Molecular Design and in Vitro Studies of Novel pH-Sensitive Hydrogels for the Oral Delivery of Calcitonin

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ABSTRACT: pH-sensitive hydrogels are suitable candidates for oral drug delivery of peptides due to their ability to respond to their environment. We have developed new hydrogels composed of poly(methacrylic acid) (PMAA) grafted with poly(ethylene glycol) (PEG) (P(MAA-*g*-EG)) which can be used as drug delivery carriers for salmon calcitonin. P(MAA-*g*-EG) hydrogels were prepared by free radical solution polymerization. The monomer mixture was diluted using a 50% w/w solution of ethanol and water. The percentage of monomer in solution was varied from 84% to 45% v/v. Swelling studies were conducted to investigate the effects of solvent content used during polymer preparation in the swelling behavior. The effects of dilution on the swelling behavior were not observed until the monomer mixture was diluted to approximately 50%. Salmon calcitonin was successfully incorporated and released in vitro from the system. Solutions of approximately 0.1 mg/mL of salmon calcitonin were used to load the protein into the gels at pH = 7 and constant ionic strength of 0.1 M. The loading efficiency was affected by the amount of solvent used during hydrogel preparation. In vitro release studies were performed at pH = 7 and 37 °C, while keeping an ionic strength of 0.1 M. The release behavior was found to be not very much affected by the amount of diluent used during polymer preparation. The transport mechanism was found to be relaxation controlled for all cases, and the diffusion coefficient was estimated using a heuristic Fickian/relaxational model.

Introduction

The delivery of peptides and proteins is usually performed by frequent injections. This results in a rapid increase and subsequent rapid decrease of the blood serum concentration levels. Therefore, the major challenge in the field of protein delivery is the design of a system capable of maintaining a blood concentration for a considerable amount of time inside the therapeutic region and to reduce the number of doses that have to be administered. As a result, oral control delivery of peptides and proteins is indeed the most convenient route of administration for the patient, as well as the most challenging.

In this work, we examine the feasibility of use of a new pH-sensitive hydrogel as a possible carrier for the oral administration of the polypeptide hormone salmon calcitonin (sCT). Calcitonin is a 32-amino-acid polypeptide hormone, which in conjunction with the parathyroid hormone and vitamin D is responsible for the calcium metabolism in human beings and other vertebrates. This polypeptide hormone is used as a therapeutic agent for bone diseases such as Paget's disease, hypercalcemia, and osteoporosis. Like other proteins it is delivered mainly by injection, leading to a limited use in the treatment of osteoporosis.

The ability of pH-sensitive hydrogels to respond to their environment makes them suitable candidates for the oral delivery of peptides and proteins. A new type of pH-sensitive hydrogel film composed of poly(methacrylic acid) grafted with poly(ethylene glycol) (PMAA-*g*-EG) has been developed which can be used as a drug delivery carrier for sCT. At low pH, these hydrogels remain collapsed, and the protein will be protected from the acidic environment of the stomach due to its limited release. On the other hand, as the formulation passes from the stomach to the upper small intestine, the drastic pH change will cause the network to swell, and the protein will be released.

Moreover, these polymers were molecularly designed to contain poly(ethylene glycol) tethered chains promoting mucosal adhesion and providing sCT protection, as well as a poly(methacrylic acid) backbone with carboxylic pendent groups (−COOH), which can act as calcium binders leading to epithelial cell junction opening.[1] In this way these hydrogels are expected to enhance the absorption of sCT through the epithelial cell monolayer of the upper small intestine.

The copolymer was first studied by Klier and Peppas.[2] In their work, they observed the pH sensitivity in the swelling behavior as well as the complex formation using NMR spectroscopy. Interpolymer complexes in this system are formed due to the interactions between the ether oxygen from the graft chain and the acidic groups.

Lowman and Peppas[3,4] further investigated this system and showed that it exhibits a reversible, pH-dependent swelling behavior. At low pH the network is collapsed due to the formation of the interpolymer complexes.[5] As the pH is increased, the interpolymer complexes dissociate due to the ionization of the pendent groups. The electrostatic repulsion causes the network to swell, and water is allowed to enter.

Lowman et al.[5] studied in detail the properties and stability of the polymer complexes formed in the P(MAA-*g*-EG) system. Major factors that affected the complexation behavior of this system were the pH, the polymer composition, and the molecular weight of the poly(ethylene glycol) (PEG) grafts. The highest degree of complexation was obtained from gels that contained a molar ratio of 1:1 repeating units of MAA per PEG group with PEG graft molecular weight of 1000. As a

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result, these hydrogels (i.e., 1:1 molar ratio and PEG molecular weight 1000) at low pH showed the lowest degree of swelling due to the high degree of complexation. On the other hand, at higher pHs these hydrogels swelled to the highest degree. The complexation phenomena were also modeled and compared to experimental results.

The application of P(MAA-*g*-EG) hydrogels in oral drug delivery was first studied by Lowman et al.[3] In this work both in vitro and in vivo studies were conducted for the delivery of insulin. P(MAA-*g*-EG) microparticles were prepared and loaded with insulin by imbibition. Insulin transport was studied both in the complexed and in the uncomplexed states in vitro. Results from these studies showed that the insulin diffusion coefficient from complexed microspheres was approximately 1000 times lower than the diffusion coefficient of insulin from uncomplexed microspheres. They also reported that the amount of PEG grafted affected the interactions between polymer and protein. The larger the amount of PEG in the polymer, the larger the complexation in the network. This phenomenon reduced the interactions between the polymer structure and the protein.

Experimental Section

Film Preparation. The monomers used were methacrylic acid (MAA, Aldrich Chemical Co., Milwaukee, WI) and methoxy-terminated poly(ethylene glycol) monomethacrylate (PEG-MA, Polysciences Inc., Warrinton, PA) with a molecular weight of 1000.

P(MAA-*g*-EG) hydrogels were synthesized by free radical solution polymerization. The monomers were mixed in a molar ratio of 1:1 repeating units. Tetra(ethylene glycol) dimethacrylate (TEGDMA, Aldrich Chemical Co., Milwaukee, WI) was incorporated as 0.75% moles of the total monomers and utilized as the cross-linking agent. Irgacure 184 (Ciba-Gaygy Corp., Hawthorne, NY) was used as the photoinitiator, using around 1 wt % of the monomer mixture. Methoxyethylhydroquinone was removed from MAA by vacuum distillation at 54 °C/25 mmHg. The monomer mixture was diluted with a mixture of 50% w/w ethanol (EtOH, 200 proof, McCormick Distillation Co., Inc., Weston, MI) and DI water (Milli-Q Plus system, Millipore). The range of dilution was varied from 84 to 45 vol % of the monomer solution (i.e., volume ratio of the monomer without solvent and the total monomer−solvent mixture).

The solution was purged with nitrogen to remove oxygen, which acts as a free radical scavenger. The mixture was then poured between microscope slides (75 × 50 × 1 mm) (Fisher, Pittsburgh, PA) separated by Teflon spacers with an approximate thickness of 1 mm. The monomer mixture in the microscope slides was then exposed to UV light. The films were washed in DI water for approximately 7 days to remove the solvent and any unreacted monomer. After the washing period, films were cut into disks of approximately 1.2 cm in diameter and 0.8 mm thickness. For the feasibility studies, film disks were preferred due to easier handling. The disks were dried under vacuum at 25 °C until they obtained a constant weight.

Swelling Studies. Buffer solutions of dimethylglutaric acid/NaOH were prepared keeping the ionic strength constant at 0.1 M. The pH range studied varied from 3.2 to 8.2. The dried polymer disks were weighted and placed into 50 mL of buffer solution and kept at 37 ± 0.5 °C. At certain time intervals polymer samples were taken out of the buffer solution, and the excess of buffer was removed by gently placing in a Kim Wipe. The weight of the wet polymer was then measured. The weight-swelling ratio (q) was calculated by the weight ratio of the wet polymer to the dried polymer.

The bioactive agent used in these experiments was salmon calcitonin (sCT, Calbiochem-Novabiochem International, San Diego, CA) obtained as a solid with 89% polypeptide content.

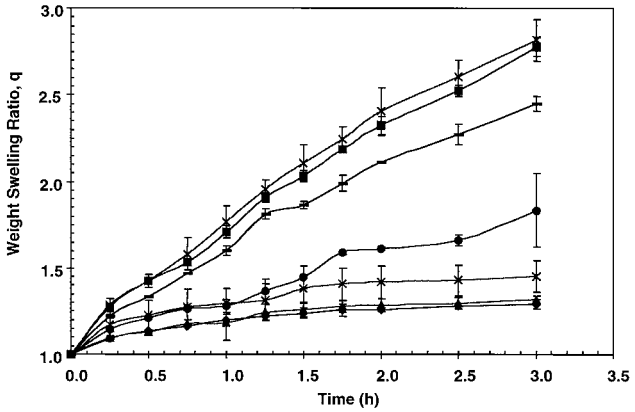

Figure 1. Dynamic swelling behavior of P(MAA-*g*-EG) for the solvent volume fraction of 0.34 in glutaric acid/NaOH buffer at $T = 37$ °C, $I = 0.1$ M at pHs 3.2 (◆), 4.0 (▲), 4.8 (*), 5.6 (●), 6.4 (—), 7.0 (■), and 8.2 (×).

sCT Assay. A polypeptide assay was performed using reverse phase high-pressure liquid chromatography (HPLC) analysis. The chromatographic data for all the sCT related experiments were obtained with a HPLC system (Shimadzu, Wood Dale, IL). The stationary phase consisted of a Hypersil BDS C18 column (4.6 × 150 mm, Phase Separations, Franklin, MA) with a pore diameter of 100 Å and a particle size of 5 μm. The mobile phase consisted of an aqueous phase composed of 0.1% tetrafluoroacetic acid (TFA) (Fisher, Pittsburgh, PA) and degassed DI water. The organic phase contained 0.08% TFA and HPLC grade acetonitrile. The linear gradient employed was 30% TFA/acetonitrile:70% TFA/water to 55% TFA/acetonitrile:45% TFA/water over a period of 15 min. The detection wavelength was 215 nm, the sample injection volume 100 μL, and the flow rate was 1.5 mL/min.

Gel Loading. Solutions of approximately 0.1 mg/mL of sCT were prepared using sterilized phosphate buffer at pH = 7, keeping the ionic strength constant at 0.1 M. The rate of microbial growth in the event of contamination was prevented by incorporating 1 mL of a 0.4 g/mL solution of sodium azide, a bacteriostatic agent, to 250 mL of buffer solution prior to sterilization. Previously dried polymer samples prepared with different polymer volume fractions were placed in 20 mL of sCT solution. The sample vials were placed in a temperature-regulated water bath at 37 ± 0.5 °C. After approximately 30 h, the samples were taken out of the solution, and the concentration of the supernatant was analyzed using HPLC.

Release Studies. Dried loaded samples were taken and placed in a sterilized 10 mL beaker with a magnetic stirrer and 4 mL of the sterilized phosphate buffer solution at pH = 7 with an ionic strength of 0.1 M. At certain time intervals the releasing medium was withdrawn from the beaker using a syringe and the medium replaced with fresh preheated buffer solution. The medium was then analyzed using the HPLC.

Results and Discussion

Dynamic Swelling Studies. Dynamic swelling studies were conducted to investigate the swelling behavior of P(MAA-*g*-EG) hydrogels prepared using a molar ratio of 1:1 repeating units of MAA per EG. Figure 1 shows an example of the dynamic swelling behavior at different pHs for hydrogels prepared with a monomer mixture that contained a solvent volume fraction of 0.34. Each datum point is the average value between three independent runs, and the error bars represent one standard deviation. Notice that as the pH is increased the swelling ratio increases due to the ionization of the pendent groups, leading to the dissociation of the interpolymer complexes. The electrostatic repulsion causes the network to swell, and water is allowed to enter. Figure 2 shows the equilibrium swelling behavior

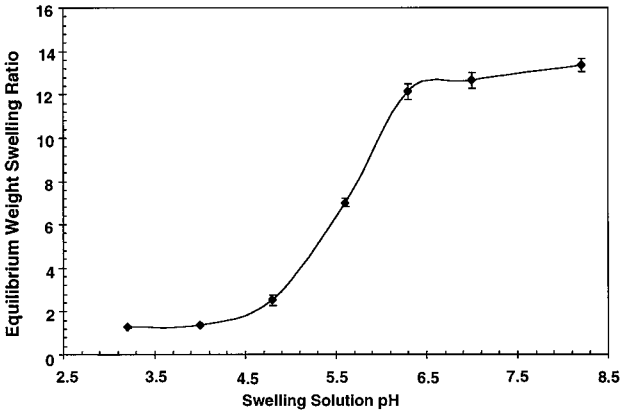

Figure 2. Equilibrium swelling behavior of P(MAA-*g*-EG) prepared using a solvent volume fraction of 0.34.

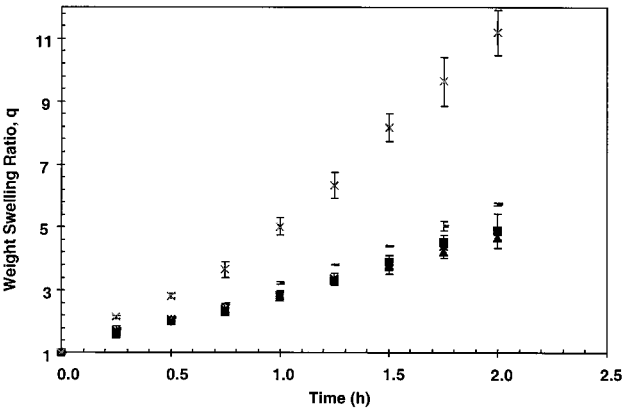

Figure 3. Swelling behavior of P(MAA-*g*-EG) for the different solvent volume fractions 0.23 (▲), 0.29 (■), 0.34 (*), 0.38 (—), and 0.57 (×) in phosphate buffer at pH = 7, I = 0.1 M, and T = 37 °C.

at different pH values, indicating the transition pH where the interpolymer complexes start to dissociate. This dissociation occurs in a very narrow pH range.

The effect of solvent incorporation prior to polymerization on the swelling behavior was also studied. The monomer solution was diluted using a solution of 50% w/w of ethanol and water and then polymerized. Figure 3 shows the dynamic swelling behavior of hydrogels prepared with different degrees of dilution. Hydrogels prepared with a solvent volume fraction of 0.23 to 0.34 (i.e., volume ratio of solvent and the total mixture) showed no significant difference in the swelling behavior. However, when the hydrogels were prepared using a volume fraction of around 50%, a dramatic change in the swelling behavior was observed.

The physical role of solvent in the final polymer properties can be explained using Figure 4, as the incorporation of a diluent will result in a change in the monomer-to-volume ratio. Films prepared using a small amount or no solvent have more monomers available, which are closer for reaction, thus creating a more compact and rigid structure. On the other hand, when higher degrees of dilution are used, the same volume will contain a smaller amount of monomers, which are farther apart, making it more difficult for two radicals to react. Therefore, a less dense structure is formed. Physically, it was observed that those hydrogels prepared with smaller amounts of diluent were stronger and more resistant to breakage, whereas those containing higher amounts of diluent were weak and fragile.

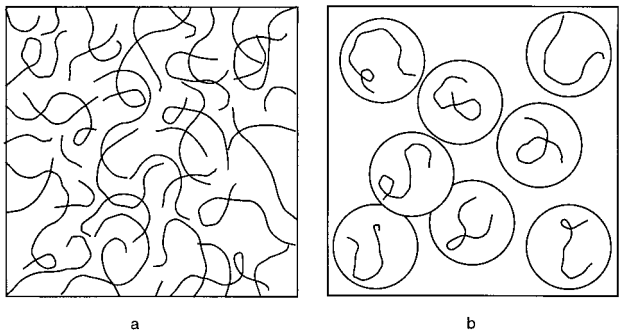

Figure 4. Idealized representation of the solvent effects in the polymerization reaction: (a) represents a concentrated monomer mixture, and (b) represents a diluted monomer mixture.

Table 1. Mesh Size Distribution for P(MAA-*g*-EG) Hydrogels Prepared Using Different Volume Fractions

solvent vol fraction	vol swelling ratio	mesh size (Å)
0.17	14	163
0.23	20	186
0.34	17	175
0.57	32	216

However, in the general sense, diluents are used in polymerization reactions to control and minimize the occurrence of the autoacceleration phenomenon. Autoacceleration occurs when the termination rate of the reaction decreases due to a dramatic increase in viscosity. Therefore, the mobility of free radicals is diminished and the termination rate is controlled by the diffusion of free radicals.[6]

To further analyze the behavior of these hydrogels in aqueous solutions, the mesh size, or the linear distance between two cross-links, was estimated using the following relationship,

$$\xi_r = Q_r^{1/3}(r_0^2)^{1/2} \tag{1}$$

where ξ_r is the mesh size (angstroms), Q_r is the volume swelling ratio, and $(r_0^2)^{1/2}$ is the end-to-end distance in the unperturbed state. For this system, the volume swelling ratio Q_r was calculated as the volume ratio of the swollen polymer at equilibrium and the dry polymer. The end-to-end distance in the unperturbed state was estimated using the following equation,

$$(r_0^2)^{1/2} = \left(\frac{2C_nM_c}{M_r}\right)^{1/2} l \tag{2}$$

where C_n is the polymer characteristic ratio (14.6 for PMAA), M_c is the molecular weight between cross-links (5700[4]), l is the carbon−carbon bond length (1.54 Å), and M_r is the molecular weight of the repeating unit (86 for MAA). However, this calculation gave an estimated result due to the fact that these equations did not take into account the effect of the graft chains. Therefore, the effective area for diffusion is smaller due to the presence of the PEG chains that need to be accommodated in the same area.

As shown in Table 1, the mesh size does not change significantly for those hydrogels prepared using up to around 0.34 solvent fraction, whereas the mesh size of hydrogels prepared using a solvent volume fraction of around 0.50 showed a significant increase in the mesh size. However, even though these values are approxi-

mate, they indicate that there is a difference in the polymer structure.

Aqueous Stability of sCT. The chemical behavior of peptides and proteins in aqueous solutions is a key factor in the design of a controlled delivery system because the three-dimensional structure of proteins, known as secondary and tertiary structure, is very sensitive to the environment. Changes in pH, temperature, ionic strength, and solvent composition can disrupt the delicate array of hydrogen bonds that keep the three-dimensional structure intact. This process of disruption of the native conformation of proteins is known as denaturation. Moreover, under extreme conditions of pH or temperature, peptide bonds can break, leading to degradation of the molecule.

Previous researchers[7,8] have studied the degradation kinetics of sCT at 70 °C and different pHs. Results showed a first-order degradation kinetics with respect to time. The degradation byproducts were different at different pHs. These were also studied and identified.[7] Salmon calcitonin is a small polypeptide. Therefore, at the specific experimental conditions of this work (i.e., 37 °C and pH = 7), which are close to physiological conditions, it was very unlikely to expect a breakage of the peptide bond. However, even though the breakage of bonds was not expected, it was necessary to study the denaturation kinetics of the molecule. The secondary structure of sCT contains a single α-helix due to the small amount of amino acid units.

The stability of sCT was determined using a reverse phase high-pressure liquid chromatography (HPLC) analysis. This method is very common in the determination of protein concentration as well as for protein sequencing. Reverse phase HPLC gives higher resolution and selectivity. Therefore, impurities and protein behavior can be observed very clearly in the chromatogram. However, the disadvantage of this procedure is that it takes a considerable amount of time.

During the course of the stability experiments, different kinetic profiles were observed. The chromatograms started to show a growing characteristic peak around 3.75 min, indicating that breakage had occurred. These inconsistencies led to further analysis of the experimental conditions, and it was concluded that the experimental conditions used were suitable for the growth and development of bacteria and fungi. When the experiments were conducted under sterile conditions, the chromatograms showed no additional peaks, indicating that peptide bonds remained intact. These results showed that after approximately 30 h of exposure to aqueous conditions, around 93% of sCT remained in solution as shown Figure 5. The remaining 7% may have been lost due to adsorption on glass, some denaturation, and possible contamination.

Loading Efficiency and Release Behavior of sCT in P(MAA-*g*-EG) Hydrogels. The loading efficiency was calculated as one minus the fraction of calcitonin remaining in solution (i.e., ratio of the final concentration and the original concentration). Table 2 shows the loading efficiency of hydrogels that contained different solvent volume fractions during preparation. Notice that the higher the solvent volume fraction, the lower the loading efficiency. This was due to the interactions between the polymer and the peptide. The isoelectric point (pI) of sCT is 8.86, which implies that at physiological conditions (i.e., pH ≈ 7) the net charge of the molecule is positive. Therefore, there are electrostatic

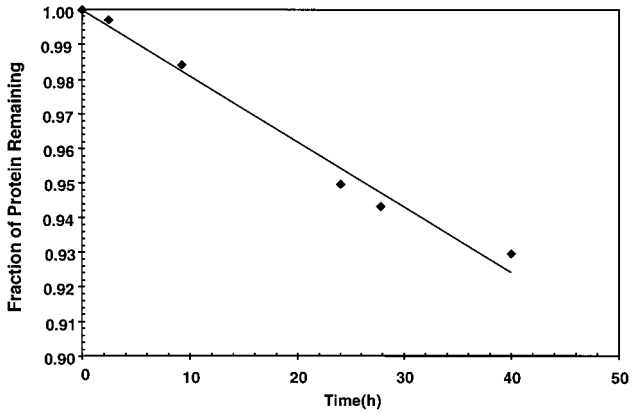

Figure 5. Denaturation kinetics of sCT in sterile phosphate buffer at pH = 7, I = 0.1 M, and 37 °C.

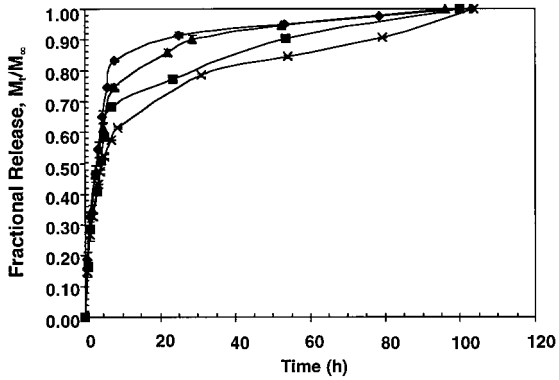

Figure 6. Release behavior of sCT in P(MAA-*g*-EG) for different solvent volume fractions 0.17 (◆), 0.23 (■), 0.34 (▲), and 0.57 (×) in phosphate buffer at pH = 7, I = 0.1 M, and T = 37 °C.

Table 2. Loading Efficiency of P(MAA-*g*-EG)Hydrogels Prepared with Different Solvent Volume Fractions

solvent vol fraction	loading efficiency
0.17	0.71
0.23	0.70
0.34	0.64
0.57	0.57

interactions between the polymer and the peptide. Hydrogels prepared with lower degrees of dilutions produced denser networks that contained a higher net charge per unit area, whereas those containing a higher amount of solvent produced a less dense polymer structure that contained less surface charge.

The release behavior of sCT was studied in vitro. Figure 6 shows the fractional release of sCT plotted as a function of time for hydrogels containing different volume fractions during preparation. The data show that the sCT release is slow and is not very much affected by the initial solvent content of the system. This was not expected due to the electrostatic interactions between the polymer and the polypeptide. Moreover, a constant release of sCT was observed for the first 7 h. This was an unusual observation, indicating that CT release was the result of a combination of polymer−protein interactions and a slow swelling process. However, the biological activity of the sCT released from these hydrogels was not tested.

To further analyze the transport behavior of sCT in the hydrogels, the diffusional phenomena were analyzed

Table 3. *n* Values, Diffusion Coefficients, and Characteristic Relaxation Times for P(MAA-*g*-EG) Hydrogels Prepared Using Different Solvent Volume Fractions

solvent vol fraction	*n* value	transport mechanism	diffusion coeff (cm^2/s) $\times 10^{-8}$	characteristic relaxation time (s) $\times 10^5$
0.17	0.88 ± 0.15	relaxation controlled	1.6	1.38
0.23	0.79 ± 0.26	relaxation controlled	1.4	1.37
0.34	0.90 ± 0.20	relaxation controlled	1.6	1.04
0.57	0.85 ± 0.16	relaxation controlled	1.3	1.85

using the following equation[9]

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left\{D\frac{\partial C}{\partial x} - vC\right\} \tag{3}$$

where C is the concentration of solute, x is distance, D is the diffusion coefficient, v is the velocity of the swelling front, and t is time. The solution of this equation characterizes the diffusional phenomena in the glassy state. One special characteristic of this equation is that it contains an additional term vC, which accounts for the non-Fickian behavior of the system. For slabs, the approximate solution for eq 3 assuming D and v constant is

$$\frac{M_t}{M_\infty} = K_1 t^{1/2} + K_2 t \quad \text{for } 0 < \frac{M_t}{M_\infty} < 0.60 \tag{4}$$

where M_t is the cumulative mass of solute released at time t, M_∞ is the total amount of solute released, and t is time. The first term is the Fickian term, and the second takes into account the non-Fickian behavior. Therefore, eq 4 can also be written in a more general sense as

$$\frac{M_t}{M_\infty} = Kt^n \quad \text{for } 0 < \frac{M_t}{M_\infty} < 0.60 \tag{5}$$

where n is the diffusional exponent. However, in some instances it is very difficult to avoid the accumulation of solute on the surface of the hydrogel. This will lead to what is called the "burst effect", where the solute accumulated in the surface goes immediately to solution, making the release profile to have an intercept. To correct for this phenomenon eq 5 can be written as

$$\frac{M_t}{M_\infty} = \alpha + kt^n \quad \text{for } 0 < \frac{M_t}{M_\infty} < 0.60 \tag{6}$$

where the term α will take into account the "burst effect" associated with the solute accumulated on the surface.

From the previous equations, the value of n can be used to determine the type of solute transport in the system. For $n = 0.5$ Fickian diffusion will dominate, for $n > 0.5$ the solute transport will be non-Fickian, and when $n = 1$ the system will be relaxation controlled. The constant α was estimated as the intercept at time zero.

Table 3 shows the results of applying eq 6 to the release data. The values of n were calculated using those data points that were under a fractional release of 0.60 (approximately 4−6 points for each case). The data points used were the averages between two separate runs. For all the cases the value of n ranges from 0.8 to 0.9, which implies that the release behavior was controlled by the chain relaxation. This phenomenon occurs when the movement of the polymer chains dominates the solute transport as the releasing agent enters in the system. Clearly, during the release of calcitonin, a relatively large molecular weight material, the macromolecular chains of the network rearrange in a strongly relaxation-controlled mechanism. The tethered PEG chains present additional barriers for this relaxational process. Moreover, the results demonstrate that the exponent n is not strongly affected by the initial content of solvent in the system. This may be due to the fact that even though there is a difference in the mesh size for hydrogels prepared with higher amounts of diluent, this difference seems to be unimportant when the molecular size is larger than water.

The solute diffusion coefficient, D, can only be estimated from these results. First, transport models for hydrogels do not take into account the interactions between the polymer and the solute. Second, the diffusion coefficient calculated from solute release from hydrogels is commonly calculated from the Fickian part of the release ($0 < M_t/M_\infty < 0.60$). Probably, an approximation could be used to calculate the diffusion coefficient, but it is not appropriate for exact diffusional analysis. However, as it was previously shown, for this system the predominant transport mechanism is chain relaxation. Thus, this approach cannot be followed.

To obtain a better approximation, an exact solution of eq 3 was used, known as the Berens and Hopfenberg model,[10,11] which establishes that

$$\frac{M_t}{M_\infty} = \phi_F\left[1 - \sum_{n=1}^{n=\infty}\frac{8}{(2n+1)\pi^2}\exp\left(\frac{-D(2n+1)^2 t}{4l^2}\right)\right] + \phi_R(1 - \exp(-kt)) \tag{7}$$

where k is the first order-relaxation constant, D is the diffusion coefficient, ϕ_F and ϕ_R are the fractions of sorption contributed by Fickian diffusion and the chain relaxation, respectively, and l is the half-thickness of the slab. This is a heuristic model that analyzes the overall release in terms of Fickian and non-Fickian contributions. This analysis can lead to the determination of not only the diffusion coefficient, D, but also a characteristic relaxation time, τ, which is defined as the reciprocal of the term k.

Equation 7 can also be recast in the following form,

$$1 - \frac{M_t}{M_\infty} = \phi_F\left[\sum_{n=1}^{n=\infty}\frac{8}{(2n+1)\pi^2}\exp\left(\frac{-D(2n+1)^2 t}{4l^2}\right)\right] + \phi_R(-\exp(-kt)) \tag{8}$$

A semilog plot of $(1 - M_t/M_\infty)$ versus linear time was used to determine the constant k, ϕ_F, and ϕ_R. At later times, the solute transport was dominated by the non-Fickian term. Therefore, the first part of eq 8 was neglected, and the parameters could be determined. The slope of the straight line formed at longer times was defined as $-k$, and the intercept at time zero was defined as $\ln \phi_R$. The value ϕ_F can be calculated using the fact that the sum of ϕ_F and ϕ_R must equal one. The

value of the integer n from eq 9 was set at three. A plot of the $\ln(1 - M_t/M_\infty)$ vs t could be used to calculate D from the slope using the first 60% of the release data.

Table 3 shows the estimated diffusion coefficients for the different hydrogels and the characteristic relaxation time. The results demonstrate that there is not a strong dependency on the amount of solvent used in the polymer preparation. Thus, the mesh size is not different enough to produce a significant change in the diffusion coefficient. Therefore, the amount of solvent used to prepare the polymer is not enough to control the rate of transport of sCT in the system.

Conclusions

The chemical stability of sCT was studied in aqueous solution at close to physiological conditions. The experimental results showed that under sterile conditions the molecule remained intact for an extended period of time. Therefore, care must be taken during experimental runs in order to avoid bacterial growth and not overestimate the concentration of sCT in solution. However, this does not imply that the molecule remains biologically active. In vivo studies are needed in order to determine the biological activity of the molecule after it has been put in contact with the system.

Swelling studies demonstrated that the swelling behavior of the hydrogels was indeed affected by the amount of solvent used during preparation. A strong effect was not observed until the monomer mixture was not diluted to approximately 50%. This was confirmed by the calculation of the mesh size.

Salmon calcitonin was successfully incorporated and released in vitro from the system. The loading efficiency was affected by the amount of solvent used during hydrogel preparation. This suggests that there is an interaction between the polymer and the hydrogel, which in this case is predominantly ionic in nature.

The transport mechanism was found to be relaxation controlled for all cases, and the diffusion coefficient was estimated using the model of Berens and Hopfenberg. In this case, the release behavior and thus the diffusion coefficient were found to be not very much affected by the amount of solvent used during preparation. Therefore, the amount of diluent used is not enough to control the polypeptide delivery rate. However, in all cases sCT release was constant for around 7 h and was completely released in approximately 3 days.

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