Synthetic Biodegradable Ionomers that Engulf, Store, and Deliver Intact Proteins

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Telechelic anionic and cationic biodegradable ionomers capable of loading, storing, and releasing proteins are presented. Two different ionomers have been synthesized with either anionic or cationic end groups. The reaction was done quantitatively as shown by ^{1}H NMR. The swelling properties of the hydrophobic poly(trimethylene carbonate) polymer are contributed to the ionic end groups that display hydrophilic properties. Depending on the molecular weight of the ionomer, and also on the ionic charge, the materials swell differently in water, from ∼50% for M_w = 12 000 g/mol to ∼500% when dealing with 2000 g/mol. The high swelling led us to believe that it would be possible to load and release proteins preferably in a still active form. As models, two different proteins were chosen: hemoglobin and cytochrome c. The swelling and release study shows that both ionomers possess the capability to adsorb and later release the proteins with retained structure. Release measurements from both the swollen and dried states have been evaluated with similar results, showing that the dried state seems to release a little bit less than the swollen one. These kinds of materials should be interesting for a wide variety of applications where drug and protein release is wanted, as well as in applications such as protein separation media.

Introduction

A wide variety of polymers have been used in drug and protein delivery applications.[1] With the recent developments in genomics and proteomics, an increase in the delivery of drugs, especially proteins, is expected.[2] Although low molecular weight drugs often are robust and resist harsh treatment, proteins are generally fragile and have limited chemical and physical stability, being susceptible to proteolysis, chemical modification, and denaturation.[3] There are a multitude of delivery and release systems, ranging from reservoir systems,[4] polymer matrixes,[5] encapsulation into micro- and nanospheres,[6] and trapping in hydrogels,[7] which might work both for drugs and proteins. The mechanism of the release might be physically controlled as for osmotic pump devices,[8] which functions as a pump driven by the osmotic pressure created in an aqueous environment. Alternatively, the drug might be dispersed in the polymer in a dehydrated state and later, when exposed to moisture, the polymer will swell and the drug will be dissolved and released.[9] There are also chemically controlled delivery systems. For example, a degradable reservoir for the drug can be mentioned, when the coating degrades the content is released, and another system where the drug is dispersed in a degradable polymer matrix that upon degradation releases the drug.[5] Such systems have been employed for the release of active growth factors from a highly porous poly(lactide-*co*-glycolide) produced with help from super critical CO_2,[10] thus avoiding harmful solvents in the preparation. Hydrogels are especially important for drug/protein delivery due to their water content, preventing protein degeneration.[11] Another notable synthetic polymer forming gels is pluronic 127, a triblock copolymer of PEG and poly(propylene oxide).[7] Recently, biodegradable analogues PEG-PLA have been employed as promising carriers.[12] Also natural gels such as gelatin have been investigated as drug carriers for controlled release.[13] For example, different growth factor proteins have been delivered with the help from gelatin to enhance bone formation. Limitations are in the control of release kinetics,[14] and problematic storage stability.[3a] Loading proteins into micro- and nanospheres is one of the most common strategies for protein delivery today.[6] Since the proteins are protected inside the sphere, it allows a somewhat extended shelf life.[15] A drawback with particles is that the production methods expose the proteins to potentially denaturating conditions such as organic solvents,[16] high temperatures,[17] detergents,[18] or agitation.[19] In that respect, hydrogels have a more gentle way of production. Release might be driven by osmosis, swelling the spheres until rupture. It can also be based on the carrier degradation or leaching out from the sphere, which may be controlled by the proper choice of polymer.[6] It is also possible to combine gels with particles as micro gels.[11] A newcomer in the drug release field is non-cross-linked week polyelectrolytes that for an anionic polyelectrolyte, carboxyalkyl methacrylate, have shown to bind cationic drugs in pure water. With a slight increase in ionic strength, the drugs have shown to be released with zero order kinetics.[20] Ionic interaction between cationic−anionic polymers forms a polyelectrolyte complex which might be used for the preparation of micro- and nanoparticles as carriers and also play a role in diffusion controlled drug loading and release.[21]

While today's systems display many of the desired features for protein delivery, there is still a need for carriers that allow (i) gentle and high efficient loading, (ii) potential protection upon storage, (iii) release rates that simply may be tuned, and (iv) delivery of intact proteins. Recently, we discovered that biodegradable oligomers with phosphoryl choline (PC) end groups, termed zwitterionomers, swell considerably in water and PBS buffers due to ionic domains and that protein could be adsorbed and released in a controlled fashion.[22]

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We have tailored our biodegradable oligomers with active end groups chosen from natures own palette. Negatively charged sulfonic acid, as in heparin,[23] and positively charged trimethylammonium, as in D- and L-carnitite,[24] functionalities were chosen. By introducing these specific functionalities, interactions in an aqueous environment could be promoted between synthetic materials and natural biomolecules, providing these anionomers and cationomers with efficient loading capabilities of charged drugs or proteins for a delayed release. Release might be deployed either from the swollen state or after a drying step. The protein chosen for the study of the anionomers is cytochrome c, a cationic protein found in the mitochondrial respiratory chains as an electron-transfer agent.[25] Since it binds to anionic polylysines, it ought therefore to be a suitable candidate for interaction with the anionic polymer. It contains the heme group which is responsible for the red color of this compound. As for protein to be delivered from the cationomers, carboxyhemoglobin (COHb) was chosen. Hemoglobin, which is found in the red blood cells, is responsible for the transport of oxygen throughout the body. Hemoglobin is an anionic protein also containing the heme group making it red in color.[26] It has an isoelectric point of 6.9 and therefore carries a net negative charge at pH 7.4. If denaturated, and consequently loses activity, these proteins either bleach or turn brownish greenish,[27] thus providing for a visual stability probe. This paper presents materials and mechanisms allowing simple loading of proteins just by swelling and sustained released of up to 99% intact proteins. As the backbone polymer, we have chosen poly(trimethylene carbonate) (PTMC), which degrades from enzymatic activation.[28] Another advantage of PTMC is that the degradation does not produce products that auto catalyze continuous degradation[29] like acetic carboxylic acids in the polyesters. The advantage of this is that only surface erosion occurs, and it is therefore easier to tailor degradation rate and physical properties during degradation.

Experimental Section

Materials. Prior to use, pyridine, acetonitrile, and 1,4-butanediol (Aldrich) were refluxed over calcium hydride, distilled, and stored under argon. Chloroform and dichloromethane (VWR) were washed with water, dried with magnesium sulfate, and distilled over calcium hydride. Stannous 2-ethylhexanoate (~95%), sulfur trioxide trimethylamine complex, hydrochloric acid (37%), sodium hydrogencarbonate (99.7%), 4-chlorobutyryl chloride, trimethylamine, *N*,*N*-dimethylformamide (DMF) (99.5%), cytochrome c (>99%), tris(hydroxymethyl)aminomethane (99.9+%) (Aldrich), benzyl alcohol (99%) (Lancaster Synthesis), disodium hydrogen phosphate dehydrate (>99.5%), sodium chloride (100%), diethyl ether (analytical grade), methanol (>98.5%), VWR, trimethylenecarbonate (Boehringer Ingelheim), and carboxyhemoglobin (COHb) from Pharmacia Biotech (GE healthcare) were used as received.

Instrumentation. ^{1}H NMR spectra were recorded using a JEOL ECP 400 MHz spectrometer with the solvent proton signal as an internal standard. For protein amount determination from the COHb solution, a TA-Instrument TGA Q-500 was used. Protein concentrations were measured with a Perkin-Elmer Lambda 35 UV−vis spectrometer. The wavelengths 482, 516, and 633 nm were used for the COHb and 365, 414, 538, 558, and 566 nm were used for cytochrome c. Size exclusion chromatography (SEC) was performed on a Waters Alliance GPCV2000 with three Styragel columns (HR1−HR3−HR5E), the detectors used were the viscometer and the RI, and Empower was the software used to evaluate the results. For cryogenic scanning electron microscopy (cryo-SEM), a JEOL 6320F scanning electron microscope, Akishima Tokyo Japan, equipped with high vacuum cryo stage, Oxford CT1500 HF (Oxford England) was used. Samples were quickly frozen in liquid N_2 at −210 °C, which is achieved by pulling vacuum on the $N_{2(lq)}$. For regular microscopy, a TE2000 with LCD camera DMX1200F from Nikon was used.

Synthesis of Poly(trimethylenecarbonate) Diol (PTMC) (1). The procedure for the 4000 g/mol oligomer is given as an example. A 50 mL = two-necked Schlenk flask equipped with a stir bar was carefully flame-dried under vacuum and purged with nitrogen before 5.0 g (49.0 mmol) of trimethylene carbonate, 25 mg (61 μmol) of $Sn(Oct)_2$, and 0.11 g (1.225 mmol) of 1,4 butanediol were added inside the glovebox for a DP of 40 (20/arm). The closed reaction mixture was stirred at 110 °C for 4 h in an oil bath. Following completion of the reaction, the PTMC was dissolved in chloroform and precipitated in 1 L of cold methanol. The precipitate was allowed to sediment and washed repeatedly with methanol and dried under vacuum at 40 °C until constant weight. Yield: 97% ^{1}H NMR ($CDCl_3$) = 1.73 (m, 2H, $-CH_2$-, initiator), 1.86 (m, 2H, -CH_2-CH_2-OH, end group,), 2.05 (m, 2H, $-CH_2$-, poly), 3.73 (t, 2H, -CH_2-OH, end group), 4.22 (t, 4H, $-CH_2$-, poly).

Synthesis of α,ω-Di(3-sulfoxy-propoxycarbonyl)poly(trimethylene carbonate) Sodium Salt (Anionomer) (2). The procedure for the 4000 g/mol oligomer is given as an example. A 50 mL two-necked Schlenk flask equipped with a stir bar was carefully flame-dried under vacuum and purged with nitrogen before 5.0 g (1.22 mmol) of polymer (**1**), 1.70 g (12.2 mmol) of sulfur trioxide trimethylamine complex, and 20 mL of DMF were added to the flask inside the glovebox. The closed reaction mixture was stirred at 50 °C for 16 h in an oil bath. Following completion of the reaction, the solution was precipitated into 2 L of diethyl ether. The precipitate was redissolved in dichloromethane, filtered, and precipitated in 1 L of cold methanol. This was done twice. The precipitate was allowed to sediment, washed repeatedly with methanol, and then dried under vacuum at 40 °C until constant weight before it was redissolved in 20 mL of DMF and 1.0 g (11.9 mmol) of solid sodium hydrogen carbonate was added. The reaction mixture was stirred at room temperature for 16 h. Following completion of the reaction, the solution was precipitated into 2 L of diethyl ether. The precipitate was redissolved in dichloromethane, filtered, and precipitated twice in 1 L of cold methanol. The precipitate was allowed to sediment, washed repeatedly with methanol, and dried under vacuum at 40 °C until constant weight. Yield: 72% ^{1}H NMR ($CDCl_3$) = 1.73 (m, 2H, $-CH_2-$, initiator), 2.05 (m, 2H, $-CH_2$-, poly), 4.22 (t, 4H, $-CH_2-$, poly).

Synthesis of α-,ω-Di(*N*,*N*,*N*-trimethyl-4-oxobutane-1-amonium) Poly(trimethylene carbonate) (Cationomer) (3). The procedure for the 4000 g/mol oligomer is given as an example. A 50 mL two-necked Schlenk flask equipped with a stir bar was carefully flame-dried under vacuum and purged with nitrogen before 5.0 g (1.22 mmol) of polymer (**1**), 0.31 g (2.94 mmol) of 4-chlorobutyryl chloride, and 0.46 g (5.87 mmol) of pyridine were dissolved in 20 mL of dichloromethane. The closed reaction mixture was stirred at room temperature for 24 h before precipitation into 1 L of cold methanol twice. The precipitate was allowed to sediment, washed repeatedly with methanol, and then dried under vacuum at 50 °C until constant weight. Before it was redissolved in 20 mL of acetonitrile and at −20 °C, the 50 mL flask was charged with 0.1 mL of trimethylamine. The closed reaction mixture was stirred at 60 °C for 24 h before precipitation into 1 L cold methanol twice. The precipitate was allowed to sediment, washed repeatedly with methanol, and then dried under vacuum at 50 °C until constant weight. Yield: 86% ^{1}H NMR ($CDCl_3$) = 1.73 (m, 2H, $-CH_2-$, initiator), 2.05 (m, 2H, $-CH_2-$, poly), 2.51 (t, $-CO-CH_2$-, end), 3.44 (s, $-N^+(CH_3)_3$, end), 3.75 (m, $-CH_2-N^+(CH_3)_3$, end), 4.22 (t, 4H, $-CH_2-$, poly).

Synthetic protocols for the molecular weights 2000 and 12000 g/mol are available in the Supporting Information.

Protein Loading. For the loading and release experiments, polymer disks were prepared by compression molding at 50 °C. A total of 1 g of polymer was compression-molded using a Teflon mould at 60 kN/100 cm^2 for 60 s to give a 16 × 60 × 1 mm plate. Round disks with a diameter of 6 mm were punched from the plate to provide the starting geometry for all of the experiments.

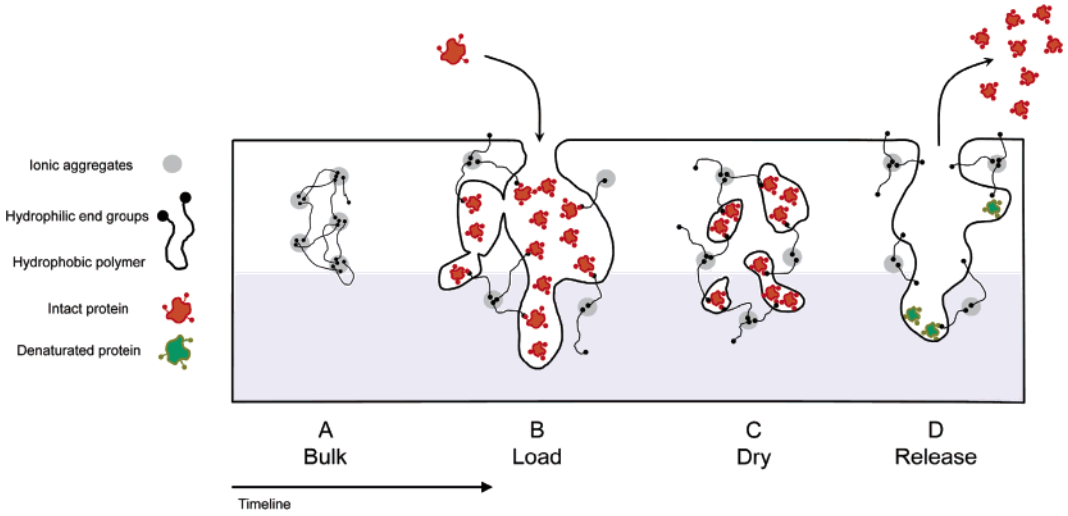


Figure 1. Schematic illustration of how protein loading and release occur in ionomeric material, from the swelling of the protein solution to the release of those contained proteins.

For the anionomer, cytochrome c was dissolved in 5 mM phosphate buffer at pH 7.0 to a final concentration of 20 mg/mL. For the cationomer, COHb was dissolved in 10 mM tris buffer at pH 8 to a final concentration of 2.5 mg/mL as determined by thermo gravimetric analysis (TGA). The disks were submerged into 0.4 mL of the protein solution for adsorption at 4 °C during 48 h for $M_w = 2000$ g/mol and $M_w = 4000$ g/mol and 168 h for the $M_w = 12\,000$ g/mol. After adsorption, the amount of protein in the remaining solution was determined spectroscopically, and the amount of protein charged into the disk was calculated.

Drying of Samples. One series of loaded disks were air-dried at 4 °C until the starting weight plus 5% was reached.

Protein Release. For release of the proteins, the protein loaded disks were submerged into release buffers. For the cationomer a 0.1 M NaCl, 10 mM tris buffer at pH 8 was used, whereas for the anionomer, a 0.1 M NaCl, 5 mM phosphate buffer at pH 7 was used. The loaded disks were submerged into 2 mL of release buffer. The concentration of protein in the release buffer was followed with a UV−vis spectrometer, measuring the adsorbance at 355, 418, 548, 582, and 633 nm for COHb and 365, 414, 538, 558, 566, and 633 nm for cytochrome c over time until the release had leveled off. Depending on the protein concentration in the solutions, different wavelengths were used to avoid concentration errors at higher or very low absorbances. We choose absorbencies between 0.2 and 0.8 in order to get as reliable of a signal as possible.[30]

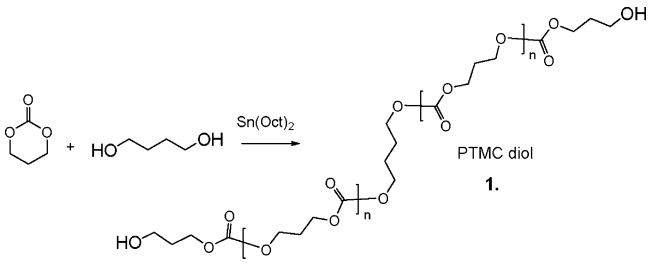
Swelling Front. To evaluate the rate of swelling, an ionomer disk was cut in half with a sharp blade to produce a straight fresh surface. This piece was then placed on a glass slide and put in the microscope. A drop of deionized water was added along the edge, and the software for the microscope was allowed to take a photo every minute. The photos reveal clearly the straight swelling front in the material. Once the front was into the material, about 5 μm the sample was put in $N_{2(l)}$ at −210 °C to quench the sample before it was analyzed with cryo-SEM.

Results and Discussion

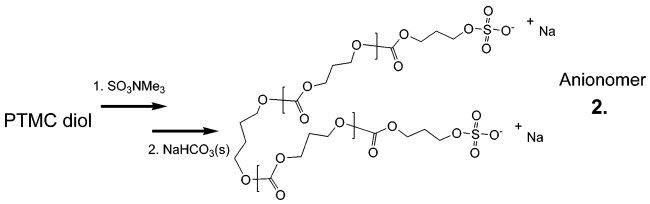
This paper presents an improved method for drug delivery from biodegradable polymers. It is based on the mechanistic findings that telechelic ionomers may open and close channels to load, store, and release their content as schematically depicted in Figure 1. Importantly this takes place in aqueous solutions and at ambient temperature, thus being compatible with gentle protein delivery.

Outlined below is the synthesis of the ionomeric materials together with the mechanism of loading and release as described for two model proteins.

Scheme 1. Synthesis of PTMC Diols



Scheme 2. Synthesis of Anionomer



Synthesis. The synthesis of the anionomer and cationomer has previously been described in detail.[31] For this study, we synthesized telechelic ionomers of polytrimethylenecarbonate (PTMC) diols and quantitatively equipped them with anionic and cationic functionalities. Cationomers with molecular weights of 2000, 4000, and 12 000 g/mol were prepared. For the anionomer, only 4000 and 12 000 g/mol were used due to the solubility of the 2000 g/mol molecular weight anionic compound in water.[31] The PTMC diols were synthesized by ring opening polymerization in bulk with stannous octanoate as the catalyst and 1,4-butane diol as the initiator, Scheme 1. For the post modification of the PTMC diol into the anionomer, carrying sodium sulfate end groups, the hydroxyls were reacted with sulfur trioxide trimethylamine complex to give the PTMC disulfate, Scheme 2. The reaction was monitored by ^{1}H NMR to ensure complete conversion of the starting ω-hydroxy terminated PTMC to the desired intermediate PTMC−$SO_3NH(CH_3)_3$, as seen by the shifting of the methylene triplet centered at 3.73−4.22 ppm. The trimethylammonium counterion was exchanged for sodium by simply stirring a solution of the polymer in DMF with solid $NaHCO_3$. Also this transformation step is quantitative and easily monitored by ^{1}H NMR, as seen by the disappearance of the trimethylammonium counterion at 2.93 ppm. The cationic ionomer was also synthesized by a two step procedure, where in the first step 4-chlorobutyryl chloride

Scheme 3. Synthesis of Cationomer

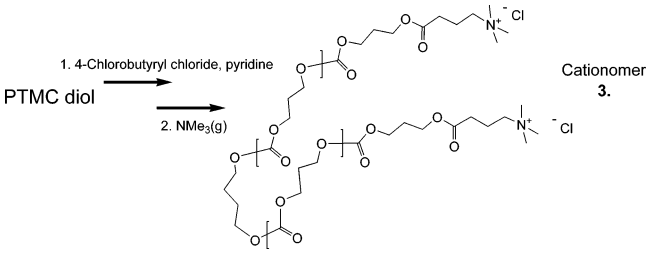


Table 1. Characteristics of PTMC Diols Synthesized[a]

sample	M/I ratio	M_n theory	M_n NMR	PDI SEC	yield (%)
PTMC 2000	20	2130	2240	1.30	95
PTMC 4000	40	4170	4088	1.33	97
PTMC 12000	120	12 330	12 626	1.30	96

[a] M/I: monomer-to-initiator ratio; M_n: number average molecular weight (g/mol); PDI: poly dispersity index.

Table 2. Selection of Ionomers Synthesized

material	M_n theory	M_n NMR	yield (%)
anionomer 4000	4580	5212	72
anionomer 12000	12 740	12 536	87
cationomer 2000	2873	2616	78
cationomer 4000	4913	4824	86
cationomer 12000	13 073	15 797	84

[a] M_n: number average molecular weight (g/mol).

was reacted with the ω-hydroxyl end-group of the PTMC mediated by pyridine and in the second step trimethylamine displace the chloride to introduce the cationic ammonium group, Scheme 3. Both of these steps were followed by precipitating of the products into cold methanol twice to give ~85% yields. Also for this synthesis ^{1}H NMR end group analysis was an easy way of ensuring complete conversion. For the acylation, ^{1}H NMR revealed the disappearance of the methylene triplet centered at 3.73 ppm, shifting to 4.22 ppm, and two new resonances appear in the intermediate as triplets, one at 3.55 ppm attributed to the methylene adjacent to the chloride and the other corresponding to protons at the α-position to the carbonyl at 2.55 ppm. In the second reaction step, the methylene triplet at 3.55 ppm shifted downfield to 3.75 ppm and lost its distinctiveness due to reduced mobility of charged groups in chloroform leading to aggregation and thus peak broadening. A new singlet peak from the 18 methyl protons present in the two ammonium groups appears at 3.43 ppm.

Using these synthetic procedures, three different molecular weights of the starting polymer with their characteristics as shown in Table 1, and five cationomers and anionomers, Table 2, were prepared in 5 g scale from the PTMC diols.

Protein Loading and Release. *Opening of Water Channels.* The ionomer is a telechelic molecule consisting of the ionic hydrophilic end groups and a hydrophobic polymer backbone. Aggregation of the ionic end groups in the bulk, Figure 1a, results in materials with rubbery behavior, typical of ionomers.[32] These could be easily compression molded into the desired shape of small disks. Loading of substances into the disks can be performed by immersion into aqueous solutions. Upon contact with aqueous solution, an interesting molecular rearrangement starts. First the hydrophilic end groups will enrich against the aqueous interface, while the hydrophobic backbone will stay in the hydrophobic bulk. Thereby the initially hydrophobic surface will turn hydrophilic, which was reported as a change in contact angle where the hydrophobic 85° was changed to the hydrophilic 25°.[31] The rearrangements do not stop at the surface but continue by channel formation into the bulk, allowing water and accompanying substances to enter, Figure 1B. Macroscopically, the water uptake occurs linearly with time through a sharp swelling front that can be seen by optical microscopy, Figure 2A. In Figure 2B, a cryo-SEM picture reveals both the swelling front and the inner co-continuous swollen structure consisting of micron sized aqueous domains held together by submicron polymer struts. Finally, the fully swollen samples carrying 50−500% water appeared as soft opaque gels.

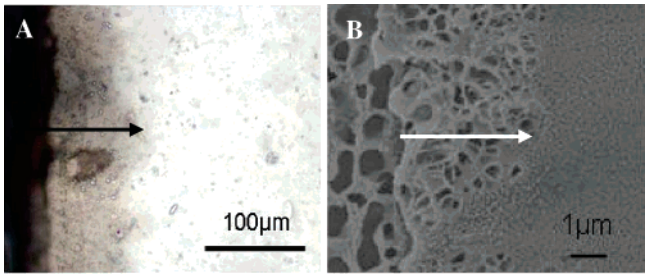


Figure 2. Aqueous swelling front in ionomer (tip of arrow), direction indicated by the arrow, shown in A with optical microscopy and in B with cryo SEM.

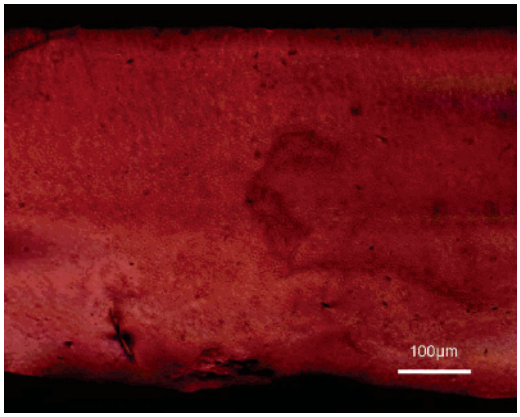


Figure 3. Cross section of a protein loaded disk shows that the COHb is evenly distributed throughout the entire cationomeric disk.

Protein Loading. Two red colored proteins were selected as models to probe both loading and release. These were carboxy-hemoglobin (COHb) and cytochrome c, both carrying the red iron containing heme group. Upon denaturation, these proteins, especially COHb, loose or change their color to green, blue, or brown.[27] Hence, the color gives the opportunity to visualize loading, release, and denaturation and quantify it with UV−visible spectroscopy. Upon immersion of disks into protein containing solutions, the proteins followed the water through the open channels into the disk. To investigate the protein distribution in the disk, it was sectioned and analyzed using light microscopy. The distribution of protein through out the ionomer disk is shown in Figure 3, where a cross section of a protein-adsorbed disk is displayed. The red color is evenly distributed throughout the disk, indicating that the protein penetrates all of the way in to the middle of the disk. This was true for both proteins. It was also observed that the cationomer even seemed to enrich protein from the solution as seen in Figure 4, where the disks released sample has a deeper red color and hence higher protein concentration than the protein solution the disk was loaded in.

Drying. The protein containing ionomers were treated in two different ways before the release experiments, (i) used directly

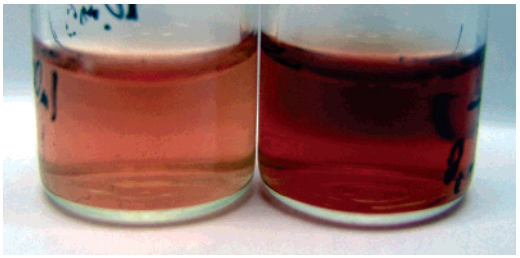


Figure 4. Loading COHb solution to the left and released COHb solution to the right.

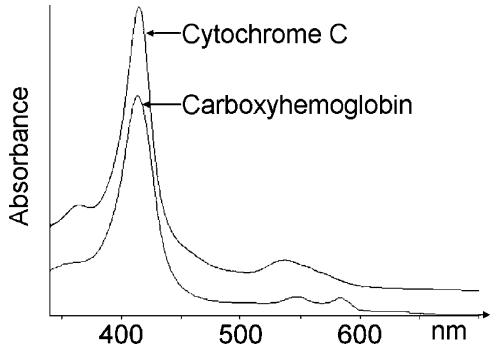


Figure 5. UV−vis spectra of cytochrome c and carboxyhemoglobin.

Figure 6. Series of cationomer disks. A, unloaded ionomer; B, COHb loaded ionomer; C, ionomer that have released approximately 95% of its protein content.

as swollen or (ii) first dried. Since the ionomer gel is viscoelastic, evaporation of water from the pores should result in their closure driven by capillary forces. Upon water removal and ionomer shrinking, the open morphology pores close to encapsulate the proteins within the ionomer matrix as schematically shown in Figure 1C. On the basis of the release findings for both COHb and cytochrome c, neither their UV−vis or IR spectra changes, and one might speculate that the proteins are retained in the polar clusters or inverse micellar domains, which should prevent development of hydrophobic interactions and subsequent protein denaturation, and enable later release.

Release. To study protein release from (i) swollen, ionomer disks carrying water and protein and (ii) dried, ionomer disks carrying only protein, the disks were immersed into a buffer solution. The release was monitored with UV−vis spectroscopy. The only visible difference between the dried and the wet sample

Table 3. Amount of Proteins Released from the Different Disks[a]

ionomer		loaded[b]	released[c] (%)	time[d] (days)
anionomer 4000	dried	2.8 mg	46	7
	wet	2.9 mg	49	8
anionomer 12000	dried	1.6 mg	16	12
	wet	1.7 mg	17	13
cationomer 2000	dried	0.69 mg	88	8
	wet	0.65 mg	95	7
cationomer 4000	dried	0.46 mg	90	11
	wet	0.61 mg	99	5
cationomer 12000	dried	32.8 μg	32	21
	wet	25.0 μg	39	18

[a] Initial dry weight of the disks is around 50 mg. [b] Amount of loaded protein. [c] Amount of loaded protein that is released. [d] Time to maximum release.

was that the dried samples release a bit less protein than the wet one. Both proteins used have a strong adsorption in the region 415−420 nm and weaker adsorptions between 500 and 600 nm as shown in Figure 5. The hydrophilic environment that dried protein containing disks are exposed to, prompts the cannels to open up again and release the contained protein, as schematically depicted in Figure 1D.

All of the proteins were released with unaltered UV−vis adsorption spectra, indicating retained 3D structure. The proteins contained in the bulk of the polymer could be visually inspected, and thereby, the conclusion that a small protein fraction had degraded could be drawn. Figure 6 displays photographs of three disks of cationomer: first the starting ionomer disk, second the COHb loaded disk, and finally a disk that has released 95% of the adsorbed proteins. In the last disk, a more greenish brownish color can be seen indicative of denaturated protein, which represents about 5% of the total loading. The COHb is only weakly anionic in nature,[26] limiting ionic interaction with the matrix, thus allowing facile release. The reason that some proteins were denaturated may be explained by hydrophobic interaction[33] with the PTMC backbone. If the charge of the ionomer and the protein are complementary, more extensive adsorption develops. This is reflected for the anionomer whose released fraction of cytochrome c is less than the cationomers release of COHb. Cytochrome c contains positively charged clusters that are essential for the docking of its reaction partners in its biological role as an electron carrier,[25] which in our materials also interacts with the negatively charged matrix. In the anionomer disk, however, the colors of denaturation cannot be seen as the disk stays red even after release, indicating that some of the protein still contained might be intact. Upon an increase in the salt concentration in the release buffer more cytochrome c is released in its intact form as judged by UV−vis spectroscopic analysis. This points to the fact that the ionic strength of the buffer plays an important role for the rate and amount of release. Salt gradually replaces the protein−

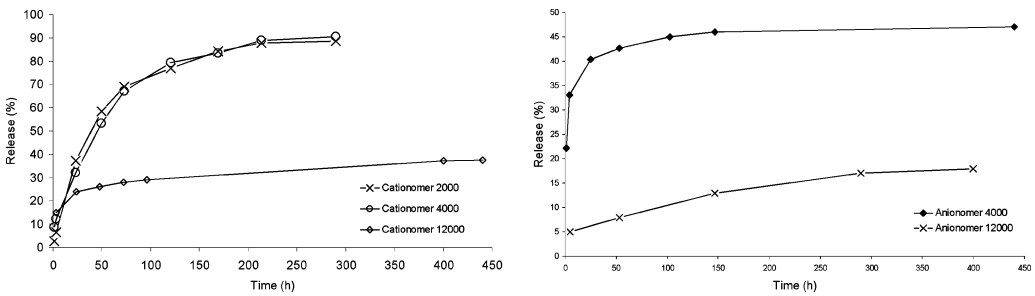


Figure 7. Curves showing the release of CPHb and cytochrome c from dried disks. On the left, cationomers of three different molecular weights 2000, 4000, and 12 000 g/mol; on the right, the release from 4000 and 12 000 g/mol anionomer.

matrix interaction, known in chromatography as "salting out".[34] The differences in amount released can be seen in Table 3, where also time to full release is presented. The drying procedure seems to affect the amount of proteins released in all cases, probably because of the increase of hydrophobic interactions when the water is evaporated. Such interactions become more pronounced with higher molecular weight. Generally, higher molecular weights also give longer release times which are a result of lower molecular mobility and fewer channels. All of the investigated ionomers shows an initial burst release, followed by a release more close to the first order release kinetics, Figure 7. It seems like the higher the molecular weight, the lower the release rate. The loading capacity also seems to be lower if the molecular weight is increased. Both of these observations are likely due to the fact that higher molecular weight gives less ionic groups per volume of material and, hence, less ionomeric properties of the material.

Conclusion

An improved method for gentle protein loading and protein delivery is presented using colored proteins and fully biodegradable cationomer and anionomer materials. The loading is simple and gentle to the proteins, by just letting the ionomer itself swell in a solution of desired protein. The protein will penetrate together with water into the ionomer carrier when the co-continuous porosity in the swollen ionomer is formed. The release can be performed immediately after adsorption, or the protein loaded ionomer might be dried, potentially allowing storage of the device, before release of up to ~95% of the adsorbed protein. The rate of release is affected by the molecular weight of the backbone but also likely by the type of backbone. Future studies should involve variation of charge of the end group to enhance or minimize ionic interactions between protein and matrix. Also, the biologic activity of encapsulated and released proteins should be determined.

Supporting Information Available. Synthetic protocols for the molecular weights 2000 and 12000 g/mol. This material is available free of charge via the Internet at http://pubs.acs.org.

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