. Lett* **3**, 413-418 (1993).
26. Simon, R. J. *et al.* Peptoids: A modular approach to drug discovery. *Proc. Natl. Acad. Sci. USA* **89**, 9367-9371 (1992).
27. Zuckermann, R. N., Kerr, J. M., Kent, S. B. H. & Moos, W. H. Efficient method for the preparation of peptoids (oligo(N-substituted glycines)) by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* **114**, 10646-10647 (1992).
28. Bunin, B. A., Plunkett, M. J. & Ellman, J. A. The combinatorial synthesis and chemical and biological evaluation of a 1,4-benzodiazepine library. *Proc. Natl. Acad. Sci. USA* **91**, 4708-4712 (1994).
29. Chen, F. Y., Freiser, H. & Muralidharan, S. Study of Extraction and Back-extraction Kinetics of Ni-1-Phenyl-2-Methyl-4-Benzoyl-5-Pyrazolone and Correlation Between Kinetics and Centrifugal Partition Chromatographic Efficiencies. *Langmuir* **10**, 2139-2144 (**1994**).
30. DeWitt, S. H. *et al.* "Diversomers": "An approach to nonpeptide, nonoligomeric chemical diversity *Proc. Natl. Acad. Sci. USA* **90**, 6909-6913 (1993).
31. Gordon, E. M. Libraries of non-polymeric organic molecules. *Current Opinion in Biotechnology* **29**, 624-631 (1995).
32. Terrett, N. K., Gardner, M., Gordon, D. W., Kobylecki, R. J. & Steele, J. Combinatorial Synthesis - The Design of Compound Libraries and their Application to Drug Discovery. *Tetrahedron* **51**, 8135-8173 (1995).

33. Shey, J. Y. & Sun, C. M. Liquid phase combinatorial synthesis of benzylpiperazines. *Bioorganic & Medicinal Chemistry Letters* **9**, 519-522 (1999).
34. Houghten, R. A. Parallel array and mixture-based synthetic combinatorial chemistry: Tools for the next millennium. *Annual Review of Pharmacology and Toxicology* **40**, 273-282 (2000).
35. Borman, S. Liquid-Phase Combinatorial Synthesis: Technique Could Ease Automation. *Chem. and Eng. News* **73**, 25 (1995).
36. Kantipuly, C., Katragadda, S., Chow, A. & Gesser, H. D. Chelating Polymers and Related Supports for Separation and Preconcentration of Trace Metals. *Talanta* **37**, 491-517 (1990).
37. Han, H. S., Wolfe, M. M., Brenner, S. & Janda, K. D. Liquid-Phase Combinatorial Synthesis. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 6419-6423 (1995).
38. Chini, M., Crotti, P., Plippin, L. A. & Macchia, F. Regiochemical Control of the Ring-Opening of 1,2-Epoxides by means of Chelating Processes. 3. Aminolysis and Azidolysis of the cic-and trans- Oxides Derived from 4-(Benzyloxy)cyclohexene. *J. Org. Chem.* **56**, 7043-7048 (1991).
39. Meltzer, C. & King, B. S. in *Advances in X-ray Analysis* 41-55 (Plenum Press, New York, New York, 1991).
40. Smiths, R. G., Kuil, M. E. & Mandel, M. *Macromolecules* **27**, 5599-5608 (1994).
41. Masuda, T., Ohta, Y. & Onogi, S. *Macromolecules* **9**, 2524 (1986).
42. Bugudu, D. C. & Rudin, A. *J. Appl. Polym. Sci.* **33**, 87 (1987).
43. Pang, S. & Rudin, A. Am. Chem. Soc., Polym. Mater. Sci. Eng. **65**, 95 (1991).
44. Van Den Berg, J. W. A., Bloys Van Treslong, C. J. & Polderman, A. *Rec. Trav. Chim.* **92**, 3 (1973).
45. Park, H. & Choi, E.-J. *Polymer* **37**, 313-319 (1996).
46. (ed. Mark, H. F. e. a.) (John Wiley, New York, 1985).
47. Einstein, A. *Ann. Phys.* **33**, 1275 (1910).
48. Raman, C. V. *Indian J. Phys* **2**, 1 (1927).
49. Debye, P. *J. Appl. Phys.* **15**, 338 (1944).
50. Zimm, B. H. *J. Chem. Phys.* **16**, 1093-1099 (1948).
51. Fixman, M. *J. Chem. Phys* **23**, 2074 (1955).
52. Moore, J. C. *J. Polym. Sci. A2*, 835 (1964).
53. Salamone, J. C., Thompson, A. M. & Su, C. H., Watterson, A. C. An investigation of the molecular dimensions of an ampholytic/amphiphilic ionomer in solution via light scattering. *Polymer Preprints* **30**, 326 (1989).
54. Billmeyer, F. W. A textbook of Polymer Science (John Wiley and Sons, New York, 1984).