1 to **2** also provides a clean, definitively characterized system in which to probe several additional questions, notably, the origin of the rearrangement's driving force,[18] its detailed mechanism, and the carbon–benzyl (C–CH_2Ph) bond energy (BDE)[19,20] in **2**, apparently *about 24 kcal/mol*—roughly one-third that of a normal C–CH_2Ph BDE—based on preliminary studies.[19] The needed studies are nearing completion and will be reported in due course.[10]

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Supplementary Material Available: Details of the X-ray structural analysis of **2**·0.5C_6H_6 including an ORTEP diagram, tables of refined atomic coordinates, bond lengths and angles, and tables of calculated hydrogen atom coordinates, and anisotropic thermal parameters (12 pages); listing of observed and calculated structure factors (16 pages). Ordering information is given on any current masthead page.

(18) The overall driving force ΔG must be small, since $K_{eq}(69°C) = 1.5$, but the ΔH and ΔS components are of interest.[10]

(19) Initial work thermolyzing **2** with TEMPO, under conditions first order in **2** and zero order in TEMPO, gives a $\Delta H^{\ddagger} = 26 \pm 2$ kcal/mol in benzene and thus a C–CH_2Ph BDE *estimate* (once radical-cage effects are taken into consideration)[20] of 24 kcal/mol.

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Ionically Cross-Linkable Polyphosphazene: A Novel Polymer for Microencapsulation

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Synthetic polymers are used increasingly in medical science due to the chemist's ability to incorporate specific properties such as strength, hydrogel characteristics, permeability, or biocompatibility, particularly in fields like cell encapsulation and drug delivery where such properties are often prerequisites. However, harsh conditions, e.g., heat or organic solvents, are always used when encapsulating with these polymers,[1] often causing difficulties in encapsulating sensitive entities, e.g., proteins, liposomes, and mammalian cells. At the opposite extreme, a natural polymer, alginate, extracted from seaweed, has been widely used for cell encapsulation.[2] This polymer can be ionically cross-linked in water to form hydrogels that fulfill many of the above requirements. However, natural polymers display variable biocompatibility and some properties can be reproduced only with difficulty.[1b]

Until now, no synthetic polymer has existed that can encapsulate sensitive entities under mild conditions. Here we report the development of a polyphosphazene that forms gel matrices by simply adding divalent cations in water at room temperature and can encapsulate mammalian cells, liposomes, and proteins.

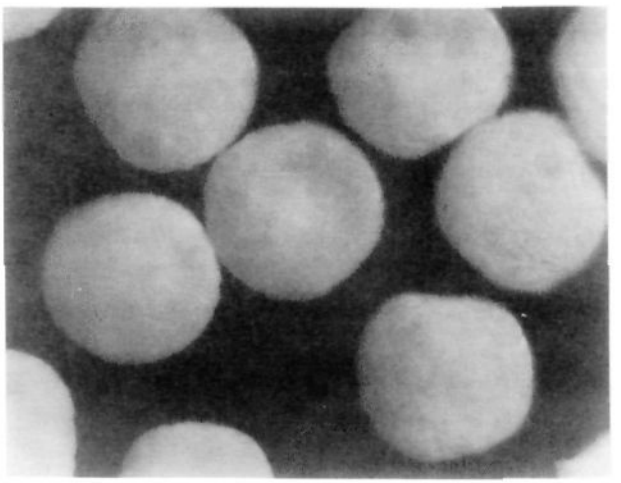
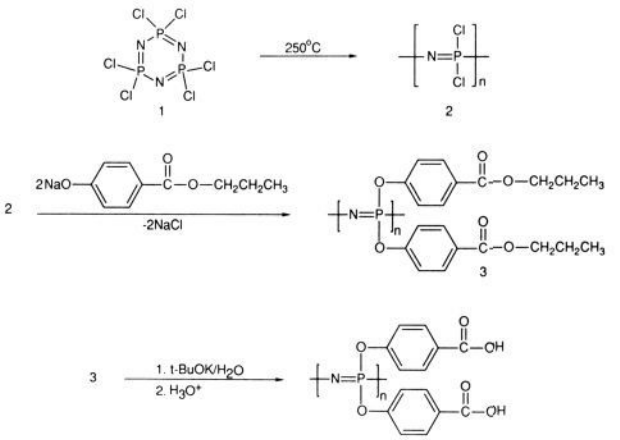


Figure 1. Ca-PCPP microspheres via phase contrast microscopy (magnification 1540×).[4a]

Scheme I. Poly[bis(carboxylatophenoxy)phosphazene] Synthesis



Poly[bis(carboxylatophenoxy)phosphazene] (PCPP (**4**)] (Scheme I) was prepared by first synthesizing poly(dichlorophosphazene) (**2**) by thermal bulk polymerization of hexachlorocyclotriphosphazene (**1**). Chlorine atoms were then replaced by carboxylate ester containing side groups, by reacting propyl *p*-hydroxybenzoate with **2**, forming poly[bis(aryloxy)phosphazene] ester **3**, followed by hydrolysis of ester groups to carboxylic acids (**4**).[3]

PCPP was insoluble in acidic or neutral solvents but soluble in basic solutions, e.g., sodium carbonate. The dissolution of 10% (w/v) PCPP in 30 mg/mL sodium carbonate caused a decrease in solution pH to 7.5–7.8 (due to polymer deprotonation), enabling mild encapsulation. When Ca^{2+} was added to PCPP, fast gelation occurred. Presumably, Ca^{2+} forms salt bridges between carboxylic groups of adjacent polymers, creating an ionically cross-linked matrix (Ca-PCPP).[3] Microspheres (Figure 1) were prepared by using a droplet-forming apparatus.[4a] Their shape and size depended on polymer and calcium ion concentrations, polymer extrusion rate, air flow, and needle diameter.[5]

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(4) (a) Microspheres were prepared by spraying aqueous PCPP (2.5% w/v) with FITC-BSA (20 mg; Sigma), or β-gal (1 mg; Sigma No. G-5635), or hybridoma cells (5 × 10^6 cells; ATCC HB123), into 7.5% w/v $CaCl_2$, using a droplet-forming apparatus.[2,5] Beads were hardened for 30 min and coated with 30 mL of 0.25% (w/v) PLL (MW 21.5 kDa; Sigma) for 30 min. (b) Release studies were performed at 37 °C, with gentle agitation, in vials containing 10 mL of phosphate-buffered saline (PBS) at pH 7.4, with 0.01% gentamicin sulfate as preservative. FITC-BSA and β-gal release was followed by absorbance at 495 nm and BCA protein assay (Pierce No. 23235), respectively. Hybridoma cell viability was followed by a trypan blue exclusion assay.

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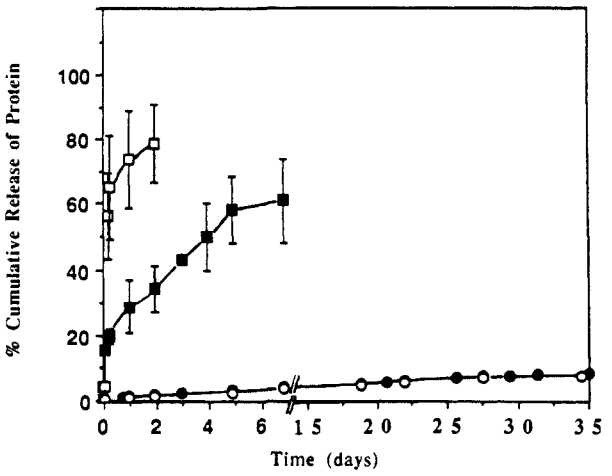

Figure 2. Release rates of FITC-BSA (□) and β-gal (■) from PCPP spheres coated with 21.5-kDa PLL.[4,5] FITC-BSA release rates from liposomes (○) and MELs (●), composed of egg hydrogenated phosphatidylcholine (PPC) and cholesterol (CH), 1:1 molar ratio.[7]

Ca-PCPP matrices efficiently entrapped fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) and β-galactosidase (β-gal), with MWs of 68 and 540 kDa (kilodaltons), respectively; 60% and 80% of FITC-BSA and β-gal, respectively, were recovered in Ca-PCPP spheres. The process enabled high retention of β-gal activity, comparable to its aqueous activity.[5,6]

Ca-PCPP spheres aggregate and adhere to glass, suggesting surface-charge effects. To neutralize charge (i.e., carboxylic groups), microspheres were reacted with the positively charged polyelectrolyte poly(L-lysine) (PLL).[4a] This not only diminished aggregation but sustained release rates of FITC-BSA (by 20%) and β-gal (by 80%) (Figure 2).[4b] Presumably, the complex PCPP-PLL creates a permselective membrane on the microsphere surface, similar to alginate-PLL membranes.[2b]

FITC-BSA release was further sustained by encapsulating it first in liposomes that were then entrapped in PCPP-PLL, providing microencapsulated liposomes (MELs) with an additional controllable barrier, i.e., lipid bilayer.[7] Liposome entrapment did not interfere with ionic cross-linking and, when coated with 21.5 kDa PLL, Ca-PCPP retained the liposomes for over 50 days. FITC-BSA release was significantly reduced and was similar to that of unencapsulated liposomes with the same lipid composition (Figure 2). The lipid bilayer is presumably rate-limiting for MELs.

PCPP degradation was studied in phosphate-buffered saline (PBS), at 37 °C for 90 days. The medium was collected periodically and assayed for backbone degradation products, i.e., ammonia, using ion activity measurements and inorganic phosphate.[8] No degradation products were detected, implying that PCPP does not degrade.

To examine cellular toxicity, liver cells[9a,b] were seeded on Ca-PCPP films coated with PLL.[5] One hour after seeding, cells had attached to the films; washing with media did not remove the cells. Microscopic inspection and viability assays[9c,d] revealed live cells. Five days later, live cells were still observed on the films. Preliminary studies also indicated that hybridoma cells survive microencapsulation.[4a,5]

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Supplementary Material Available: Details of the encapsulation process and the preparation of Ca-PCPP films, a graph showing the β-gal activity in PBS and Ca-PCPP matrices, and a figure showing hybridoma cells in PCPP spheres via phase contrast microscopy (3 pages). Ordering information is given on any current masthead page.

(5) Supplementary material available: detailed encapsulation process (1 paragraph); preparation of Ca-PCPP films (1 paragraph); β-gal activity in PBS and Ca-PCPP matrices (1 figure); hybridoma cells in PCPP spheres via phase contrast microscopy (1 figure).

(6) β-Gal activity assay using *o*-nitrophenyl β-galactopyranoside as a substrate.

(7) (a) Liposomes of PPC (Avanti Polar Lipids) and CH (Sigma), 1:1 molar ratio, were prepared by reverse-phase evaporation.[7b] To prepare MELs, 1 mL of (FITC-BSA)-laden liposomes (66-88 μmol of lipid) was mixed with 1 mL of 5% (w/v) PCPP and the mixture was sprayed as microdroplets into the $CaCl_2$ solution.[4,5] (b) Szoka, F.; Papahadjopoulos, D. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4194.

(8) Phosphate was quantified colorimetry by reducing a molybdenum phosphate heteropolyacid with ascrobic acid (Sigma, kit No. 670).

(9) (a) Liver cells were isolated from male fisher rats.[9b] (b) Selgen, P. O. In *Methods in Cell Biology*; Prescott, E., Ed.; Academic Press: New York, 1976; p 13. (c) Cell viability was followed by tetrazolium salt assay.[9e] (d) Mosmann, T. J. *Immunol. Methods* **1983**, *65*, 55.

Solution-Phase Reactivity as a Guide to the Low-Temperature Chemical Vapor Deposition of Early-Transition-Metal Nitride Thin Films

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Recently there has been an interest in using inorganic and organometallic compounds as precursors for the synthesis of inorganic thin films by chemical vapor deposition (CVD).[1] In the CVD process, reactions are thought to occur both in the gas phase and at the gas phase-substrate surface interface.[2] It remains to be demonstrated that well-developed solution reaction chemistry is relevant to such complicated systems,[3-6] and concurrently, whether or not solution reactivity can be used as a guide in the selection of precursors for the designed synthesis of target materials.

In the early 1960s, Bradley and co-workers reported that dialkylamido complexes undergo facile transamination reactions in solution (eq 1).[7] With primary amines, reaction 1 leads to imido-bridged oligomers if the alkyl substituent is small (eq 2)[8,9] and to dimer formation in the case of *t*-$BuNH_2$ (eq 3).[8,10] The formation of formal M-N multiple bonds by successive amine elimination reactions in eqs 2 and 3 suggests that a combination of transamination and amine elimination reactions involving volatile metal amido complexes could be the basis for the CVD synthesis of stoichiometric metal nitride thin films if ammonia were the co-reactant.[11,12]

$$MNR_2 + HNR'_2 \rightarrow MNR'_2 + HNR_2 \quad (1)$$

$$M(NMe_2)_4 + RNH_2 \rightarrow [M(=NR)_2]_x + HNMe_2 \quad (M = Ti, Zr) \quad (2)$$

$$2M(NMe_2)_4 + 2t\text{-}BuNH_2 \rightarrow [M_2(NMe_2)_2(\mu\text{-}NR)]_2 + 4HNMe_2 \quad (3)$$

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