CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Drug Delivery System (DDS)

Drug delivery system (DDS) is the method or the process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals. Drug delivery technologies are patent protected formulation technologies that modify the drug release profile, the absorption, the distribution and elimination for the benefit of improving product efficiency and safety, as well as patient convenience and compliance. The DDS for a controlled drug release is the process of introducing a drug into a body at a specific rate and at a proper time. The design of a DDS is usually based on the drug's physicochemical and pharmacokinetic properties. The effect of environment is considered to affect the DDS. A drug is incapable of releasing until it is placed in an appropriate biological environments such as pH, ionic concentration, and electrical strength. Most of the materials used in a swelling controlled release system are based on hydrogels: they are polymers that will swell without dissolving when placed in water or other biological fluids. A diffusion occurs through the polymer that exhibits the controlled release (Gupta *et al.*, 2002).

2.2 Controlled Release Mechanisms

The control release is helpful for maintaining constant drug levels in the target tissues or cells. The classification of controlled release polymeric systems according to the mechanism that controls the release of the therapeutic agent is as shown in table 2.1.

Table 2.1 Classification of controlled release systems (Niamlang, 2008; http://www.authorstream.com/Presentation/vamsikrishnareddy-255033-drug-release-mechanisms-kinetics-mechanism-science-technology-ppt-powerpoint/)

Type of System	Rate-Control Mechanism
Dissolution Controlled System	
-Encapsulation dissolution controlled system	-Microencapsulation techniques
-Matrix dissolution controlled system	-Dispersed throughout medium
Diffusion Controlled Systems	
-Reservoir controlled systems	-Diffusion through membrane
-Matrix controlled systems	-Diffusion through bulk polymer
Water Penetration Controlled Systems	
-Swelling systems	-Water penetration into glassy polymer
-Osmotically controlled systems	-Osmotic pressure
Chemically Controlled Release Systems	
-Erodible systems	-Erosion of polymer
·Bulk erosion	·Polymer degradation occur through bulk
·Surface erosion	·Degradation only at surface of polymer
-Pendent chain system	-Hydrolysis or enzymatic degradation of the linkages

2.2.1 Dissolution Controlled System

In dissolution controlled systems, the rate controlling step is dissolution. The drug is embeded in slowly dissolving or erodible matrix or by coating with slowly dissolving substances. That consists of two types: encapsulation dissolution controlled system; matrix dissolution controlled system.

2.2.1.1 Encapsulation Dissolution Controlled System

The drug particles are coated or encapsulated by microencapsulation techniques with slowly dissolving materials, the dissolution rate of coat depends upon the solubility and thickness of the coating.

2.2.1.2 Matrix dissolution controlled system

Matrix systems are homogeneously dispersed with a drug throughout a rate controlling medium. Waxes such as beeswax, carnauba wax, hydrogenated castor oil, and etc. are used; they control the drug dissolution by controlling the rate of dissolution fluid penetrating into the matrix by altering the porosity.

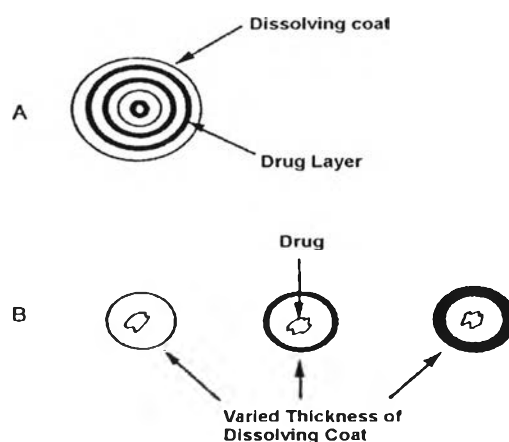

Figure 2.1 (A) matrix dissolution controlled system; and (B) encapsulation dissolution controlled system. (http://www.authorstream.com/Presentation /vamsikrishnareddy-255033-drug-release-mechanisms-kinetics-mechanism-science-technology-ppt-powerpoint/).

2.2.2 Diffusion Controlled Systems

Diffusion systems are characterized by the release rate of drug, depending on its diffusion through the water insoluble membrane barrier. The diffusion occurs when a drug or other therapeutic agent passes through the controlled-release device. That can pass between polymer chains on a macroscopic

scale as through pores in the polymer matrix or on a molecular level. Figure 2.2 shows the examples of the diffusion-release systems.

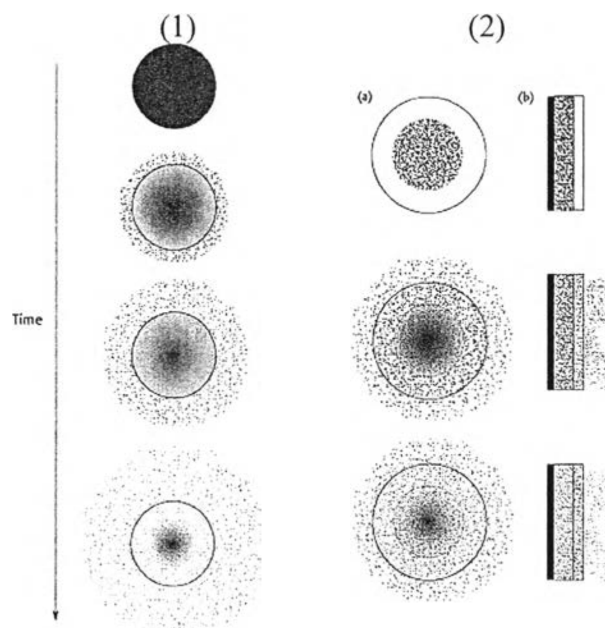

Figure 2.2 Drug delivery from (1) a typical matrix drug delivery system and (2) typical reservoir devices: (a) implantable or oral systems; and (b) transdermalsyst-ems (http://pharmacrunch.net/archives/2011/06/12/novel-drug-delivery-system/).

In Figure 2.2, the homogeneous mixture of a polymer and a therapeutic agent is referred to as the matrix system. The diffusion of drug occurs when it passes from the polymer matrix through the external environment. As the release continues, its rate normally decreases with this type of system, since the therapeutic agent has a progressively longer distance to travel and therefore requires a longer diffusion time to release. For the reservoir systems shown in Figures 2.2(2a) and 2.2(2b), the drug delivery rate can remain fairly constant. In this design, a reservoir whether solid drugin a dilute solution, or highly concentrated drug solution within a polymer matrix is surrounded by a film or membrane of a rate-controlling material. The only structure effectively limiting the release of the drug is the polymer layer surrounding the reservoir. Since this polymer coating is essentially uniform and

of a non-changing thickness, the diffusion rate of the active agent can be kept fairly stable throughout the lifetime of the delivery system. The system shown in Figure 2.2(2a) is a representative of an implantable or oral reservoir delivery system, whereas the system shown in Figure 2.2(2b) illustrates a transdermal drug delivery system, in which only one side of the device will actually be delivering the drug. Once the therapeutic agent has been released into the external environment, one might assume that any structural control over drug delivery has been relinquished. However, this is not always the case. For the transdermal drug delivery, the active pharmaceutical ingredients permeate the skin. They then pass through the stratum corneum, go into the epidermis, penetrate into the dermis, and finally are absorbed into peripheral capillary vessels to be distributed into the rest of the body, are shown in Figure 2.3.

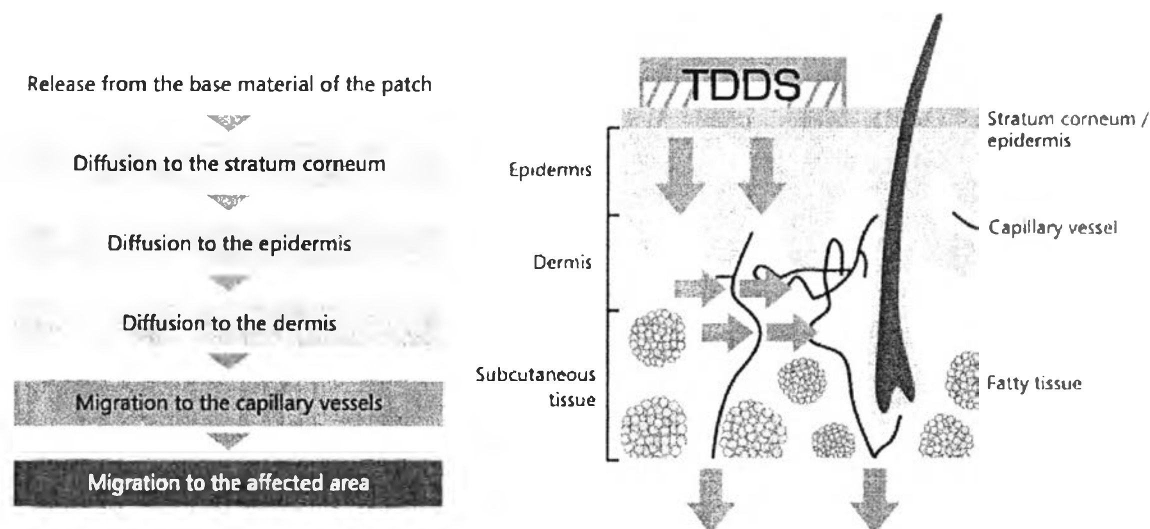

Figure 2.3 Transport processes in transdermal drug delivery. (http://www.hisamitsu.co.jp/english/company/operations/tdds.html).

For the diffusion-controlled systems described by the combinations of polymer matrices and chosen bioactive agents, they must allow the drug to diffuse through the pores or macromolecular structure of the polymer without inducing any change in the polymer itself. The drug delivery device is fundamentally stable in the

biological environment and does not change its size either through swelling or degradation (Niamlang, 2008).

2.2.3 Water Penetration Controlled Systems

It is also possible for a drug delivery system to be designed so that it is incapable of releasing its agent or agents until it is placed in an appropriate biological environment. Swelling-controlled release systems are initially dry, but when placed in the body they will absorb water or other body fluids and swell. The swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size, enabling the drug to diffuse through the swollen network into the external environment. Examples of these types of devices are shown in Figures 2.4a and 2.4b for the reservoir and matrix systems, respectively. Most of the materials used in swelling-controlled release systems are based on hydrogels, which are polymers that will swell without dissolving when placed in water or other biological fluids. These hydrogels can absorb a great deal of fluid, at equilibrium, and typically comprise of 60–90% fluid and only 10–30% polymer (Niamlang, 2008).

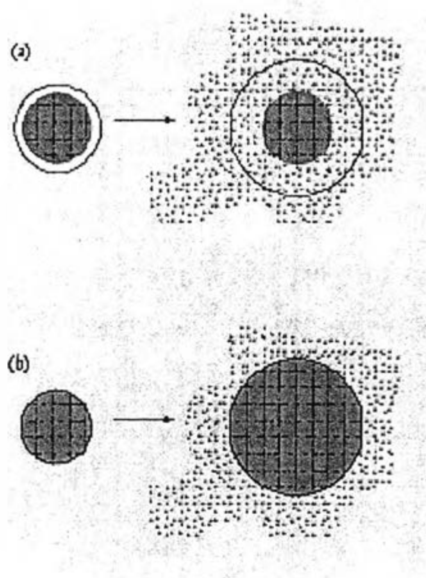

Figure 2.4 Drug delivery from: (a) reservoir ; and (b) matrix swelling-controlled release systems (Peppas, 1997).(http://www.pharmainfo.net/raghanaveen/biodegr-adable-polymers-controlled-drug-delivery-ii).

2.2.4 Chemically Controlled Systems

All of the previously described systems are based on polymers that do not change their chemical structure beyond what occurs during swelling. These materials degrade within the body as a result of natural biological processes, eliminating the need to remove a drug delivery system after release of the active agent has been completed. Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable, and progressively smaller, compounds. Degradation may take place through bulk hydrolysis, in which the polymer degrades in a fairly uniform manner throughout the matrix, as shown schematically in Figure 2.5a. For some degradable polymers, most notably the polyanhydrides and polyorthoesters, the degradation occurs only at the surface of the polymer, resulting in a release rate that is proportional to the surface area of the drug delivery system (see Figure 2.5b) (Niamlang, 2008).

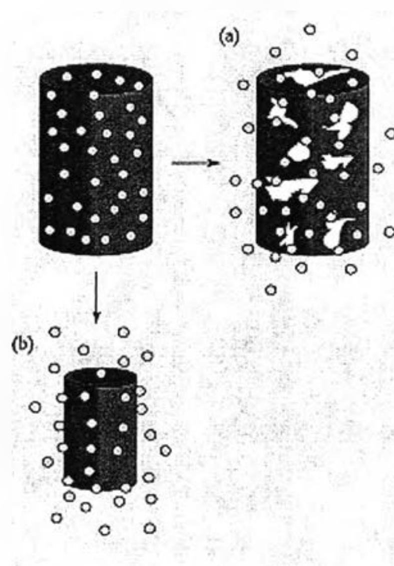

Figure 2.5 Drug delivery from: (a) the bulk-eroding; and (b) the surface-eroding biodegradable systems (http://www.pharmainfo.net/raghanaveen/biodegradable-polymers-controlled-drug-delivery-ii).

2.3 Transdermal Drug Delivery System (TDDS)

Transdermal drug delivery system (TDDS) has gained an attention in recent years. TDDS is applied to a skin to allow a drug to pass through the skin to impart a systemic or local therapeutic effect. The factors that control the iontophoretic drug delivery across the skin are the current density and the donor drug concentration; both of which are directly related to the drug flux. TDDS has many advantages of avoiding hepatic first pass metabolism, maintaining constant blood levels for a longer period of time, decreasing side effects, composition relatively invariant in use, possible to be fabricated in a desired size and shape, easy to be used, a decrease in the gastrointestinal effect that occurs during chronic treatment using conventional oral route, and the improved compliance. The rate of release depends on the polymer composition, the drug diffusion, and the thickness of membrane (Niamlang, 2008).

In 2002, Gupta *et al.* studied the use of hydrogels (a class of polymeric systems) in controlled drug delivery, and their application in the stimuli responsive, especially pH-responsive, drug release. The success of hydrogels as delivery systems can be judged by several preparations. In the present scenario, the major considerations during the formulation of hydrogel-based drug products are their mechanical strength and response-time in a physiological environment. Fast-responding hydrogels releasing maximal drug in less time while maintaining the structural integrity in a biological system will be the more appreciated delivery systems. Moreover, a high level of in vitro–in vivo correlation in their performance will determine their future success. The exploitation of these polymeric networks for improved therapeutic efficacy will open newer arenas in drug delivery

In 2008, Liu *et al.* studied the drug release behavior following electrostimulation from nanocomposite hydrogels of montmorillonite (MMT) in a chitosan (CS) matrix. MMT was used to enhance the anti-fatigue property and corresponding long-term stable release kinetics. The exfoliated silica nanosheets were able to act as cross-linkers to form a network structure between the CS and MMT. The difference in the cross-linking density strongly affects the release of vitamin B12, and the release of mechanism was changed from a diffusion-controlled mode to a swelling controlled mode under electrostimulation. An increase of MMT

content resulted in reducing both the diffusion and the responsiveness of the nanohydrogel to the electrostimulation. In addition, the repeated ''on'' and ''off'' operation showed that the electro responsiveness of the nanohydrogel was reduced with higher MMT concentrations, but its anti-fatigue behavior was improved. In this work, the nanohydrogel with 2 wt% MMT achieved a mechanically reliable and practically desirable pulsatile release profile and an excellent anti-fatigue behavior, compared with that of the pure CS. So, the release rate and the cumulative drug amount from pure CS hydrogel or the nano hydrogels upon electrostimulation were higher than that of pure CS with no electric field applied. The ejection of the drug from the electro-sensitive hydrogels was a result of deswelling and syneresis. Furthermore the nanoscale dispersion of MMT did not strongly influence the swelling of the CS matrix and the subsequent hindrance of the molecules from the diffusion. In particular, the result was in good agreement with the fact that the MMT nanoplates were well dispersed in the CS matrix.

In 2010, Oh *et al.* synthesized size controlled magnetic carbon nanoparticles for the drug delivery. The carbonized polypyrrole nanoparticles (CPyNs) exhibited an embedding capability using pyrene as a typical hydrophobic fluorescent molecule. In addition, ibuprofen was incorporated into the carbon nanoparticles and the drug-loaded carbon nanoparticles. CPyNs showed highly microporous structures compared to a zeolite. The loading guest molecules into CPyNs was based on the phase separation. Due to their superiorities such as the microporous structure, monodispersity, magnetism, and biocompatibility, it was expected that the CPyNs open the way to use in the fields such as the biomaterials science, including the bioimaging and as magnetic induced drug carriers.

2.4 Hydrogel

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 due to 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 by physically or chemically crosslinking reaction, especially like gelatin which is a kind of a well-defined hydrogel matrix (Schacht, 2004).

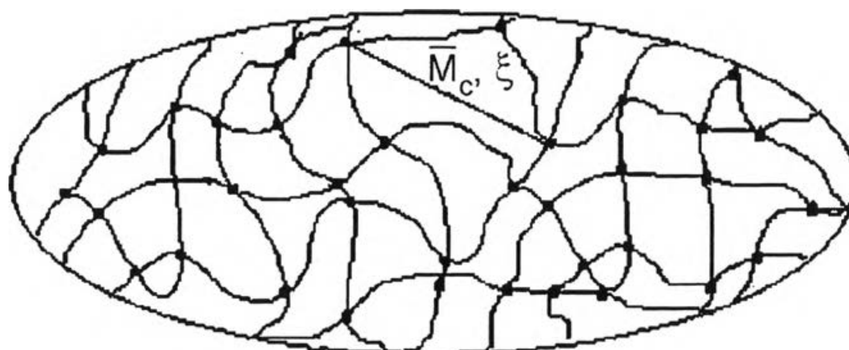

Figure 2.6 Schematic representation of the cross-linked structure of a hydrogel. $\overline{M}_c$ is the molecular weight of the polymer chains between cross-links and ξ is the molecular mesh size (Ganjil *et al.*, 2010).

The structure of an idealized hydrogel is shown in Figure 2.6 The most important parameters that define the structure and properties of swollen hydrogels are the polymer volume fraction in the swollen state, $\upsilon_{2,s}$, the effective molecular weight of the polymer chain between cross-linking points, $\overline{M}_c$, and the correlation distance between two adjacent cross-links, ξ (Ganjil *et al.*, 2010).

The favorable property of hydrogels is their ability to swell, when put in contact with a thermodynamically compatible solvent. When a hydrogel in its initial

state of contact with solvent molecules, the latter attacks the hydrogel surface and penetrates into the polymeric network (Ganjil *et al.*, 2010).

The gelatin hydrogels are analyzed by swelling studies immediately after crosslinking, according to the method of (Gudeman *et al.*, 1995). The degree of swelling and the weight loss of the gelatin hydrogels are measured in an acetate buffer solution at 37 °C for 48 h (Taepaiboon *et al.*, 2006) using the following equations (2.1-2.2):

$$\text{Degree of swelling (\%)} = \frac{M_s - M_d}{M_d} \times 100 \tag{2.1}$$

and

$$\text{Weight loss (\%)} = \frac{M_i - M_d}{M_i} \times 100 \tag{2.2}$$

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, $\overline{M}_c$, the mesh size, ξ, and the crosslinking density, ρ_x, the sample of gelatin film is cut immediately after crosslinking (1 cm^2). This sample is then weighted in air and heptane. The sample is then placed in distilled water at 37 °C for 5 days that allows it to swell to equilibrium, and then is weighted in air and heptane again. Finally, the sample was dried at 25 °C in vacuum oven for 5 days. Once again, it is weighted in air and heptane. The volumes of the polymer sample in the dry, relaxed, and swollen states are calculated by using equations (2.3) - (2.5), respectively.

$$V_d = \frac{W_{a,d} - W_{h,d}}{\rho_h} \tag{2.3}$$

$$V_r = \frac{W_{a,r} - W_{h,r}}{\rho_h} \tag{2.4}$$

$$V_s = \frac{W_{a,s} - W_{h,s}}{\rho_h} \quad (2.5)$$

where, $W_{a,d}$ is the weight of the dry polymer in air, $W_{h,d}$ is the weight of the dry polymer in heptane, $W_{a,r}$ is the weight of the relaxed polymer in air, $W_{h,r}$ is the weight of the relaxed polymer in heptane, $W_{a,s}$ is the weights of the swollen polymer in air and $W_{h,s}$ heptane, ρ_h is the density of heptanes, V_d is the volume of the polymer sample in the dry states, V_r is the volume of the polymer sample in the relaxed states and V_s is the volume of the polymer sample in the swollen states.

The calculattion of the polymer volume fraction in the relaxed, $\upsilon_{2,r}$, and swollen states, $\upsilon_{2,s}$, are by using equations (2.6) and (2.7), respectively (Peppas *et al.*, 1998).

$$\upsilon_{2,r} = \frac{V_d}{V_r} \quad (2.6)$$

and

$$\upsilon_{2,s} = \frac{V_d}{V_s} \quad (2.7)$$

The molecular weight between crosslinks, $\bar{M}_c$, can be calculated from the swelling data by using equation (2.8) (Peppas *et al.*, 1998).

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} - \frac{\frac{\bar{\upsilon}}{\bar{V}_1}[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi\upsilon_{2,s}^{2}]}{\upsilon_2[\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)^{1/3} - \frac{1}{2}\left(\frac{\upsilon_{2,s}}{\upsilon_{2,r}}\right)]} \quad (2.8)$$

where $\bar{M}_n$ is the number averaged molecular weight of the polymer before cross linking, $\bar{\upsilon}$ is the specific volume of gelatin ($\bar{\upsilon}$ = 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 number averaged molecular weight of the polymer before cross linking, $\overline{M}_n$, can be determined through the intrinsic viscosity, $[\eta]$. The intrinsic viscosity, $[\eta]$, is determined by the measurements of solution viscosity. The measurements are described by comparing the elution times (t = elution time of polymer solution, t_0 = elution time of solvent) in the viscometer. The times are proportional to the viscosity of the polymer solution, η, and solvent, η_o, respectively (Abrusci *et al.*, 2004). The specific viscosity and relative viscosity are calculated by the following equations (2.9) and (2.10) (Abrusci *et al.*, 2004):

$$\eta_{rel} = \frac{\eta}{\eta_o} \tag{2.9}$$

and

$$\eta_{sp} = \frac{\eta - \eta_o}{\eta_o} = \frac{t - t_o}{t_o} = \eta_{rel} - 1 \tag{2.10}$$

The intrinsic viscosity, $[\eta]$, is calculated by extrapolation to infinite dilution of the equation of Huggins (2.11) and Kramer (2.12) (Derosa 2008).

$$\frac{\eta_{sp}}{c} = [\eta] + k'[\eta]^2 c \tag{2.11}$$

and

$$\frac{\ln \eta_{rel}}{c} = [\eta] + k''[\eta]^2 c \tag{2.12}$$

where η_{sp} is the specific viscosity, η_{rel} is the relative viscosity, c is concentration of polymer in grams per deciliter (g/dL), η_{sp}/c is defined as reduced viscosity ,η_{red}, $\ln\eta_{rel}/c$ is defined as inherent viscosity, η_{inh}, and k' and k'' are the constants of Huggins and Kramer, respectively.

Molecular weight, M_w, of the gelatin are determined by the capillary viscometer. The relation between $[\eta]$ and M_w can be described in terms of the Mark–Houwink–Kuhn–Sakurada (MHKS) equation (2.13) (Enrione *et al.*, 2011).

$$[\eta] = KM_w{}^a \tag{2.13}$$

where K and a are the constant values of gelatins.

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 (2.14) (Peppas *et al.*, 1996).

$$\xi = \upsilon_{2,s}{}^{-1/3}\left[C_n\left(\frac{2\bar{M}_c}{\bar{M}_r}\right)\right]^{1/2} \cdot l \tag{2.14}$$

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).

The crosslinking density of the hydrogel, ρ_x, is calculated by using equation (2.15) (Peppas *et al.*, 1996).

$$\rho_x = \frac{1}{\bar{\upsilon}\bar{M}_c} \tag{2.15}$$

In order to study drug transport mechanism from various hydrogels, three diffusion models are generally used to fit the experimental data is described by the Ritger-Peppas equation (2.16) (Venkatesh *et al.*, 1992):

$$\frac{M_t}{M_\infty} = k_1 t^n \tag{2.16}$$

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

For a thin hydrogel film, when n = 0.5, the drug release mechanism is the Fickian diffusion. This mechanism is based on the Higuchi's equation. Model 1 is

based on the Higuchi's equation (2.17) (Serra *et al.*, 2006) and described the Fickian diffusion of the drug:

$$\frac{M_t}{M_\infty} = k_H t^{1/2} \tag{2.17}$$

where M_t/M_∞ is the fractional drug release, k_H is a kinetic constant (with the unit of T^{-n}) and t is the release time.

When n = 1, Case II transport occurs, leading to the zero-order release. Model 2 represents a zero-order model and is expressed by the following equation (2.18). (Serra *et al.*, 2006):

$$\frac{M_t}{M_\infty} = k_H t \tag{2.18}$$

When $0.5 < n < 1$, the anomalous transport is observed. Model 3 represents a first-order model and is expressed by the following Eq. (2.19) (Liu *et al.*, 2003):

$$M_t = M_\infty[1 - exp(-t/\tau)] \tag{2.19}$$

where M_t and M_∞ are the amounts of drug release at time t and infinity, respectively, and t is the release time.

The diffusion coefficients of drug from the hydrogels are determined from the slopes of plots of drug accumulation versus square root of time according to Higuchi's equation (2.20) (A-sasutjarit *et al.*, 2005):

$$Q = 2C_0(Dt/\pi)^{1/2} \tag{2.20}$$

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).

In 1987, Ritger *et al.* introduced the relation $M_t/M_\infty = kt^n$ which may be used to describe the Fickian and the non-Fickian release behaviors of swelling-

controlled release systems which swelled to a moderate equilibrium degree of swelling, and they were prepared by incorporation of a drug in a hydrophilic, initially glassy polymer. Again the diffusional exponent, n, is an important indicator of the mechanism of transport of a drug through the polymer. Analysis was presented for a solute release from sheets, cylinders, spheres and polydisperse samples.

In 1995, Gudeman *et al.* synthesized poly(vinyl alcohol) and poly(acrylic acid) interpenetrating network and also investigated the permeation of solutes of various size of drug including urea, guaiacolglyceryl ether, L-tryptophan, vitamin B_{12} and dextrans. They found that for L-tryptophan and urea, the diffusion coefficient was smaller at a pH of 3 than at a pH 6. For FITC-dextran and vitamin B_{12}, dextran permeation was rejected while the smaller solute was transported through the membrane.

In 2001, Jensen *et al.* studied the size of the guest molecule as an important parameter in the electrically-stimulated drug release. Further work obviously was needed to be carried out with a larger range of molecular weights and conformations by using Chondroitin 4-sulphate (CS) hydrogels as a matrix cross-linked with ethylene glycol diglycidylether for the electro-controlled delivery of peptides and proteins. Three positively charged molecules of different molecular weights (vasopressin M_w 1,084, aprotinin M_w 6,512 and lysozyme M_w 14,400), and one negatively charged protein (bovine serum albumin M_w 67,000) were used as the model solutes. The guest molecules were loaded passively by diffusing in the water phase of the gel. So, the efficiency of loading was observed to increase with decreasing molecular size of solute and the electro-stimulated releases of the loaded peptide and proteins were followed for a period of 3 h during applied electric field in pulses of 5 V with the pulse interval of 20 min. The releases of lysozyme and aprotinin from CS hydrogels responded to electrical pulses. Vasopressin and albumin were largely released by the passive diffusion and their releases could not be electrically controlled.

In 2008, Juntanon *et al.* synthesized the electrically controlled release of sulfosalicylic acid from crosslinked poly(vinyl alcohol) hydrogels. They also found that the diffusion coefficients of drug-loaded PVA hydrogels decreased with increasing crosslink ratio. Moreover, the diffusion coefficients of the charged drug in

the PVA hydrogels depended critically on the electric field strength between 0 and 5 V as well as on the electrode polarity. Thus, the release rate of sulfosalicylic acid can be altered and controlled precisely through the electric field stimulation. Hence, they concluded that by varying crosslinking density, the electric field, or by changing the electrode polarity, they can control and modulate the drug release rate.

2.5 Gelatin

Gelatin, one of the most popular biopolymers, is widely used in food, pharmaceutical, cosmetic, and photographic applications because of its unique functional and technological properties. In the pharmaceutical and medical fields, gelatin is used as a matrix for implants, in injectable drug delivery microspheres, and in intravenous infusions (Karim *et al.*, 2009). Generally, gelatin has been separated into 2 types; A and B that is produced from the skin or bone collagen by acid or alkali treatment, respectively. Gelatin is mainly extracted from mammals, poultries, and fish in which they are primarily consisted of polydisperse polypeptides. 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. The main different between the mammalian and fish gelatins is their gelation temperature (Chiou *et al.*, 2008) and water permeability (Avena-Bustilos *et al.*, 2006). Fish gelatin from cold-water has lower gelation temperature than the mammalian gelatins. Since the lower of proline and hydroxyproline concentration are approximately 30% for mammalian gelatins and 17% for cold-water fish gelatin (Karim *et al.*, 2009). The amino acid composition of cold-water fish and pork skin are shown in table 2.2.

Table 2.2 Amino acid composition (% mole) of cold-water fish and pork skin gelatins (Avena-Bustilos *et al.*, 2006)

Amino aicid	Cold-water fish skin		Pork skin
	Pollock	Salmon	
Alanine	10.88	12.49	11.06
Arginine	5.18	5.06	5.08
Aspatic acid	5.21	5.12	4.66
Cystine	0.14	0.08	0.09
Glutamic acid	7.17	7.25	7.44
Glycine	35.74	35.54	31.73
Histidine	0.80	0.87	0.50
Homocystine	0.16	0.12	0.03
Hydroxylysine	0.61	0.76	0.70
Hydroxyproline	5.30	5.56	9.78
Isoleusine	1.07	0.97	1.05
Leusine	2.10	1.83	2.50
Lysine	2.78	2.47	2.80
Methoinine	1.13	1.00	0.29
Phenylalanine	1.20	1.27	1.37
Proline	10.09	10.79	12.12
Serine	5.85	4.73	3.39
Therosine	2.68	2.55	1.87
Tyrosine	0.24	0.13	0.37
Valine	1.67	1.41	2.18

Gelatin chains in solution may be covalently cross-linked to form a matrix; suitable of swelling in the aqueous solutions, forming designated gelatin hydrogels to obtain a hydrophilic polymer insoluble at 37 °C for drug delivery field. The chemical cross-linkers used may be either relatively small bifunctional molecules or polyfunctional macromolecules like, for instance, glutaraldehyde (GTA), carbodiimide, genipin, and polyvinyl alcohol, which bind either the amino or carboxylic residues of the polypeptide chains (Deiber *et al.,* 2009). The GTA treated hydrogels are slightly yellowish and insoluble even in hot water, due to the chemical crosslinking of gelatin with GTA. The gelatin crosslinking with GTA is a two-steps reaction, occurring through the formation of N-substituted imines (Schiff-base) between the e-amino groups from the protein chain and the carbonyls from GTA. Basically the reaction starts with a nucleophilic addition of the amine to the carbonyl

carbons of GTA resulting in the protonation of oxygen and deprotonation of nitrogen ending with unstable intermediates (carbinolamines); carbinolamines undergo a water elimination forming a double bond generating imines. These mechanisms occur at the two terminal aldehyde reacting groups in GTA forming the Schiff-base linkages and thus crosslinking the polypeptide (Stancu *et al.*, 2010) as displayed in Figure 2.7.

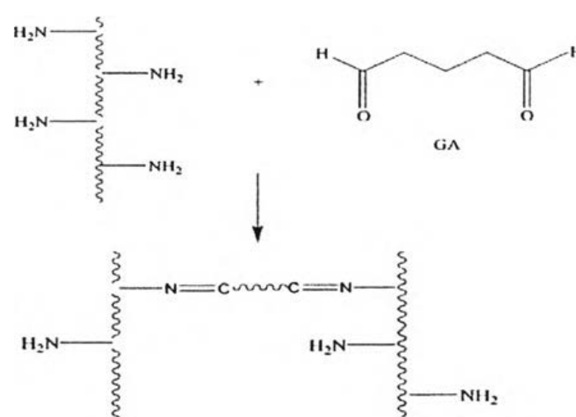

Figure 2.7 Chemical crosslinking through Schiff-base formation between GTA and NH_2 from gelatin chains (Stancu *et al.*, 2010).

In 2006, Avena-Bustillos *et al.* studied the water vapor permeability (WVP) of cold- and warm-water fish skin gelatins films and compared with different types of mammalian gelatins. WVP was determined from the differences on percent relative humidity (%RH) at the film underside. They found that WVP of cold-water fish gelatin films (0.93 gmm/m2hkPa) was significantly lower than warm-water fish and mammalian gelatin films (1.31 and 1.88gmm/m2hkPa, respectively) at 25 °C, 0/80 %RH through 0.05-mm thickness films. This was related to increased hydrophobicity due to the amounts of proline and hydroxyproline in cold-water fish gelatins (17% mole) lower than warn-water fish (22-25% mole) and mammalian gelatin (30% mole).

In 2007, Sutter *et al.* studied the recombinant gelatin hydrogels for the sustain release of proteins by preparing (HU4) hydrogels for the sustained release of

proteins. HU4 gelatin was modified with methacrylate residues for the chemical crosslinking and gel formation. Methacrylated gelatins with degrees of substitution (DS; defined as fraction of methacrylate residues with respect to the total number of primary amines) of 0.24, 0.67, 0.82, and 0.97 were synthesized by a radical polymerization. Mesh size (ξ) was $\geq$26 nm, as determined by the dynamic mechanical analysis. Release of the incorporated model proteins lysozyme and trypsin inhibitor occurred by diffusion and was nearly complete. Protein diffusion coefficients in the gel were between 5.0×10^{-7} and 4.0×10^{-8} cm^2 s^{-1}, up to 100 fold lower than in water. Release under physiological conditions was effectively controlled by varying hydrogel mesh size and protein–gelatin charge interactions, which demonstrated that recombinant gelatins were a versatile class of biopolymers for the preparation of hydrogels for protein delivery. The result is shown by increasing of DS and gelatin concentration in the gel , Molecular weight between crosslink (Mc) decreased to values below the molecular weight of HU4 gelatin. And the electrostatic repulsion between the negatively charged trypsin inhibitor and the negatively charged HU4 gelatin matrix, as well as electrostatic attraction between the positively charged lysozyme and the negatively charged gelatin matrix was therefore expected. In agreement with these expectations, the diffusion coefficient of trypsin inhibitor in gels composed of methacrylated HU4 gelatin of DS 0.97 was 3–4 times higher than that of lysozyme in corresponding gels. The amount of protein released was about the same for lysozyme and trypsin inhibitor (80–90%), suggesting that the electrostatic interactions did not lead to a permanent immobilization of protein in the hydrogel matrix, and that unreleased protein (10–20%) was possibly entrapped in very dense parts of the hydrogels. Hence, they concluded that the modification of HU4 gelatin with methacrylate leading to hydrogels with non-degradable crosslinks. Under physiological conditions, the release of encapsulated protein occurs by diffusion and can be effectively steered by the DS of the gelatin, the polymer volume fraction in the swollen state ($\upsilon_{2,s}$), and charge interaction between the protein and the hydrogel matrix.

In 2008, Chiou *et al.* studied the cold water fish gelatin films and the effect of cross-linking on the thermal, mechanical, barrier, and biodegradation properties. The fish gelatin films were tested for the tensile, thermal, water vapor permeability,

oxygen permeability, and biodegradation properties and were compared to those of the bovine and porcine gelatin films. In addition, fish gelatin films were cross-linked with glutaraldehyde. The fish gelatin, especially those from cold water species, had a much lower gelation temperature than those of other species. This is due to the fish gelatin having lower concentrations of proline and hydroxyproline. They also found that the lower tensile strength and the elongation might have been due to lower structural gelatin levels present in the fish gelatin films.

In 2008, Yu and Xiao investigated the gelatin based pharmaceutical hydrogels by use of oxidized konjac glucomannan (DAK) as a macromolecular cross-linker. FTIR, XRD, SEM, swelling and mechanical properties experiments were performed to confirm the effect of DAK and evaluate the relationship of the structure and morphology of the hydrogels. The results indicated that DAK promoted the formation of gelatin network. More interestingly, gelatin hydrogels treated by DAK slowed down prominently the release of the model drug ketoprofen, and the release rate could be tailored by the DAK/GL ratio and pH value of buffer solutions. The results shown at the same pH, the swelling ration value rapidly decreased with the increase of the DAK content. At a different pH buffer, the pH value of the buffer solution (pH 9.0) was far higher than the isoelectric point (PI) of GEL (PI 4.0–5.0), the carboxyl groups were de-protonized to carry negative charges, which made molecular chains repulsed to each other. The network became looser and it was easy for the water molecules to diffuse into the crosslinked network. The release rates of ketoprofen from hydrogels were slightly at different crosslinked ratios. The high content of DAK in the hydrogel matrix caused a delay in the kinetics of drug release. When compared with the release profile, at pH 9.0 had a more rapidly release of ketoprofen than at pH 4.0. Since, at pH 9.0 the relatively high swelling degrees of DKG hydrogels resulted in higher release rates. Hence, they concluded that all the hydrogels showed sustained release properties, and the dependence of release rate on the equilibrium swelling ratio of hydrogels and pH value of medium.

In 2009, Gomez *et al.* studied the most recent scientific literature dealing with films based on gelatins from different fish species and considered various strategies intended to improve the physical properties of such films by combining fish gelatins with such other biopolymers. However, the physical properties of the

fish gelatin films were highly dependent on gelatin attributes, which were in turn dependent not only on intrinsic properties related to the fish species used but also on the process employed to manufacture the gelatin.

In 2010, Farris *et al.* investigated the chemical functional groups involved in the cross-linking reaction between glutaraldehyde and gelatin molecules. The results suggested that, at high pH (11.0) values, the cross-linking reaction was mainly governed by the well-known Schiff base formation. At low pH (4.5), the reaction might also involve the -OH groups of hydroxyproline and hydroxylysine. The formation of different types of covalent bonds during the cross-linking reaction might contribute to the remarkably different properties of the resulting gelatin films. The films cross-linked with glutaraldehyde (0.3% w/w) at low pH (4.5) exhibited the elastic modulus and elongation at break lower and higher than those of non-cross-linked films, respectively. At the same time, it was found that the addition of glutaraldehyde did not result in any significant change in either glass transition temperatures (Tg) or melting temperatures (Tm) of gelatin films. Hence, they concluded that the gelatin-based matrices cross-linked at different pH conditions exhibited distinctive mechanical, thermal, and water resistance properties.

2.6 Drug

The drug characteristics such as the size, shape, and ionization of the drug affect its diffusion through the polymer membrane are important parameters.

2.6.1 <u>Salicylic Acid</u>

Salicylic acid is the colorless crystalline organic acid and widely used in organic synthesis. Salicylic acid is a key ingredient in many skin-care products for the treatment of acne, psoriasis, calluses, keratosis pilaris, and warts. Molecular weight = 138, Molecular size =3.28 Å (Niamlang, 2008).

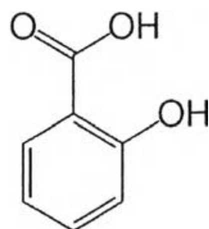

Figure 2.8 Chemical structure of salicylic acid.

2.6.2 5-Sulfosalicylic Acid

Sulfonic acid derivative are the sulfa drugs which are important as antibiotics such as Sulfadiazine, an antibiotic drug that is used in animals. Molecular weight = 254.22, molecular size = 9.25 Å (Juntanon *et al.*, 2008).

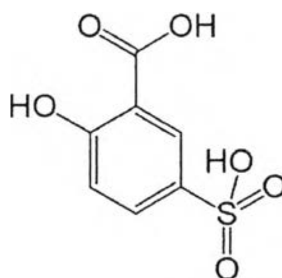

Figure 2.9 Chemical structure of 5-sulfosalicylic acid.

In 2006, Lee and Lee *et al.* inevestigated the swelling behavior of poly(N-isopropylacrylamide), poly (NIPAAm), and gelatin. The hybrid gels were crosslinked through a two-step process with genipin or glutaraldehyde. The effects of gelatin on the drug release profile were demonstrated. The results showed the ionicity of hybrid gels strongly influenced the release of phenol red (Mw = 354) (anionic) and neutral red (Mw = 288) (cationic). But, the releases of sulfanilamide (Mw = 172), caffeine (Mw = 194), and vitamin B12 (Mw = 1355) were not influenced by the ionicity of hybrid gel. The drug released from the gels crosslinked with genipin was significantly smaller than that released from the gels crosslinked with glutaraldehyde. That was because the swelling ratios of GP series gels were lower than those for GA series gels; heterocyclic structure of GP might cause the

gels relaxing more difficult than those gels crosslinked with linear GA. The release profiles of nonionic drugs decreased with increasing drug molecular weight. Hence, they concluded that drug release profile from the NIPAAm/gelatin hybrid gels was mainly determined by the ionicity of hybrid gels and drug types. The fractional release of nonionic drugs, such as sulfanilamide, caffeine and vitamin B12, in the hybrid gels was not affected by the ionicity of hydrogels. However, the swelling ratio of the hybrid gels and molecular size of the drug influence their release profile. The anionic solute (phenol red) strongly interacted with cationic hybrid gel, so the fractional release of phenol red in cationic gels was very low. On the other hand, the cationic solute, neutral red, only adsorbed on the skin layer of the cationic hydrogel due to the charge repulsion and released quickly. Therefore, the fractional release is the highest for the combination cationic hydrogel and cationic drug.

In 2006, Pal *et al.* they designed and developed the novel membrane of hydrogels prepared by esterification of polyvinyl alcohol with gelatin. The hydrogel was characterized by a hemolysis test and by the water vapor transmission rate. Diffusion coefficient of salicylic acid (SA) and gatifloxacin through the membrane was determined. The diffusion coefficient of SA and gatifloxacin through the membrane was found to be 1.49×10^{-5} and 3.97×10^{-6} cm^2/s respectively. The SA was an anionic drug while gatifloxacin was cationic in nature. Both the drugs were water-soluble. The molecular weight of SA and gatifloxacin were 138.1 and 384.4, respectively. They suggested that as the molecular weight of the drug increased, its diffusion coefficient through the hydrogel membrane decreased. Thus the membrane is permeable to both anionic and cationic drugs. Hence, they concluded that the diffusion of low molecular weight drug appears better than the higher molecular weight drug.