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Research paper

A novel approach for the control of drug release rate through hydrogel membrane: I. Effect of drug immobilization on drug release rate by copolymerization method

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Abstract

Precise control of drug release rate in hydrogel drug delivery systems to better mimic physiological condition is a challenging research topic in development of Advanced Drug Delivery Systems. One of the major issues with bioresponsive drug delivery systems is the excessive 'leakage' of drug while the system is in the 'off' state, which leads to shortening of the device life-time and potential overdose problem for the patient. In the present study, a new approach, based on partition effects, termed drug immobilization via copolymerization, is proposed to control the drug release rate of membrane-based drug delivery systems. In this method, a certain level of drug is pre-immobilized in the membrane through copolymerization. The immobilized drug contributes to the overall chemical potential of drug molecules in the membrane but their mobility is restricted, hence will not be released. At equilibrium, the amount of drug from donor that dissolved in the membrane is reduced due to contribution of immobilized drug, resulting in an effective reduction in partition coefficient and hence the release rate. The testing of the method by bovine serum albumin (BSA) as a model drug confirmed the controllability of the method: almost 35% reduction of the drug leakage in the 'off-state' was observed when 20 mg BSA was immobilized in the pH-sensitive hydrogel membrane. The mathematical model of the drug partition in the membrane was modified to describe the new partition phenomenon (mobile drug and immobilized drug in the membrane) in this study.

Keywords: Controlled drug delivery; Hydrogel; Release rate control; Burse release; Partition coefficient

1. Introduction

One of the major unsolved problems in drug delivery concerns the development of the so-called bioresponsive systems, i.e. systems that deliver drug in response to changes in the physiological environment [1]. Such delivery clearly is optimal for many disease conditions, particularly in the case of diabetics, who need insulin only when their sugar levels are higher than normal [2]. However, precise control over release rate to better mimic physiological condition is still a challenging topic in the development of Advanced Drug Delivery Systems (ADDS).

Diffusion is one of the most important mechanisms used to control drug release from pharmaceutical devices [3,4]. Control of Fickian transport for drug release is widely performed by the use of polymer membranes or matrices [5]. Variation of the release rates is controlled by several factors, i.e. structure and composition of the matrix, pore size, pore enhanced modification to alter drug–matrix/membrane interaction [6], size and shape of the matrix [7], surface to volume ratio [8], etc.

The solution-diffusion model [9] is one of the most popular mathematical models to describe the drug release through polymeric membranes. Most research effort for drug release focuses on how the diffusion step is dependent

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on material variables. One such example is the excellent model developed by Peppas et al. [10,11] based on free volume theory [12].

With these control methods, however, burst release [13] and uncontrollable off-state leakage are usually observed. The burst release refers to the sudden initial release upon administration because of the partitioning of drug to the surface of the membrane based device during storage, whereas the off-state leakage is the drug release when the device is supposed to be 'shut-down', contributed by measurable drug diffusion even in the unswollen (or partially-swollen) state. For bioresponsive systems, burst release in the beginning of administration and leakage during the off-state are problems that have not been satisfactorily resolved to date. Without resolution of this issue, it is unlikely that bioresponsive systems will be viable commercially.

Although protein partition behavior in Aqueous Two Phase Systems (ATPS) has been studied for decades in the area of protein separation and purification [14], and study of hydrogel as a separation medium in ATPS was reported more than ten years ago [15,16], the partition effect on the drug release rate is usually taken for granted. Tada et al. [17,18] first immobilized albumin in a hydrogel structure. One of their purposes was to make use of the binding ability of albumin to various drugs, so as to control their release rate. Their method was equivalent to alter the drug–membrane interactions (through protein–drug binding); therefore to control the amount of drug entering into the membrane and its diffusivity in it. The method works for proteins that bind to albumin, however, it is not applicable to proteins without (or with low) albumin affinity. Moreover, the affinity of drug to albumin is not a controllable parameter, so the controllability of this method is poor.

A device that controls release rates based on partitioning is a more attractive option. In drug partitioning, two factors determine the amount of drug that dissolves in the membrane (Eq. (1)), they are steric factor (Eq. (2)) and the chemical potential equilibrium (Eq. (3)) [15,16].

$$K_p = K_{p\text{-non}} + K_{p\text{-elec}} \tag{1}$$

$$K_p = \exp\left[-\phi_p\left(1+\frac{a_s}{a_p}\right)\right] \tag{2}$$

and

$$\ln K_{p\text{-elec}} = \ln\frac{x_{p,membrane}}{x_{p,bulk}} = -\ln\gamma'_{p,membrane} - \frac{\pi v_p}{RT} \tag{3}$$

where $x_{p,membrane}$ and $x_{p,bulk}$ are solute mole fractions at the donor side of the membrane and donor bulk solution, respectively. $\gamma'_{p,membrane}$ is the solute activity coefficient in the membrane, which is a measurement of degree of non-ideality of the solute (forces experienced by the solute molecule and spatial arrangement of atoms). a_s is the solute radius and a_p is the radius of voids available in the membrane. R and T are gas constant and absolute temperature, respectively. π is the osmotic pressure, which measures the pressure difference between the solution and hydrogel. The osmotic pressure of the hydrogel can be calculated based on gel elasticity [15]:

$$\pi_{elastic} = -C_{cross}RT\left(\frac{\phi_p}{\phi_{p,synthesis}}\right)^{1/3} \tag{4}$$

where C_{cross} is the concentration of crosslinks (mol/m^3), ϕ_p and $\phi_{p,synthesis}$ are the volumetric fractions of the hydrogel at fully swollen state and as synthesized, respectively.

The steric effect is a function of intrinsic properties of membrane and solute, and hence more difficult to control. A better option is to use the effect of chemical potential on the partition coefficient, because the chemical potential difference between the solutes in the donor and in the membrane is the driving force of the partition step. As the amount of drug in the membrane increases, the chemical potential of the drug in the membrane increases, until the chemical potential of the drug in the membrane is equal to the chemical potential of the drug in the donor solution.

In the present study, we demonstrate the feasibility to control the drug release rate at the partition (solution) step by drug (which is the same drug as in the donor solution) immobilization through copolymerization. The immobilized drug is expected to contribute to the chemical potential of the drug in the membrane, but not able to release from it. Our objective is to control the off-state leakage through proper selection of amount of drug immobilized in the hydrogel membrane. The method presented can also be used for control of burst release.

2. Experimental

2.1. Materials

The monomers used for the pH-sensitive hydrogel were polyethylene glycol monomethacrylate (PEGMA, EG repeating unit molecular weight 400; Polysciences, Warrington, PA), and 2-(diethylamino)ethyl methacrylate (DEAEMA, Sigma). Tetraethylene glycol diacrylate (TEGDA, Sigma) was used as crosslinking agent and the photoinitiator used was 2,2-dimethoxy-2-phenyl acetophenone (DMPA, Sigma). A well-studied protein, bovine serum albumin (BSA, Sigma), was chosen to be the model drug in this study to prove the concept. FITC-BSA (Sigma) was used to visualize the immobilization of protein in the membrane. Acryloyl chloride (Merck) was used for protein functionalization and attachment to the hydrogel. Phosphate Buffered Saline (PBS, Sigma) was used as buffer solution. All the chemicals were used as received.

2.2. Bovine serum albumin functionalization

In order to immobilize BSA in the hydrogel membrane during polymerization, BSA molecules need to be functionalized first by introducing C=C bonds. The functionalization process was similar as previously described by

Peppas et al. [19]. Briefly, 1 ml of 20 mg/ml BSA solution was prepared in pH 7.4 PBS, 2 μl acryloyl chloride was then added and stored at 4 °C for 2 h. Acrylate groups will be covalently bound to the nitrogen atoms of the peptide bonds in the BSA molecule after this process.

2.3. BSA immobilization through copolymerization

The immobilization of functionalized BSA was also similar to the immobilization of Glucose Oxidase (GOx) and catalase to pH-sensitive hydrogel described by Peppas et al. [19]. One milliliter of DI water was added into 1 ml ethanol (improving compatibility of water with DEAEMA) followed by 4 g PEGMA, and 2 ml DEAEMA. Crosslinking density was set to be 1% per mole of monomer (PEGMA + DEAEMA). The monomer mixture was stirred overnight. Just before synthesis, 3% w/w (total monomer) DMPA and 1 ml functionalized BSA solution (added dropwise) were added and stirred for 2 h. The monomer mixture was put in an ultrasonic bath for 10 min in order to remove dissolved oxygen, which acts as a free radical scavenger [20]. One hundred microliter of monomer was then pipetted between two silane treated glass slides, which were separated by 200 μm spacer. The system was then exposed to UV light (Crosslinker, 365 nm) for 2 min on each side at the intensity of 3 mW/cm^2. The control pH-sensitive hydrogel membranes, which did not contain immobilized BSA, were synthesized in a similar way with 1 ml PBS (pH 7.4) instead of the functionalized BSA solution.

The synthesized membranes were soaked in DI water for a week to remove unreacted proteins and monomers. The soaking water was changed daily to ensure freshness.

2.4. Fluorescence study of protein immobilized membrane

The purpose of protein immobilization was to affect the partition behavior of free protein drugs in the donor, but the immobilized drugs should not be released. In order to prove the protein can be retained in the hydrogel by copolymerization method, the fluorescence labeled BSA (FITC-BSA) was functionalized and immobilized according to the presented procedure. After soaking the FITC-BSA immobilized membrane in DI water for a week with water changed daily (stored at 4 °C), the membrane was imaged by a fluorescence microscope (Carl Ziess, Germany), with 10× objective lens and 40 ms exposure time. The excitation wavelength of the fluorescence was 490 nm, and the emission at 521 nm was recorded. If immobilization failed, no or very weak signal at 521 nm (green in color) shall be observed after soaking.

2.5. Swelling experiment

The hydrogel membranes were dried in a vacuum oven (1 mBa) at 37 °C for 2 days. The pH response of the membrane was studied in phosphate buffer solutions with pH values between 4.5 and 7.4. To study the dynamic swelling behavior of the membrane, samples were weighted and immersed into PBS of pH 2.4 and pH 7.4 at room temperature for 10 min. The sample was taken out every minute and weighted after carefully removing surface water. The equilibrium swelling ratio was obtained by leaving the membrane in the PBS for 24 h. Both static and dynamic experiments were duplicated. Swelling ratio was calculated according to

$$\text{swelling\_ratio} = \frac{m_{\text{swollen}} - m_{\text{dry}}}{m_{\text{dry}}} \tag{5}$$

where m_{swollen} is the membrane mass at swollen state and m_{dry} is the mass when the membrane is dry.

The dynamic swelling behavior was characterized by fitting the data into an empirical relationship:

$$\frac{M_t}{M_\infty} = kt^n \tag{6}$$

where M_t is the amount of solvent absorbed at time t and M_∞ is the amount of solvent absorbed at equilibrium. K and n are both constants.

2.6. Protein permeation experiment

pH 7.4 corresponded to the 'off-state' of the system as suggested in the swelling experiment. So the effect of protein immobilization on the 'off-state' leakage was studied at pH 7.4.

The membranes used for the protein permeation experiment were equilibrated in PBS of pH 7.4 for at least 2 days. The permeation experiment was conducted using a pair of home-made side-by-side diffusion cell with inner diameter of 1 cm. The diffusion cell consists of two glass chambers with volume of 3 ml each. To determine the effective diffusion coefficient of solute in 'off-state', precisely weighed BSA was dissolved in PBS of pH 7.4 and stored at 4 °C before use. Samples that were equilibrated at that pH were used for the permeation experiment. Two milliliter of 5 mg/ml BSA solution was loaded into the donor chamber and 2 ml fresh PBS of the same pH was loaded into the receptor chamber. Receptor solution was completely taken out at predetermined interval for measurement and fresh solution of the same volume was refilled into the receptor and restart timing. Donor solution was also changed at the end of each interval to guarantee infinite source condition. The sampling interval chosen was 0.5, 1, 1.5, 2, 2.5, and 3 h. Collected drug in receptor solution was measured using a UV–Vis spectrophotometer (Shimadzu UV-1700) at 278.5 nm, the concentration was obtained from a standard curve calibrated at 278.5 nm. The permeation for each type of membrane at a given pH value was repeated five times.

The flux entering the receptor was calculated by

$$F = C_a \cdot V_a/(A \cdot t) \tag{7}$$

where C_a and V_a are the receptor protein concentration and receptor volume, respectively, A is the effective area for diffusion, and t is time.

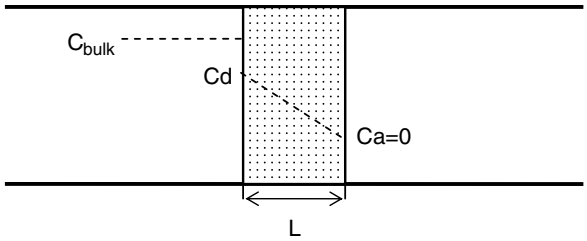

Fig. 1. Drug distribution in the membrane.

The diffusivity of the solute in the membrane and the partition coefficient were calculated from the permeation experimental data by [21,22]

$$\ln(t^{0.5}F_t) = \ln\left\{2C_d\left(\frac{D}{\pi}\right)^{0.5}\right\} - \frac{L^2}{4Dt} \quad (8)$$

where F_t is the flux at time t, L is the thickness of the membrane, D is the diffusivity of the solute in the hydrogel membrane, and C_d is the effective drug concentration at the donor side of the membrane, which was used for partition coefficient calculation according to the following relationship:

$$K = \frac{C_d}{C_{bulk}} \quad (9)$$

where C_{bulk} is the drug concentration in the donor chamber.

2.7. Partition coefficient measurement

To confirm the calculated partition coefficient from permeation experiment, an experimental approach was applied to measure the partition coefficient directly. Membranes after each permeation experiment were rinsed once by dipping into DI water to remove the absorbed BSA on the membrane surface, followed by removing the surface water of the membrane using lab tissue; the membrane was then immersed into 3 ml PBSA solution (unloading medium) at the same pH level as the permeation experiment. After 12 h, the BSA concentration in the unloading medium was measured by UV–Vis spectrophotometer to determine the total amount of protein dissolved in the membrane during permeation experiment.

The average BSA concentration in the membrane was calculated by dividing the total amount of BSA dissolved it by the total solvent volume in the membrane. Under steady-state, assuming infinite source (donor concentration is constant) and perfect sink condition (receptor concentration is zero) as in the permeation experiment, a linear concentration gradient (assuming constant diffusivity in the membrane,) is established in the membrane (Fig. 1); hence the solute concentration at the donor side is twice the average solute concentration in the membrane. The solute concentration at the donor side was used for calculation of partition coefficient rather than the average solute concentration, this was because the chemical potential equilibrium occurred only at the solution–membrane interface, but not inside the membrane; therefore, partition coefficient due to chemical potential equilibrium shall only be calculated using the boundary values.

3. Results and discussion

3.1. Fluorescence study of protein immobilized membrane

The fluorescence images of membranes with and without FITC-BSA immobilization are shown in Fig. 2. The strong and uniform green[1] color of the FITC-BSA immobilized membrane suggested that great amount of FITC-BSA were still present in the membrane even after one week soaking in DI water, hence immobilization was successfully done. Uniformity of the fluorescence also indicated that the distribution of immobilized FITC-BSA in the membrane was uniform by the copolymerization method.

The fluorescence image of FITC-BSA immobilized membrane right after synthesis (without soaking) was not used to compare the effectiveness of immobilization (the amount of BSA remained in the hydrogel after soaking) because the intensity of fluorescence depended on the concentration of FITC-BSA, whereas the concentration of FITC-BSA in the membrane before and after soaking was not comparable due to swelling of hydrogel in soaking medium.

3.2. Kinetics of swelling

pH-sensitivity changed after BSA immobilization (Fig. 3). At high pH, there was no significant difference in swelling rate; however, it swelled much faster after BSA immobilization at lower pH value. This was because the BSA molecule was also positively charged at low pH values (Table 1), hence greater electrostatic repulsion resulted and the membrane swelled faster.

To understand the effect of BSA immobilization on kinetics of swelling behavior of the membrane, we fitted the swelling data of first five minutes (corresponds to $\frac{M_t}{M_\infty} < 0.6$) to a power law equation (Eq. (6)) and results are summarized in Table 2.

Two mechanisms contribute to hydrogel swelling rates: diffusion and network relaxation. The value of n indicated which mechanism dominated the swelling process. A value of 0.5 meant swelling was dominated by diffusion; whereas $n = 1$ meant swelling was dominated by network relaxation. In all cases (before and after BSA immobilization), n increased as pH decreased, suggesting network relaxation contribution to swelling was more prominent at low pH. The swelling rate can be obtained by differentiating Eq. (6) with respect to time.

[1] For interpretation of the references to color in this text, the reader is referred to the web version of this article.

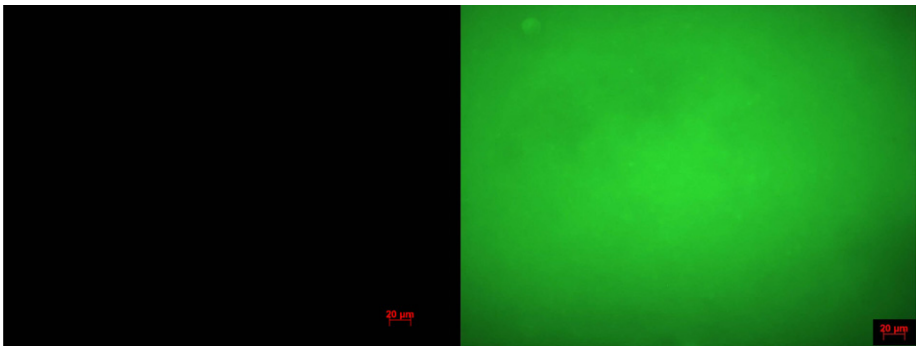

Fig. 2. Membranes with immobilized FITC-BSA after rinsing for one week (right image) as compared to a membrane with no BSA immobilization (left image), scale bar is 20 μm.

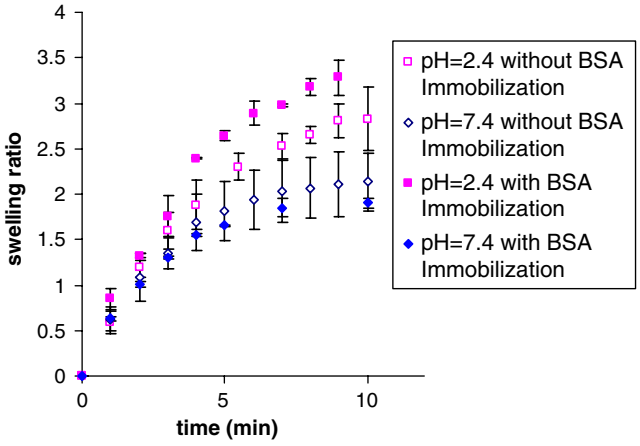

Fig. 3. Dynamic swelling.

Table 1
BSA parameters [23]

Parameter	Value
M_p	69,000 g/mol
σ_p	6.26 nm
ε_l/k_B	91.3 K
z_p	+20 at pH 4.0, −13.5 at pH 6.0, −18.8 at pH 7.0, −22.9 at pH 8.0, $I = 1.0$ mol/L −20.4 at pH 7.4, −9.1 at pH 5.4, +4.5 it pH 4.7, $I = 0.15$ mol/L

Table 2
Summary of dynamic swelling data

Sample	pH	k	n	R^2
Without BSA immobilization	7.4	0.257	0.671	0.992
	2.4	0.200	0.813	0.995
With BSA immobilization	7.4	0.275	0.601	0.993
	2.4	0.207	0.655	0.999

After BSA immobilization, n was closer to 0.5, which meant that the immobilized BSA tended to restrict relaxation. This effect could be explained by calculating the number of acrylate groups per BSA molecule. Twenty milligram of BSA corresponded to 3×10^{-7} mol BSA molecules, whereas 2 μl acryloyl chloride corresponded to 0.000123 mol. Assuming reaction was complete and random, each BSA molecule would have 408 acrylate groups attached (586 amino acids per BSA). Therefore, each BSA molecule would act as a multi-functional 'crosslinker' during copolymerization (initial crosslinker: 0.000182 mol TEGDA). With more crosslinks, network relaxation was restricted.

The swelling rate of the hydrogel with/without BSA immobilization could be obtained by differentiating Eq. (6) with respect to time (results not shown). The swelling rate of hydrogel with BSA immobilization was higher in the early stage of swelling due to electrostatic repulsion; however its swelling rate decreases rapidly when the hydrogel swells more due to the restrictive effect of crosslinks.

3.3. Equilibrium swelling experiment

Poly (DEAEMA-g-PEG) hydrogels showed sharp transition between pH 7 and pH 7.4 as reported by Peppas and co-workers [19]. Although the crosslinking density was increased after immobilization, the equilibrium swelling ratio of hydrogels was not affected by immobilization (Fig. 4). The underlying reason would be discussed in the latter section.

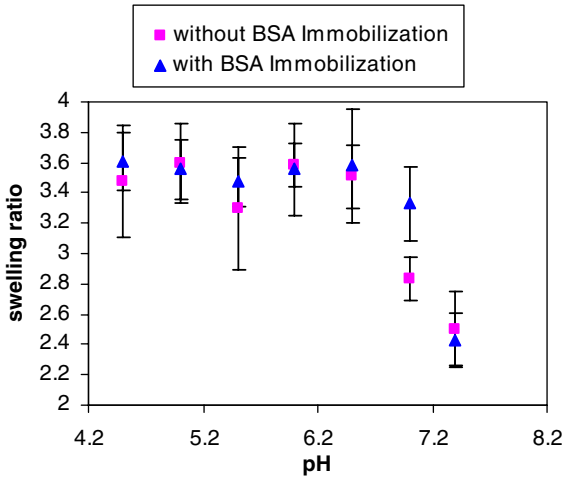

Fig. 4. pH-response of hydrogels before and after BSA immobilization.

At pH 7, around which transition took place, hydrogel swelled more after BSA immobilization, which meant that pH transition was sharpened by BSA immobilization. This was because of the lower osmotic pressure (Eq. (4)) in the membrane (calculated osmotic pressure in the membrane was shown in Fig. 5).

3.4. Discussion on swelling rate versus swelling ratio

In hydrogels, whose crosslinking densities were usually adjusted to realize a desired release rate, swelling rate, and swelling ratio are usually positively correlated (low swelling rate corresponded to small swelling ratio), because restrictive force of swelling was affected by crosslinking but the expansion forces were usually not affected. However, when protein was immobilized in the hydrogel structure, both restrictive and expansion forces were affected, therefore, swelling rate and swelling ratio might behave differently compared to the conventional cases.

Three functions that immobilized BSA served can be identified so far, namely, extra crosslinks, supply chargeable groups and decrease in osmotic pressure in the membrane. The first function tends to restrict swelling, however, the last two functions [which are related, not independent] provide expansion force for swelling. In the early stage of swelling when the polymeric chains are tangled, expansion force dominates, therefore membranes with more chargeable groups and lower osmotic pressure swell faster. As membrane continues swelling to the extent that polymeric chains are extended, the restrictive effect due to crosslinks takes place until expanding and restrictive forces are balanced. Therefore, for the particular composition of the protein immobilized hydrogel in this study, although hydrogels with BSA immobilization swelled faster in the early stage of swelling at low pH, the equilibrium swelling ratio was not affected by protein immobilization due to the balance of the competing forces when equilibrium was reached.

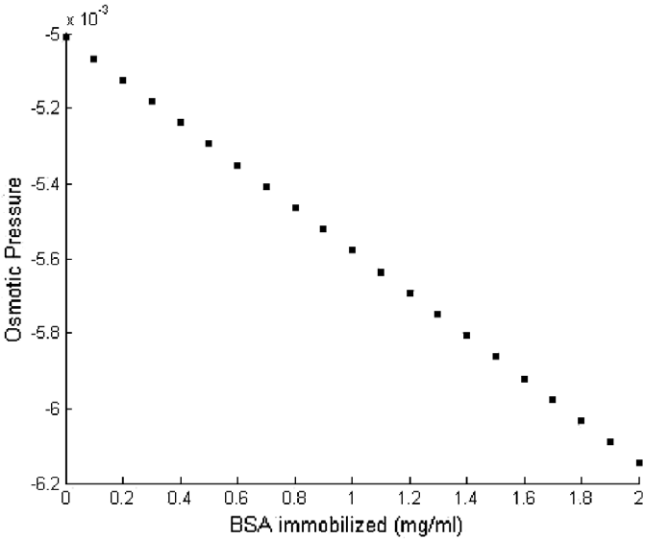

Fig. 5. Osmotic pressure changes after BSA immobilization, assuming all the BSA has been immobilized, and each BSA acts as 100 di-functional crosslinks.

3.5. Protein permeation experiment

The calculated flux is shown in Fig. 6 (values shown are average value from five experiments; error bar indicates one standard deviation):

Significant difference was observed after performing the statistical analysis (t-test, $p > 0.05$ using Excel) and the flux was reduced by 34.7% after protein immobilization.

Reduction in flux was observed after BSA immobilization, however, in the calculation, the effect of protein immobilization on solution (partition) and diffusion steps is still unknown. The diffusivity and partition coefficient are therefore needed.

The diffusivity of the mobile BSA in the membrane and its partition coefficient could be extracted by applying Eq. (8) and (9). The thickness of the membrane after equilibration was 0.42 mm measured by a micrometer. By plotting $\ln(t^{0.5}J)$ versus $1/t$, the diffusivity and partition coefficient could be determined from the intersection of the line with y axis and its slope (Table 3).

Statistical analysis by t-test showed no significant difference as $p > 0.05$ of the diffusivity of mobile BSA in the membrane after immobilization, however, partition coefficient was decreased by 30%. Therefore, the reduction of release rate was contributed by partition step rather than diffusion step.

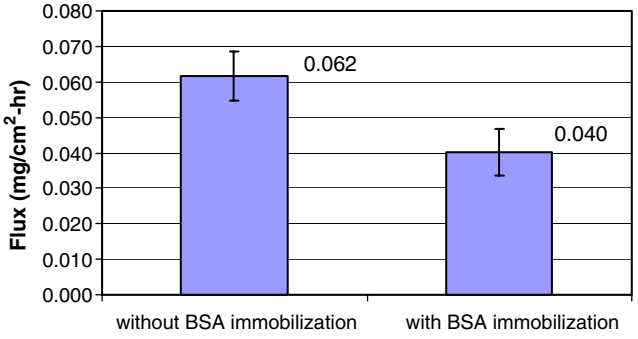

Fig. 6. Flux comparison.

Table 3
Comparison of partition coefficient and diffusivity before and after BSA immobilization

Membranes	Partition coefficient (average ± SD)	Diffusivity (cm^2/s) × 10^7 (average ± SD)
Without BSA immobilization	1.219 ± 0.155	1.325 ± 0.311
With BSA immobilization	0.826 ± 0.348	1.447 ± 0.456

SD, standard derivation.

3.6. Prediction of the structure of the BSA immobilized hydrogel

Since swelling ratio remained unchanged after BSA immobilization the diffusivity of solute in the membrane should not change, according to free volume theory (Eq. (10)) [10,11], the void size was unaffected after BSA immobilization (Eq. (10)), so was the number of repeating units between crosslinks.

$$\frac{D}{D_0} = f(\bar{M}_c)\exp\left(\frac{-v_{solute}}{V_{solvent}\left(\frac{1}{\phi_p}-1\right)}\right) \quad (10)$$

where D_0 is the diffusivity of the solute in water. $\bar{M}_c$ is the number average molecular weight between crosslinks. $f(\bar{M}_c)$ is the probability function of finding a void big enough to allow diffusion, v_{solute} is solute volume, and $V_{solvent}$ is the free volume of solvent.

Because BSA acted as extra multi-functional crosslinks, if each BSA molecule was considered as a number of difunctional crosslinks, there must be more than two free amino acids (not crosslinked) between two functionalized amino acids (crosslinked) to maintain the number of average repeating units between crosslinks.

Conventional crosslinking agents are usually small molecules, therefore their function is just to crosslink different molecular chains, but their own size is usually ignored. However, randomly functionalized proteins are big in size, when they are functionalized and used as crosslinks, the unfunctionalized portion will serve as spacers between crosslinks.

3.7. Partition coefficient measurement

To verify the partition coefficient predicted from the permeation experiment, a direct experimental method was applied. The estimated partition coefficient at the donor side of membrane is given in Table 4.

The partition coefficient estimated from this method was smaller compared to previous results, which was possibly because not all the solute dissolved in the membrane would be unloaded. Therefore, the exact value of this calculation did not reflect the exact amount of protein partitioned into the membrane, but it was meaningful as a comparison parameter since all the experiments were conducted following the same procedure. Partition coefficient was reduced by 53.7% based on this calculation.

3.8. Discussion on the reduction of partition coefficient

In the study of Tada et al. [17,18], the protein that was immobilized in the membrane was different from the permeating drugs. And the parameter that they actually changed was in fact the activity coefficient of the solute in the membrane by using a large amount of albumin (albumin: monomer = 11:1 w/w) as a binding agent to affect the interaction between solute and membrane. Minor increase in partition coefficient was observed due to drug binding to the albumin [18]. However in our study, a reduction in partition coefficient of BSA was observed after immobilizing small amount (BSA: monomers < 1% w/w) of the same type of protein (BSA) as the drug in the donor. Another advantage of the proposed method was that it could be applied to control the 'off-state' leakage for any protein drugs because partition behavior was independent of protein types.

To find out the effect of protein immobilization on the partition mechanism, the partition coefficient equation (Eq. (1)) was analyzed term by term. First of all, the mesh size did not change after BSA immobilization (as discussion in previous section), suggesting steric effect was not affected (Eq. (2)); secondly, a more negative osmotic pressure was observed from the swelling experiment, which should increase the partition coefficient (Eq. (3)).

To find out the influence of protein immobilization on solute activity coefficient, the UNIQUAC model [24] and solute partition model in hydrogels [15,16] were applied to predict the changes of solute activity coefficient. From the UNIQUAC model, even if 200 mg/ml BSA was used during synthesis, the change in solute activity coefficient was less than 1% (calculation based on UNIQUAC model is shown in Fig. 7). Furthermore, the model predicted that solute activity coefficient in the membrane decreased after immobilization, which caused an increase in partition coefficient. The second model, the solute partition model in hydrogels, predicted that the partition coefficient should decrease after immobilization due to electrostatic repulsion, the reduction in partition coefficient was also less than 1% (calculation was not shown).

Table 4
Comparison of partition coefficient

Membranes	Partition coefficient (average ± SD)
Without BSA immobilization	0.946 ± 0.166
With BSA immobilization	0.438 ± 0.116

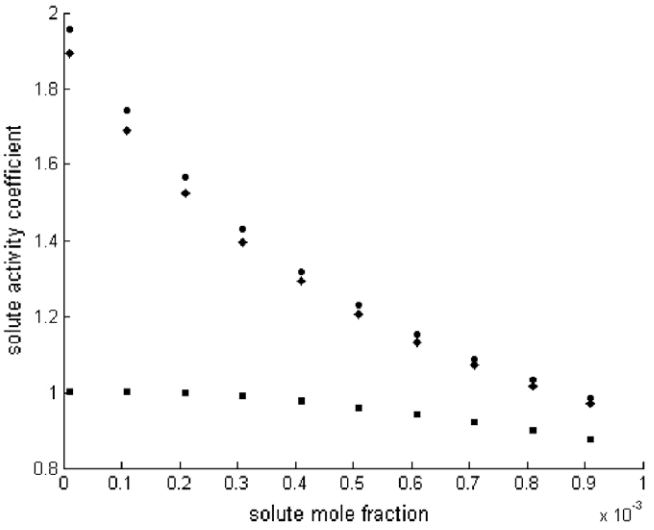

Fig. 7. BSA activity coefficient. ■, In bulk solution; ●, in membrane; ♦, in membrane with 200 mg/ml added during polymerization in membrane.

Based on the discussions on the changes of osmotic pressure and activity coefficient after protein immobilization, the right-hand side of Eq. (3) increased after BSA immobilization, so the solubility of BSA in hydrogel ($\ln\frac{x_B}{x_A}$) should increase slightly, which seemed contradictory with experimental observation. However on the left-hand side of the Eq. (3), there were in fact two types of proteins in the BSA immobilized membrane, namely, mobile protein ($x_{\text{m-mobile}}$) and immobilized protein ($x_{\text{m-immob}}$). The total chemical potential of protein in the membrane should be affected by both.

Therefore, the total fraction of protein in the membrane was

$$x_{\text{m-immob}} = x_{\text{m-immob}}^{\text{mobile}} + f\left(b \times x_{\text{m-immob}}^{\text{immobilized}}\right) \tag{11}$$

where b is a factor that accounts for the changes in thermodynamic properties of protein after polymerization reaction. If the total fraction is a linear combination of mobile and immobilized protein the partition coefficient of mobile protein after BSA immobilization becomes:

$$\ln\left(K_{\text{p}} + b\frac{x_{\text{p,membrane}}^{\text{immob}}}{x_{\text{p,bulk}}}\right) = -\ln\gamma'_{\text{p,membrane}} + C_{\text{cross}}v_{\text{p}}\left(\frac{\phi_{\text{p}}}{\phi_{\text{p,synthesis}}}\right)^{1/3} \tag{12}$$

So, even the solubility of BSA in membrane increased slightly after protein immobilization, because of the existence of immobilized protein, the partition coefficient of free protein was reduced (as shown in Fig. 8). The acceptable release rate at off-state could be realized by immobilizing suitable amount of drug molecules in the hydrogel according to the above equation. Although the effect of protein immobilization on the 'on' state was not examined experimentally in this preliminary study, theoretical analysis suggested that the reduction effect at 'on' state was less prominent than the 'off' state due to the diluting effect when the hydrogel swelled more at 'on' state (since the amount of immobilized protein was fixed, $x_{\text{p,membrane}}^{\text{immob}}$ was smaller at 'on' state compared to 'off' state due to greater swelling ratio at 'on' state). The detailed comparison of the effects of protein immobilization on the release behavior between 'on' and 'off' states will be presented in the near future.

4. Conclusion

In this preliminary study, it has been shown that proteins can be successfully immobilized in hydrogel with good uniformity by copolymerization method, and drug release rate can be controlled, especially reduced at the partition step by immobilizing certain amount of drug molecules in the membrane. Although the immobilization increases the solubility of protein slightly due to osmotic pressure changes, the effective amount of mobile solutes for diffusion is reduced, giving rise to the reduction of release rate because the immobilized solute molecules contribute to the overall chemical potential equilibrium of solute molecules in the membrane. By immobilizing merely 20 mg BSA, the 'off' state leakage was reduced by about 35%. Since the control method proposed in this study is based on thermodynamic property of drug molecules, it is applicable to all types of protein drugs. Moreover, the copolymerization method utilized in this study can be used to immobilize all types of proteins because of the existence of peptide bonds in all proteins. The effect of different amount of immobilized protein on the release rate at both 'on' and 'off' states will be studied in our future work.

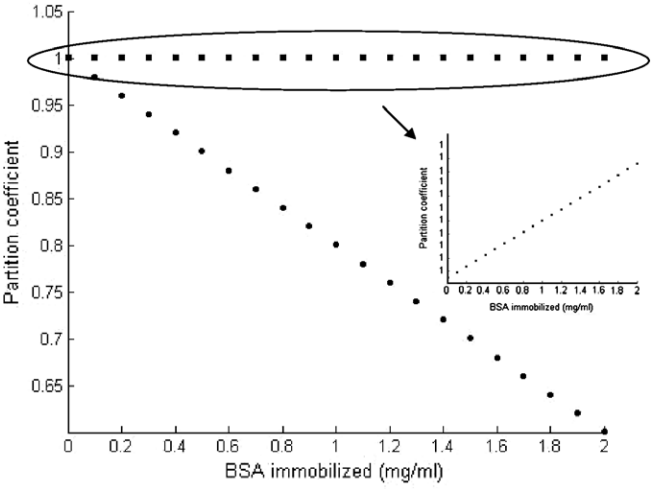

Fig. 8. BSA partition coefficient as a function of amount of immobilized BSA. ■, Partition coefficient of mobile BSA; ●, Partition coefficient contributed from osmotic pressure change; assuming $b = 1$ and activity coefficient of mobile BSA in the membrane is one.

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