CHAPTER IV

Controlled Release of Drugs from Gelatin Hydrogels

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4.1 Abstract

This study evaluates and characterizes the use of porcine and fish gelatin hydrogels as the matrix in a controlled drug delivery system. The drug-loaded gelatin hydrogels were prepared by solution-casting using salicylic acid and 5-sulfosalicylic acid as the model drugs and glutaraldehyde as the crosslinking agent. The average molecular weight between crosslink, the crosslinking density, and the mesh size of the gelatin hydrogels were determined using the equilibrium swelling theory, as well as by scanning electron microscopy. The release mechanisms and the diffusion coefficients of the hydrogels were determined by using a modified Franz-Diffusion cell in an acetate buffer (at pH 5.5 and at a temperature of 37 °C for 48 hours) in order to investigate the effect of the crosslinking ratio. The diffusion coefficient of the drug was determined through the Higuchi equation at various crosslink ratios and different drug size. The diffusion coefficients of drug in the gelatin hydrogels decrease with increasing crosslink ratio due to the smaller mesh sizes of gelatin hydrogels. The diffusion coefficient of a smaller drug size is higher than that of a larger drug size. The diffusion coefficients obey the power law of the drug size over the mesh size ratio with the scaling exponent m equal to 0.45.

Keywords: Gelatin hydrogels; Crosslink; Diffusion coefficient; Controlled drug release

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4.2 Introduction

The routes of drug introducing into the body are oral, injection, and transdermal administration. The benefit of oral and injection routes is to provide the maximum tolerable dose, however the dose level decreases over a short period (Im *et al.*, 2010).Transdermal drug delivery system (TDDS) is a candidate when the frequent administration is required. Besides, TDDS provides the advantages of avoiding the first-pass metabolism, maintaining of blood level for a long period of time, and reducing side effects with a painless (Kim *et al.*, 2006).

Hydrogels are a unique class of macromolecular networks that may contain a large fraction of an aqueous solvent within their structure (Ganji *et al.*, 2008; Hosseinkhani *et al.*, 2006, 2009). They are particularly suitable for biomedical and tissue engineering applications because of their ability to simulate biological tissues. The hydrophilicity of the network is the presence of chemical residues such as hydroxylic (–OH), carboxylic (–COOH), amidic (–CONH–), primary amidic (–$CONH_2$), sulphonic (–SO_3H), and others that can be found within the polymer backbone or as lateral chains (Ganjil *et al.*, 2010). Hydrogels have been widely studied in the application of the controlled drug release because they are three-dimensional crosslinked structures through water-soluble polymers. Many hydrogel forms are available to fabricate, for examples, slabs, microparticles, nanoparticles, coatings, and films. Their properties strongly depend on their building blocks and the preparation procedures. Biopolymers are also available to form hydrogels via physically or chemically crosslinking reaction, especially like gelatin that is a kind of well-defined hydrogel matrix (Schacht *et al.*, 2004).

Gelatin is mainly extracted from mammals, poultries, and fish in which they are primarily consisted of polydisperse polypeptides obtained from either acid or alkaline collagens. The well-known sources are bovine hides, pig and fish skins (Deiber *et al.*, 2009). Recently, the bovine bone gelatin has been raised on a special issue regarding to a risk of contracting bovine spongiform encephalopathy (BSE), even if the possibility could be controlled by safe manufacturing steps (Hidaka *et al.*, 2003) and foot-and-mouth (FMD) (Songchotikunpan *et al.*, 2008). Thus, the gelatin products from a porcine and fish are candidates to avoid the problem of BSE and

FMD. Normally, gelatin is a soluble polymer. Thus it has to be modified to obtain a hydrophilic polymer insoluble at 37 °C for the transdermal drug delivery. The chemical crosslinking has been used to form the interconnected chains (Vandeli *et al.*, 2001). It has been known that the cross-linking of gelatin is easily fabricated by glutaraldehyde via the unprotonated ε-amino groups of lysine and hydroxylysine, and the amino groups of the N-terminal amino acid in gelatin structure (Farris *et al.*, 2010).

The aim of this work includes the preparation, the characterization and the comparison the properties of porcine and fish gelatin hydrogels for the controlled drug release employing different drug sizes through the investigation of the morphology, the swelling, the diffusion, and the drug releasing rate under the effect of the matrix crosslinking ratio.

4.3 Materials and Methods

4.3.1 Materials

Porcine gelatin 180 g Bloom (PorGel) and cold-water fish gelatin (FishGel) used as a matrix, were supplied from (AR grade) Fluka and Sigma, respectively. Salicylic acid (SA) and 5-sulfosalicylic acid (SSA) used as the model drugs were purchased from (AR grade) Fluka. Glutaraldehyde (50% in water AR grade) was purchased from Fluka and used as the crosslinking agent. Sodium acetate and Glacial acetic acid were purchased form Ajax Finechem and (AR grade) Merck, respectively. These were used as the buffer solutions.

4.3.2 Preparation of Drug-loaded Gelatin Hydrogels

PorGel and FishGel powder were dissolved in deionized water at 60 °C for 1 hr to prepare a gelatin solution at a fixed concentration of 10% w/v. After the solution was cooled down to room temperature, the model drugs (SA/SSA) were loaded into the gelatin solution at 1 wt% (based on the weight of gelatin powder) under constant stirring for 1 h. Then the glutaraldehyde as the crosslinking agent was added into the solution in order to cross-link the gelatins at various crosslinking ratios of 0.25, 0.50, 0.75, 1.00, 3.00, and 7.00% (based on weight of PorGel powder)

for PorGel and 1.00, 3.00, and 7.00% (based on weight of FishGel powder) for FishGel. The solution was mixed very slowly to prevent the formation of air bubbles (Peppas *et al.*, 1998). Immediately after mixing the solution, the mixture was cast on the petridish (diameter 9 cm, film thickness 0.45-0.50 mm) and then cooled to room temperature.

4.3.3 Characterizations

The FTIR spectrometer (Bruker, Equinox 55/FRA 1065) was used to identify the functional group of salicylic acid (SA) and 5-sulfosalicylic acid (SSA). The ATR-FTIR spectroscopy (Thermo Nicolet) was used to investigate the polymer/model drug interaction in the drug-loaded gelatin hydrogels. A scanning electron microscope or SEM (JEOL, model JSM-5200) was used to investigate morphology of each crosslinked PorGel and FishGel hydrogels. The hydrogels was immersed in distilled water at 37 °C, before it was rapidly frozen in liquid nitrogen then dried it in the vacuum chamber at -50 °C. The scanning electron micrographs of crosslinked PorGel and FishGel hydrogels were obtained by using an acceleration voltage of 15 kV at magnifications of 3000x.

The degree of swelling and the weight loss of the gelatin hydrogels were measured in an acetate buffer solution at 37 °C for 48 h (Taepaiboon *et al.*, 2006) using the following equations (4.1-4.2):

$$\text{Degree of swelling (\%)} = \frac{M_s - M_d}{M_d} \times 100 \qquad (4.1)$$

and

$$\text{Weight loss (\%)} = \frac{M_i - M_d}{M_i} \times 100 \qquad (4.2)$$

where M_s is the weight of the sample after submersed in the buffer solution, M_d is the weight of sample after submersed in the buffer solution as dry state, M_i is the initial weight of the sample without submersed in the buffer solution as dry state.

To determine the molecular weight between crosslinks, $\bar{M}_c$, the mesh size, ξ, and the crosslinking density, ρ_x, A sample of gelatin film was cut immediately after crosslinking (1 cm^2). This sample was weighted in air and heptane.

The sample was then placed in distilled water at 37 °C for 5 days that allow it swelling to equilibrium, then weighted in air and heptane again. Finally, the sample was dried at 25 °C in vacuum oven for 5 days. Once again, it was weighted in air and heptane.

The molecular weight between crosslinks, $\bar{M}_c$, was calculated from the swelling data by using equation (4.3) (Peppas *et al.*, 1998):

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\frac{\bar{v}}{\bar{V}_1}\left[\ln(1-v_{2,s}) + v_{2,s} + \chi v_{2,s}^{\,2}\right]}{v_2\left[\left(\frac{v_{2,s}}{v_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{v_{2,s}}{v_{2,r}}\right)\right]} \tag{4.3}$$

where $\bar{M}_n$ is the number averaged molecular weight of the polymer before cross linking ($\bar{M}_n$ = 69,480 g/mol for porcine gelatin and 36,800 g/mol for fish gelatin), $\bar{v}$ is the specific volume of gelatin ($\bar{v}$ = 0.69 cm^3/g of gelatin) (Sutter *et al.*, 2007), $\bar{V}_1$ is the molar volume of water ($\bar{V}_1$ = 18.1 mol/cm^3), χ is the Flory interaction parameter of gelatin (χ = 0.49) (Bohidar, 1998) and the dissociation constant p*K*a is 4.7.

The hydrogel mesh size, ξ, defines the linear distance between consecutive crosslinks. It indicates the diffusional space available for solute transport and was calculated by using equation (4.4) (Peppas *et al.*, 1996):

$$\xi = v_{2,s}^{\,-1/3}\left[C_n\left(\frac{2\bar{M}_c}{\bar{M}_r}\right)\right]^{1/2} \cdot l \tag{4.4}$$

where C_n is the Flory characteristic ratio for gelatin (C_n = 8.8) (Deiber *et al.*, 2009), $\bar{M}_r$ is the molecular weight of repeating unit of gelatin ($\bar{M}_r$ = 100 g/mol) (Sutter *et al.*, 2007), and l is the carbon–carbon bond length (l = 1.54 Å) (Naimlang et al., 2009).

4.3.4 Drug Release Experiments

4.3.4.1 Preparation of Acetate Buffer

Acetate buffer was chosen to simulate human skin pH condition of 5.5. To prepare 1000 ml of the acetate buffer solution, 150 g of sodium acetate was dissolved in distilled water. Then 15 ml of glacial acetic acid was added very slowly into the aqueous sodium acetate solution. The solution was poured into the receptor chamber of a modified Franz-Diffusion cell.

4.3.4.2 Spectrophotometric Analysis of Model Drug

A UV-Visible spectrophotometer (TECAN, Infinite M200) was used to determine the spectra of model drugs. Each model drug, in aqueous solution was prepared for scanning the maximum absorption wavelength and the characteristic peak was observed. The absorbance value at the maximum wavelength of the model drug was read that can be related with the model drug, so the calibration curves with various model drugs were generated.

4.3.4.3 Actual Drug Content

The actual amount of drug in the drug-loaded gealtin hydrogels (circular disc about 2.5 cm in diameter, thickness 0.45-0.50 mm) was quantified by dissolving the sample in 4 ml of dimethyl sulfoxide (DMSO) and then 0.5 ml of the solution was added into 8 ml of the acetate buffer solution. The amounts drugs in the solution were measured by used the UV-Visible spectrophotometer at a wavelength of 298 nm for salicylic acid and 5-sulfosalicylic acid.

4.3.4.4 Thansdermal Transport Studies

The custom built modified Franz-diffusion cells were used. A diffusion cell consists of two compartments. The first is a water jacket compartment that is expose an ambient condition. Other is a receptor chamber that used to contain an acetate buffer solution pH 5.5 and maintained at 37 °C by a circulating water bath. Drug-loaded gelatin hydrogels with various crosslinking ratios (0.25, 0.50, 0.75, 1.00, 3.00, and 7.00) of PorGel and (1.00, 3.00, and 7.00) of FishGel, were placed over the nylon net (mesh size = 2.25 mm^2) on the receptor chamber which contained the acetate buffer solution. The nylon net was allowed to come into contact with the buffer solution. The buffer was magnetically stirred during the experiment period (48

h) at temperature 37 ± 2 °C. The drugs diffused through the polymer matrix and the net towards the buffer solution. 0.1 ml of the sample was withdrawn at various time intervals and simultaneously replaced with an equal volume of the fresh buffer solution. The amount of drug in solution was determined by the UV-Visible spectrophotometer.

4.4 Results and Discussion

4.4.1 Characterizations

4.4.1.1 Fourier Transforms Infrared Spectroscopy (FTIR)

The absorption infrared spectra of pure gelatin hydrogel and salicylic acid (SA) powder are shown in comparison with 1% and 15% of SA-loaded gelatin hydrogel in figure 4.1. For pure SA, peaks at 867 and 1483 cm^{-1} can be observed. The characteristic peaks are the C-H out of plane bending and C-C ring stretching, respectively (Mohan, 2004). For the gelatin hydrogel, peaks at 1400, 1540, and 3290 cm^{-1} are observed. These characteristic peaks are assigned to the O-H bending (Stancu *et al.*, 2010), the N–H bending of amide II (Muyouga *et al.*, 2004), and the N-H stretching from the primary amine (Stancu *et al.*, 2010), respectively. For the SA-loaded gelatin hydrogel, the spectra show the characteristic peaks at 856 and 1476 cm^{-1} for the 15% SA-loaded gelatin hydrogel, and a peak more evident at 1400 cm^{-1} which slightly shifts to 1405 cm^{-1} for the 1% SA-loaded gelatin hydrogel. This result suggests that the created hydrogen bonds between the COOH group of SA and the O-H group of gelatins.

4.4.1.2 Swelling Behaviour of Drug-loaded Gelatin Hydrogels

The gelatin hydrogels were prepared at the various crosslinking ratios (base on gelatin powder) 0.25, 0.50, 0.75, 1.00, 3.00, and 7.00% for PorGel, 1.00, 3.00, and 7.00% for FishGel by using glutaraldehyde as a crosslinking agent. The swelling behavior, the molecular weight between crosslink, $\bar{M}_c$, the mesh size, ξ, and the drug diffusion ability are investigated. Figure 4.2 shows the degree of swelling and the weight loss of crosslinked gelatin hydrogels at various crosslinking ratios after immersing in the acetate buffer solution at 37 °C for 5 day. The results show that the degree of swelling and the weight loss of both PorGel and

FishGel decrease with increasing crosslinking ratio. Since glutaraldehyde reacts with the ε-amino group of lysine (Chiou *et al.*, 2008), hydroxylysine, and the amino group of the N-terminal amino acid (Farris *et al.*, 2010), this leads to the increase in the crosslinking density o resulting a limited swelling capacity and improved gel strength. So, a lower of crosslinked hydrogel has a longer gelatin strand between crosslinks along with a looser network for easier diffusion (Juntanon et al., 2007; Naimlang et al., 2009). At the same crosslinking ratio (1%), the degree of swelling and the weight loss of a PorGel are higher than that of a FishGel because the PorGel have hydroxyproline and proline contents higher than those compares to FishGel. This creates the hydrophilic structure leading to easier water penetration and more swelling for the PorGel hydrogels (Avena-Bustillos et al., 2006).

The swelling data are used to evaluate the crosslinked structure of these hydrogels. The molecular weight between crosslink, $\bar{M}_c$ and the mesh size, ξ parameters used for characterization the porous structure of hydrogel for drug delivery system. These values of each hydrogel matrix are determined using the equilibrium swelling theory developed by Peppas (Peppas *et al.*, 1998). Table 4.1 shows the $\bar{M}_c$ and ξ, of each crosslinked the gelatin hydrogels at various crosslinking ratios. The $\bar{M}_c$ and ξ values of both PorGel and FishGel hydrogels are larger at lower crosslinking ratios. The mesh sizes of PorGel and FishGel vary between 148 Å to 9 Å (with crosslinking ratio 0.25% to 7%) and 27 Å to 9 Å (with crosslinking ratio 1% to 7%), respectively, as shown in table 4.1. At the same crosslinking ratio (1%), the mesh size of PorGel hydrogel is larger than FishGel hydrogel because the molecular weight average, M_w of PorGel is higher than FishGel. At the higher M_w, it has a higher degree of entanglements which tends to screen the crosslinking agents from penetrating and reacting with the polymer chain, resulting in a lower of crosslinking point for PorGel when compared with FishGel. Figure 4.3 shows morphologies of PorGel and FishGel hydrogels in SEM micrographs. The pictures show porous structures and the pore sizes which are larger at lower crosslinking ratios. And at the same crosslinking ratio (1%) (see in figure 4.3b and 4.3d), the PorGel has the pore size larger than the FishGel. Since the PorGel has a lower crosslinkng degree than that of the FishGel.

4.4.2 Release Kinetics of Drug from Drug-loaded Gelatin Hydrogels

The actual amount of drug present in the sample is reported as the percentage of the weight of drug loaded over the weight of crosslinked gelatin in the gelatin solution. The actual amount of SA and SSA presented in the sample are about 91.75 ± 5.51 % and 92.83 ± 3.96 %, respectively.

To study SA and SSA transport mechanism from the PorGel and FishGel hydrogels, the experimental data were analyzed by two diffusion models. The released drug mechanism is described through the Ritger-Peppas equation (Venkatesh *et al.*, 1992), describing the released drug from a polymeric system following equation (4.5). Model 1 is called the Ritger-Peppas equation:

$$\frac{M_t}{M_\infty} = k_1 t^n \tag{4.5}$$

where M_t/M_∞ is the fractional drug release, k_1 is a kinetic constant (with the unit of T^{-n}), t is the release time, and n is the scaling exponent which can be related to the drug transport mechanism.

A thin hydrogel film, when $n = 0.5$, the drug release mechanism is the Fickian diffusion. When $n = 1$, the Case II transport occurs, corresponding to the zero-order release. When $0.5 < n < 1$, the anomalous transport is observed. Model 2 is based on the Higuchi's equation (4.6) (Serra *et al.*, 2006) and described by the Fickian diffusion of the drug:

$$\frac{M_t}{M_\infty} = k_H t^{1/2} \tag{4.6}$$

where M_t and M_∞ are the masses of drug released at times equal to t and infinite time, respectively, and k_H is the Higuchi constant (with the unit of T^{-n}).

The diffusion coefficients of the model drugs from the PorGel and FishGel hydrogels are determined from the slopes of plots of drug accumulation

versus square root of time according to the Higuchi's equation (4.7) (A-sasutjarit *et al.*, 2005):

$$Q = 2C_0(Dt/\pi)^{1/2} \quad (4.7)$$

where Q is the amount of material flowing through a unit cross-section of barrier (g/cm^2) in unit time, t (s); C_0 is the initial drug concentration in the hydrogel (g/cm^3); and D is the diffusion coefficient of a drug (cm^2/s).

4.4.2.1 Effect of Crosslinking Ratio

The amounts of drug released from drug-loaded gelatin hydrogels at various crosslinking ratios versus time, t, during 48 h. The result shows the amount of drug release increases very rapidly over the first 1 hr and then they reach equilibrium values. Figure 4.4 shows the log-log plots between fractional drug release, M_t/M_∞, and time, t, which are used to determine the n exponential from slope. The exponential values n of all systems are tabulated in table 4.2; they are quite close to the Fickian exponential (n = 0.5). Therefore, the Fickian diffusion mechanism appears to control the drug diffusion for all of systems. The slopes of these plots of the Higuchi's equation provide the diffusion coefficients (see figure 4.5). From figure 4.5, the amount of drug released increases with decreasing crosslinking ratio due to the larger pore size or the lower crosslinked hydrogel, which can be related to the highly susceptibility to swelling in an aqueous medium. Figure 4.6 shows the diffusion coefficients of SA from the both types of gelatin hydrogels versus crosslinking ratios and mesh size at 37 °C. The diffusion coefficients, D, increase with decreasing crosslinking ratio for the both PorGel and FighGel hydrogels due to the easier drug movement in this pathway because of the lower crosslinking ratio or larger pore size of the swollen gelatin hydrogels at the lower crosslinking ratio (Paradee *et al.*, 2012).

4.4.2.2 Effect of Types of Gelatin

Figure 4.6 shows D of SA from SA-loaded FishGel and PorGel hydrogels at various crosslinking ratios and mesh sizes. From this figure, D of SA from the PorGel is lower than that of the FishGel because the hydroxyproline

and proline as the amino acid group in the PorGel structure exist at higher contents than those FishGel (Avena-Bustilos *et al.*, 2006; Chiou *et al.*, 2008; Karim *et al.*, 2009). These two groups can create the hydrogen bonding between the drug and the gelatin matrix which can retard the drug diffusion from the gel.

4.4.2.3 Effect of model drug

Figure 4.7 shows D of SA (Molecular size =3.28 Å) (Niamlang 2008) and SSA (Molecular size = 9.25 Å) (Juntanon *et al.*, 2008) from drug-loaded PorGel hydrogels at various crosslinking ratios and mesh sizes. The result shows that D of SA is higher than that od SSA because ofthe smaller size of SA resulting an easier diffusion from gel.

The log-log plots of the D as a function of the ratio of drug size over mesh size, a/ξ, of the cross-linked gelatin hydrogels are shown in figure 4.8. The scaling exponent m was determined from the following equation (4.8):

$$D = D_0(a/\xi)^{-m} \tag{4.8}$$

where D is the diffusion coefficient of a drug, D_0 is the diffusion coefficient for a very small drug size, a is the drug size, ξ is the mesh size of hydrogels, and m is the scaling exponent (Juntanon *et al.*, 2008).

The scaling exponent m and D_0 values of the SA and SSA diffusion through the crosslinked PorGel and FishGel hydrogels is 0.45 and 1.48 × 10^{-6} cm^2/s, respectively. The result shows that D decreases with increasing a/ξ because the drug molecules can diffuse easier with a smaller drug size and a larger mesh size.

Table 3 shows the comparison of the D of the SA and SSA from the gelatin hydrogels with the previous work that consist of the D of SA release from PAAM (Niamlang *et al.*, 2009), SSA release from PVA (Juntanon *et al.*, 2008), and Lysozyme and Trypsine release from gelatin (HU4) hydrogels (Sutter *et al.*, 2007). The D of SA and SSA from the both types of gelatin (PorGel and FishGel) hydrogels decrease with decreasing mesh size of the gelatin hydrogel or increasing crosslinking agent. D of SSA is higher than SA because the SA size is smaller than

the SSA size (SA =3.28 Å and SSA = 9.25 Å). For the comparison of the different type of gelatin hydrogels, *D* of FishGel is higher than PorGel. Because the lysine and hydroxylysine groups can create the hydrogen bonding interaction between the FishGel gelatin and the drug; the interaction is lower than the interaction of PorGel. *D* of SA from PAAM (Niamlang *et al.*, 2009) is higher than the *D* from the both gelatins because the mesh size of PAAM hydrogels is higher than the gelatin hydrogels. *D* of SSA from PVA (Juntanon *et al.*, 2008) is lower than those of both gelatins. From these results, it can be suggested that the hydrogen bonding interaction between the SSA and vinyl alcohol is higher than that compared to the gelatin. At 100 g/mol of gelatin and PVA, the amino acid group of gelatin has a –OH group or none. On the other hand, vinyl alcohol of PVA has the two –OH group to 100 g/mol of PVA which can be used to create more hydrogen bounds between the drug and the PVA structure and thus lower *D* of the drug. *D* of protein as model drug from the gelatin (HU4) decreases with increasing gelatin concentration and DS crosslinking ratio (DS; defined as fraction of methacrylate residues with respect to the total number of primary amines) because the increases of the gelatin content and DS ratio result in the decrease of swollen gelatin hydrogels. The electrostatic interaction between model drug and gelatin hydrogel affect the diffusion coefficient Sutter *et al.*, (2007) studied the release behavior of lysozyme and trypsin from gelatin (HU4) hydrogel. The electrostatic repulsion occured between the negatively charged trypsin and the negatively chargeg gelatin hydrogel. On the other hand, the lysozome is positively charged which creates the attractive force between this charge and the negatively charged gelatin results in a lower *D* value of lysozome when compared with trypsin. (Sutter *et al.*, 2007).

Thus, the above results confirm that the diffusion coefficients of the substances from the hydrogel depend on and can be controlled by many factors such as the drug molecular size, the charged drug, the chemical composition of drug, the polymer matrix, the interaction of drug-matrix, and the experiment set up (Paradee *et al.*, 2012).

4.5 Conclusions

The drug-loaded gelatin hydrogels were prepared at various crosslinking ratios to investigate and compare the release mechanism and the diffusion coefficient of the drugs from the drug-loaded porcine and fish gelatin hydrogels. Each hydrogel was characterized for the swelling ability and mesh size. The degree of swelling, the weight loss, and the mesh size of the both gelatin hydrogels decreased with increasing crosslinking ratio. The effects of the crosslinking ratio, mesh size, drug size, and type of gelatin were investigated on the diffusion coefficients. The diffusion coefficients of the drug from drug-loaded gelatin hydrogels decrease with increasing crosslinking ratio since the mesh size of gelatin hydrogel is larger. The diffusion coefficients of drug from drug-loaded gelatins decrease with increasing drug size, at the same crosslinking ratio and type of the gelatin matrix. The diffusion coefficients of drugs from drug-loaded fish gelatin hydrogels are higher than those compared with porcine gelatin at the same crosslinking ratio, because the fish gelatin hydrogel has the lower hydrogen bounding interaction between the gelatin and the drug compared with that of the porcine gelatin hydrogel.

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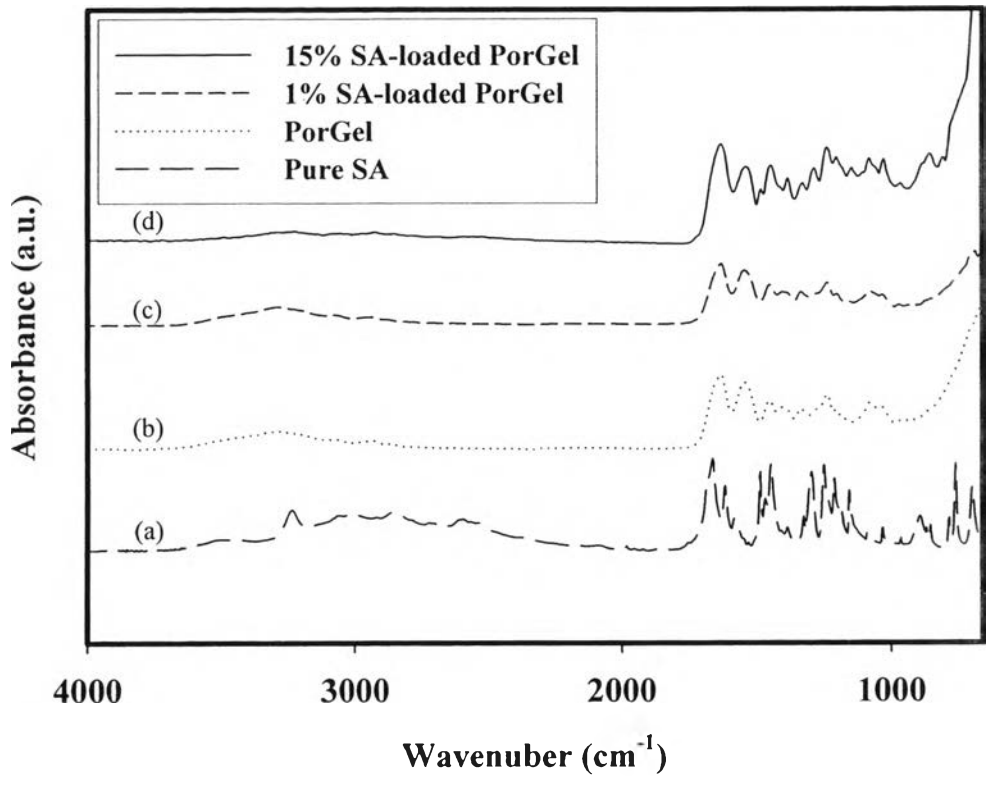

Figure 4.1 Absorption infrared spectra of pure SA and SA-loaded PorGel hydrogel: (a) Pure SA; (b) PorGel hydrogel ; (c) 1% SA-loaded PorGel hydrogel; and (d) 15% SA-loaded PorGel hydrogel.

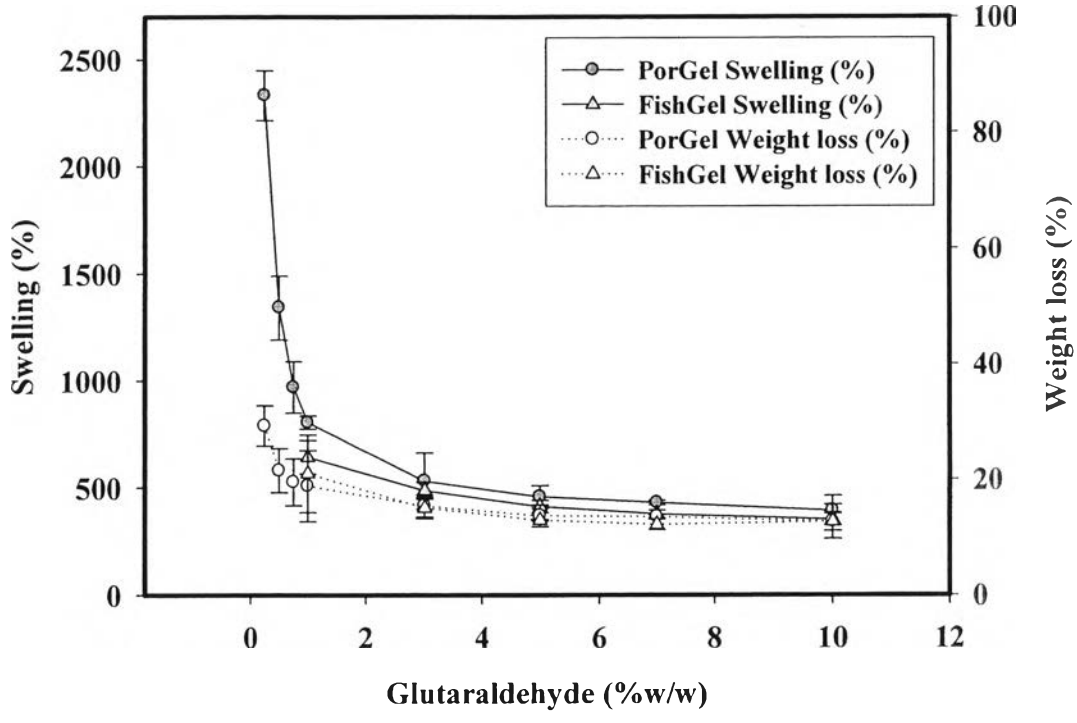

Figure 4.2 The degree of swelling (%) and the weight loss (%) of PorGel hydrogels at various crosslinking ratios between 0.25 and 10.00 %w/w, and FishGel hydrogels at various crosslinking ratio between 1.00 and 10.00 %w/w (Number of samples = 3 and thickness of films = 0.45-0.5 mm).

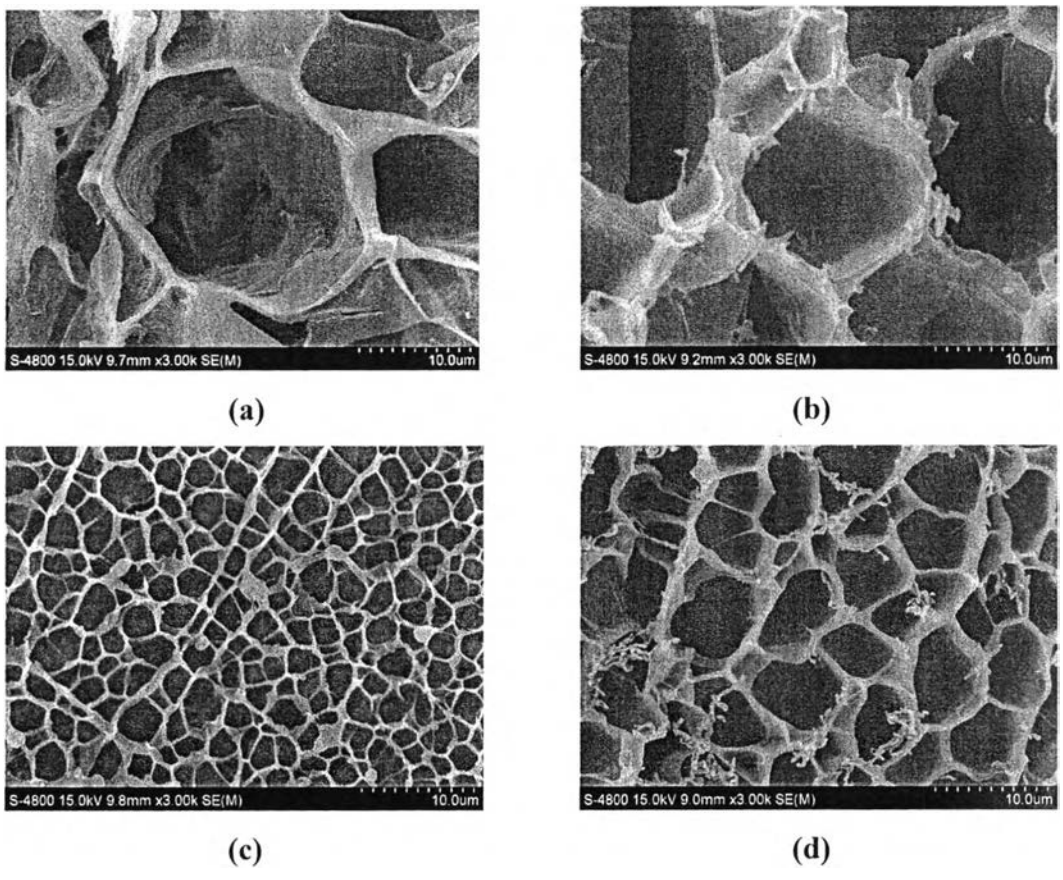

Figure 4.3 The morphologies of PorGel and FishGel samples after swelling: (a) PorGel_0.25; (b) PorGel_1.00; (c) PorGel_3.00; and (d) FishGel_1.00 at magnification of 3000X.

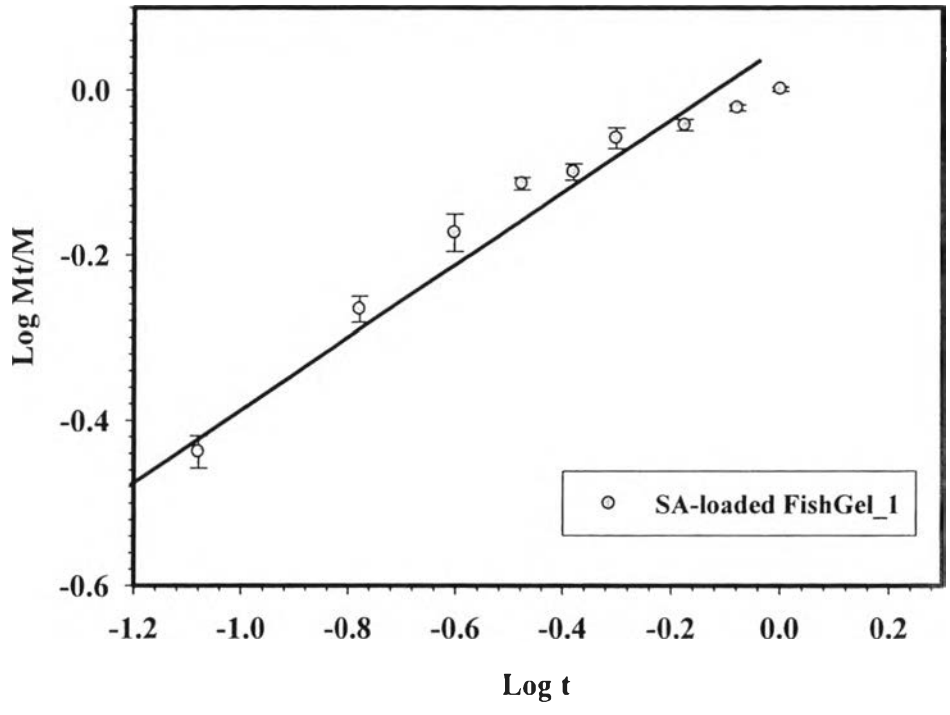

Figure 4.4 Log-log plots between fractional drug release of SA release from SA-loaded FishGel hydrogel and time at crosslinking ratio 1%, pH 5.5, and at 37 °C, number of samples = 3.

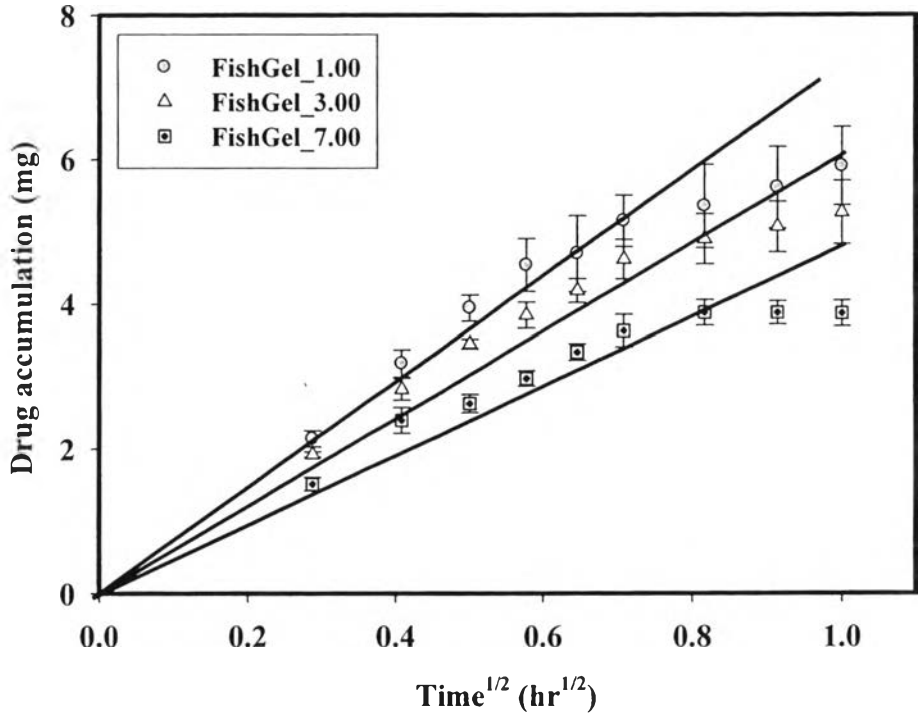

Figure 4.5 Amounts of SA release from SA-loaded FishGel hydrogel versus time$^{1/2}$ at various crosslink ratios, pH 5.5, and at 37 °C, number of samples = 3.

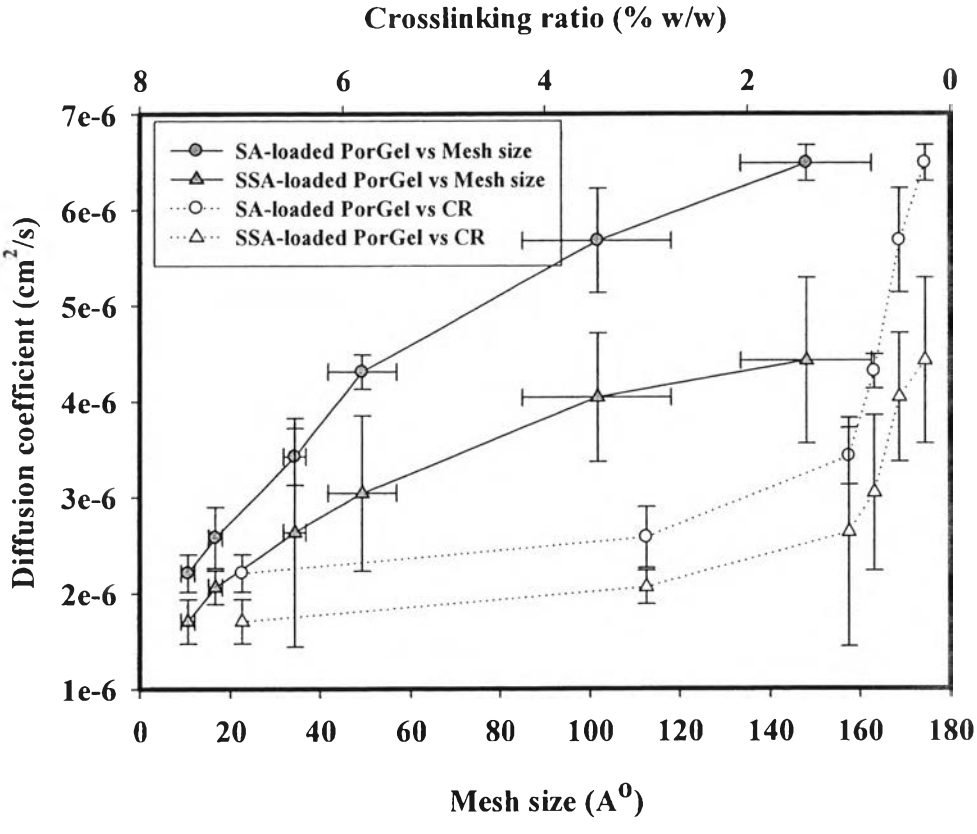

Figure 4.6 Diffusion coefficient of SA and SSA from drug-loaded PorGel hydrogels versus crosslinking ratios and mesh sizes, pH 5.5, and at 37 °C, number of samples = 3.

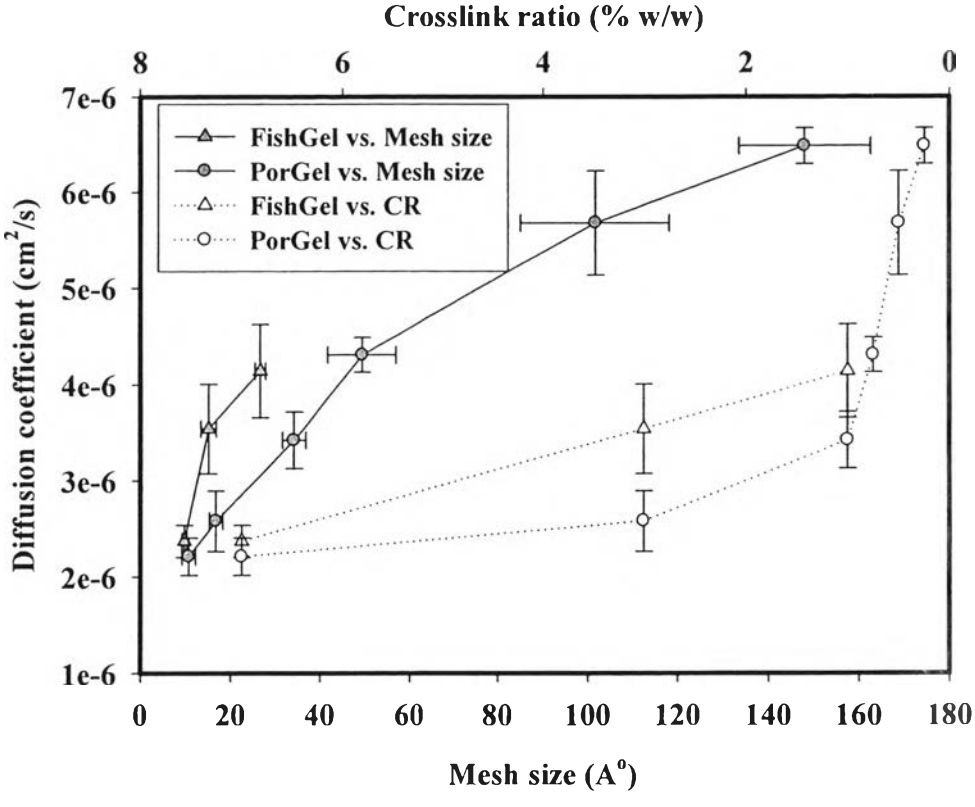

Figure 4.7 Diffusion coefficient of SA from SA-loaded PorGel and FishGel hydrogels versus crosslinking ratios and mesh sizes, pH 5.5, and at 37 °C, number of samples = 3.

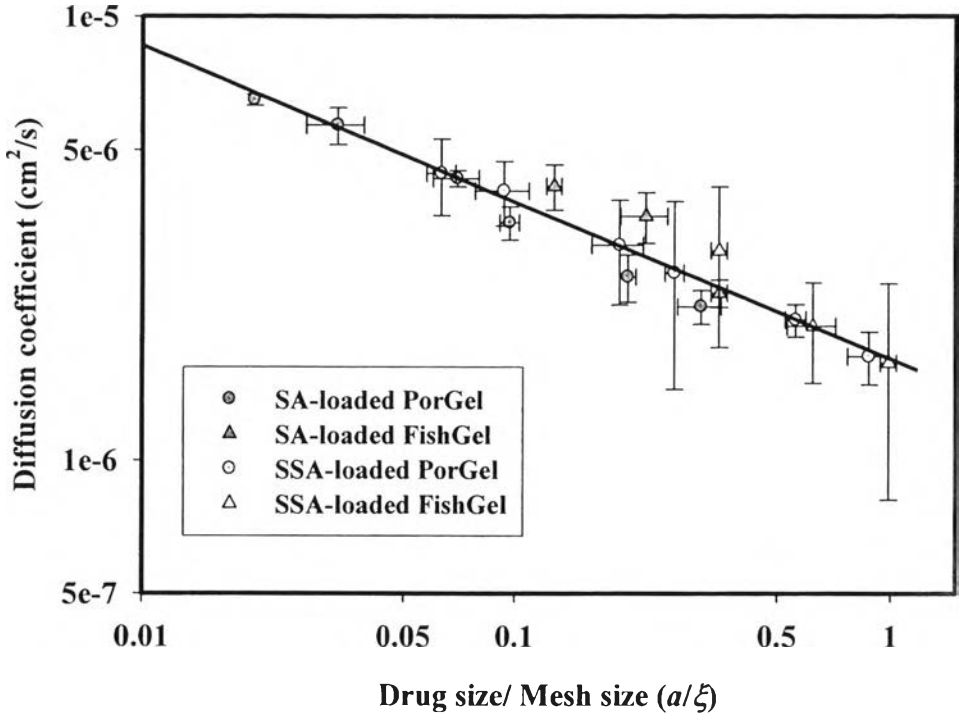

Figure 4.8 Log-log plots of the diffusion coefficient as a function of drug size over mesh size of SA and SSA from drug-loaded PorGel and FishGel hydrogels, pH 5.5, and at 37 °C, number of samples = 3.

Table 4.1 Summary of the molecular weights between crosslink, $\bar{M}_c$, mesh size, ξ, of PorGel and FishGel hydrogel at various crosslinking ratios

Crosslinking ratio, X	**Number-average molecular weight between crosslinks, $\bar{M}_c$ (g/mol)**		**Mesh size, ξ (Å)**	
	PorGel	**FishGel**	**PorGel**	**FishGel**
0.25%	16673 ± 2070	-	148 ± 15	-
0.50%	8851 ± 2371	-	101 ± 16	-
0.75%	2950 ± 739	-	49 ± 8	-
1%	1610 ± 143	1103 ± 113	35 ± 2	27 ± 1
3%	529 ± 66	467 ± 136	17 ± 1	15 ± 2
7%	254 ± 41	220 ± 17	11 ± 1	9 ± 0

Table 4.2 Release parameters of the drugs from PorGel and FishGel hydrogels at various crosslinking ratios

Drug	Crosslinking ratio (%w/w)	PorGel		FishGel	
		Diffusional exponent (n)	Kinetic constant k_{II} (h^{-n})	Diffusional exponent (n)	Kinetic constant k_{II} (h^{-n})
SA	CR_0.25	0.4228	0.9944	-	-
	CR_0.50	0.4304	1.0659	-	-
	CR_0.75	0.4533	1.0852	-	-
	CR_1	0.4672	1.1928	0.4615	1.1418
	CR_3	0.4750	1.2608	0.4918	1.2049
	CR_7	0.5025	1.3171	0.5300	1.3153
SSA	CR_0.25	0.5555	1.0603	-	-
	CR_0.50	0.5638	1.0770	-	-
	CR_0.75	0.5714	1.0924	-	-
	CR_1	0.5882	1.1145	0.6154	1.1733
	CR_3	0.5970	1.1505	0.6250	1.2116
	CR_7	0.6154	1.2069	0.6452	1.2822

Table 4.3 The diffusion coefficients of the model drugs from gelatins, PVA, and PAAM hydrogels at various conditions

solute	M_w	Drug size (Å)	Mesh size, ξ, (Å)	D (cm^2/s)	T (°C)	pH	Remarks
SA	138	3.29	148	6.49×10^{-6}	37	5.5	PorGel_crosslink ratio = 0.25
			101	5.68×10^{-6}	37	5.5	PorGel_crosslink ratio = 0.50
			49	4.31×10^{-6}	37	5.5	PorGel_crosslink ratio = 0.75
			35	3.42×10^{-6}	37	5.5	PorGel_crosslink ratio = 1.00
			17	2.58×10^{-6}	37	5.5	PorGel_crosslink ratio = 3.00
			11	2.21×10^{-6}	37	5.5	PorGel_crosslink ratio = 7.00
			27	4.14×10^{-6}	37	5.5	FishGel_crosslink ratio =1.00
			15	3.54×10^{-6}	37	5.5	FishGel_crosslink ratio =3.00
			9	2.37×10^{-6}	37	5.5	FishGel_crosslink ratio =7.00
SSA	254	9.25	148	4.42×10^{-6}	37	5.5	PorGel_crosslink ratio = 0.25
			101	4.04×10^{-6}	37	5.5	PorGel_crosslink ratio = 0.50
			49	3.04×10^{-6}	37	5.5	PorGel_crosslink ratio = 0.75
			35	2.64×10^{-6}	37	5.5	PorGel_crosslink ratio = 1.00
			17	2.07×10^{-6}	37	5.5	PorGel_crosslink ratio = 3.00
			11	1.71×10^{-6}	37	5.5	PorGel_crosslink ratio = 7.00
			27	2.96×10^{-6}	37	5.5	FishGel_crosslink ratio =1.00
			15	2.00×10^{-6}	37	5.5	FishGel_crosslink ratio =3.00
			9	1.65×10^{-6}	37	5.5	FishGel_crosslink ratio =7.00
SA	138	3.29	252	8.46×10^{-5}	37	5.5	PAAM_crosslink ratio = 2.0×10^{-3}
			158	5.85×10^{-5}	37	5.5	PAAM_crosslink ratio = 5.0×10^{-3}
			128	3.70×10^{-5}	37	5.5	PAAM_crosslink ratio = 1.0×10^{-2}
			85	2.00×10^{-5}	37	5.5	PAAM_crosslink ratio = 1.6×10^{-2}
			75	3.52×10^{-6}	37	5.5	PAAM_crosslink ratio = 2.4×10^{-2}
SSA	254	9.25	232	2.08×10^{-9}	37	5.5	Uncrosslink of PVA
			143	1.08×10^{-9}	37	5.5	PVA_crosslink ratio = 0.5
			71	5.13×10^{-10}	37	5.5	PVA_crosslink ratio = 2.5
			36	2.76×10^{-10}	37	5.5	PVA_crosslink ratio = 5.0
Lysozyme (+)charge	-	~40	-	2.33×10^{-7}	37	7.4	10% of Gelatin concentration_crosslink ration (DS 0.24)
			-	1.94×10^{-7}	37	7.4	10% of Gelatin concentration_crosslink ration (DS 0.67)
			-	1.05×10^{-7}	37	7.4	10% of Gelatin concentration_crosslink ration (DS 0.82)
			-	1.03×10^{-7}	37	7.4	10% of Gelatin concentration_crosslink ration (DS 0.97)
			-	2.19×10^{-7}	37	7.4	5% of Gelatin concentration_crosslink ration (DS 0.97)
			-	0.69×10^{-7}	37	7.4	15% of Gelatin concentration_crosslink ration (DS 0.97)
			-	0.43×10^{-7}	37	7.4	20% of Gelatin concentration_crosslink ration (DS 0.97)
Trypsin (-)charge	-	~40	-	3.87×10^{-7}	37	7.4	10% of Gelatin concentration_crosslink ration (DS 0.97)