Photoinitiated Graft Poly(organophosphazenes): Functionalized Immobilization Substrates for the Binding of Amines, Proteins, and Metals

Harry R. Allcock,* Constance J. Nelson, and William D. Coggio

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

Received July 15, 1993. Revised Manuscript Received January 18, 1994®

Functionalized poly(organophosphazenes) have been prepared via photoinitiated graft polymerization with bifunctional monomers. Molecular level modification of poly[bis(*p*-methylphenoxy)phosphazene] (MPP) was accomplished by ultraviolet irradiation of films of the polymer which contained an unsaturated monomer and benzophenone. The grafting behavior of a number of unsaturated monomers that bear two reactive functionalities was studied. The choice of bifunctional monomers enabled the incorporation of reactive sites, which were subsequently employed for the immobilization of amines, biologically active compounds, and metal species. For example, glycidyl methacrylate (GMA) was photoinitiation grafted to MPP to prepare the MPP-*graft*-GMA films, which were later used to immobilize protein A via a ring-opening reaction of the epoxy groups. This substrate-bound protein A retained its activity for the subsequent immobilization of the immunoglobulin IgG. Similarly, the use of 4-vinylpyridine (VP) to synthesize the MPP-*graft*-VP films afforded a substrate with reactive coordination sites for the attachment of metal carbonyl species. Molecular structural characterization for the poly(organophosphazene) precursors was achieved by ^{1}H, ^{13}C, and ^{31}P NMR, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and elemental microanalyses. The photoinitiation grafted immobilization substrates were characterized via attenuated transmission reflectance infrared spectroscopy (ATR-IR), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and X-ray microanalysis, contact angle measurements, DSC, and elemental microanalyses.

The preparation of functionalized polymers via irradiation techniques is a topic of considerable interest.[1-3] Such reactive polymers have potential uses as immobilization substrates for binding catalysts and biologically active compounds.[4,5] Immobilized protein systems, in particular, are of interest for use in medical devices.

Grafting is an attractive methodology for polymer property modification since it allows the incorporation of a broad range of functional groups. In this study the modification of poly(organophosphazenes) at the molecular level, via a photoinitiated graft polymerization of bifunctional monomers, was explored. Various techniques (anionic, thermal, and UV or γ-radiation) have been reported for the modification of phosphazene polymers via grafting reactions.[6] The photochemical behavior and stability of poly[(aryloxy)phosphazenes] have been described previously.[7] One of the principal advantages of polyphosphazenes for use in photochemical grafting reactions is the transparency of the backbone in the visible region and to 220 nm in the ultraviolet. Thus, photochemical backbone cleavage is less of a problem than with many totally organic polymers.

Typically, the first step in a photoinitiated grafting process involves the formation of macromolecular radicals by exposure of a substrate polymer, in the presence of a graft monomer or polymer, to γ-rays or ultraviolet (UV) radiation.[8] The UV irradiation is usually carried out in the presence of a photoinitiator such as benzophenone, and has advantages over γ-irradiaton in selectivity and convenience. Benzophenone forms a reactive triplet excited state during UV exposure and is able to abstract a proton from a polymeric substrate. The resultant macromolecular radicals then attack an unsaturated monomer to initiate graft chain growth.[9] The UV grafting of monomers onto polymers in the presence of benzophenone was first studied by Oster et al.[10]

An inherent limitation of conventional grafting processes is the necessity for the use of unsaturated monomers which

® Abstract published in *Advance ACS Abstracts,* February 15, 1994.

(1) Clough, R. L., Shalaby, S. W., Eds. *Radiation Effects on Polymers*; ACS Symposium Series 475; American Chemical Society: Washington, DC, 1991.

(2) Hoyle, C. E., Kinstle, J. F., Eds. *Radiation Curing of Polymeric Materials*; ACS Symposium Series 417; American Chemical Society: Washington, DC, 1990.

(3) Allen, N. S., Rabek, J. F., Eds. *New Trends in the Photochemistry of Polymers*; Elsevier: New York, 1985.

(4) Sherrington, D. C. In *Syntheses and Separations Using Functional Polymers*; Sherrington, D. C., Hodge, P., Eds.; Wiley: Chichester, 1988; Chapter 11.

(5) Hartmeier, W. *Immobilized Biocatalysts*; Springer-Verlag: Berlin 1988.

(6) For examples of grafted poly(organophosphazenes) see the following, and references therein: (a) Wisian-Neilson, P.; Islam, M. S. *Macromolecules* **1989**, *22*, 2026. (b) Lora, S.; Carenza, M.; Palma, G.; Caliceti, P. *Radiat. Phys. Chem.* **1990**, *35*, 117. (c) Minto, F.; Scoponi, M.; Fambri, L.; Gleria, M.; Bortolus, P.; Pradella, F. *Eur. Polym. J.* **1992**, *28(2)*, 167. (d) Gleria, M.; Minto, F.; Scoponi, M.; Pradella, F.; Carassiti, V. *Chem. Mater.* **1992**, *4*, 1027.

(7) (a) O'Brien, J. P.; Ferrar, W. T.; Allcock, H. R. *Macromolecules* **1979**, *12*, 108. (b) Gleria, M.; Minto, F.; Flamigni, L.; Bortolus, P. *Macromolecules* **1987**, *20*, 1766.

(8) Allcock, H. R.; Lampe, F. W. *Contemporary Polymer Chemistry*, 2nd ed.; Prentice-Hall: Englewood Cliffs, NJ, 1990; p 156.

(9) For a review of the photochemistry of benzophenone, see: Rabek, J. F. *Mechanisms of Photophysical Processes and Photochemical Reactions in Polymers*; Wiley: New York, 1987; pp 272-295.

(10) Oster, G.; Oster, G. K.; Moroson, H. *J. Polym. Sci., Polym. Symp.* **1959**, *34*, 671.

0897-4756/94/2806-0516$04.50/0

undergo radical addition polymerization. Typically, these monomers contain no secondary functionality; hence subsequent reactions on the grafted system are difficult to carry out. A solution that has been employed by Ranby and co-workers to expand this chemistry is the use of unsaturated monomers that bear a second, reactive, functional group.[11] Once the bifunctional monomer has been incorporated by a graft polymerization reaction, the secondary reactive site allows the subsequent linkage of a variety of compounds.

The use of two such monomers, glycidyl methacrylate (GMA) and 4-vinylpyridine (VP), was investigated extensively in this work. The photoinitiation grafted immobilization substrates, MPP-*graft*-GMA and MPP-*graft*-VP, were prepared via UV irradiation of films of MPP in which were dissolved the monomer and the photoinitiator benzophenone (BP). The photoinitiation grafted MPP-*graft*-GMA films were then used to bind amines and biologically active compounds via a ring opening of the epoxy group. Multifunctional amines were also immobilized, and these substrates were later utilized to bind metal species. In addition, the functionalized MPP-*graft*-VP films were utilized to coordinatively bind metal carbonyl groups.

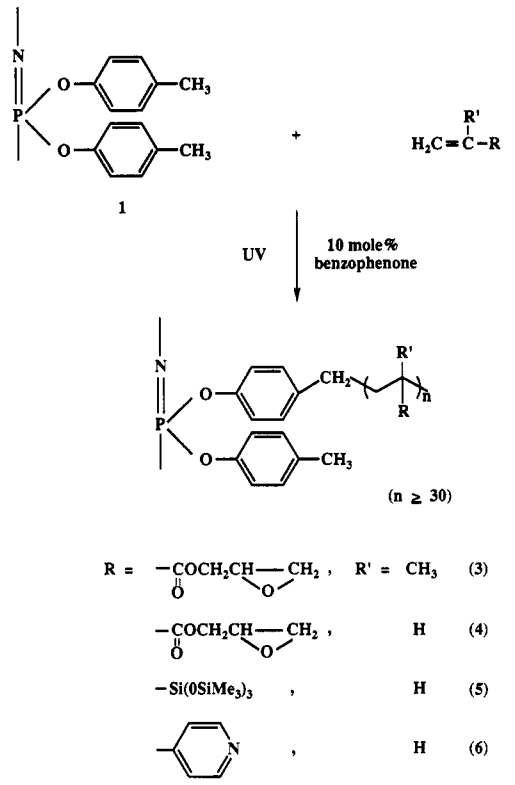
Results and Discussion

General Synthetic Approach. Polyphosphazenes are unique in the sense that replacement of the highly reactive chlorine atoms in the macromolecular intermediate, poly(dichlorophosphazene), allows the properties of the resultant derivative polymers to be modified over a wide range.[12] Poly[bis(*p*-methylphenoxy)phosphazene] (1) is a hydrophobic, fiber- and film-forming polymer, with a glass transition temperature (T_g) of −3.9 °C. Polymer 1 forms free-standing films of good integrity. The *p*-methyl groups provide potential sites for monomer grafting reactions.

Initial studies showed that this polymer is susceptible to ultraviolet induced cross-linking reactions, even in the absence of a photoinitiator. This indicated that the free radicals that are responsible for the cross-linking might, instead, be used for grafting reactions. Qualitatively, a similar degree of cross-linking was achieved with 3-fold less UV exposure when benzophenone was present as a photoinitiator. Thus, benzophenone was used in all of the grafting reactions described below.

Solid-state modification of films of 1 was accomplished by the UV-initiated graft polymerization of dissolved glycidyl methacrylate (GMA) in the presence of benzophenone, as shown in Scheme 1. This process results in the free radical polymerization of the GMA monomer and subsequent coupling between the acrylate radicals and the benzyl radicals of the polymer, thus forming the MPP-*graft*-GMA.[11] This technique maximizes the degree of graft polymerization within the material. The use of 4-vinylpyridine to prepare the photoinitiation grafted films, MPP-*graft*-VP, was also investigated. These last materials proved useful for the subsequent coordinative

Scheme 1



R = $-COCH_2CH(O)CH_2$, R' = CH_3 (3)
$-COCH_2CH(O)CH_2$, H (4)
$-Si(OSiMe_3)_3$, H (5)
N, H (6)

binding of metal carbonyl groups. Additional unsaturated monomers that were studied at a preliminary level include glycidyl acrylate and vinyltris(trimethylsiloxy)silane (see Experimental Section).

A rough estimate of the degree of polymerization of the grafted organic units was obtained by means of a control experiment that made use of a polyphosphazene that lacked the *p*-methyl groups needed for facile grafting. Films which contained poly[bis(phenoxy)phosphazene] (**2**), 2 equiv of GMA, and 10 mol % BP were exposed to UV radiation with use of standard procedures. The use of polymer **2** allowed the subsequent extraction of the polyacrylate via sonication and precipitation techniques. An estimate of the polyGMA molecular weight was obtained by the use of gel permeation chromatography. The extracted polyGMA had a very broad polydispersity, with an estimated average degree of polymerization of ≥30.

Characterization of the Photoinitiation Grafted Film MPP-*graft*-GMA. Scanning electron micrographs of the surface of several polymer films are shown in Figure 1. The "air-side" of a film of the non-cross-linked, parent polymer 1 (**a**) was quite regular in form, with a considerable degree of microporosity. By comparison, a UV cross-linked film of polymer 1 (20 min in the presence of 10 mol % benzophenone) after extraction of the photoinitiator and non-cross-linked MPP (**b**) showed a broken surface in which the porosity was clearly evident. The MPP-*graft*-GMA (**3**) film surface (**c**) had marked differences from both MPP films. The sculptured surface of the photoinitiation grafted system is obvious from the micrograph.

The contact angle of a sessile drop of water on the film surface of both the cross-linked and the parent polymer (1) was 93°. By contrast, a value of 82° was measured for the MPP-*graft*-GMA (**3**) film. This decrease in the contact angle is indicative of an increase in the surface free energy of the grafted material.

(11) (a) Ranby, B.; Gao, Z. M.; Hult, A.; Zhang, P. Y. In *Chemical Reactions on Polymers*; Benham, J. L., Kinstle, J. F., Eds.; ACS Symposium Series 364; American Chemical Society: Washington, DC, 1988; Chapter 13. (b) Allmer, K.; Hult, A.; Ranby, B. *J. Polym. Sci. A: Polym. Chem.* **1989**, *27*, 1641. (c) Allmer, K.; Hilborn, J.; Larsson, P. H.; Hult, A.; Ranby, B. *J. Polym. Sci. A: Polym. Chem.* **1990**, *28*, 173.

(12) Allcock, H. R. *Phosphorus-Nitrogen Compounds*; Academic Press: New York, 1972, and references therein.

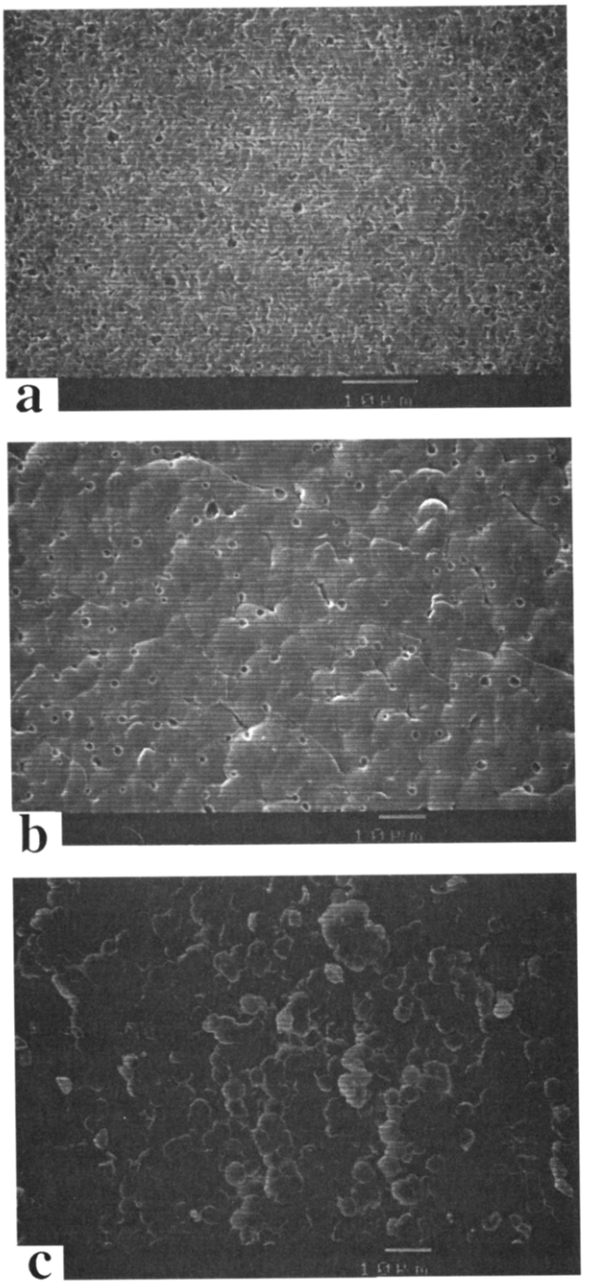


Figure 1. Scanning electron micrographs of a film of polymer **1** (**a**), an ultraviolet radiation cross-linked film of **1** (**b**), and the photoinitiation grafted film **3** (**c**).

The parent polymer **1** and the grafted film **3** were also analyzed by XPS methods (see Figure 2). The XPS spectra for the O(1s) region of polymer **1** contained a single peak at 532 eV. This peak was broadened and shifted slightly to a higher binding energy for the MPP-*graft*-GMA film. This shift reflects the incorporation of the O(O=C—O) and O(C—O—C) groups of the glycidyl methacrylate in the grafted film. For the C(1s) region, the single peak at 285 eV for polymer **1** was assigned to carbon atoms bonded only to other carbon and hydrogen atoms. In the MPP-*graft*-GMA spectrum this same peak is present, plus a new shoulder at approximately 286 eV, due to the (C—O—C) and (C—O) species. Furthermore, a new peak centered at 289 eV was assigned to the (O=C—O) groups of the grafted GMA.

ATR-IR spectroscopy provided further evidence to confirm the incorporation of the grafted organic species. As shown in Figure 3, analysis of the cross-linked film of polymer **1** showed peaks assignable to the ring C=C stretch

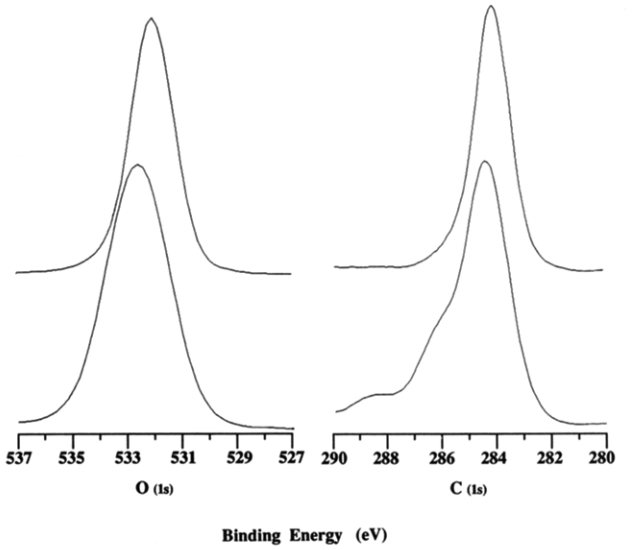


Figure 2. XPS spectra of the O(1s) and C(1s) region of films of polymer **1** (upper) and the photoinitiation grafted polymer **3** (lower).

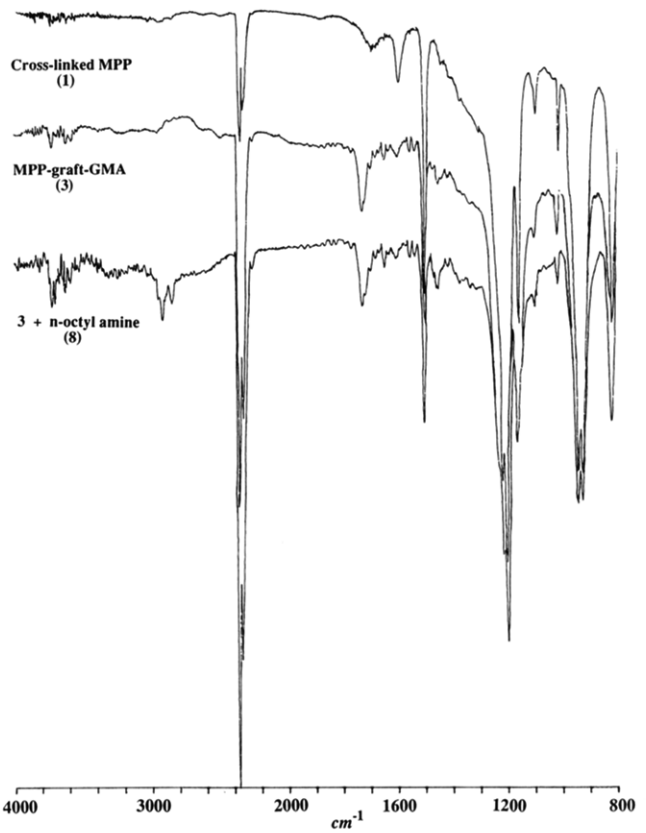
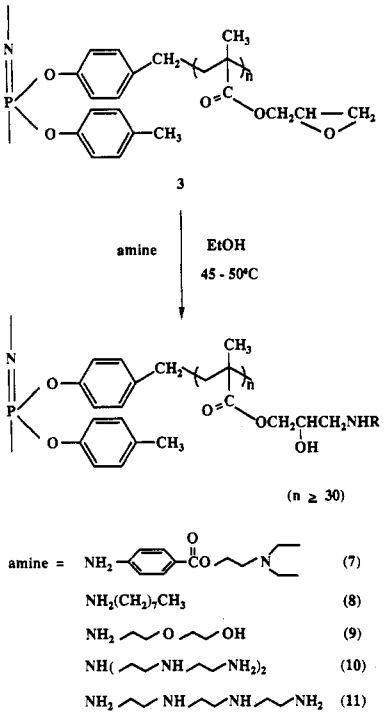


Figure 3. ATR-IR spectra (45° Ge crystal, 200 scans in air) of films of the parent polymer **1**, the grafted polymer **3**, and the amine-immobilized substrate **8**.

at 1610 and 1520 cm^{-1} and the P=N stretch at 1200 cm^{-1}.[12] The ATR-IR spectrum of the MPP-*graft*-GMA film contained a new peak at 1730 cm^{-1}. This peak corresponds to the carbonyl group of the grafted GMA. Due to the complexity and presence of overlapping peaks from **1** in the fingerprint region, the assignment of peaks due to the epoxy groups could not be accomplished.

The effects of the photoinitiated grafting reaction on the glass transition temperatures of these materials was studied by use of DSC analysis. A slight increase in the glass transition temperature (T_g) was detected following

Scheme 2



the grafting reaction. For example a T_g of 3.1 °C was measured for films of the MPP-*graft*-GMA as compared to the parent polymer **1** (T_g = −3.9 °C). This modest increase can be attributed to reduced polymer mobility due to the incorporation of the bulky grafted polyorganic species and the formation of cross-link sites within the methylphenoxy polymer.

Elemental microanalysis values showed a 1.6-fold increase in the %C/%P ratio for the MPP-*graft*-GMA polymer when compared to the parent polymer **1**.

Reactions of MPP-*graft*-GMA with Amines. Reaction of the pendent epoxy rings in the MPP-*graft*-GMA with primary amines is possible, as outlined in Scheme 2. The relative reaction rates depend on the reactivity of the amine, the reaction time, the surface area of the polymer, the extent of substrate swelling in the chosen solvent, and the degree of loading of epoxy groups.[11]

In a typical reaction, films of the MPP-*graft*-GMA substrate were dried to constant weight, then treated under sonication bath conditions with known concentrations of the amine in an ethanol solution, to allow coupling between the pendent epoxy units and the amine. Controlled exposure of the films to the amine solutions allowed the change in binding efficiency over time to be determined. After purification, these amine immobilized MPP-*graft*-GMA films were dried to constant weight for gravimetric analysis. This allowed an estimate to be made of the grams of amine bound for each gram of MPP-*graft*-GMA polymer film that was exposed for a given reaction time.

As shown in Figure 4, the choice of amine played a major role in determining the immobilization efficiency with this substrate. For example, use of the reactive, aliphatic *n*-octylamine (**a**) resulted in an initial very rapid coupling followed by a leveling to a plateau after approximately 10 h of reaction time. By comparison, no reaction was detected before 4 h for the sterically hindered, aromatic amine, procaine, depicted in (**b**). Even after prolonged reaction times this biologically active amine was immobilized to a lesser extent than *n*-octylamine.

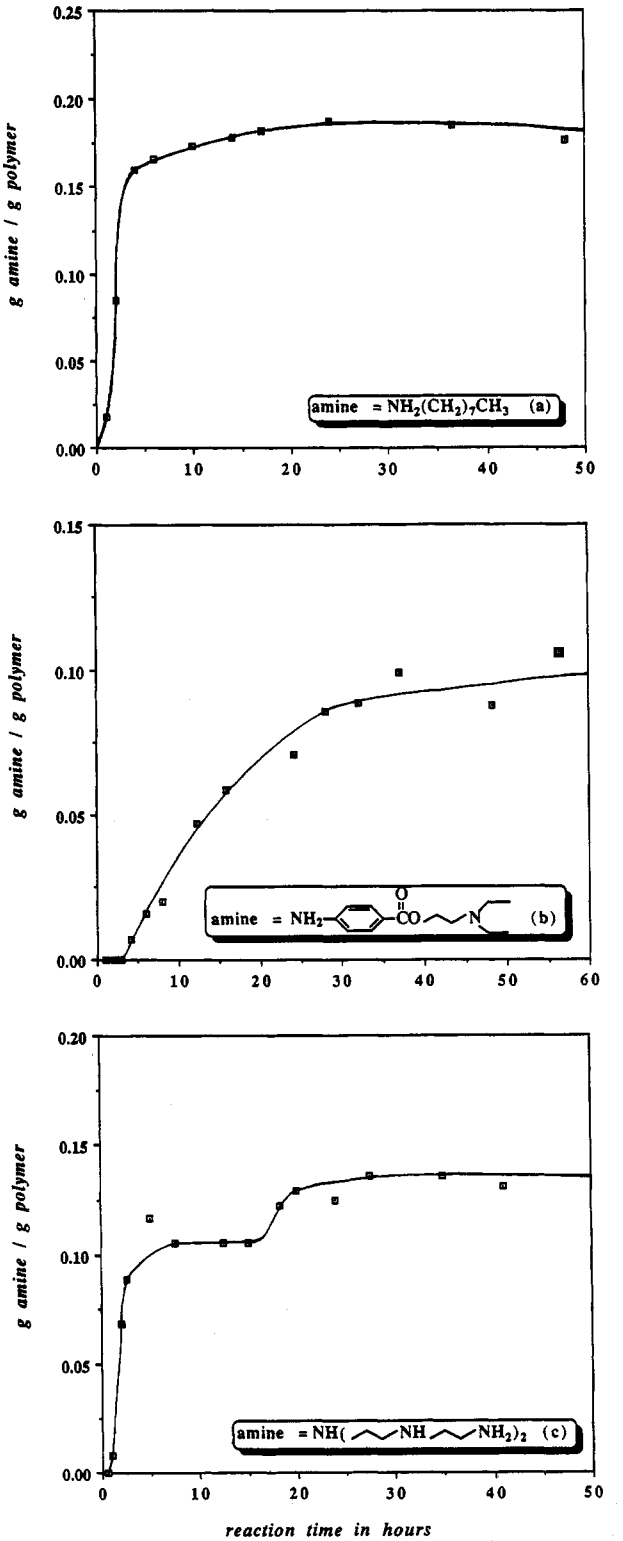
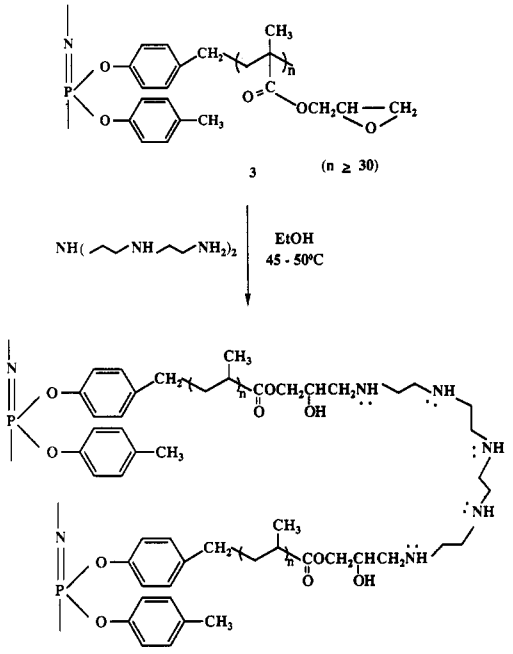


Figure 4. Graphed plots of the gravimetrically determined binding efficiencies of *n*-octylamine (**a**), procaine (**b**), and tetraethylenepentamine (**c**) with the grafted polymer films **3**.

Amines with multiple reaction sites, such as tetraethylenepentamine (tepa), underwent a more complex binding profile, as shown in (**c**). One explanation for this behavior is that the primary amino groups react rapidly in the early stages of the interaction, a process that would account for the high initial measured weight increase. Once a number of epoxy sites have reacted in this fashion, the immobilized amines may continue to react via the secondary amino sites and through any unreacted primary amino sites.

Scheme 3

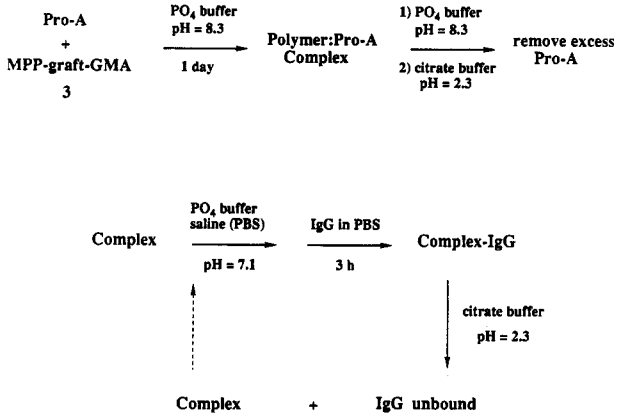


Although the secondary amines are less reactive, once the amino unit is bound to the MPP-*graft*-GMA substrate, their proximity to the remaining epoxy units may provide a positional advantage over the primary amine sites in unreacted free amine molecules. These reactions would consume epoxy groups without the additional uptake of amine molecules, as seen in the graphed gravimetric analysis data. After several hours this equilibrium shifts and a renewed binding of free amine molecules is detected. This sequential reaction pattern is mirrored in the graphed data set.

The amine-immobilized films were also characterized by use of ATR-IR spectroscopy. As shown in Figure 3, a new peak at 2900 cm^{-1} appears which was assigned to the aliphatic C–H stretch of the *n*-octylamine immobilized by MPP-*graft*-GMA substrate (system 8). Amine immobilization via ring-opening the epoxy groups was also reflected in a small decrease in the T_g from 3.1 °C for the MPP-*graft*-GMA to −1.6 °C for 8. However, for the multifunctional amines, a noticable *increase* in T_g was measured. For example, immobilization of the tepa group yielded a material (**10**) with a T_g of 17.9 °C. These data support the idea that both the primary and secondary amine sites react to yield a network system (see Scheme 3).

Immobilization of Protein A and IgG. The application of the amine immobilization process to the binding of protein A was also investigated.[13] Studies have shown that recombinant protein A specifically binds immunoglobulins (primarily IgG) with high affinity.[14,15] The preparation of a biocompatible polymeric support which could immobilize high levels of protein A, while not deactivating it to the subsequent binding of IgG, is an

Scheme 4



important target in biomedicine. An application for such a system is in the development of a blood dialysis system for the selective removal of IgG. High levels of this immunoglobulin have been linked to organ transplant rejection and hemophilic activity.

Suitable polymeric supports for this purpose must generate no cytotoxic side effects and should be inherently stable to changes in pH. A high intrinsic surface area and material porosity is also advantageous for the efficient immobilization of the protein. The preparation of this polymer:pro-A complex is outlined in Scheme 4.

After the formation of this complex, and removal of any residual, noncovalently bound protein A, the quantity of protein immobilized was determined. Lowry protein analysis was used to measure the amount of protein which was removed from a solution of known concentration after it had been placed in contact with a weighed amount of the solid polymeric support (see Experimental Section).

A weighed amount of the resultant polymer:pro-A complex was then exposed to a known concentration of human IgG in phosphate buffer saline (PBS) solution at body pH of 7.1. The resultant IgG–protein A:polymer complex was disrupted by washing with citrate buffer solution at pH of 2.3, and the amount of IgG eluted was measured. This process allowed (1) the determination of the level of protein A activity and calculation of the binding efficiency, and (2) the reactivation of the polymer:pro-A complex for repeated cyclized binding with IgG (see Scheme 4).

For the MPP-*graft*-GMA substrate, where the

$$\text{binding efficiency} = \text{IgG removed/protein A bound} \quad (1)$$

a relatively low binding efficiency of approximately 1.0 (3.2 mg/3.1 mg) was determined. One approach to increase this efficiency is to reduce the hydrophobic character of polymer **1**, in order to improve its reactivity under aqueous conditions. Thus, the synthesis of the cosubstituent poly[(*p*-methylphenoxy)(2-(2-methoxyethoxy)ethoxy)phosphazene] (**12**) was carried out. The single substituent poly[bis(2-(2-methoxyethoxy)ethoxy)phosphazene] (MEEP) is a water-soluble material that can be cross-linked by exposure to ultraviolet radiation.[16]

This photoinitiated graft substrate (MPP:MEEP-*graft*-GMA) was prepared, and its affinity for binding protein A and subsequently IgG was evaluated. Although a higher overall binding efficiency (ca. 2.0) was determined for this

(13) The protein A and IgG immobilization work was carried out by our collaborators Drs. L. F. Pelosi and J. P. O'Brien of duPont Fibers Division at the Experimental Station.

(14) Zeltzer, P. M.; Pepose, J. S.; Bishop, N. H.; Miller, J. N. *Infect. Immun.* **1978**, *21(1)*, 163.

(15) Coleman, P. L.; Walker, M. M.; Milbrath, D. S.; Stauffer, D. M.; Rasmussen, J. K.; Krepski, L. R.; Heilmann, S. M. *J. Chromatogr.* **1990**, *512*, 345.

(16) Nelson, C. J.; Coggio, W. D.; Allcock, H. R. *Chem. Mater.* **1991**, *3*, 786.

substrate, the relative quantities of protein A immobilized (≪1.0 mg) and IgG bound (1.5 mg) were greatly reduced. Further development of this process will depend on changes to the surface structure of the polymeric material.

Characterization of the Photoinitiation Grafted Film MPP-*graft*-VP. Films of MPP-*graft*-VP (**6**) were prepared by the previously described photoinitiation graft methodology (see Scheme 1). These films were analyzed by the use of SEM. They possessed surfaces very similar in appearance to the films of **3**. ATR-IR analysis confirmed the presence of the grafted 4-vinylpyridine groups. The aromatic C—H stretch was detected at 3080 and 3030 cm^{-1} and the aliphatic C—H stretch at 2950 and 2860 cm^{-1}. The C—C and C—N ring stretching peaks assigned to the pyridine were present between 1640 and 1400 cm^{-1}. The aromatic C=C stretch of the MPP at 1510 cm^{-1} and the P=N stretch at 1200 cm^{-1} were also identified.

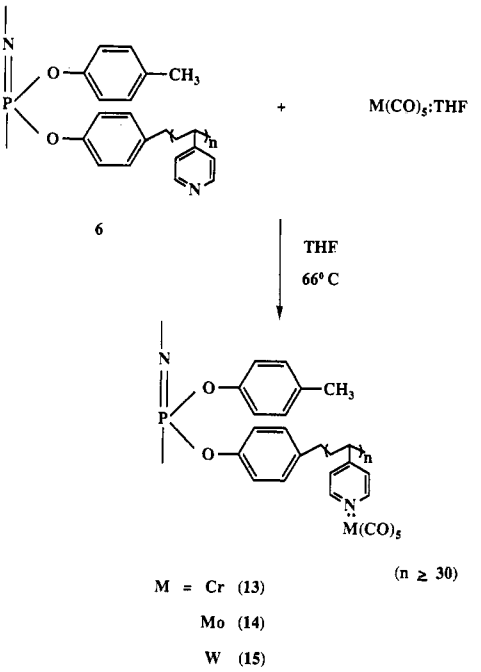
Differential scanning calorimetry revealed a T_g at 4.3 °C for the grafted film (**6**), an increase of approximately 8 °C above that of the parent polymer **1** (T_g = −3.9 °C). Again, this increase can be attributed both to reduced side-group mobility with grafting of the polyVP and the presumed cross-linking of the parent MPP. Elemental microanalysis of films of the MPP-*graft*-VP (**6**) revealed an increase in the %C/%P ratio and a decrease in the %C/%N ratio from the parent polymer **1**.

Reactions of MPP-*graft*-VP with Metal Carbonyls. The functionalized MPP-*graft*-VP (**6**) films were examined as immobilization substrates for organometallic species, with the pendent pyridine units providing potential metal coordination sites. An initial model synthesis of $Cr(CO)_5$:NC_5H_5 via the reaction of $Cr(CO)_5$:THF with pyridine was carried out. This was then followed by an investigation of the coordination behavior of the graft polymers (see Experimental Section). Films of MPP-*graft*-VP were allowed to react with $M(CO)_5$:THF, where M = Cr, Mo, and W, at reflux in tetrahydrofuran solution as shown in Scheme 5.[17] This resulted in the coordinative linkage of organometallic groups to the polymeric support (see Experimental Section).

The extent of metal coordination varied with the choice of metal carbonyl and typically increased with increasing reaction times. The chromium species was immobilized more efficiently than the molybdenum or tungsten analogues. The coordinative efficiency of the films was defined as the weight of the organometallic species bound divided by the initial weight of the polymer film. For example, after 84 h of exposure of the MPP-*graft*-VP (**6**) film to $Cr(CO)_5$:THF, 41.5 mg of the organometallic species was coordinated, a binding efficiency of approximately 1. By comparison, only 6.4 mg of the tungsten analogue (efficiency of ca. 0.1) was coordinated.

A dramatic color change was detected after immobilization of the organometallic species. For example, the color changed from off-white to a deep blue when chromium (**13**) was coordinated, to a dark brown for molybdenum (**14**), and a dark yellow for tungsten (**15**). An increase in the T_g of the organometallic bound substrates was detected for the chromium immobilized film (T_g = 13.0 °C), presumably due to the high loading achieved.

Scheme 5



X-ray microanalysis of the organometallic bound films confirmed the presence of the metallo species. ATR-IR analyses of the films (45° Ge and KRS crystals) revealed peaks assignable to the MPP-*graft*-VP (**6**) films as described previously. However unambiguous assignment of the $M(CO)_5$ carbonyl peaks was not possible due to the extreme complexity of this spectral region.[18] One explanation for this complexity is a possible side reaction which involves coordination of the $M(CO)_5$:THF species with the polymer to form η^6-arene $M(CO)_3$ groups.[19] To examine this possibility, a series of control reactions was performed in which UV cross-linked films of polymer **1** were exposed to $M(CO)_5$:THF species in THF at reflux. Although no change in film color was detected after prolonged reaction times, trace amounts of the metal species were detected by use of X-ray microanalysis.

Reactions of MPP-*graft*-GMA Bound Amines with Metal Salts. The MPP-*graft*-GMA (**3**) films with immobilized amines offered an opportunity to utilize these secondary reactive sites for the coordination of metal salts. Of primary interest were the tetraethylenepentamine (tepa) and triethylenetetramine (teta) species which have the potential to mimic chelation sites and display pseudo crown ether behavior.[20]

Initially, an investigation was carried out of the dependence of the binding efficiency of the MPP-*graft*-GMA:tepa substrate (**10**) on (1) reaction *temperature* and method of film agitation, (2) reaction *solvent*, and (3) solvent *pH*. Films of **10** were exposed to solutions of $CuCl_2$ for 24 h, purified via sonication, and their binding efficiencies (g of metal/g of amine) determined by gravimetric analysis.

The effect of *temperature* was explored when the reaction was performed in EtOH with the use of a

(17) For relevant examples of group VIA metal carbonyl complex reactions, see: Kertley, S. W. In *Comprehensive Organometallic Chemistry*; Wilkinson, G., Stone, F. G. A., Abel, E. W., Eds.; Pergamon: Oxford, 1982; Vol. 3, pp 817–825, 1097–1105, 1271–1274, and references therein.

(18) Predicted ν_{CO} patterns for $LM(CO)_5$ species are discussed in: Lukehart, C. M. *Fundamental Transition Metal Organometallic Chemistry*; Wadsworth Inc.: Monterey CA, 1985; Chapter 3.

(19) Allcock, H. R.; Dembek, A. A.; Klingenberg, E. H. *Macromolecules* **1991**, *24*, 5208.

(20) (a) Cowie, J. M.; Wadi, N. M. A. *Polymer* **1985**, *26*, 1566. (b) Arpadjan, S.; Mitewa, M.; Bontchev, P. R. *Talanta* **1987**, *34(11)*, 953. (c) Suh, J.; Cho, Y.; Lee, K. J. *J. Am. Chem. Soc.* **1991**, *113*, 4198.

mechanical shaker at 25 °C, a sonication bath at 45–50 °C, and in the boiling solvent at 78 °C. An almost 2-fold increase in binding efficiency occurred under sonication bath conditions. The role of the *solvent* used was also studied. The binding efficiency of **10** for $CuCl_2$ with various solvents decreased as EtOH > MeOH > EtAc > THF > H_2O under sonication bath conditions. When the solvent used was H_2O, a variation of the reaction *pH* from 1 to 11 produced no measurable effect (within experimental error) on the calculated binding efficiency of the substrate films **10**. Thus 100% EtOH and sonication bath conditions were utilized throughout the remainder of the investigation.

The binding efficiency of films of MPP-*graft*-GMA:tepa (**10**) and MPP-*graft*-GMA:teta (**11**) for a variety of alkali, alkaline, and transition metal halides was studied. A complementary series of control reactions with films of UV cross-linked polymer **1** (no binding detected) and MPP-*graft*-GMA (**3**) (some binding detected, probably via ring-opening of the epoxy groups)[21] was also explored. After exposure to the metal halide solutions, these films were characterized by gravimetric analysis. Substrates with a measured weight increase were examined further by use of X-ray microanalysis to confirm the presence of the metal species.

The binding efficiencies of alkali and alkaline metal chlorides with ionic radii that increase in the order Mg < Li < Ca < Na < K were determined.[22] The film substrates **10** and **11** showed an affinity for all of these salts except for potassium chloride (metal ionic radius = 138 pm). Presumably this exclusion behavior can be attributed to the size of the metal ion. Those metal halides which bound to the substrates contained metals with ionic radii between 72 and 102 pm.

Coordination of a series of transition metal halides Cr(II) through Zn(II), Mo(V), W(IV), Cd(II), and Hg(II) was also studied.[22] The teta-immobilized substrate **11** had the highest binding efficiency for the WCl_4 species, which has a metal ionic radius of 66 pm. In contrast, the tepa bound substrate **10** showed a marked preference for the $CrCl_2$ species which has a 73-pm ionic metal radius. These results suggest the potential for selective binding of metals based on the relative *ring size* possible with the MPP-*graft*-GMA bound tetra- or pentafunctional amine. However, both substrates **10** and **11** showed some affinity for all the transition metal halides studied. Thus, any presumed specificity, based on difference in ring size for the bound teta and tepa amines, was not sufficient to bring about a clean separation of metals with ionic radii between 61 and 102 pm.

On the basis of these data it was concluded that, for binding to occur, a probable upper limit value for the ionic radius of the metal species would be between 102 and 138 pm. Thus, with the potential for binding selectivity for either substrate, based on *cation size*, the immobilization of Hg(I) (119 pm) and Hg(II) (102 pm) chloride was explored. Both of the substrates **10** and **11** showed a greater binding affinity for the smaller Hg(II) species. However a measurable amount of the Hg(I) halide was also immobilized. Although this limits the potential uses of these substrates, it is clear that the polymers can be used for the separation of metal halides with ionic radii <120 pm from those with larger radii (ca. 138 pm). Apart from the question of selectivity, these immobilization substrates are useful for the *general* binding of a wide variety of alkali, alkaline, and transition metal halides.

(21) (a) Onaka, M.; Kawai, M.; Izumi, Y. *Chem. Lett.* **1985**, *6*, 779. (b) Ciaccio, J. A.; Heller, E.; Talbot, A. *Synlett* **1991**, *4*, 248. (c) Crivello, J. V.; Fan, M. *Makromol. Chem., Macromol. Symp.* **1992**, *54/55*, 179.

(22) Metal ionic radii are quoted from: Greenwood, N. N.; Earnshaw, A. *Chemistry of the Elements*; Pergamon Press Ltd.: Oxford, 1986.

Experimental Section

Equipment. ^1H NMR (360.0 MHz), ^{13}C NMR (90.0 MHz), and ^{31}P NMR (145.8 MHz) spectra were obtained using a Bruker WM-360 MHz spectrometer. Chemical shifts are relative to tetramethylsilane at $\delta = 0$ for proton and carbon. The phosphorus chemical shifts are relative to 85% H_3PO_4 at $\delta = 0$, with positive shift values downfield from the reference. All heteronuclear NMR spectra were proton decoupled. Molecular weights were determined using a Hewlett-Packard HP 1090 gel permeation chromatograph equipped with an HP-1037A refractive index detector and Polymer Laboratories PL gel 10-μm columns. The samples were eluted with a 0.1 wt % solution of tetra-*n*-butylammonium bromide in THF. The GPC column was calibrated with polystyrene standards (Waters) and with fractionated samples of poly[bis(trifluoroethoxy)phosphazene] provided by Drs. R. Singler and G. Hagnauer of the U.S. Army Materials Technology Laboratories, Watertown, MA.

A 450-W Canrad-Hanovia medium-pressure, quartz, mercury vapor lamp equipped with a water-cooled quartz immersion well was utilized. The typical emitted ultraviolet radiation for the system is between 220 and 400 nm. A Branson Ultrasonic bath (50/60 Hz, 117 V) was used for film purification and specified immobilization reactions. Infrared spectra were recorded using a Perkin-Elmer Model 1710 FTIR spectrophotometric instrument interfaced with a Perkin-Elmer 3600 Data Station. A Harrick variable-angle twin parallel mirror reflection attachment equipped with a 45° Ge or KRS crystal (50 × 10 × 3 mm) was used to obtain all ATR-IR spectra. A Rame'-Hart Model 100 Goniometer equipped with an environmental chamber maintained at 100% relative humidity was used to measure the contact angle of a sessile drop of deionized water on the surface of the films. The reported values are the average of repeated measurements taken at different locations on the film surface. The electron microscopy work was performed at the Electron Microscopy Facility for the Life Sciences in the Biotechnology Institute at The Pennsylvania State University. Scanning electron microscopy (SEM) was carried out using a JEOL JSM-5400 scanning electron micrograph with X-rays accelerated at 20 kV. X-ray microanalyses were determined via the SEM interfaced with a Princeton Gamma-Tech (PGT) IMIX (integrated microanalyzer for imaging and X-ray) system. X-ray photoelectron spectra were obtained at the Perkin-Elmer Physical Electronics Laboratory. An Mg anode (Mg Kα = 1486 eV) operated at 600 W (15 kJ, 40 mA) was used as the primary excitation source. Charge compensation was accomplished by flooding the sample surface with low energy (<2 eV) electrons. Thermal analyses were carried out by use of a Perkin-Elmer Thermal Analysis System 7 equipped with a Perkin-Elmer 7500 computer. For the determination of glass transition temperatures by differential scanning calorimetry, a heating rate of 10–20 °C/min under a nitrogen atmosphere was used with a sample size between 10 and 30 mg.

Materials and Procedures. Where specified, reactions were carried out under an atmosphere of dry argon (Linde), with use of standard Schlenk and drybox techniques. Tetrahydrofuran (Omnisolv) and dioxane (Aldrich) were dried and distilled from sodium benzophenone ketyl. Fractional sublimation at 30 °C/10 μm was utilized to purify the hexachlorocyclotriphosphazene, which was provided by Ethyl Corp. Poly(dichlorophosphazene) was prepared by the thermal ring opening polymerization of the trimer at 250 °C.[23] The phenol (Aldrich) and the *p*-methylphenol (Aldrich) were purified by recrystallization from pentane and sublimation at 10 °C/10 μm and were then stored in vacuo. The 2-(2-methoxyethoxy)ethanol (Aldrich) was distilled in vacuo and was stored under an inert atmosphere. Benzophenone (Aldrich)

(23) Allcock, H. R.; Kugel, R. L. *J. Am. Chem. Soc.* **1965**, *87*, 4216.

was purified via recrystallization from EtOH and was dried under high vacuum. The graft monomers used [glycidyl methacrylate, glycidyl acrylate, 4-vinylpyridine (Aldrich), and vinyltris(trimethylsiloxy)silane (Petrarch)] were purified by fractional vacuum distillation. The 100% EtOH (Midwest Grain Products Co.) was stored over molecular sieves before use. The amines (Aldrich) were purified by fractional vacuum distillation except for the procaine which was used as received. The $M(CO)_6$ species (Strem, Aldrich) were purified by sublimation at 25 °C/2 μm with use of a dry ice/acetone cooled cold finger. The alkali and alkaline metal halides (Baker, Aldrich, Fisher) and the transition metal halides (Strem, Aldrich, Alfa Inorganics) were used as received. Elemental analyses were performed by Galbraith Laboratories in Knoxville, TN, and Schwarzkopf Microanalytical Laboratory in Woodside, NY.

Poly[bis(*p*-methylphenoxy)phosphazene] (1). Poly(dichlorophosphazene) (17.0 g, 0.15 mol) dissolved in dry dioxane (500 mL) was added to a solution that contained an excess of sodium *p*-methylphenoxide (3 equiv/PCl) in dry dioxane (1000 mL) in a 2-L three-necked round-bottomed flask equipped with a condenser and a mechanical stirrer. The reaction solution was boiled at reflux for 4 days under argon and the progress of the P–Cl bond replacement was monitored by use of ^{31}P NMR spectroscopy. After completion of the reaction, the solution was concentrated to approximately 200-mL total volume and the product polymer was isolated by precipitation into water. Purification of polymer 1 was accomplished via Soxhlet extraction with 100% EtOH (2 days), hexane (1 day), and 1:1 H_2O:95% EtOH (2 days). The polymer was dried under vacuum to yield a white, fibrous material (70%). For 1: ^{31}P NMR ($CDCl_3$) δ −18.8 ppm (s); ^1H NMR ($CDCl_3$) δ 6.8–6.4 (m, Ar), 2.2–2.0 ppm (m, CH_3); ^{13}C NMR ($CDCl_3$) δ 149.6, 132.3, 129.2, 121.1 (Ar), 20.6 ppm (CH_3); T_g = −3.9 °C; $M_w = 5.4 \times 10^6$, $M_n = 3.3 \times 10^6$, M_w/M_n = 1.6. Anal. Calcd: C, 64.86; H, 5.44; N, 5.40; Cl, 0.00. Found: C, 64.50; H, 5.46; N, 5.02; Cl, 0.52.

Poly[bis(phenoxy)phosphazene] (2). Poly(dichlorophosphazene) (5.0 g, 43.1 mmol) was dissolved in dry dioxane (250 mL) and was added dropwise to an excess of sodium phenoxide (3 equiv/PCl) in dry dioxane (600 mL). This reaction solution was boiled at reflux for 5 days under argon. The solution was concentrated to an approximate volume of 100 mL and was precipitated into hexane and H_2O. Purification of the product polymer was accomplished by use of Soxhlet extraction with 95% EtOH (2 days) and hexane (2 days). The polymer was dried in vacuo to yield a white, fibrous material (81%). For 2: ^{31}P NMR ($CDCl_3$) δ −19.1 ppm (s); ^1H NMR ($CDCl_3$) δ 6.9–6.7 ppm (m, Ar); ^{13}C NMR ($CDCl_3$) δ 151.6, 128.8, 123.6, 121.2 ppm (Ar); T_g = −8.4 °C; $M_w = 3.2 \times 10^6$, $M_n = 1.8 \times 10^6$, M_w/M_n = 1.8. Anal. Calcd: C, 62.34; H, 4.36; N, 6.06; Cl, 0.00. Found: C, 61.39; H, 4.00; N, 5.75; Cl, 0.22.

Poly[(*p*-methylphenoxy)(2-(2-methoxyethoxy)ethoxy)phosphazene] (12). Sodium *p*-methylphenoxide (0.5 equiv/PCl) was prepared by the reaction at 66 °C of *p*-methylphenol (9.3 g, 86.2 mmol) with sodium (2.2 g, 94.8 mmol) suspended in dry THF (150 mL) for 12 h. This sodium salt solution was then transferred via cannula to an addition funnel and was added in a dropwise fashion to a solution of poly(dichlorophosphazene) (10.0 g, 86.2 mmol) in dry THF (200 mL). The reaction solution was stirred with use of a mechanical stirrer and was heated to reflux for 48 h. Subsequently, dropwise addition of sodium 2-(2-methoxyethoxy)ethoxide (0.6 equiv/PCl) in dry THF (150 mL) to the cooled polymer solution was carried out. The reaction solution was heated at reflux for 3 days until no residual P–Cl units were detected by use of ^{31}P NMR spectroscopy. The reaction solution was then concentrated to 100-mL total volume and the product polymer was isolated by precipitation into water and hexane. Further purification of polymer 12 was accomplished by Soxhlet extraction with hexane (3.5 days). The adhesive, off-white polymer was dried under high vacuum to yield the product 12 (40%). For 12: ^{31}P NMR ($CDCl_3$) δ −20.3, −15.1, −9.9 ppm (s); ^1H NMR ($CDCl_3$) δ 7.2–6.7 ppm (Ar), 4.2–3.7, 3.7–3.2 ppm (OCH_2CH_2, OCH_3), 2.4–2.1 ppm (CH_3); ^{13}C NMR ($CDCl_3$) δ 149.8, 132.6, 129.3, 121.0 ppm (Ar), 71.8, 70.1, 65.7, 65.1 ppm (OCH_2CH_2), 58.8 ppm (OCH_3), 20.7 ppm (CH_3); T_g = −59.0 °C; $M_w = 8.0 \times 10^5$, $M_n = 3.1 \times 10^5$, M_w/M_n = 2.6. Anal. Calcd: C, 51.15; H, 6.77; N, 5.21; Cl, 0.66. Found: C, 50.98; H, 6.80; N, 4.94; Cl, 0.71.

Preparation of Photoinitiation Grafted Films: General Procedure. The polymer films were prepared by a solution-casting method in which a solution, that contained 1 equiv of MPP, 2 equiv of monomer, and 10 mol % of benzophenone as the photoinitiator, was poured onto a glass plate with the film boundaries defined by an aluminum frame. The solvent was allowed to evaporate slowly in a near-solvent-saturated, dust-free environment. The films were then removed from the glass plate, cut into small sections, and placed in quartz reaction tubes. These tubes were purged with argon for 1 min and were then sealed with rubber septa. The quartz tubes were placed in a rotating carousel at an approximate distance of 7 cm from the unfiltered 450-W quartz mercury vapor lamp and were irradiated for 20 min. The resultant photoinitiation grafted films were purified via sonication in THF (2 days), hexane (1 day), and finally 100% EtOH (2 days), cut into smaller sections approximately (20 × 8 × 0.1 mm) and then dried, and initially flattened between glass slides and later dried under high vacuum, to constant weight.

Preparation of MPP-*graft*-GMA. Polymer 1 (3.0 g, 11.6 mmol, 1 equiv) was dissolved in dry THF (90 mL) with warming of the stirred solution. To this solution was added glycidyl methacrylate (3.2 mL, 23.1 mmol, 2 equiv) and benzophenone (0.21 g, 1.2 mmol, 0.1 equiv) to yield a homogeneous solution. Preparation and purification of the photoinitiation grafted films (MPP-*graft*-GMA) were carried out as described above. These opaque, white films were dried, initially flattened between weighted-down glass slides and later under high vacuum, to constant weight. Characterization data for 3: vide infra.

A similar methodology was employed for the preparation of all of the photoinitiation grafted films. Characterization data for MPP-*graft*-GA (4): ATR-IR (C=O stretch at 1734 cm^{-1}); DSC (T_g = 3.3 °C). For MPP-*graft*-VMS (5): ATR-IR (Si–O–Si stretch at 1062 cm^{-1}, Si–CH_3 stretch at 1253 cm^{-1}); DSC (T_g = 4.0 °C). For MPP-*graft*-VP: vide infra.

MPP-*graft*-GMA Immobilization of Amines. The grafted films 3 of known weight were immersed in solutions of the amine (8.5 mmol/film) in 100% EtOH with a total volume of 12.7 mL in Wheaton reaction vials. These vials were sealed with pierced caps and were sonicated in a water bath with an average temperature of 45–50 °C. The films were removed at various time intervals, rinsed with 100% EtOH, and purified by sonication in THF (3 days) and 100% EtOH (3 days). The amine-immobilized films were then flattened between weighted glass slides and were allowed to dry in air before high-vacuum drying. Gravimetric analysis (±0.1 mg) was used to determine the binding efficiency (g of metal/g of amine) of the polymer films 3 for the amines.

MPP-*graft*-GMA Immobilization of Protein A and Binding of IgG.[13] *Background*: Protein A is a stable protein of molecular weight approximately 42 000 daltons. Recombinant protein A specifically binds immunoglobulins (primarily IgG) at the Fc region of the molecule with high affinity. Recombinant protein A (Calbiochem) was obtained as a salt-free, lyophilized powder of greater than 98% purity, as determined by SDS gel electrophoresis and HPLC analysis. The specific activity of recombinant Protein A is equivalent to most protein A preparations extracted from *S. aureus* cultures with a dissociation constant to rabbit IgG of 10^{-9} M at pH 7.4 as measured by solid-phase radioimmunoassays. Each vial contained 100 mg of recombinant protein A with an extinction coefficient (1% at 280 nm) of 1.6.

Unconjugated human IgG whole molecule (Organon Tekniika) extracted from pooled human serum was purified chromatographically. The IgG was filtered and lyophilized and contained 0.01 M sodium phosphate (pH 7.3) with 0.07 M sodium chloride when restored with distilled H_2O to yield fractions of approximately 90–95% IgG. Each vial contained 50 mg of IgG with an extinction coefficient (1% at 280 nm) of 1.4.

Lowry and UV absorption at 280 nm were used for protein analysis. Calibration curves were determined with a Milton Roy Co. Spectronic 1201 UV/visible spectrophotometer with an autosipper. All unknown samples were centrifuged before analysis. The estimated error in the protein analyses was from 5 to 10%

and resulted from (1) the small aliquots sampled, (2) the different color development of different proteins, and (3) errors from the absorbance intensity on the Lowry calibration curve.

The Lowry protein assay (Sigma Chemical kit) was carried out directly on the protein solutions. The method is based on the complex formation of the alkaline cupric tartrate reagent with the peptide bonds. This forms a soluble purple–blue color when the phenol reagent is added and the absorbance is measured at 750 nm. Calibration curves were determined with use of bovine serum albumin (BSA), protein A (measured A-280 mg/mL = 0.159, reported extinction coefficient = 0.16), and IgG (measured A-280 mg/mL = 1.42, reported extinction coefficient = 1.4).

Proteins absorb at 280 nm solely due to the tyrosine and tryptophan amino acids in their backbone. Since the content of these amino acids is variable for each protein, their absorbance at a particular concentration will also vary. Protein A has a very low absorbance in the UV since it contains a low level of tyrosine residue. Thus a calibration curve for the UV method was measured with use of IgG.

Evaluation: The immobilization of protein A to a solid support was determined by measurement of the disappearance of the protein from the solution in contact with the solid support. The amount of protein immobilized was determined as the difference between the initial and final concentration. All protein supports and samples were stored at 4 °C.

Support pretreatment and controls: Weighed amounts of untreated supports were loaded into tared columns and were washed (with 30 times their volume) to remove surface contaminants. Thus 1 g of the support, that had been ground to a very small particle size, was washed with (1) methanol, (2) citrate buffer at pH = 2.3, (3) distilled H_2O, and (4) phosphate buffer at pH = 8.3. The excess PO_4 buffer was flushed out of the column and the weight of the damp support was determined. The weight of retained buffer was used later to obtain a more accurate analysis of the protein concentration. The untreated damp supports were used as control samples.

Protein A immobilization: This procedure is outlined in Scheme 4. (1) A weighed amount of protein A solution of known concentration (ca. 5 mg/mL) in PO_4 (pH 8.3) buffer was added to the weighed damp support. The mixture was rolled or rocked back and forth for 24 h at 25 °C. Duplicate aliquots were removed at 1, 3, and 24 h for Lowry protein analysis. (2) After 24 h the protein solution in the column was flushed out and collected. The support was washed six times with 5 mL each of PO_4 (pH 8.3) buffer and these solutions were also analyzed for Protein A. (3) The polymer:Pro-A complex was then washed six times with 5-mL portions of citrate (pH 2.3) buffer. This was done to elute any protein A which might elute later under the acidic conditions used to determine the activity of the immobilized protein A. Lowry protein analyses of these fractions typically detected very little protein A. (4) Finally, the polymer:Pro-A complex was washed with 30 times its volume of phosphate buffer saline (PBS) of pH 7.1 to establish body pH levels. All of the PBS was discarded and the weight of the damp protein A support was redetermined.

Determination of protein A activity: As shown in Scheme 4, (1) a weighed amount of human IgG solution of known concentration (ca. 5 mg/mL) in PBS solution was added to the damp polymer:Pro-A complex. The mixture was agitated by rolling at 25 °C for 3 h, and duplicate aliquots were then removed from the protein solution at 1-, 2-, and 3-h intervals for Lowry and UV protein analyses. (2) After 3 h the protein solution was flushed out of the column and collected. The complex support was washed six times with 5 mL each of PBS, and the fractions were analyzed for IgG content. (3) The polymer:Pro-A:IgG complex was then disrupted by lowering the pH to 2.3 with citrate buffer. Six fractions (5 mL each) were collected and analyzed by Lowry and UV protein analyses. The amount of protein eluted under these conditions represents the amount of IgG complexed with protein A on the polymer support. This gave a measure of the level of protein A activity and allowed calculation of the binding efficiency. (4) The polymer:Pro-A complex support was then washed with 30 times its volume of PBS solution. This established a pH of 7.1 and reactivated the protein A for complex formation with IgG. Typically, the supports were retested two to three times to detect large changes in the binding capacity of the protein A support after exposure to acidic conditions.

MPP-*graft*-VP Immobilization of Metal Carbonyl Species. Preparation of the $M(CO)_5$:THF species, where M = Cr, Mo, W was accomplished by heating $M(CO)_6$ (17.0 mmol/film) in dry THF (25 mL) at 66 °C under an inert atmosphere for 24 h. The reaction solution became dark yellow/orange in color, and this was indicative of formation of the $M(CO)_5$:THF species. This adduct was also detected by FTIR analysis of the reaction solution. Grafted films **6** of known weight were added to each reaction solution with several boiling chips to provide agitation, and the solution was heated to 66 °C for various times (24–96 h). The metal carbonyl bound films were rinsed with 100% EtOH and were purified by sonication in hexane (1 day), THF (1 day), and 100% EtOH (1 day). The films were flattened between glass slides before vacuum drying for gravimetric analysis measurements and characterization. M = Cr: T_g = 13.0 °C, dark blue color, 41.5 mg immobilized after 3.5-day reaction (coordination efficiency ca. 1). M = Mo: T_g = 1.4 °C, dark brown color, 4.0 mg immobilized after 3.5-day reaction (coordination efficiency ca. 0.1). M = W: T_g = 2.5 °C, dark yellow color, 6.4 mg immobilized after 3.5-day reaction time (coordination efficiency ca. 0.1).

Metal Halide Bound MPP-*graft*-GMA:amine Substrates. Solutions of the metal halide (1.0 g/film) in 100% EtOH (25 mL) were prepared under atmospheric conditions in Wheaton vials. Films of the MPP-*graft*-GMA substrate with immobilized "tepa" (**10**) and "teta" (**11**) were immersed in the metal halide solutions and the vials were sealed with pierced caps. These reaction vials were sonicated for 24 h in a water bath. The films were then removed, rinsed with 100% EtOH, and purified by sonication in THF (2 days), H_2O (2 days), and 100% EtOH (2 days). The films were dried flattened under ambient conditions before high-vacuum drying and analysis. For the transition metal halides, the highest binding efficiencies (g of metal halide/g of amine) were found for $CrCl_2$ (3.4) with the tepa substrate (**10**) and for WCl_4 (1.3) with the teta substrate (**11**). The measured binding efficiencies for the alkali/alkaline earth metal chlorides ranged from 0.2 with $CaCl_2$ (substrates **10** and **11**) to 0.03 with LiCl (for substrate **11**). X-ray microanalysis confirmed the presence of Mg and Ca but could not be used reproducibly to assess the quantities present. Elemental microanalysis was employed to identify the presence of Li and Na in the samples.

Conclusions

The preparation of photoinitiation grafted poly(organophosphazenes) via the UV irradiation of films of MPP which contain bifunctional monomers is described. These functionalized immobilization substrates were then utilized to bind amines, biologically active compounds, and metal species. Protein A immobilized on the MPP-*graft*-GMA substrate was found to retain its activity for the subsequent binding of IgG. Further, multifunctional immobilized amines were exploited to bind metal halides, and these systems show some potential for ion filtration and ion separations.

Acknowledgment. We thank the Office of Naval Research for the support of this work. The authors appreciate the collaborative efforts of Drs. J. P. O'Brien and L. F. Pelosi of the duPont Co. We are also grateful to Dr. P. Maslak and his co-workers at Penn State for use of the UV photolysis facilities, R. Walsh for assistance with the X-ray microanalyses and SEM data, and Drs. R. J. Fitzpatrick and L. Salvati, who performed the XPS analyses.