PREPARATION, CHARACTERIZATION AND DRUG RELEASE BEHAVIOR OF THERMO- AND pH-RESPONSIVE HYDROGELS AND MICROSPHERES

by

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ABSTRACT

PREPARATION, CHARACTERIZATION AND DRUG RELEASE BEHAVIOR OF THERMO- AND pH-RESPONSIVE HYDROGELS AND MICROSPHERES

In the present work, N-isopropylacrylamide/itaconic acid and N-isopropylacrylamide /maleic acid copolymeric hydrogels were prepared by irradiating the ternary mixtures of N-isopropylacrylamide/diprotic acid moieties (itaconic or maleic acid)/water by γ-rays at ambient temperature.

The influence of external stimuli such as pH and temperature of the swelling media on the equilibrium swelling properties were investigated. The hydrogels showed both temperature and pH responses. The effect of comonomer concentration and irradiation dose on the swelling equilibria and phase transition were studied. For the characterization of network structures of these hydrogels, molecular weight between cross-links and cross-linking densities were also determined. PNIPAAm microspheres (71-500μm) were prepared by using inverse suspension polymerization technique. The selected fraction (180-250μm) was used for preparing NIPAAm/itaconic acid graft copolymer by radiation-induced surface modification technique.

Finally, methylene blue, sildenafil citrate and lidocaine were used as model drugs for the investigation of drug adsorption and controlled release behaviour for the hydrogels and micospheres. Specific adsorption capacity of hydrogels are found to increase with increasing amount of itaconic acid in the gel system. The effect of the initial drug loading amount, temperature and pH of the solution on the controlled release behaviour of the hydrogels were investigated. The release studies show that some of the basic parameters affecting the drug release behaviour of the hydrogels are pH and temperature of the solution.

ÖZET

SICAKLIK VE pH'A DUYARLI HİDROJEL VE MİKROKÜRELERİN HAZIRLANMASI, KAREKTERİZASYONU VE İLAÇ SALINIM DAVRANIŞI

Bu çalışmada, N-izopropilakrilamid-ko-itakonik asit ve N-izopropilakrilamid-ko-maleik asit hidrojelleri, N-izopropilakrilamid /diprotik asit (itakonik veya maleik asit)/su ile oluşan üçlü karışımın gama kaynağı ile oda sıcaklığında ışınlanmasıyla hazırlanmıştır.

Çözelti pH'ı ve sıcaklığı gibi dış ortam şartlarının dengedeki şişme davranışına olan etkileri incelendi. Hidrojeller hem pH'a hem de sıcaklığa duyarlılık gösterdi. Komonomer konsantrasyonu ve ışınlama dozunun şişme davranışına ve faz geçişine olan etkileri incelendi. Ayrıca, bu hidrojellerin ağ yapılarının karakterizasyonu için çapraz bağlar arası molekül ağırlık ve çapraz bağlama yoğunluğu tayin edildi.

PNIPAAm mikroküreleri (71-500μm) ters süspansiyon polimerizasyon tekniği ile hazırlandı. Seçilen fraksiyon(180-250μm), radyasyon yüzey modifikasyon tekniği ile NIPAAm-ko-IA kopolimeri oluşturmakta kullanıldı.

Bu hidrojel ve mikroküreleri, ilaçları adsorplaması ve kontrollü ilaç salım davranışlarını incelemek için Metilen Mavisi, Sildenafil Sitrat ve Lidokain ilaçları model olarak kullanıldı. Hidrojellerin spesifik adsorpsiyon kapasiteleri jel sistemindeki itakonik asit miktarını arttırmakla arttığı bulundu. Başlangıç ilaç yükleme miktarının, çözelti sıcaklık ve pH'ının hidrojellerin salım davranışlarına olan etkileri incelendi. Salım çalışmaları, hidrojellerin salım davranışı etkileyen temel parametrelerin bazılarının çözelti pH ve sıcaklığının olduğunu gösterdi.

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LIST OF SYMBOLS

a_1	Activity of solvent
a_i	Activity of component i
c	Concentration
d	Density
d_1	Density of water
D	Final equilibrium diameter of the gel
D_o	Initial diameter of the gel
f	Average number of ionic units between succesive junctions in a network chain
ΔG	Gibbs free energy change of the system
ΔG_m	Gibbs free energy change of mixing
ΔG_{el}	Gibbs free energy change of elastic deformation
ΔG_i	Gibbs free energy change of electrostatic interactions
$\overline{\Delta G}$	Partial molar free energy of dilution
ΔH	Entalpy change
ΔH_m	Heat of mixing
$\overline{\Delta H}$	Partial molar heat of dilution
I	Number of electronic charges per polymer unit in a polyelectrolyte
k	Boltzmann's constant
k_s	The swelling rate constant.
l	Bond length
M	Molecular weight of polymer
$\overline{M_c}$	Number average molecular weight between successive junctions in a network chain
$m_{initial}$	The initial weight of the dry gel
m_{final}	The final weight of the extracted gel after drying
m_0	The mass of the dry gel at the time 0
m_t	The mass of swollen gel at time t

m_∞	The mass of swollen gel at equilibrium
n_1	Mole fraction of solvent molecules in a solution
n_2	Mole fraction of polymer network in a solution
n_i	Mole fraction of component i in a solution
N	The number of segments in a network chain
N_A	Avagadro's number
N_1, N_2	Mole fractions of molecules 1 and 2 in a solution
P	Pressure
P_1	Partial pressure of solvent in a solution
P_1^0	Partial pressure of pure solvent
pK_{a1} , pK_{a2}	The first and second dissociation constants
Q	The equilibrium degree of swelling
q_w	Swelling ratio by weight
q_m	Equilibrium swelling ratio
R	The gas constant
ΔS	Entropy change
ΔS_m	Entropy change for mixing
ΔS_{el}	Entropy change for elastic deformation
ΔS_m^{conf}	Configurational entropy change
$\overline{\Delta S}$	Partial molar free energy of dilution
T	Absolute temperature
T_c	Critical temperature
T_{tr}	Transition temperature
V	Volume of the polymer network at the equilibrium degree of swelling
V_o	Volume of the network in the state of formation
v_1	Molar volume of solvent
Δw_{ij}	Interaction free energy change associated with the formation of a contact between a pair of segments of molecules of i and j
x	The number of "segment" in a solvent molecule
z	The lattice coordination number

α	Factor expressing the linear deformation of a polymer molecule owing to solvent-polymer interaction
α_s	Factor expressing the linear deformation of a network structure by isotropic swelling
$\alpha_x, \alpha_x, \alpha_x$	Linear deformation factor with reference to x, y and z coordinates
θ	Ideal temperature
κ_1	Heat parameters
μ	Number of junctions
μ_1, μ_2	Chemical potential of solvent, polymer network, respectively
μ_1^0	Chemical potential in standard states
$(\mu_1-\mu_1^0)_E$	Excess chemical potential of the solvent
μ_i	Chemical potential of component i
ν	Number of network chains
ν_e	Effective crosslinking densities of polymer networks
ν_i	Number of ways of arranging the i-th polymer molecule on a lattice to which i-1 polymer molecules havee been added previously
ν_1	Volume fraction of solvent
ν_2	Volume fraction of polymer network
ν_2'	Volume fraction of polymer network in the less swollen phase
ν_2''	Volume fraction of polymer network in the more highly swollen phase
ν_{2m}	Volume fraction of polymer network at the equilibrium degree of swelling
ν_2^0	Volume fraction of polymer network after preparation
ξ	Cycle rank
π	Osmotic pressure
ρ	Density of polymer
ϕ	Average functionality
χ_1	Parameters expressing the first neighbor interaction free energy, divided by kT, for solvent with the polymer
χ_{ij}	Corresponding parameter for the interaction of component i with j in a polycomponent system

Ψ_1	Entropy parameter
Ω	The total number of configurations

1. INTRODUCTION

Hydrophilic polymers when crosslinked chemically or physically forming three dimensional networks swell but do not dissolve in water. They are termed hydrogels when the amount of water retained is between 20-100 per cent of the total weight, and when water content exceeds 100 per cent these hydrogels are called superadsorbent hydrogels. A hydrogel can be considered as a container of water made of a three dimensional mesh. Many materials, both naturally occurring and synthetic fit the definition of the hydrogels. Dextrans, strach, alginates, and collagens are examples of natural polymers that can be crosslinked to form hydrogels. Hydrogels based on synthetic polymers include poly(acrylamide), poly(ethylene oxide), poly(n-vinyl 2-pyrolidone), poly(vinyl alcohol). Wichterle and Kim were the first to suggest that a hydrogel based on poly(2-hydroxy ethyl methacrylate) could be a synthetic biocompatible material [1].

Recently hydrogels have found a wide range of biomedical applications including controlled drug delivery systems, replacement blood vessels, wound dressings, soft tissue substitution, contact lenses and variety of other related and potential uses [2-11]. Hydrogels are found to be very well tolerated when implanted in vivo and can be easily tailored to suit many functions of prosthetics in contact with blood or tissues. The success of hydrogels as biomaterials lies in their resemblance to living tissue because of their relatively high water content which minimizes the frictional irritation of surrounding tissue. Additional advantages of hydrogels are their non-toxicity, nonantigenicity, nonirritability and chemical stability. High water content, permeability to small molecules, and cross-linked structure allow residual monomers and initiators to be efficiently extracted from the gel network before usage. The high solute permeability of hydrogels made them ideal materials of choice as devices for the controlled release of drugs and other active agents. In addition to the inertness and good biocompatibility of hydrogels, their ability to release entrapped drug in an aqueous medium and the ease of regulating such drug release by controlling water swelling and cross-linking density

make hydrogels particularly suitable as drug carriers in controlled delivery applications. By proper design of hydrogels it is possible to control the kinetics of delivery of active ingredients.

The most characteristic property of hydrogels is their ability to swell in the presence of water and to shrink in the absence of it. The two most important factors controlling the extent of swelling are the hydrophilicity of polymer chains and the crosslink density. By incorporating some stimuli-responsive comonomers either into the backbone of the network structure or as pendant groups it is possible to prepare hydrogels with responsive properties. These hydrogels possess the ability to swell, shrink, bend or even degrade in response to a signal. These stimuli responsive hydrogels are called intelligent hydrogels. They reversibly swell or shrink with small changes in environmental conditions. The most common environmental factors that cause an abrupt volume change in hydrogels are pH, temperature, electric field, ionic strength and type of salt.

Hydrogels are typically synthesized by one of the two well established procedures: polymerization and simultaneous or postpolymerization crosslinking of hydrophilic monomers and modification or hydrophilization of existing polymers with potential hydrogel properties. There is renewed interest in radiation induced polymerisation and cross-linking in polymeric hydrogels. The advantages of radiation methods are that they are relatively simple and do not require addition of any extra materials for polymerisation and cross-linking. Moreover, the degree of cross-linking, which strongly determines the extent of swelling in hydrogels can be controlled easily by varying the dose rates. Therefore these methods are found to be very useful in preparing hydrogels for medical applications, where even a small contamination is undesirable. By this method, it is possible to combine into one step the synthesis and sterilization of the product, and it is economically competitive when compared to conventional methods [12]. Recently, Nagaoka et. al. have reported for the first time the synthesis of poly(N-isopropylacrylamide) (PNIPAAm) hydrogel by γ-radiation technique [13].

PNIPAAm hydrogels are attracting more and more interest in biomedical applications, because they exhibit a well-defined lower critical solution temperature (LCST) in water, around 31-34°C, which is close to the body temperature. PNIPAAm hydrogels swell when cooled below the LCST, and they collapse when heated above the LCST [14]. This hydrogel has received much attention recently and has been used as a model system to demonstrate the validity of theories describing coil-globule transition, swelling of networks, and folding and unfolding of biopolymers [15,16]. It has also been proposed for various applications ranging from controlled drug delivery to solute separation [3, 4, 10, 11].

By preparing hydrogels from a mixture of the monomers it is possible to synthesize copolymeric building blocks. During the last decade, a number of papers published from different laboratories showed clearly the irradiating aqueous monomer solutions to synthesize copolymeric hydrogels [17-24]. Incorporation of diprotic acid moieties (itaconic acid or maleic acid) into the gels as a comonomer will impart different level of pH sensitivity to the gels [18-23]. Much attention has been directed in recent years at hydrogels that undergo large volume changes in response to small variation in external stimuli such as pH and temperature [25-27]. Temperature and pH have been the solution variables of great importance, because these variable change in typical physiological, biological, and chemical systems.

The objectives of this study are first to prepare N-isopropylacrylamide/itaconic acid and N-isopropylacrylamide/maleic acid copolymeric hydrogels by irradiating the ternary mixtures of N-isopropylacrylamide/diprotic acid moieties (itaconic or maleic acid)/water by γ-rays at ambient temperature.

Studying swelling behaviour and phase transition of these temperature and pH sensitive gels as a function of pH and temperature were also aimed. The dependence of swelling properties on the comonomer concentration, irradiation dose and temperature were investigated. For the characterization of network structure of these hydrogels, molecular weight between crosslinks and crosslink density were determined.

For drug delivery applications, the hydrogel cylinders prepared by the radiation induced polymerisation process are useful only in topical and implant application. To be useful for oral dosage forms, hydrogels would have to be in granules prepared by cutting or granulating hydrogel sheets or cylinders followed by sieving into proper particle sizes. Such hydrogel granules are generally irregular in shape which may be objectionable not only from the standpoint of product aesthetic, but also from the standpoint of reproducibility in controlling the drug release. For this reason, PNIPAAm microspheres (71-500μm) were prepared by using inverse suspension polymerization technique. The particles were sieved using ASTM sieves. The selected fraction (180- 250μm) was used for radiation-induced surface modification of PNIPAAm with itaconic acid (IA).

Finally, after the characterization of these hydrogels, adsorption capacities of the NIPAAm/IA copolymeric hydrogelss and NIPAAm/IA copolymeric microspheres for model drugs (methylene blue, lidocaine and sildenafil citrate) and their drug release systems were investigated.

In Chapter 2, some information on the following subjects are given: thermodynamics of polymer solutions and gels, swelling of network structures and phase equilibria in polymer systems, the drug loading and release characteristics of hydrogel systems, the radiolysis of water and polymer. The experimental studies carried out are presented in Chapter 3 and the results are discussed in Chapter 4. Finally, conclusions and recommendations for future work are given in Chapter 5.

2. THEORY OF POLYMER SOLUTIONS

In the beginning of this chapter, general information about thermodynamics of polymer solutions and gels, and also swelling of network structures and phase equilibria in polymer systems is given. Next, general information about the drug loading and release characteristics of hydrogel systems is presented. Finally, the radiolysis of water and polymer are briefly summarized.

2.1. Thermodynamics of Polymer Solutions and Gels

Polymers, by virtue of their high molecular weight, are soluble only in selected solvents. Although the basic principles of physical chemistry are applicable to all molecules, the size and conformations of dissolved polymer molecules require special theoretical treatment to explain their solution properties. In macromolecule solutions, only at extreme dilutions the ideality can be achieved. At concentrations exceeding a few percent, deviation from ideality usually become so great that the ideal law is of little value as a basis for rationality correlating the thermodynamic properties of polymer solutions. Some other relationship is therefore needed [28-30].

2.1.1. Thermodynamics of Simple Liquid Mixtures

For a system at equilibrium, the chemical potential, μ_i, of component i is related to its activity, a_i,

$$\mu_i - \mu_i^0 = RT \ln a_i \qquad (2.1)$$

where μ_i^0 is the chemical potential of component i in its standard state and $\mu_i - \mu_i^0$ is the partial molar Gibbs free energy change, $\overline{\Delta G_i} = \left(\frac{\partial \Delta G_i}{\partial n_i}\right)_{P,T,n_j}$, for the formation of the system.

Ideal solutions are defined as those in which the activity a_i of each component should equal to its mole fraction n_i. In a binary solutions consisting of solvent and a polymer having molecular weight a thousand times or more that of the solvent, only a very small percentage by weight of the solvent is sufficient to bring its mole fraction n_1 very close to unity. Hence according to Raoult's law (with $a_1 = P_1 / P_1^0$) the partial pressure P_1 of the solvent in the solution should be very nearly equal to that of the pure solvent P_1^0 over the greater portion of the composition range.

In ideal solutions, the molecules of solvent and polymer are identical size and for which the energies of like and unlike molecular interactions are equal. The latter condition leads to athermal mixing (i.e. $\Delta H_m = 0$), which also means that there are no changes in the rotational, vibrational and translational entropies of the components upon mixing. Thus ΔS_m depends only upon the configurational entropy change, ΔS_m^{conf}, which is positive because the number of distinguishable spatial arrangements of the molecules increases when they are mixed.

The evaluation of configurational entropy is usually performed by placing the n_1 identical molecules of the solvent and n_2 identical molecules of the solute on the lattice comprising $n_0 = n_1 + n_2$ cells. The number of distinguishable arrangements is

$$\Omega = (n_1 + n_2) ! / n_1 ! n_2 ! \qquad (2.2)$$

from which we obtain

$$\Delta S_m = k \ln \Omega = - k (n_1 \ln N_1 + n_2 \ln N_2) \qquad (2.3)$$

where N_1 and N_2 are mole fractions of molecules 1 and 2, respectively.

The conditions for ideal mixing imply that the heat of mixing is zero. Then, since $\Delta G = \Delta H - T \Delta S$, the free energy of mixing is given by,

$$\Delta G_m = kT (n_1 \ln N_1 + n_2 \ln N_2) \qquad (2.4)$$

This important equation provides the fundamental basis from which standard thermodynamic relationships for ideal solutions can be derived [29, 31].

2.1.2. Entropy of Mixing in Polymer Solutions

Deviation from ideality in binary mixtures of the same size and shape may arise mainly from the non-athermal mixing conditions. ($\Delta H_m \neq 0$) However, the polymer solutions show deviations from ideal solution behavior, even when $\Delta H_m = 0$. These are the natural result of the large difference in molecular size between the two components. When one component of the solution is a macromolecule the connectivity of the chain segments imposes a special restriction on the arrangement of the two components in space. Paul Flory and Maurice Huggins independently derived expressions on this basis for the total number of configurations of a liquid mixture formed from n_1 (number of molecules of solvent) and n_2 (number of molecules of solute). They estimated the number of distinguishable ways in which n_1 solvent molecules and n_2 macromolecular chains could be placed on a lattice.

Each polymer chain is represented by x segments, with the molar volume of the segment equal to that of the solvent molecules v_1. There are a total of $(n_1 + xn_2) = n_0$ lattice sites. The n_2 polymer molecules are added one by one to the lattice before adding the solvent molecules. The individual numbers of possible placements are calculated for each segment of the chain, and by multiplying these numbers together, ν, the total number of possible conformations of the polymer molecule in the lattice is obtained,

$$\nu_{i+1} = \{(n_0 - x_i) ! / [n_0 - x_{(i+1)}] ! \} [(z - 1) / n_0]^{x-1} \qquad (2.5)$$

where z is the coordination number of the lattice. The total number of distinguishable spatial arrangements of the mixture is given by,

$$\Omega_{12} = \frac{1}{n_2!} \prod_{i=1}^{n_2-1} \nu_{i+1} \qquad (2.6)$$

where n_2 is the number of identical polymer molecules. The number of Ω_2 by which chains can be placed on xn_2 sites is obtained by setting $n_1=1$ in Equation (2.6). The configurational entropy of mixing is,

$$\Delta S_m = k \ln (\Omega / \Omega_2) = - k (n_1 \ln \nu_1 + n_2 \ln \nu_2) \quad (2.7)$$

where k is boltzmann's constant and ν_1 and ν_2 are volume fractions of solvent and the polymer defined as

$$\nu_1 = \frac{n_1}{n_1 + xn_2} \qquad \nu_2 = \frac{xn_2}{n_1 + xn_2} \quad (2.8)$$

Comparison of Equation (2.3) with (2.7) reveals an interesting analogy to the ideal entropy of mixing; mole fractions occurring in the ideal expression are replaced with volume fractions in the formula for mixing of molecules dissimilar size. The ideal mixing "law" can be derived only for the case in which solvent and solute molecular volumes are equal. When this is true, the volume fractions and mole fractions are identical and Equations (2.3) and (2.7) are then equivalent [28, 29].

2.1.3. Heat and Free Energy of Mixing

Intermolecular interactions are large in the liquid state owing to the close proximity of the molecules. Since the pure solvent and pure liquid polymer are taken as reference states for the treatment of the solution, it is interested only in the difference between the total interaction energy in the solution as compared with that of the pure liquid components. In particular, it is needed to express the dependence of this difference, that is heat of mixing, on the concentration. The calculations is simplified by restriction to first neighbour interactions on the basis that the forces between uncharged molecules are known to decrease rapidly with their distance of separations. Therefore, the heat of mixing can be considered to originate in the replacement of some of the contacts between like species in the pure liquids with contacts between unlike species in the solution.

Three types of contact need to be considered. For every two solvent- segment contacts of pure components are lost. Thus the change in the internal energy for the formation of an unlike molecular pair (solvent-polymer or 1-2) contacts given by the mean field expression as,

$$\Delta w_{12} = w_{12} - (1/2)(w_{11} + w_{22}) \tag{2.9}$$

where w_{ij} is the energy of i-j contacts. The expression that Flory and Huggins gave for the enthalpy of mixing is,

$$\Delta H_m = z n_1 x_1 \nu_2 \Delta w_{12} \tag{2.10}$$

where z is the lattice coordination number or number of cells that are first neighbours to a given cell, x_1 represents the number of "segment" in a solvent molecule for consideration of the most general case. Since z and Δw_{12} have the character of empirical parameters, it is convenient to define a single energy parameter called Flory interaction parameter, χ_{12}, given as,

$$\chi_{12} = z x_1 \Delta w_{12} / kT \tag{2.11}$$

The interaction parameter is a dimensionless quantity that characterizes the interaction energy per solvent molecule (having x_1 segments) divided by kT. As Equation (2.11) indicates, χ_{12} is inversely related to temperature but is independent of concentration. The expression for the enthalpy of mixing then be written by combining Equation (2.10) and Equation (2.11) as,

$$\Delta H_m = kT \chi_{12} n_1 \nu_2 \tag{2.12}$$

The quantity $kT\chi_{12}$ represent merely the difference in energy of a solvent molecule immersed in the pure polymer ($\nu_2 \cong 1$) compared with one surrounded by molecules of its own kind, i.e., in the pure solvent.

Well known Flory-Huggins expression for the Gibbs free energy of mixing is simply obtained by combining Equations (2.7) and (2.11). That is,

$$\Delta G_m = \Delta H_m - T\Delta S_m \tag{2.13}$$

$$\Delta G_m = kT ([n_1 \ln \nu_1 + n_2 \ln \nu_2] + \chi_{12} n_1 \nu_2) \tag{2.14}$$

Using the Flory-Huggins Equation it is possible to account for the equilibrium thermodynamic properties of polymer solutions, particularly phase-separation and fractionation behavior, melting point depression in crystalline polymers and swelling of networks [28, 31-32].

2.1.4. Partial Molar Quantities and Chemical Potential

For multicomponent systems such as polymer solutions, it is important to know how thermodynamic parameters vary when a small amount of one component is added to the system whilst maintaining the pressure, temperature and amounts of all other components of the system constant. This requires the use of partial molar quantities.

The most commonly encountered one in the thermodynamics of polymer solutions are partial molar Gibbs free energies, G_i. This quantities are called chemical potentials, μ_i , which therefore are defined by

$$\overline{G_i} = \mu_i = \left(\frac{\partial G_i}{\partial n_i}\right)_{P,T,n_j} \tag{2.15}$$

The chemical potential μ_1 of the solvent in the solution relative to its chemical potential μ_1^0 in the pure liquid is obtained by differentiating the free energy of mixing ΔG_m with respect to the number of n_1 of the solvent molecules. Differentiation of Equation (2.14) for ΔG_m with respect to n_1 to obtain chemical potential per mole gives

$$\mu_1 - \mu_1^0 = RT [\ln (1- \nu_2) + (1- 1 / x) \nu_2 + \chi_{12} \nu_2^2] \tag{2.16}$$

Differentiation of Equation (2.14) with respect to n_2 yields for the chemical potential of the polymer relative to the pure liquid polymer as standard state.

$$\mu_2 - \mu_2^0 = RT [\ln \nu_2 - (x - 1) (1 - \nu_2) + \chi_{12} x (1 - \nu_2)^2] \qquad (2.17)$$

From the chemical potential an expression for the osmotic pressure π of the solution may be written, using standard relations of thermodynamics. For the osmotic pressure $\pi v_1 = - (\mu_1 - \mu_1^0)$,

$$\pi = -\frac{RT}{v_1} [\ln (1 - \nu_2) + (1 - 1 / x) \nu_2 + \chi_{12} \nu_2^2] \qquad (2.18)$$

where χ_{12} is interaction energy per solvent molecule (having x segments), ν_2 is volume fraction of the polymer and v_1 is the molar volume of the solvent [28].

2.1.5. Dilute Polymer Solutions

In dilute solutions, coils of flexible macromolecules, representing high local concentration of segments, are separated by large regions of polymer free solvent. The inhomogeneity in segment distribution in solution makes the Flory-Huggins theory inapplicable because the assumption that the probability of occupancy of a lattice site is equal to the overall fraction occupied sites becomes clearly invalid. Flory and Krigbaum have provided a model to describe the thermodynamics of a dilute polymer solution in which individual polymer chains are isolated and surrounded by regions of solvent molecules. In contrast to the case of a semi dilute solution addressed by the Flory-Huggins theory, segmental density can no longer be considered to be uniform. In their development Flory & Krigbaum viewed the dilute solution as a dispersion of clouds consisting of polymer segments surrounded by regions of pure solvent [30].

Let assume that such a model in which each cloud of segments is approximately spherical with an average density which is maximum at the center and decreases in a continuous manner with the distance from the center. Each molecule in a very dilute

polymer solution in a good solvent (low χ_{12}) will tend to exclude all others from the volume which it occupies. Conversely, the excluded volume decreases in pure solvent. Therefore excluded volume is related to the interaction parameter. In evaluating the excluded volume, Flory and Krigbaum considered the centers of two coils approaching each other so that a volume element δV contains segments from both molecules.

Let assume that the number of polymer molecules in the volume element is zero. It is as if the molecular weight were infinite, in which case would be zero. Then the free energy of mixing of polymer segments with solvent in the volume element δV is,

$$\delta(\Delta G_m) = kT[\delta n_1 \ln (1 - \nu_2) + \chi_{12} \delta n_1 \nu_2] \qquad (2.19)$$

where δn_1 is the number of solvent molecules in the volume element. The volume fraction ν_2 appearing here and in the following Equations refers to the volume element and not to the solution as a whole.

The chemical potential of the solvent in the volume element, obtained by differentiating Eq.(2.19), is

$$(\mu_1 - \mu_1^0)_E = RT [\ln (1- \nu_2) + \nu_2 + \chi_{12} \nu_2^2] \qquad (2.20)$$

which also follows directly from the previous Equation (2.16) when x is taken to be infinite. Expanding in series

$$(\mu_1 - \mu_1^0)_E = - RT [(1 / 2 - \chi_{12}) \nu_2^2 + \nu_2^2 / 3 + \ldots] \qquad (2.21)$$

These expressions comprise the nonideal terms in the previous equations for the chemical potential. They may therefore be regarded on the excess relative partial molar free energy or chemical potential. If the concentration of polymer in the volume element is very small, higher terms in such a series expansion may be neglected and the Equation is rewritten as,

$$(\mu_1 - \mu_1^0)_E = - RT (\kappa_1 - \Psi_1) \nu_2^2 \qquad (2.22)$$

where κ_1 and Ψ_1 are heat and entropy parameters such that the excess enthalpy and excess entropy is

$$\Delta H_1 = RT \kappa_1 \nu_2^2 \qquad (2.23)$$

$$\Delta S_1 = R \Psi_1 \nu_2^2 \qquad (2.24)$$

χ_{12} of the preceding treatment may be related to these parameters by comparing Equations (2.20) and (2.21). Then

$$\kappa_1 - \Psi_1 = \chi_{12} - 1 / 2 \qquad (2.25)$$

The ratio of ΔH_1 and ΔS_1, is defined by Flory as the " ideal" temperature θ,

$$\theta = \kappa_1 T / \Psi_1 \qquad (2.26)$$

such that

$$\Psi_1 - \kappa_1 = \Psi_1 (1 - \theta / T) = 1 / 2 - \chi_{12} \qquad (2.27)$$

Hence the excess chemical potential may be written,

$$(\mu_1 - \mu_1^0)_E = - RT \Psi_1 (1 - \theta / T) \nu_2^2 \qquad (2.28)$$

At temperature T equal to θ, the excess enthalpy and entropy contributions cancel each other. The θ point can be reached by changing either the temperature of the solution or the nature of the solvent. The size of the macromolecule in θ solvent corresponds to the unperturbed dimensions [28, 29].

2.2. Swelling of Network Structures

A three-dimensional network polymer such as vulcanized rubber, although incapable of dispersing completely, may nevertheless absorb a large quantity of a suitable liquid with which it is placed in contact. Swelling occurs under these conditions for the same reason that the solvent mixes spontaneously with an analogous linear polymer to form an ordinary polymer solutions; the swollen gel is in fact a solution, although an elastic rubber rather than a viscous one. Thus an opportunity for an increase in entropy is afforded by the added volume of the polymer throughout which the solvent may spread. This mixing tendency, expressed as the entropy of dilution, may be augmented ($\chi_{12} < 0$) or diminished ($\chi_{12} > 0$) by the heat (or first neighbour interaction free energy) of dilution. As the network is swollen by the absorption of solvent, the chains between network junctions are required to assume elongated configurations, and a force akin to the elastic retractive force in rubber consequently develops in position to the swelling process. As swelling proceeds, this force increases and the diluting force decreases. Ultimately a state of equilibrium swelling is reached in which these two forces are in balance.

A close analogy exists between swelling equilibrium and osmotic equilibrium. The elastic reaction of the network structure may be interpreted as pressure acting on the solution, or swollen gel. In the equilibrium state this pressure is sufficient to increase the chemical potential of the solvent in the solution so that it equals that of the excess solvent surrounding the swollen gel. Thus the network structure performs the multiple role of solute, osmotic membrane and pressure-generating device [28, 33-40].

2.2.1. Definition for Topological Structure of Networks

A network chain (a chain between two junction points) forms the basis of the elementary molecular theory of amorphous polymeric networks. [28, 41-43].

In general, network chains exhibit a distribution of molecular weights about an average, which serves as a representative reference quantity in describing network

structure. The number of chains meeting at each junction is called the functionality ϕ of that function. A network may have two or more sets of junctions with different functionalities; it may then be characterized by an average functionality. A chain connected to a junction of the network at only one end is called a dangling chain, and one having both its ends attached to the same junctions is called a loop. A network with no dangling chains or loops and in which all junctions have a functionality greater than two is called a perfect network can never be obtained in reality, it forms a simple reference structure around which molecular theories are constructed [42, 44].

The topological structure of a perfect network may be derived by various parameters: the average molecular weight between junctions, M_c; the average functionality ϕ ; the number of network chains ν; the number of junctions μ; and the cycle rank ξ, which denotes the number of chains that have to be cut in order to reduce the network to a three with no closed cycles. These five parameters are not independent, however, and are related by three equations [42].

For the network to be perfect the number of chain ends, 2ν, has to be equal to the number of functional groups, $\phi\mu$, on the end- linking molecules, Thus,

$$\mu = 2\nu/\phi \tag{2.29}$$

which states, for example , that to get ν chains in a perfect tetrafunctional network only $\mu = \nu / 2$ junctions are required. The other two Equations are,

$$\xi = (1-\frac{2}{\phi})\nu \tag{2.30}$$

$$\frac{\xi}{V_0} = \frac{(1-\frac{2}{\phi})\rho}{M_c / N_A} \tag{2.31}$$

where V_0 is the volume of the network in the state of formation, ρ is the density of the network in the state of formation and N_A is the Avagadro's number [28, 42, 43].

2.2.2. Theory of Swelling

The thermodynamics of the system may be described by the change in the Gibbs Free Energy ΔG of the system. The total change results from the change in the elastic free energy ΔG_{el} of the network upon isotropic (i.e.unstrained) dilation with the introduction of the solvent, and from the change in the free energy of mixing ΔG_{mix} of the solvent molecules with the chains constituting the network. [28, 33-38, 41].

It is assumed that the change in total free energy is the direct sum of ΔG_{el} and ΔG_{mix}. Thus,

$$\Delta G = \Delta G_{mix} + \Delta G_{el} \tag{2.32}$$

For the free energy of elastic deformation ΔG_{el} , several theories are available [45-50]. In the simplest affine network model, the junction points are assumed to be embedded in the network. As a result of this assumption, components of each chain vector transforms linearly with macroscopic deformation. Elastic free energy of the affine network,

$$\Delta G_{el} = 1 / 2 \, \nu_e \, k \, T \, (\alpha_x^2 + \alpha_y^2 + \alpha_z^2 - 3 - \ln \alpha_x \alpha_y \alpha_z) \tag{2.33}$$

where ν_e is the effective number of chains in the network; α_x ,α_y and, α_z are linear deformation factor with reference to x, y and z coordinates.

$$\alpha_x^2 = < x^2 > / < x^2 >_0 , \quad \alpha_y^2 = < y^2 > / < y^2 >_0 , \quad \alpha_z^2 = < z^2 > / < z^2 >_0 \tag{2.34}$$

If we let α_s represent the linear deformation factor, then by the condition of isotropy $\alpha_s = \alpha_x = \alpha_y = \alpha_z$, and Equation (2.33) expressed as,

$$\Delta G_{el} = 1 / 2\ \nu_e\ k\ T\ (\ 3\alpha_s^{\ 2} - 3 - \ln \alpha_s^{\ 3}) \qquad (2.35)$$

$$\alpha_s = (\ V_m / V_0)^{1/3} \equiv (\ \nu_2^{\ 0} / \nu_{2m})^{1/3} \qquad (2.36)$$

where ν_e is the effective number of chains in the network, V_m is the volume of solvent plus polymer, V_0 is the volume of network in the state of formation, ν_{2m} is the volume fraction of polymer network at equilibrium degree of swelling when exposed to excess solvent and $\nu_2^{\ 0}$ is the volume fraction of the polymer network after preparation.Substituting Equation (2.36) into Equation (2.35), respectively, leads to,

$$\Delta G_{el} = 3 / 2\ \nu_e\ k\ T\ [\ (\nu_2^{\ 0} / \nu_{2m}\ )^{2/3} - 1 - \ln (\nu_2^{\ 0} / \nu_{2m}\ )^{1/3}\ ] \qquad (2.37)$$

The second term of Equation (2.32), ΔG_{mix}, for mixing polymer chains with solvent is given by the Flory-Huggins relationship [28, 51].

$$\Delta G_{mix} = kT\ (\ n_1 \ln \nu_1 + n_2 \ln \nu_2 + \chi_{12}\ n_1\ \nu_2\ ) \qquad (2.38)$$

where n_1 and n_2 are the numbers of solvent and polymer molecules, respectively. For a crosslinked network, $n_2 = 1$. The quantity χ_{12} is the interaction parameter for the solvent - polymer system [41].

Introduction of solvent molecules into the system results in an increase in ΔG_{el} due the decrease of the entropy of the network chains upon dilation, and a decrease in ΔG_{mix} primarily due to the increase in entropy of mixing the solvent molecules with the network chains. A state of equilibrium swelling is obtained when the two chains balance each other. Mathematically this state is expressed as,

$$(\frac{\partial \Delta G}{\partial n_1})_{T,P} = (\frac{\partial \Delta G_{mix}}{\partial n_1})_{T,P} + (\frac{\partial \Delta G_{el}}{\partial n_1})_{T,P} = 0 \qquad (2.39)$$

where the subscripts, T, P indicate that the differentiation are made at constant temperature and pressure. Performing the differentiations indicated in Equation (2.39) one obtain,

$$\ln (1- \nu_{2m}) + \nu_{2m} + \chi_{12} \nu_{2m}^2 + \nu_1 (\nu_e / V_0) (\nu_{2m}^{1/3} - \nu_{2m} / 2) = 0 \qquad (2.40)$$

or, adopting the terminology

$$\rho \nu_1 / M_c = N^{-1} = \nu_e \nu_1 / V_0 \qquad (2.41)$$

$$\ln (1- \nu_{2m}) + \nu_{2m} + \chi_{12} \nu_{2m}^2 + (\nu_1 / \nu M_c) (1 - 2 M_c / M) (\nu_{2m}^{1/3} - \nu_{2m} / 2) = 0 \qquad (2.42)$$

where M_c is the molecular weight between junctions, ρ is the density of the polymer network and N is the number of segments in a network chain.

The left hand side of Equation 2.42 represents the lowering of the chemical potential owing to mixing of polymer and solvent; that on the right side gives the increase from the elastic region of the network. The latter corresponds to the increase πv_1 in the chemical potential resulting from an osmotic pressure π at equilibrium. It is customary to employ the swelling ratio, q, equal to the ratio V / V_0 of the volumes of the swollen and unswollen structures. This $q = 1 / \nu_2$. At swelling equilibrium, $1 / \nu_{2m}$ may be replaced by q_m, the subscript m indicating equilibrium swelling.

At large M_c values of 10000 or more, q_m in a good solvent will exceed 10. Then $\nu_{2m} / 2$ is considerably smaller than $\nu_{2m}^{1/3}$ and can be neglected. Also the higher terms in the series of expansion of the first term member of Equation (2.40) may be neglected. the swelling equilibrium Equation may then be solved for $\nu_{2m} = 1 / q_m$ with the following result.

$$q_m^{5/3} \cong (V_0 / \nu_e) (1 / 2 - \chi_{12}) / \nu_1 \qquad (2.43)$$

This simplified relationships offer a clear insight into the dependence of the equilibrium swelling ratio q_m on the quality of the solvent as expressed by χ_{12}, and on the extent of crosslinking. Because of the nature of the approximations introduced to obtain Equation (2.43), their use as quantitative expressions must be limited to networks of very low degrees of crosslinking in good solvents [28, 33-40].

2.2.3. Swelling of Ionic Networks

If the polymer chains making up the network contain ionizable groups, the swelling forces may be greatly increased as a result of the localization of charges on the polymer chains. The character of the behaviour of the ionic network depends essentially on the fact whether network chains carry charged units of the same or opposite sign. It is possible to consider two limiting cases.

When network chains contain only charged units of the same sign and oppositely charged counterions move freely within the sample, it is called a polyelectrolyte network. In the opposite case, when network chains carry both negative and positive charges in equal quantities and counter ions are absent, the network is called an isoelectric network [51].

Crosslinked polyacrylic acid and polymethacrylic acid afford appropriate examples for polyelectrolyte network. When neutralized with sodium hydroxide, the negatively charged carboxyl groups attached to the polymer chains set up an electrostatic repulsion which tends to expand the network.

The exchange of ions and solvent between a swollen ionic network and the surrounding electroyte is represented in Figure 2.1, where the fixed ion is taken to be a cation. It is apparent that the equilibrium between the swollen ionic gel and its surroundings closely resembles Donnan membrane equilibria. The polymer acts as its own membrane preventing the charged substituents from diffusing into outer solution. The swelling force resulting from the presence of these fixed charges may be identified with the swelling pressure or osmotic pressure, across the semipermeable membrane in

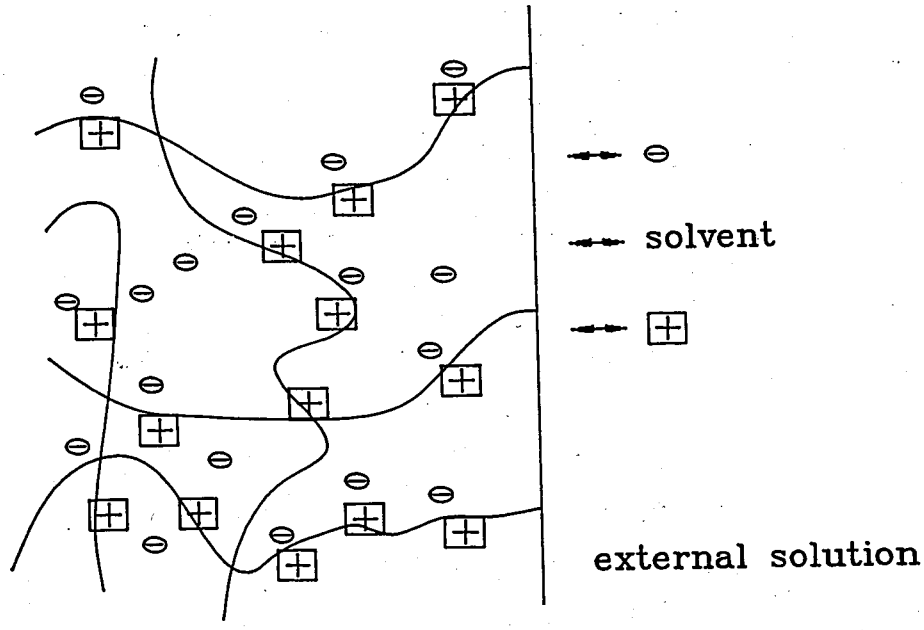


Figure 2.1. Diagram of swollen ionic gels in equilibrium with electrolyte solution. Fixed charges are represented by + [28]

a typical Donnan equilibrium. The free energy of such a network can be written as the sum of at least three terms:

$$\Delta G = \Delta G_{el} + \Delta G_{mix} + \Delta G_i \qquad (2.44)$$

where ΔG_i is the free energy of electrostatic interactions. In case of a weakly charged polyelectrolyte network, in order to describe the effect of charges it is sufficient to take into account only the translational entropy of counterions. If i represents the number of ions in $(xn_2 + i)$ units, then the mean number of ionic units between successive junctions in a network chain, f can be written as:

$$f = N\, i / xn_2 + i \qquad (2.45)$$

where N is the number of segments in a network chain. Then the free energy of electrostatic interactions may be written as follows [28, 33-39, 52] :

$$\Delta G_i = RT\,(f / N)\,(\nu_2 / \nu_1)\, n_1 \ln (f\, \nu_2 / N) \qquad (2.46)$$

In the dilute polymer solution approximation, which may not necessarily be appropriate if the concentration of electrolyte in the external solution is relatively large. Then, the swelling equilibrium equation in Equation 2.43 changes to,

$$q_m^{5/3} \cong (V_0 / \nu_e) \left[(i / 2\nu_u S^{*1/2})^2 + (1/2 - \chi_{12}) / v_1 \right] \qquad (2.47)$$

where ν_e is the effective number of chains in the network, i/ν_u is the concentration of fixed charge referred to unswollen network, S^* is the ionic concentration in the external solution, $(1/2 - \chi)/v_1$ is the affinity of hydrogel with solvent, ν_e/V_o is the crosslinking density of hydrogel.

2.3. Phase Equilibria in Polymer-Solvent Systems

The mutual solubility of a polymer-solvent system can be best understood by referring to the free energy of the system as a function of composition. If two separate phases were present in the system, they will always correspond to a higher free energy and hence, are unstable with respect to the homogeneous phase. For total miscibility, it is necessary that both

$$\Delta G_{mix} < 0 \qquad (2.48)$$

and that the second derivative of ΔG_{mix} with respect to the volume fraction of solvent or polymer be greater than zero.

$$\left(\frac{\partial^2 \Delta G_{mix}}{\partial \nu_2^{\,2}} \right)_{P,T} > 0 \qquad (2.49)$$

over the entire compositon range.

The phase equilibrium is strongly affected by solution temperature. If the solvent chosen for a given polymer becomes progressively poorer as the temperature is lowered, eventually a temperature may be reached below which the solvent and the

polymer no longer are miscible in all proportions. At each lower temperature, mixtures of the polymer and solvent over a certain composition range will separate into two phases. If the polymer consists of a sufficiently narrow range of molecular species then the mixtures of solvent and polymer may be treated as binary solution system. The temperature-composition phase diagram consists of a boundary curve distinguishing the homogeneous region and it is called the binodal. The binodal is the loci of points that satisfy the conditions for thermodynamic equilibrium of a binary mixture. The maximum in the binodal curve is a critical point for the binary system; the compositions of the coexisting phases merge at this point, and at temperatures above the critical temperature, T_c, the system is homogeneous for all compositons [30, 31].

2.3.1. Binary Polymer- Solvent Systems

The conditions for equilibrium between two phases in a binary system are expressed by stipulating equality of the chemical potentials in the two phases; that is

$$\mu_1 = \mu_1' \tag{2.50}$$

$$\mu_2 = \mu_2' \tag{2.51}$$

where the prime is adopted as the designation more concentrated phase. Equations (2.16) and (2.17) express the chemical potentials μ_1 and μ_2 as a function of the volume fraction ν_2 of polymer. The single parameter χ_{12} occurs in these functions. There must be two concentrations at which the chemical potential μ_1 pass through a minimum and then a maximum as a ν_2 is increased from zero to unity. Since μ_1 and μ_2 are derived by differentiating the same free energy function, Eq. (2.14), it is easy to show that the one must pass through a maximum where the other is at its minimum.

Confining attention to μ_1, the inflection point characterized by the condition of zero curvature $(\partial^2\mu_1 / \partial \nu_2^2)_{T,P} = 0$ must necessarily occur between the minimum and the maximum in the curve. At both the maximum and the minimum $(\partial \mu_1 / \partial \nu_2)_{T,P} = 0$. If the curve representing μ_1 as a function of composition decreases monotonically with

ν_2 total miscibility is assured. In passing to progressively poorer solvents, eventually a minimum and a maximum will appear in the curves, their appearance signifies incomplete miscibility.

The critical solution temperature T_c, is an important quantity. At this temperature incipient phase separation will be encountered. Hence the conditions for incipient phase separation are,

$$(\partial \mu_1 / \partial \nu_2)_{T,P} = 0 \tag{2.52}$$

$$(\partial^2 \mu_1 / \partial \nu_2^2)_{T,P} = 0 \tag{2.53}$$

Application of these critical conditions to Equation (2.16) leads to the first derivative of that equation:

$$(1 - \nu_{2c})^{-1} - (1 - 1 / x) - 2\chi_{1c} \nu_{2c} = 0 \tag{2.54}$$

while the second derivative is,

$$(1 - \nu_{2c})^{-2} - 2\chi_{1c} = 0 \tag{2.55}$$

where the subscript c denotes critical conditions. The critical compositions at which phase separation is first detected is then,

$$\nu_{2c} = 1 / (1 + x^{1/2}) \tag{2.56}$$

$$\chi_{1c} = 1 / 2 (1 + x^{-1/2})^2 \tag{2.57}$$

It is seen that for very large values of x, the above expressions reduce to,

$$\nu_{2c} = x^{-1/2} \tag{2.58}$$

$$\chi_{1c} = 1 / 2 \tag{2.59}$$

The critical value of χ_1 approaches a limiting value of 1 / 2 for infinite molecular weight. This corresponds to the θ point in Eq. (2.27), i.e.,$T_c = \theta$ for $x \rightarrow \infty$, Equation (2.27) can be rewritten as

$$1 / 2 - \chi_{1c} = \Psi_1 (1 - \theta / T_c) \tag{2.60}$$

Combination with Equation (2.57) gives,

$$\frac{1}{T_c} = \frac{1}{\theta}\left[1 + \frac{1}{\psi_1}\left(\frac{1}{x} + \frac{1}{2x}\right)\right] \tag{2.61}$$

which predicts a linear relation between $1 / T_c$ and $(1 / x^{1/2} + 1 / 2x)$. It can be seen from Equation (2.61) that the theta temperature is also the critical missibility temperature in the limit of infinite molecular weight [28, 30, 31].

2.3.2. Critical Phenomena and Gel Collapse

The osmotic compressibility or the chemical potential of a network depends on temperature and solvent activity. If the network chains carry ionic groups, then the pH, ionic strength of the solvent or an electric field acting across the gel, also affects the osmotic compressibility. It has been shown experimentally that very large changes in the state of a gel may be induced by small changes in the external conditions.

When certain critical conditions are reached, the osmotic compressibility becomes infinitely large indicating that the network shrinks strikingly and can continue almost indefinitely under a slight indefinitely in external pressure. At this state, pure solvent phases appear in the network and their sizes reach macroscopic dimensions. This indicates the onset of large fluctuations in the system. The first and second derivatives of the solvent chemical potential with respect to ν_2 equate to zero at the critical point for a polymer- solvent system. Thus,

$$\Delta\mu_1 = RT \ln a_1, \quad \frac{\partial \Delta\mu_1}{\partial \nu_2}=0, \quad \frac{\partial^2 \Delta\mu_1}{\partial \nu_2^2}=0 \tag{2.62}$$

A network-solvent system may also exhibit phase separation where three phases of different concentrations may exist in equilibrium inside the swollen network. One of the phases is pure solvent ($\nu_2 = 0$). The remaining two phases are a less swollen and a highly swollen phase. Such a state is referred to as triphasic equilibrium, described by Equations;

$$\Delta\mu_1 (\nu_2') = \Delta\mu_1 (\nu_2'') = RT \ln a_1 \tag{2.63}$$

$$\int \Delta\mu_1 dn_1 = 0 \tag{2.64}$$

where ν_2' and ν_2'' represent the two coexisting polymer concentration. This state may be reached by varying the temperature, which in turn varies χ_1 (T) and χ_2 (T). Alternatively, triphasic conditions may be obtained by varying the activity or the pH of the solvent in which the gel immersed. The results of typical calculations are shown in Figure 2.2 for triphasic equilibrium obtained by varying the temperature.

The horizontal line identifies the temperature at which the transition takes place. At higher temperatures $\nu_2 < \nu_2'$ and the network is highly swollen. At lower temperatures, $\nu_2 > \nu_2''$ and the network is collapsed. The width of the horizontal line at the transition depends on various parameters such as the presence of ionic groups on the network chains, the degree of crosslinking and the amount of solvent during cross-linking [42].

2.3.3. Fundamental Interactions for Volume Phase Transition of Gels

Four fundamental molecular interactions play an essential role in determining the structures and specific functions of macromolecules and their assemblies. These are van der Waals, hydrophobic, hydrogen bonding and electrostatic interactions (Figure 2.3) [53]. A gel can be one macroscopic molecule. Because of this reason, the

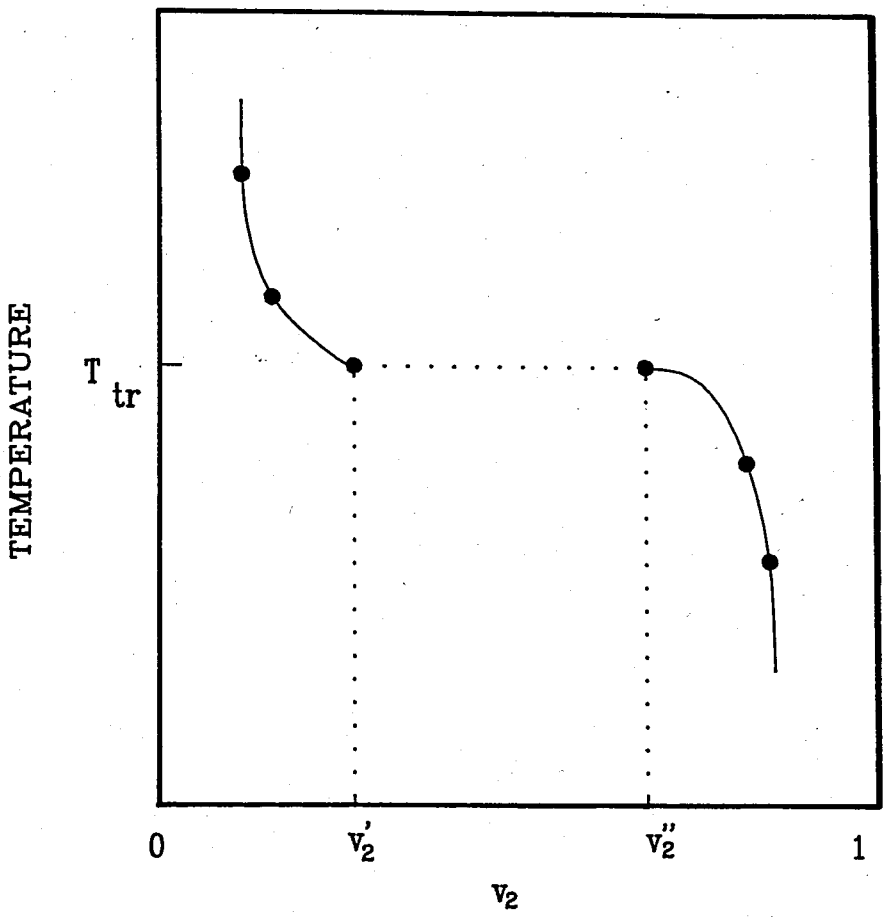


Figure 2.2. Temperature-composition diagram for a swollen network showing a transition [42]

size of a gel is very sensitive to the change in the molecular interactions. Therefore, by measuring the size of a gel as a function of external intensive variables, such as temperature, pH, solvent composition, one can investigate the local environment of polymer chains. Since gels are simply obtained by crosslinking these polymer chains, gels can be a good system for studying molecular interactions [54].

2.3.3.1. Van der Waals Interaction. A partially hydrolyzed polyacrylamide gel undergoes a phase transition in acetone/water mixtures [36]. The main polymer-polymer affinity is due to the van der Waals interaction. Acetone, a nonpolar poor-solvent had to be added to water in order to increase the attractive interaction between polymers in the networks to induce the transition. The transition is also observed when

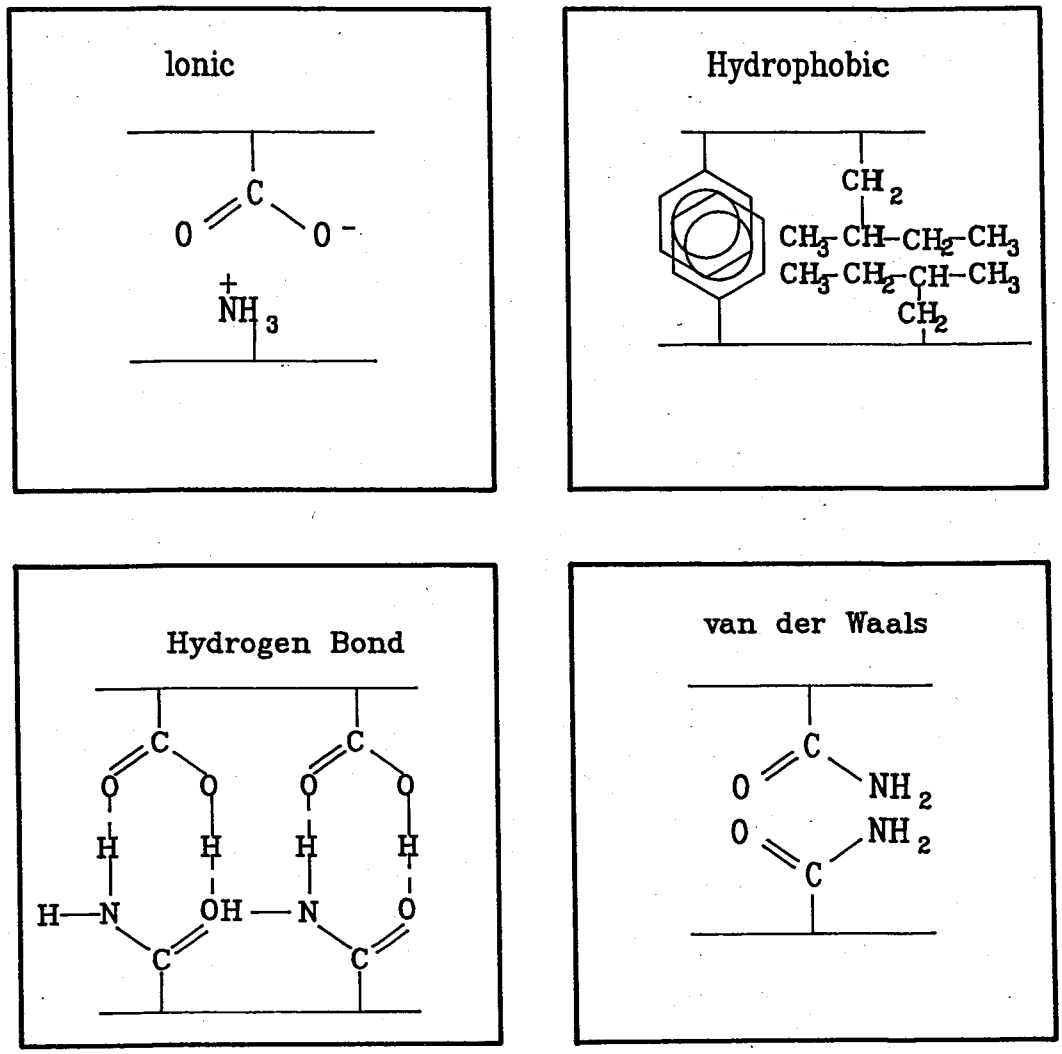


Figure 2.3. Schematic representation showing four fundamental molecular interactions

the temperature was varied while the solvent composition fixed near the transition threshold at room temperature. The gel swells at higher temperatures and shrinks at lower temperatures.

2.3.3.2. Hydrophobic Interactions. In an attempt to find a gel that undergoes a volume phase transition in pure water Hirokawa studied with N-isopropylacrylamide gels which had side groups with more hydrophobicity than that of acrylamide [37]. They observed the collapsing transition at approximately 33.2°C by increasing temperature. It is interesting to observe that gels swell at lower temperatures and collapse at higher temperatures. This temperature dependence, which is opposite to the transition induced

by van der Waals interaction, is due to the hydrophobic interaction of the polymer network and water. At higher temperatures the polymer network shrinks and becomes more ordered, but the water molecules excluded from the polymer network become less ordered [38].

2.3.3.3. Hydrogen Bonding. A volume phase transition via the hydrogen bonding interaction was demonstrated with an interpenetrating polymer network (IPN) consisting of two independent networks intermingled with each other [55]. For example, one network is poly(acrylic acid) and the other polyacrylamide. The gel was originally designed and developed by Okano and his colleagues who found that the gel is shrunken at low temperatures in water, and the volume increased as temperature rose. There was a sharp but continuous volume change at about 30 °C.

These researchers identified the main interaction to be hydrogen bonding and also pointed out the importance of the so-called "zipper" effect, which described the cooperative nature of the interaction between two polymers [56]. By slightly ionizing the gel, Ilmain, Tanaka, and Kokufata succeeded in inducing the discontinuous volume transition of the IPN in pure water [55].

2.3.3.4. Electrostatic Interaction. The electrostatic interaction is a long-range order interaction. The electrostatic interaction is inversely proportional to the dielectric constant of the medium.

In synthetic polymers, either positive or negative charges is introduced on the polymer chains by copolymerization or partial ionization, which give rise to a strong repulsive interaction. Since a free movement of the charges is not allowed because the charges are fixed on the polymer chain, the counter ions have to be localized near the polymer chains so as to keep the electroneutrality. As a result donnan potential is created between the inside and outside of the gel, resulting in an increase in the osmotic pressure [54].

2.3.4. Phase Diagram of a Gel

Phase diagram of a gel (Figure 2.4) is constructed by calculating the osmotic pressure for many combinations of volume and either temperature or solvent concentration.

In the upper diagram this information is presented as a series of isotherms, which are curves that specify all possible combinations of pressure and volume at a given temperature (or solvent composition). The lower diagram was derived from the upper, but it gives the volume of the gel as a function of temperature or solvent concentration.

The complete phase diagram for the gel has three regions. Below the swelling curve the gel has a negative osmotic pressure and is always stable; it will expel fluid and shrink until it reaches the zero-pressure swelling curve. Another region represents states with a positive osmotic pressure, which are stable only if there is no excess fluid the gel could absorb in order to expand. Between these realms is the dominant of dual stability, where some parts of the gel shrinks and another part swells.

The boundary of this region is the coexistence curve for the two phases. The difference in volume between the two phases is greatest along the swelling curve, and it decreases steadily at higher temperature ultimately the coexistence curve reaches a maximum temperature where the two phases are identical or in other words where they cease to exist as distinguishable phases. That is critical point of the gel [57].

2.4. Synthetic Hydrogels For Drug Delivery

Recent development in polymeric delivery systems for the controlled release of therapeutic agents has demonstrated that these systems can not only improve drug stability both in vitro and in vivo by protecting labile drugs from harmful conditions in the body, but also increase residence time at the application site and enhance the activity of duration of short half-life drugs. Therefore, compounds which otherwise would have

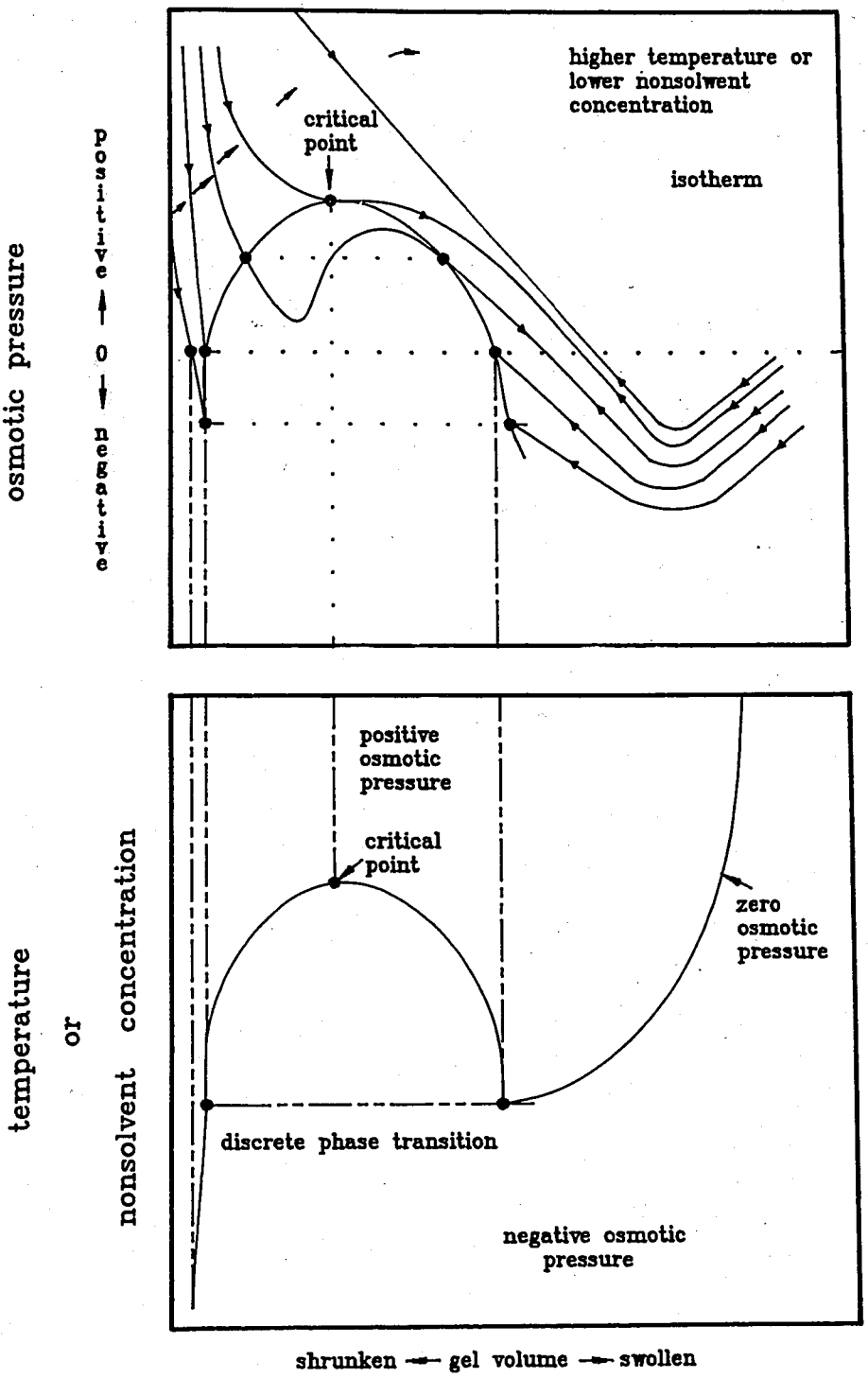


Figure 2.4. Phase diagram of a gel [57]

to be discarded due to stability and bioavailability problems may be rendered useful through a proper choice of polymeric delivery system. Furthermore, polymeric systems can provide predictable and reproducible drug release for extended duration in meeting a specific therapeutic requirement, thereby eliminating side effects, frequent dosing, and waste of drugs.

One such polymer system for drug delivery applications, which has attracted significant recent attention, is based on hydrogels. Hydrogels are hydrophilic network polymers which are glassy in the dehydrated systems and swollen in presence of water to form an elastic gel. Although hydrogels are either natural or synthetic origin, it is the covalently crosslinked synthetic hydrogels that have been gaining increasing popularity in various biomedical applications [2-10].

2.4.1. Structure of Synthetic Hydrogel

In general, hydrogels for drug delivery applications are prepared by the polymerisation or copolymerisation of certain hydrophilic monomers with a cross-linking agent using free radical initiators. Useful hydrogels are also obtained by copolymerizing and cross-linking of both hydrophilic and hydrophobic monomers to balance the desired level of equilibrium water absorption in the hydrogel. Examples of hydrophilic monomers useful for hydrogel preparation are hydroxyalkyl acrylates or methacrylates, acrylamide derivatives (e.g.,N-isopropylacrylamide), N-vinyl-2-pyrrolidone. Hydrophobic monomers potentially useful for hydrogel preparation are acrylics, vinyl acetate, acrylonitrile and styrene.

2.4.2. Preparation of Hydrogels

2.4.2.1. Bulk Solution Polymerization. Hydrogels are commonly prepared by the bulk/solution polymerisation process where a mixture of monomer, initiator, and an optional solvent, usually water, is polymerized in a mold to form sheets and cylinders [58]. The sample thickness for the bulk/solution polymerization process is generally kept small (millimeter range) because of need to dissipate the heat of polymerisation.

Localized inhomogeneity and opaqueness are generally observed in thick, bulk-polymerized samples due to lack of efficient polymerisation of heat generated from the reaction exotherm. Since oxygen inhibits the free-radical polymerisation process, degassing of the monomer mixture and nitrogen purging are often needed for the polymerisation process. Several studies have been reported in the literature by first incorporating drugs of interest into the monomer mixture followed by polymerisation, thereby entrapping the drug within the hydrogel matrix. However, this is not a good way of making drug delivery systems because of the possible side reactions between drug and the monomer and the inability to remove the potentially toxic material the residual monomer and initiator while keeping the entrapped drug intact. The preferable way is to polymerize the hydrogel in the absence of drug. Subsequently, the hydrogel can be extracted in a good swelling solvent to remove residual monomer, initiator and other impurities. The drug loading can then be achieved by equilibrating the cleaned hydrogel in a concentrated drug solution prepared with a good swelling solvent.

For drug delivery applications, the hydrogel sheets and cylinders prepared by the bulk polymerisation process are useful only in topical and implant application. To be useful for oral dosage forms, hydrogels would have to be in granules prepared by dicing or granulating hydrogel sheets or cylinders followed by sieving into proper particle sizes. Such hydrogel granules are generally irregular in shape which may be objectionable not only from the standpoint of product aesthetic, but also from the standpoint of reproducibility in controlling the drug release.

2.4.2.2. Suspension Polymerization. A more elegant and preferred way of preparing hydrogel particles is the suspension polymerisation process which is capable of producing spherical hydrogel beads with particle size ranging from approximately 100 μm up to 5 mm in diameter [3, 59]. Suspension polymerisation is similar to bulk polymerisation with respect to reaction kinetics; however, the monomer phase is dispersed by mechanical agitation into droplets in a nonsolvent medium with the aid of a protective colloid as a suspension stabilizer. The monomer droplets, which are generally larger than those of a true emulsion, are then polymerized through a heat-induced decomposition of the free radical initiator. The dissipation of heat involved

during polymerisation is more efficient in suspension polymerisation because the suspending medium, mostly water, act as a sink. This process yields spherical beads with uniform composition in a one-step process. The hydrogel beads obtained from this process can easily be separated from the suspending aqueous phase and extracted with a good solvent, before being used for drug loading.

2.4.3. Equilibrium Swelling Properties

Hydrogels can be considered as having two components, namely, a solid component of polymer network and a variable aqueous component which can undergo exchange with the environment. The network is held together mostly by covalent cross-links and, to a lesser extent, by physical cross-links such as chain entanglement and crystallites.

The driving force of swelling is the water chemical potential difference between the network and external aqueous phase. In other words, the osmotic pressure due to polymer segment in the gel network causes the swelling. Thermodynamically, the swelling properties of a hydrogel network are controlled by the combination of free energies of mixing between water (or any other solvent) and polymer chains and by the elastic response of the network to volume increase due to water absorption. At swelling equilibrium, the elastic response of the network exactly balances the difference in chemical potential of water between the swollen network and external aqueous phase.

The equilibrium water content (EWC) is generally affected by the nature of the hydrophilic monomers used in the hydrogel preparation, the nature and density of cross-links, and factors such as temperature, osmolarity, and pH of the hydrating medium.

2.4.4. Drug Loading Properties

In order to develop a useful hydrogel drug delivery system, one has to achieve a sufficiently high loading in the polymer while at the same time providing a release rate consistent with biological requirements in terms of the magnitude and duration of drug

release. From the safety standpoint, it is necessary to extract all leachable by-products and residual monomers left after polymerization in order to make hydrogels for drug delivery applications. Therefore, for all practical purposes, one is limited to polymerize hydrogels in the absence of the drug and to carry out drug loading with the extracted hydrogels. In this case, a high degree of swelling in water is desired to control the rate of diffusional drug release.

When there are no substantial variations of drug solubilities over the solvent composition range, one can choose the solvent composition at maximum swelling as the loading solvent. A concentrated drug solution prepared in such solvent composition should allow a maximum amount of drug to be imbided into the hydrogel at swelling equilibrium. Subsequent separation, rinsing, and drying will result in glassy, dehydrated hydrogel matrices containing uniformly dissolved or dispersed drug.

2.4.5. Characterization of Diffusion Properties in Swollen Hydrogels

Generally, the diffusion properties of solutes in polymers can be evaluated by either the membrane permeation method or the sorption/desorption method. In the former case, the permeation time-lag experiment has been widely used for the measurement of diffusion coefficient. However, this requires the elimination of boundary layer resistances and materials strong enough to be made into membranes. Hydrogels, being more fragile, especially when the equilibrium water content is high, may not be able to meet the latter requirement. Furthermore, for drugs with large molecular sizes and small diffusion coefficients, it often takes an impractically long time to establish the permeation time lag and steady state in a permeation experiment. Therefore, it is more convenient to characterize diffusion properties in swollen hydrogels using sorption/desorption method.

The simplest way to obtain drug diffusion coefficients is to measure the initial rate of desorption of drug from a swollen hydrogel membrane or sheet of thickness l into an infinite volume. By plotting the fractional amount of drug desorbed, M_t/M_∞, vs. t, the

drug diffusion coefficient, D, can be evaluated from the slope of the initial linear plot using the following Equation [60]:

$$M_t/M_\infty = (4 / l) [Dt/\pi]^{1/2} \quad (2.65)$$

which is accurate to within 1% for up to approximately 60% of the total amount released.

When the hydrogel membrane or sheet contains dispersed drug, the desorption kinetics can be analysed by the familiar Higuchi Equation [61]:

$$M_t = [C_s(2A-C_s) Dt]^{1/2} \quad (2.66)$$

which has been applied to drug delivery systems whenever the initial drug loading per unit volume, A, is greater than the drug solubility in the matrix, C_s.

A set of simple, accurate approximate solutions for different geometries recently given by Lee is much more convenient to used than the existing solutions [62]. It enables one to evaluate the diffusion coefficient from simple algebraic Equations based on sorption data. For an absorption experiment, the Equation for flat sheet configuration is,

$$F(C)= [(C_0^2 - C^2)/2C^2 + \ln (C/C_0) (3 \lambda^2/16)] = Dt/l^2 \quad (2.67)$$

where C_0 is the original drug concentration in the finite volume of external solution, C the corresponding concentration at time t during the absorption, and l the thickness of the hydrogel sheet. The effective volume ratio is defined as:

$$\lambda= C_\infty / (C_0 - C_\infty) = V/SK\, l \quad (2.68)$$

with V being the total liquid volume, S is the area of each side of the sheet, K the partition coefficient, and C is the equilibrium drug concentration in the external solution

after the completion of the absorption experiment. The concentration change is further related to the fraction absorbed by:

$$C/C_0 = 1 - [1/(1+\lambda)] (M_t/M_\infty) \quad (2.69)$$

Therefore, in a finite volume absorption experiment, the monotonic decrease of drug concentration in the solution external to a hydrogel material fabricated in sheet form is followed as a function of time. The left-hand side of 2.69 is then calculated and plotted against time. From the slope of such a linear plot, the diffusion coefficient can be evaluated.

2.4.6. Kinetics of Swelling and Drug Release from Dry Hydrogels

In many applications, especially oral delivery, drug-loaded hydrogels are usually stored in a dry, glassy state before usage to stability and dosing requirement. The release of water-soluble drugs from initially dry hydrogel matrices generally involves the simultaneous absorption of water and desorption of drug via a swelling-controlled mechanism [63]. Thus, as water penetrates a glassy hydrogel matrix containing dissolved or dispersed drug, the polymer swells and its glass transition temperature is lowered.

In most cases, a sharp penetrating solvent front separating the glassy from the rubbery phase, in addition to a volume swelling, is also observed. In terms of drug distribution, this solvent front also separates the undissolved core from the partially extracted region, with the dissolved drug diffusing through this swollen rubbery region into external releasing medium. Depending on relative magnitude of the rate of polymer relaxation at the penetrating solvent front and the rate of diffusion of the dissolved drug, the release behavior during the initial stage of the solvent penetration may range from Fickian to non-Fickian (anomalous), including the so-called Case II diffusion.

Typically, for a polymer slab, Fickian diffusion is characterized by a square-root-of-time dependence in both the amount diffused and the penetrating diffusion front position. On the other hand, Case II transport, which is completely governed by the rate of polymer relaxation, exhibits a linear-time dependence in both the amount diffused and the penetrating front position. In most cases, the intermediate situation, which is often termed non-Fickian diffusion and polymer relaxation are comparable. Phenomenologically, it is possible to express the fraction released, M_t/M_∞, as a power function of time t, for at least the short time period,

$$M_t/M_\infty = k\, t^n \qquad (2.70)$$

where k is a constant characteristic of the system and n is an exponent characteristic of the mode of transport. For n=0.5, the solvent diffusion or drug release follows the well-known Fickian diffusion mechanism. For n > 0.5, non-Fickian or anomalous diffusion behavior is generally observed. The special case of n=1 gives rise to a Case II transport mechanism, which is of particular interest because the drug release from such devices having constant geometry will be zero order.

2.5. Radiation Chemistry of Polymers in Solution

2.5.1. Radiation Sources

There are primarily two types of large-scale sources: those based on the radioactive decay of radioactive Cobalt 60 with a half-life of 5.3 years and electron accelerators.

The radioactivity of a typical Cobalt 60 research installation would lie between 1 and 50 kcurie, corresponding to a gamma power output of 1.48 and 74 watts. These gamma sources often consist of a series of cobalt rods which can move together from a safe position into the chamber in which specimens to be irradiated. Most useful feature is range of dose rates is available, from several Mrads (tens of kGy) per hour downwards.

2.5.2. Radiolysis of Water and Aqueous Solutions

Radiation-chemical reactions taking place in water and in aqueous solutions have been intensively studied because of their importance in chemical processes, biological systems, and the development of nuclear technology. As a result of all the work done, many of the aspects of the radiation chemistry of water and aqueous solutions are now reasonably well understood [64-68].

The principal experimental facts have already begun to acquired by the early days of the twentieth century. Liquid water itself, when highly purified and irradiated under conditions where gas cannot escape from solution, resembles water vapour in that it does not decompose significantly under irradiation with low Linear Energy Transfer (LET) radiation such as X-rays. On the other hand water decomposes into hydrogen, hydrogen peroxide, and oxygen on irradiation with high LET radiation like α-particles. Among the other facts, many of which were confirmed by the radiation chemists of the United States atomic bomb project [65], is that decomposition under low LET irradiation is very much enhanced by impurities. Oxygen is one such impurity: it causes water to give hydrogen peroxide and some hydrogen on irradiation with low LET radiation. Hydrogen peroxide itself enhances radiolysis by low LET radiation, but hydrogen tends to suppress radiolysis. Correspondingly if water is irradiated under conditions where hydrogen can escape, for example, in contact with a large evacuated space, or while boiling, then it undergoes decomposition. The radiation chemistry of pure water may be represented by the general Equation [68] :

$$H_2O \Rightarrow e^-_{aq},\ H,\ H_2, OH,\ H_2O_2,\ H_3O^+$$

Once the primary species (right side of above Equation) have been introduced into a solution by means of radiation, they will begin to react chemically. Molecular hydrogen, hydrogen peroxide, and hydrogen ions are relatively inert, but hydrated electrons, hydroxyl radicals and hydrogen atoms are highly reactive. The Equations imply the transient formation of OH as well as other species, and this species has been detected experimentally [68]:

$$H_2O^+ + H_2O \longrightarrow H_3O^+ + OH$$

$$e^-_{aq} + e^-_{aq} \xrightarrow{+2H_2O} H_2 + 2OH^-$$

$$e^-_{aq} + H_3O^+ \longrightarrow H + H_2O$$

$$e^-_{aq} + H \xrightarrow{+H_2O} H_2 + OH^-$$

$$2\,H \longrightarrow H_2$$

$$2\,OH \longrightarrow H_2O_2$$

$$e^-_{aq} + OH \longrightarrow OH^-$$

$$H + OH \longrightarrow H_2O$$

When dose-rate is low and solute concentration not too small, the concentration of hydrated electrons, hydroxyl radicals and hydrogen atoms will be kept to a low level, and there will be little reaction of these primary species with each other. At high dose-rates the concentration of primary species can be high, so that interactions between primary species become more important.

2.5.3. Radiolysis of Polymers in Solution

The irradiation of biopolymers in an aqueous environment has received considerable attention because of its interest in radiobiology [69-72]. The parallel but simpler model of a polymer in water show many features which may help to interpret the radiobiology work, but which are of great interest in their own right.

Many water-soluble polymers can be crosslinked by radiation, even though the individual molecules may be some distance apart. The irradiated polymeric system can become crosslinked above a gelation dose r_g, forming a network swollen with water. At first the amount of swollen polymer increases with dose above r_g, and then decreases approximately as $r^{0.6}$ as required by the conventional swelling theory. The earlier rise is of course due to the swelling of only a fraction of the polymer, namely that fraction (gel) which is forming the network.

The second feature is that the lower the polymer concentration, the lower is the dose needed to form a crosslinked network. In other words the further apart the individual molecules, the easier it is to link them together. The explanation is that one is also irradiating the water molecules the fragments into which this broken can attack the polymer to give radicals capable of linking them together. Obviously the fewer polymer molecules, the more each one can be affected by these fragments-termed the indirect effect. The net effect is therefore due to crosslinking by direct and indirect effects.

A third feature is that below a certain concentration (typically about 1%), it is no longer possible to form a network, even at very high doses. This is not because there is any change in the radiation-induced chemical changes. The explanation is that crosslinks can be formed by radiation, either between the separate, but neighbouring, polymer molecules (external links) or between two parts of the same molecule which happen to swim into each others proximity. Such internal links, have the same chemical nature as the external links, but simply serve to bind together parts of the same molecule to for a micronetwork. As such internal links increase with dose, this micronetwork shrinks, and therefore moves further away from other molecules.

The aspect of polymers in aqueous solution has not received adequate study. The formation of a water-swollen network has been utilised for medical purposes, the pores can be readily and reproducibly controlled by radiation dose, and may act as a selective membrane.

3. EXPERIMENTAL WORK

In the first sections of this chapter, the preparation of poly(N-isopropylacrylamide) gels by different techniques, namely, radiation synthesis, solution and inverse suspension polymerisation are described. Next, a summary on the synthesis of poly(N-isopropylacrylamide-co-itaconic acid) and poly(N-isopropylacrylamide-co-maleic acid) gels is given. Finally, the characterization methods of the gels and controlled release of drugs from the gels are presented.

3.1. Materials

N-isopropylacrylamide (NIPAAm) was purchased from Aldrich Chemical Company. N,N,N',N'-tetramethylethylenediamine (TEMED), N-N'-methylenebisacrylamide, ammonium peroxodisulfate, methylene blue and paraffin oil were purchased from Merck AG. Itaconic acid (IA), maleic acid (MA) were purchased from Fluka Chemical Company. Lidocaine and sildenafil citrate were obtained from Marmara University.

3.2. Experimental Set-up and Equipment

The experimental set-up and equipment used for this work consisted of an analytical balance, an electronic digital calliper, a vacuum oven, a shaking water bath, a mechanical strirrer and a reactor system and other auxilary equipments such as tubes, pipettes, volumetric flasks, vials sieves (porosity between 71μm-500μm). In this work, UV-Spectrophotometer, Fourier Transform Infrared (FT-IR), optical microscopy and scanning electron microscopy (SEM) were used. The specifications of the equipment mentioned above are as follows:

Spectrophotometer: Spectrophotometric analysis was carried out using a Shimadzu Model UV-160A spectrophometer.

Fourier Transform Infrared (FT-IR) Spectroscopy: The IR spectra of the polymer samples were recorded using a Jasko 5300 Model FT-IR spectrophotometer.

Optical Microscopy: Optical micrograph of the Poly(N-isopropylacrylamide) gel particles was obtained using Reichert Universal 'MeF2' type microscope. Magnification = 40x

Scanning Electron Microscope (SEM): Micrographs were obtained using XL30 ESEM FEG type scanning electron microscope.

Mechanical Strirrer and a Reactor System: Gelation of Poly(N-isopropylacrylamide) was conducted in a 500 ml of round bottom, four neck flask, fitted with a mechanical stirrer, nitrogen inlet and pipette outlet. A Janke &Kunkel IKA-WERK RW 18 Nr type mechanical stirrer was used with a speed range from 25-2500 rpm. The experiments were done in the laboratories of TÜBİTAK Marmara Research Center. A diagram for the apparatus is shown in Figure 3.1.

3.3. Preparation of Polymer Gels

The synthesis of poly(N-isopropylacrylamide) gels, N-isopropylacrylamide/itaconic N-isopropylacrylamide/maleic acid copolymeric gels are described below:

3.3.1. Preparation of Poly(N-isopropylacrylamide) Gels by Different Techniques

3.3.1.1. Radiation Induced Polymerization. The synthesis was done by irradiating a 10 per cent w/w aqueous solution of NIPAAm, in a glass tube with 5 mm inner diameter. All irradiations were carried out under oxygen atmosphere at 25°C with a Gammacell 220 type ^{60}Co gamma irradiator with doses up to 104 kGy at a dose rate of 3 kGy/hour. All irradiations were carried out in the laboratories of Ankara Nuclear Research and Training Centre.

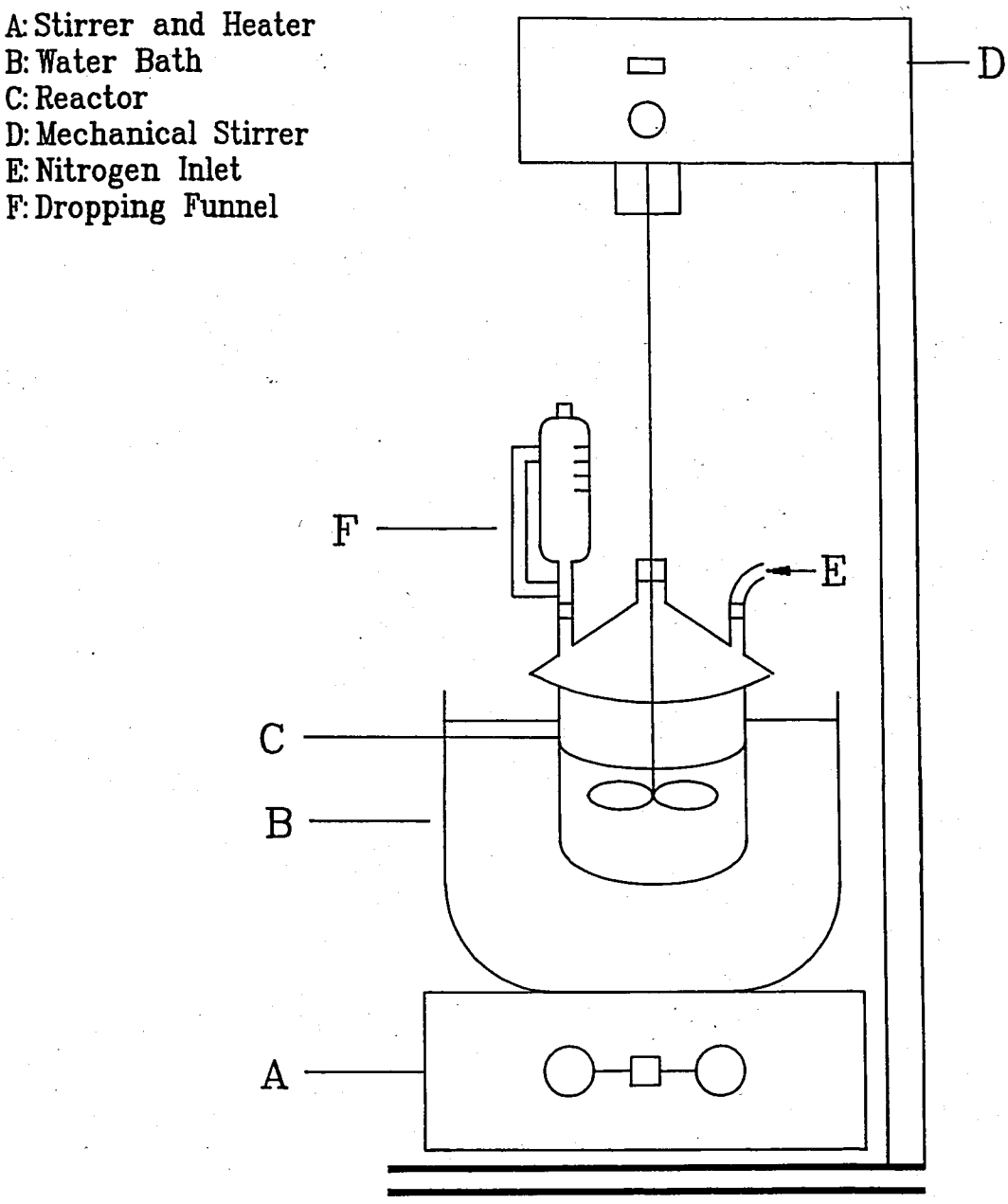


Figure 3.1. Mechanical stirrer and a reactor system for suspension polymerization

After polymerization the gels were removed from tubes and the hydogels obtained in long cylindirical shapes were cut into pieces of approximately 1 mm. They are thoroughly washed with distilled deionized water for three weeks. The gel fraction was measured after Soxhlet-type extraction in water for 24 h and drying the extracted gel in vacuum to constant weight.

3.3.1.2. Solution Polymerization. The poly(N-isopropylacrylamide) gels (PNIPAAm) were prepared by the free-radical cross-linking copolymerisation of NIPAAm with a small amount of N,N'methylenebis(acrylamide) (MBA) in aqueous solution. Ammonium peroxodisulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) were, respectively, the initiator and the accelerator. The reactions were carried out at room temperature and the gels were prepared according to the following method [52]:

8.0 g of the monomer mixture (NIPAAm and MBA) and 80 mg of APS were dissolved in double distilled water to give a total volume of 100 ml. The solution was then purged nitrogen gas for 10 min to eliminate dissolved oxygen in the system. After addition of 0.24 ml of TEMED, the solution was transferred to small tubes of 5 mm in diameter. After 2 hours of polymerization, the gels were cut into specimens of approximately 10 mm in length and immersed in a large excess of water to wash out any unreacted monomer and intiators.

Polymerization of NIPAAm monomer and production of a gel proceeds by way of a chain reaction. The first step in the polymerization is a reaction between ammonium peroxodisulfate and TEMED in which TEMED molecule is left with an unpaired valance electron. The activated TEMED molecule can combine with an NIPAAm or N,N '-methylenebisacrylamide monomer; in the process the unpaired electron is transferred to the NIPAAm unit, so that it in turn becomes reactive.

As the chain of NIPAAm unit grows, the active site shifts to the free end. Another monomer can therefore be attached and activated in the same way. The polymer can continue growing indefinitely (or until the supply of monomer is exhausted), with the active center being continually shifted to the free end of the chain. A N,N'-methylenebis(acrylamide) molecule can be incorporated into two growing chains simultaneously and forms a permanent link between them. N,N'-methylenebis(acrylamide) leads to the formation of cross-links between chains. With an abundance of cross-links the polymer of a gel has a topologically complex configuration, with loops, branches and inter connections [57]. The polymerization mechanism can be seen in Figure 3.2.

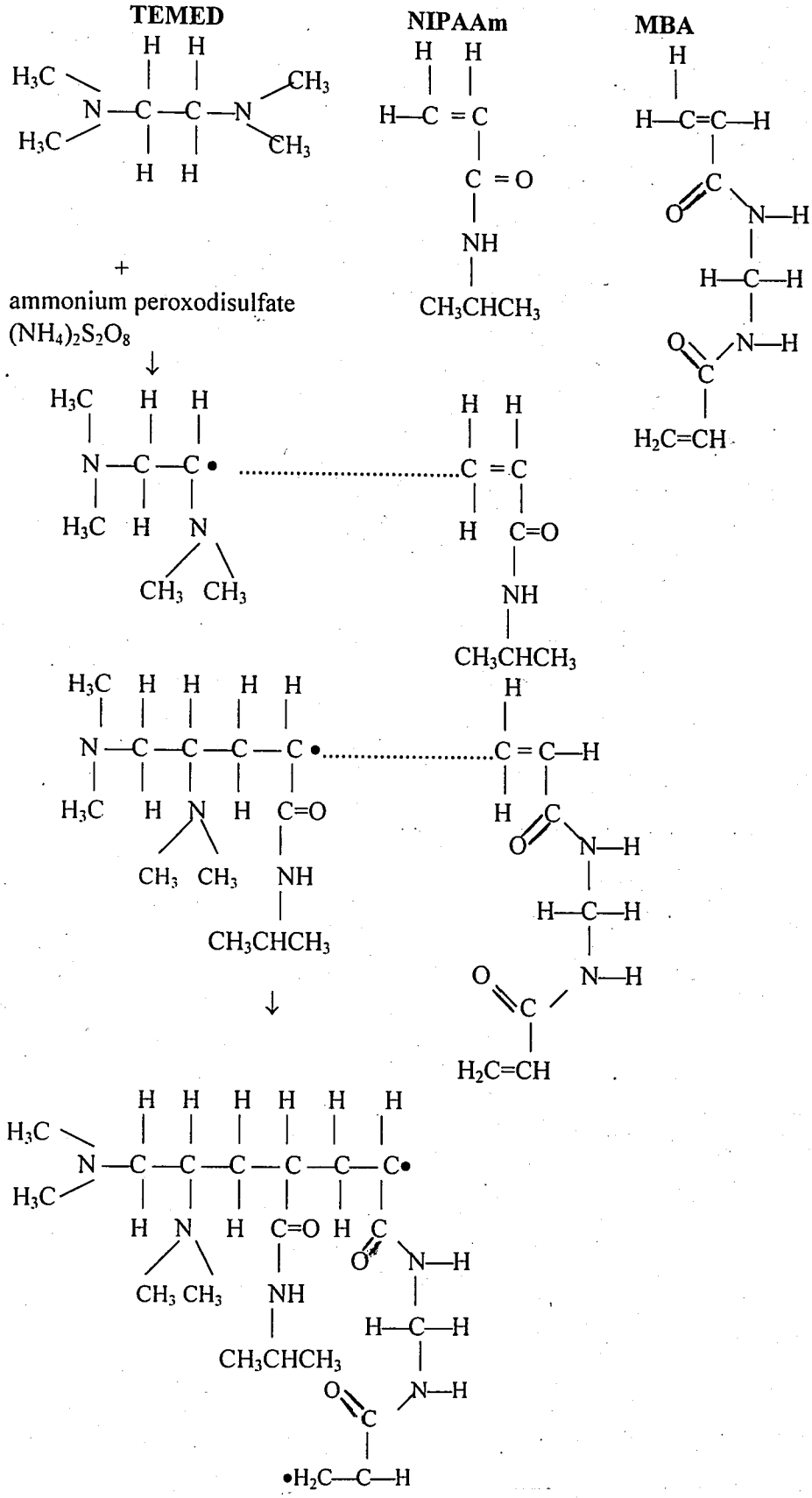


Figure 3.2. The polymerisation mechanism

3.3.1.3. Inverse Suspension Polymerization. Polymerization was conducted in a 500 ml round bottom, four-neck flask, fitted with a mechanical stirrer, nitrogen inlet and pipette outlet. Clean PNIPA gel beads were prepared without using an external emulsifier in a water in a water insoluble continuous phase. Paraffin oil was used as the continuous phase as reported by Park and Hoffman [3, 73]. 200 ml of paraffin were first introduced into the reactor and, after 1 min, TEMED (=0.12 ml) was added to the mixture to initiate the polymerisation. The reaction was followed to proceed for 3 h. After polymerisation, the beads were separated from the oil phase and washed several times with acetone and water. The diameter of the hydrogel beads in the swollen state ranged from 71 μm to 500 μm. The particles were sieved using ASTM sieves and those of selected fractions were used for further experiments.

3.3.2. Preparation of N-isopropylacrylamid/Itaconic Acid and N-isopropyl acrylamide / Maleic Acid Copolymeric Hydrogels

3.3.2.1. Radiation Induced Polymerization. The hydrophilic NIPAAm monomer was used as a base monomer in the synthesis of hydrogels. The comonomers carrying diprotic acid groups were itaconic acid and (IA) and maleic acid (MA), respectively. The chemical formula of these monomers is given in Figure 3.3.

Aqueous solutions of PNIPAAm (%10 w/w) were prepared in distilled water. Different amounts of IA or MA were added to 1 ml of PNIPAAm solution (NIPAAm/IA mole ratios, 100:0, 99:1, 98:2, 97:3). Monomer solutions thus prepared were placed in a glass tube with 5 mm inner diameter.

All irradiations were carried out under oxygen atmosphere at 25°C with a Gammacell 220 type gamma irradiator in Ankara. The dose range is between 48 and 104 kGy at a dose rate of 3 kGy/hour. After polymerization, crosslinked copolymers were removed from tubes and the hydogels obtained in long cylindirical shapes were cut into pieces of approximately 1 mm. They are thoroughly washed with distilled deionized water for three weeks. The gel fraction was measured after Soxhlet-type extraction in water for 24 hour and drying the extracted gel in vacuum to constant weight.

3.3.2.2. Preparation of Radiation Induced Surface Modified P(N-isopropylacrylamide) with Itaconic Acid as a Graft Copolymer. 1.0 g of the selected fraction of PNIPAAm microspheres (180 μm to 250 μm) synthesized by using inverse suspension polymerization technique were put into a 10 % w/w aqueous solution of itaconic acid and waited at 4 °C for 24 hours [3, 74]. Then, the PNIPAAm microspheres was removed from the solution and transferred to small glass tubes for irradiating with a ^{60}Co gamma source at 25°C in atmosphere at different irradiation dose (5, 24 and 48 kGy). After irradiation, the PNIPAAm microspheres were immersed into in a large excess of water to wash out any unreacted itaconic acid and finally, they are dried in vacuum to constant weight.

3.4. Characterization Methods

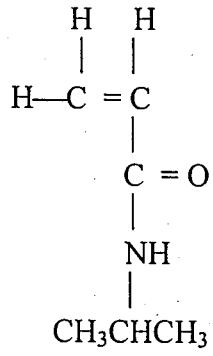
3.4.1. Composition of Gels

Irradiated mixtures were dried in a vacuum at 30°C to constant weight and subjected to Soxhlet extraction with water as solvent. During the extraction process the solvent was refreshed at least three times. Uncrosslinked polymer and/or residual monomer were removed with this extraction from the gel structure. Extracted gels were dried again vacuum oven at 30°C to constant weight. The amount of uncrosslinked IA and MA was determined by titration of extract against NaOH to phenolphtlaein end point.

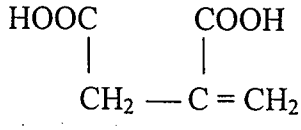
3.4.2. Swelling Measurements

Dried hydrogels (1 mm thickness, 5 mm diameter) were immersed in vials (100 ml) filled with pure water. The vials were set in a temperature-controlled bath at 25 ±0.1 °C. In order to reach the equilibrium degree of swelling, the gels were immersed in solutions for at least one week. Two different methods were used to measure the equilibrium swelling ratio of the gels, V/V_o, where V and V_o are the volumes of gel at equilibrium and reference conditions, respectively. First, the diameter of the gels was measured by a calibrated digital compass and their swelling ratio was calculated as:

N-isopropylacrylamide (NIPAAm)



Itaconic Acid (IA)



Maleic Acid (IA)

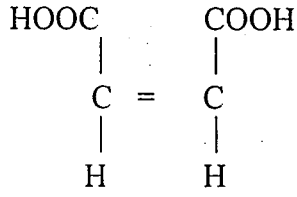


Figure 3.3. The chemical formula of the monomers

$$V/V_o = (D / D_o)^3 \quad (3.1)$$

where D and D_o are the diameter of the gels after equilibrium swelling and after synthesis, respectively. Second, the swelling ratio was determined by weighing the specimens in swollen and dry states. Swollen gels removed from the water at regular intervals were dried superficially with filter paper, weighed placed in the same bath. The measurements were continued until constant weight was reached for each sample. The swelling ratio was calculated as:

$$V/V_o = \nu_2^0 (1 + \frac{(q_w - 1)\rho}{d_1}) \quad (3.2)$$

where ν_2^0 is the volume fraction of the polymer network after preparation, q_w is the ratio of the weights of the network in the swollen state and the dry state, ρ and d_1 are the densities of PNIPAAm and water, respectively. q_w was used to calculate the volume faction of polymer network at equilibrium degree of swelling (ν_{2m}) and equilibrium degree of swelling (EDS), Q, of the sample equilibrated in the solution. The equilibrium degree of swelling (EDS), Q, was defined as :

$$\nu_{2m} = 1 / [\, 1 + \rho / d_1 \, (q_w - 1) \,] \quad (3.3)$$

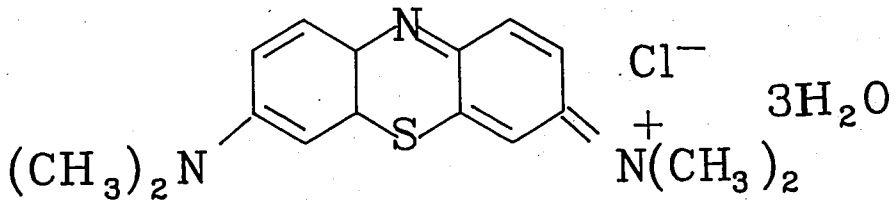
$$Q = 1 / \nu_{2m} \quad (3.4)$$

3.4.3. Drug Loading

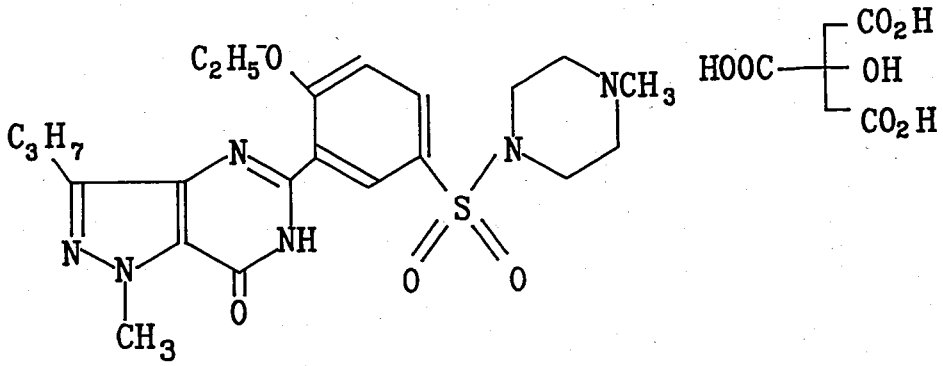
Methylene blue, sildenafil citrate (viagra) and lidocaine were used as model drugs for the investigation of drug release behaviour of PNIPAAm and P(NIPAAM/IA) hydrogels pr microspheres. Dry polymer discs (1 mm thickness, 5 mm diameter) were loaded with one of the above mentioned drug prepared in phosphate buffer at pH 7.4 at 4°C for one week. The chemical formula of the drugs is given in Figure 3.4.

3.4.4. Controlled Release of Drugs from Gels

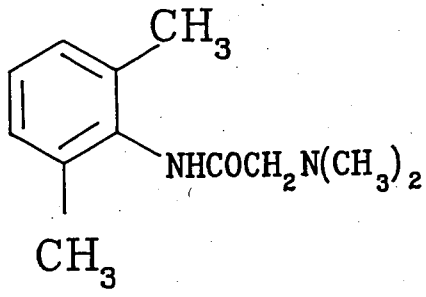
3.4.4.1. Methylene Blue Release Experiment. The dry gels (hydrogels or microspheres) were equilibrated in 500 ppm (mg/L) of methylene blue (MB) prepared in phosphate buffer at pH 7.4 at 4°C for one week. After incubation the polymer rods or microspheres were removed from the solution and rinsed in cold buffer. The MB release experiments were carried out by transferring previously incubated drug gels in a vessel containing 50 mL of phosphate buffer at pH 7.4 at 37°C at a constant shaking rate. At various times aliquots of 3 mL were drawn from medium to follow MB release and placed again into the same vessel so that the liquid volume was kept constant.



Methylene Blue



Sildenafil Citrate



Lidocaine

Figure 3.4. The chemical formula of the drugs

MB release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophometer at 664 nm. The controlled release of non-specifically adsorbed MB was followed at pH 7.4, pH 5.5, pH 4.0 and pH 2.0 were used for the controlled release of specifically bonded MB from the gels. After release at pH 2, the gels were immersed in a 0.1 mole/L HCl for 2 days to remove any remaining MB in the gel system.

3.4.4.2. Sildenafil Citrate Release Experiment. The dry gels (hydrogels or microspheres) were equilibrated in 500 ppm (mg/L) of sildenafil citrate (viagra) (VG) prepared in phosphate buffer at pH 7.4 at 4°C for one week. After incubation the polymer rods or microspheres were removed from the solution and rinsed in cold buffer. The VG release experiments were carried out by transferring previously incubated drug gels in a vessel containing 50 mL of phosphate buffer at pH 7.4 at 37°C at a constant shaking rate. At various times aliquots of 3 mL were drawn from medium to follow VG release and placed again into the same vessel so that the liquid volume was kept constant.

VG release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophometer at 291 nm. The controlled release of non-specifically adsorbed VG was followed at pH 7.4. pH 5.5, pH 4.0 and pH 2.0 were used for the controlled release of specifically bonded VG from the gels. After release at pH 2, the gels were immersed in a 0.1 mole/L HCl for 2 days to remove any remaining VG in the gel system.

3.4.4.3 Lidocaine Release Experiment. The dry gels (hydrogels or microspheres) were equilibrated in 5000 ppm (mg/L) of lidocaine (LD) prepared in phosphate buffer at pH 7.4 at 4°C for one week. After incubation the polymer rods or microspheres were removed from the solution and rinsed in cold buffer. The LD release experiments were carried out by transferring previously incubated drug gels in a vessel containing 50 mL of phosphate buffer at pH 7.4 at 37°C at a constant shaking rate. At various times aliquots of 3 mL were drawn from medium to follow LD release and placed again into the same vessel so that the liquid volume was kept constant.

LD release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophometer at 262 nm. The controlled release of non-specifically adsorbed LD was followed at pH 7.4, pH 5.5, pH 4.0 and pH 2.0 were used for the controlled release of specifically bonded LD from the gels. After release at pH 2, the gels were immersed in a 0.1 mole/L HCl for 2 days to remove any remaining LD in the gel system.

3.4.5. Morphology

In polymer science, the term morphology generally refers to form and organization on a size scale above the atomic arrangement but smaller than the size and shape of fillers and additives and the size, distribution and association of the structural units within manostructure. Microscopy is the study of the fine structure and the morphology of objects with the use of optical, tranmisson electron and scanning electron microscopes for the investigation of the morphology of polymer sample.

3.5. Irradiation Conditions

All irradiations were carried out under oxygen atmosphere at 25°C with a Gammacell 220 type ^{60}Co gamma irradiator with dose range 48 between 104 kGy at a dose rate of 3 kGy/hour. All irradiations were done in the laboratories of Ankara Nuclear Research and Training Centre.

4. RESULTS AND DISCUSSION

In the first sections of this chapter, the experimental results on the radiation synthesis of N-isopropylacrylamide/itaconic acid and N-isopropylacrylamide/maleic acid copolymeric hydrogels are presented. The dependence of swelling equilibria and phase transition on the comonomer concentration, irradiation dose and temperature were summarized. Next, the results on molecular weight between cross-links and crosslinking density for the characterisation of network structures of these hydrogels are given. Finally, drug release behaviour of these hydrogels are presented.

4.1. Radiation Synthesis of N-isopropylacrylamide / Itaconic Acid N-isopropylacrylamide / Maleic Acid Copolymeric Hydrogels

N-isopropylacrylamide/itaconic acid and N-isopropylacrylamide/maleic acid copolymeric hydrogels were prepared by irradiating the ternary mixture of N-isopropylacrylamide/diprotic acid moieties (itaconic acid or maleic acid)/water by γ-rays at ambient temperature.

4.1.1. Composition of Hydrogels

In the present work, poly(N-isopropylacrylamide) hydrogels were synthesized by radiation induced polymerization. First of all, 1.0 g of the pure N-isopropylacrylamide monomer was dissolved in double distilled water to give a total volume of 10 mL. These solutions were transferred to small glass tubes of 5 mm in diameter and irradiated at different doses in air at ambient temperature in a ^{60}Co Gammacell 220 type γ irradiator. All irradiations were carried out in the laboratories of Turkish Atomic Energy Authority in Ankara.After the polymerization, the gels were removed from the tubes and cut into pieces. Then, they are dried in a vacuum at 30°C to constant weight and subjected to Soxhlet extraction with water as solvent. Uncrosslinked polymer and/or residual monomer were removed with this extraction from the gel structure. Extracted gels were dried again in vacuum.

The percentage soluble fraction and gelation were calculated as:

$$\text{Percentage Soluble Fraction} = \frac{m_{initial} - m_{final}}{m_{initial}} \times 100 \quad (4.1)$$

$$\text{Percentage Gelation} = 100 - \text{Percentage Soluble Fraction} \quad (4.2)$$

where $m_{initial}$ is the initial weight of the dry gel and m_{final} is the final weight of the extracted gel after drying.

Table 4.1 and Figure 4.1 show the gelation percent as a function of the irradiation dose for 10 per cent w/w aqueous solutions of NIPAAm monomer. As the irradiation dose increases, conversion of NIPAAm monomer into gel increases.

Table 4.1. Percentage gelation as a function of the irradiation dose for 10 per cent w/w aqueous solutions of NIPAAm monomer

Irradiation Dose (kGy)	$m_{initial}$ (g)	m_{final} (g)	Percentage gelation
6	0.074	0.058	78.0
12	0.018	0.016	89.5
24	0.022	0.021	94.9
48	0.023	0.022	96.9
82	0.026	0.022	97.4
104	0.020	0.019	97.5

When the monomer is irradiated in aqueous solution, monomer radicals are mostly generated by the indirect effect based on the reactions of the products of water radiolysis with the monomer [69]. The radiolysis of water leads to formation of hyrated electrons, hyroxyl radicals and hydrogen atoms as explained in Section 2.5.2. The reaction of these radicals (R•) with the monomer (M) generates the monomeric radical. This may be expressed as:

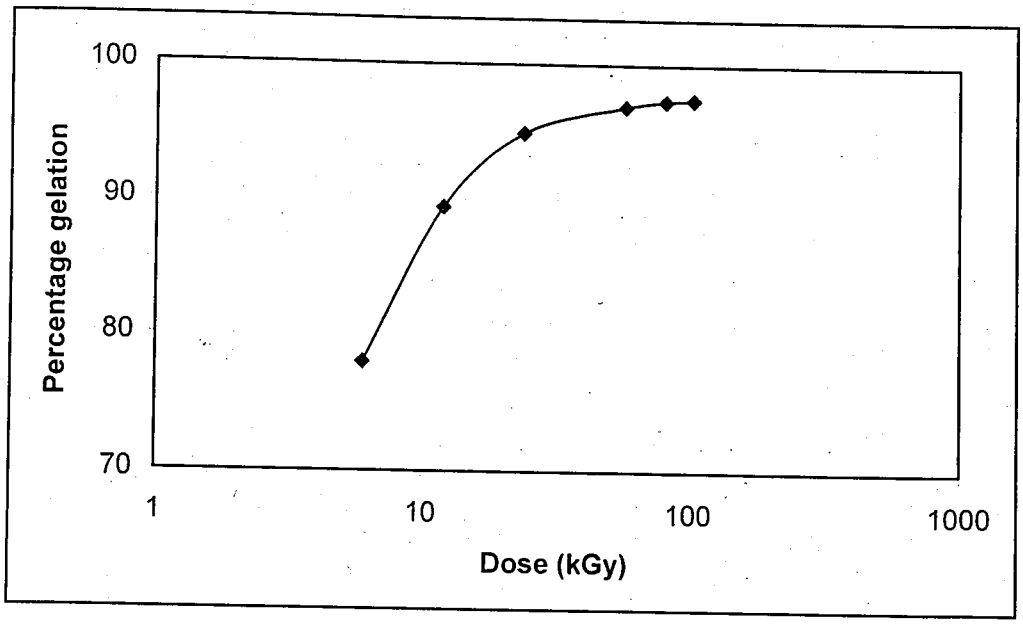


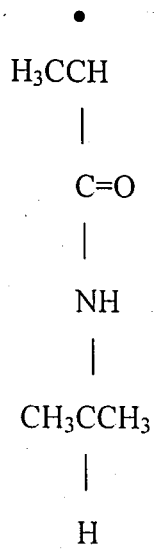
Figure 4.1. Percentage gelation as a function of the irradiation dose for 10 per cent w/w aqueous solutions of NIPAAm monomer

$$M + R\bullet \rightarrow M\bullet + RH$$

After these initial processes, monomer radicals combine and propagate to form a linear but soluble polymer until the dose reaches the gel point. Once the gel point is reached (at dose=D_g), the amount of the gel grows rapidly due to propagation of gel radicals with the monomers and recombination of sol-gel radicals. Nagaoka et.al. found that D_g was 71 Gy for an aqueous solution of pure NIPAAm [13].

According to the literature, the monomeric radical which is the most probably responsible for the initiation of the polymerization and cross-linking is the α-carboxyl alkyl radical [13].

Besides this carboxyl alkyl radical, an isopropyl radical is also produced, based on the indirect effect of radiation, by a hydrogen abstraction on the side chain. But it was found that this radical is stable and less likely to participate in the polymerization and cross-linking reactions [13].



In this work, total dose required for an approximately 100 per cent gelation of NIPAAm/IA and NIPAAm/MA hydrogels has been found to be 48 kGy when the comonomer (IA or MA) was used in the range of 1.0-3.0 per cent in the initial mixture.

The irradiated copolymers were subjected to Soxhlet extraction with water as solvent. The amount of IA or MA in the copolymeric gels was determined by titration of the extract against NaOH (0.01N) to phenolphtalein end point. Percentage gelation was based on the total weight of the diprotic acid and the monomer in the initial mixture. Mole percentages of monomers in the initial mixtures and in the copolymeric gels and percentage gelation are summarized in Tables 4.2-4.3. These results show that increasing mole percentage of IA or MA causes a decrease in the extent of gelation from monomer to gel.

The maximum solubility of IA and MA was found to be 90 mg in 1 g NIPAAm/1 ml water. However, the hydrogels prepared from these initial compositions were not mechanically stable and disintegrated into small parts during swelling. So only hydrogels with three compositions could be investigated in this study. The considerations for selecting the particular feed compositions are firstly the solubility of diprotic acids in aqueous monomer solution and the shape stability of swollen hydrogels.

Table 4.2. Mol per cent of IA in the feed and in the gel system and gelation per cent

Gel name	Mole per cent of IA		Irradiation dose (kGy)	Gelation per cent
	In feed	In gel		
PNIPAAm(1)	0.0	0.0	48	95.0
P(NIPAAm/IA)-1	1.0	1.0	48	92.3
P(NIPAAm/IA)-2	2.0	2.0	48	91.1
P(NIPAAm/IA)-3	3.0	3.0	48	90.0
P(NIPAAm/IA)-4	1.0	1.0	82	94.5
P(NIPAAm/IA)-5	2.0	2.0	82	93.4
P(NIPAAm/IA)-6	3.0	3.0	82	92.9
P(NIPAAm/IA)-7	1.0	1.0	104	96.7
P(NIPAAm/IA)-8	2.0	2.0	104	95.4
P(NIPAAm/IA)-9	3.0	3.0	104	94.9

Table 4.3. Mol per cent of MA in the feed and in the gel system and gelation per cent

Gel name	Mole per cent of MA		Irradiation dose (kGy)	Gelation per cent
	In feed	In gel		
PNIPAAm(1)	0.0	0.0	48	95.0
P(NIPAAm/MA)-1	1.0	1.0	48	91.1
P(NIPAAm/MA)-2	2.0	2.0	48	90.6
P(NIPAAm/MA)-3	3.0	3.0	48	90.2
P(NIPAAm/MA)-4	1.0	1.0	82	94.3
P(NIPAAm/MA)-5	2.0	2.0	82	93.5
P(NIPAAm/MA)-6	3.0	3.0	82	92.9
P(NIPAAm/MA)-7	1.0	1.0	104	98.5
P(NIPAAm/MA)-8	2.0	2.0	104	97.6
P(NIPAAm/MA)-9	3.0	3.0	104	96.9

4.1.2. pH Sensitivity of Hydrogels

Figures 4.2 and 4.3 represent pH dependence of the equilibrium degree of swelling for NIPAAm/IA and NIPAAm/MA hydrogels at 25°C in phosphate buffer solution from pH 2 to 8. The equilibrium degree of swelling was calculated according to Equation 3.4 as explained in Section 3.4.2.

Consistent with poly-electrolyte behaviour, swelling of hydrogels was found to increase with pH. In all compositions maximum extents of swelling were reached at pH 7, this being due to the complete dissociation of acidic groups of itaconic acid and maleic acid at this pH value. The first and second dissociation constants of IA and MA are pK_{a1}=3.85, pK_{a2}=5.44 and pK_{a1}=1.85, pK_{a2}=6.06, respectively [75]. Since two dissociation constants for IA are rather close, the consecutive swellings at around these pH values overlap and only single-step swelling versus pH curves are observed in Figure 4.2. For NIPAAm/MA hydrogels, however, due to large difference in pKa values, swelling takes place in a stepwise manner, as shown in Figure 4.3. The swelling shows sudden increase at the pH values around corresponding pKa values.

The equilibrium degree of swelling for pure PNIPAAm is not affected by varying the pH of the swelling medium since PNIPAAm is non-ionic hydrogel and does not have any group that could be ionized in aqueous solution. With the introduction of the diprotic acid groups into the main chain, pH of the solution becomes an even more important factor determining swelling kinetics and equilibrium swelling value. The percentage mass swelling, as a function of time for NIPAAM-IA and NIPAAm-MA copolymeric hydrogels in several pH buffer solutions are shown in Figures 4.4-4.5. The percentage mass swelling was calculated from the following equation:

$$\text{Percentage mass swelling} = [(m_t - m_o)/ m_0] \times 100 \qquad (4.3)$$

where m_0 is the mass of the dry gel at the time 0 and m_t is the mass of swollen gel at time t.

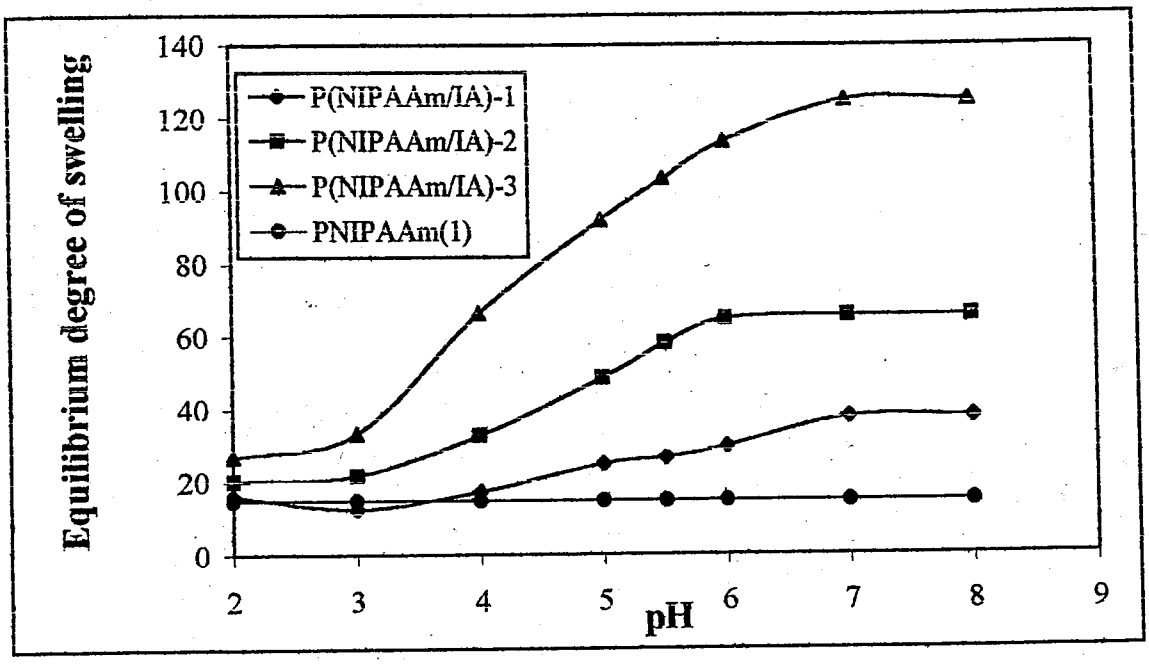


Figure 4.2. Effect of pH on the equilibrium degree of swelling of NIPAAm/IA copolymeric hydrogels:

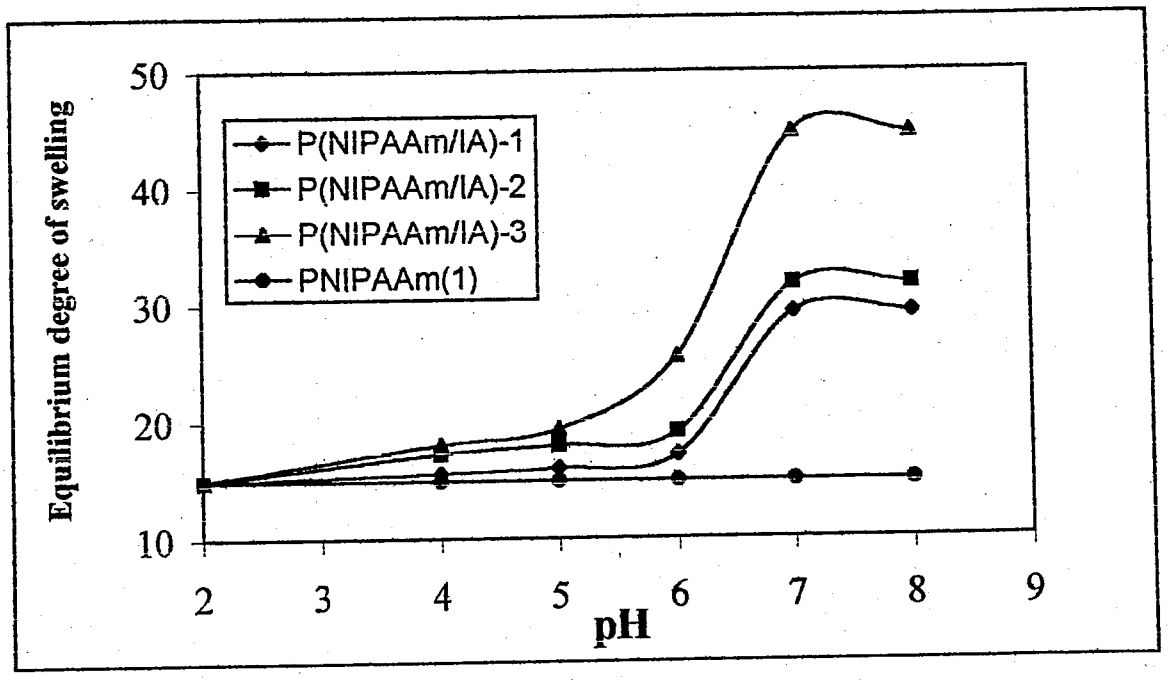


Figure 4.3. Effect of pH on the equilibrium degree of swelling of NIPAAm/MA copolymeric hydrogels:

The results indicate that under acidic conditions, anionic carboxylate groups are protonated, and the copolymeric network collapsed. At the high pH values, the concentration of anionic groups in the polymer network increases. This occurrence makes the percentage mass swelling of the hydrogels increase with an increase in ionizable constituent. The maximum percentage mass swelling occurred at pH 7, indicating the complete neutralization of carboxylic acid groups.

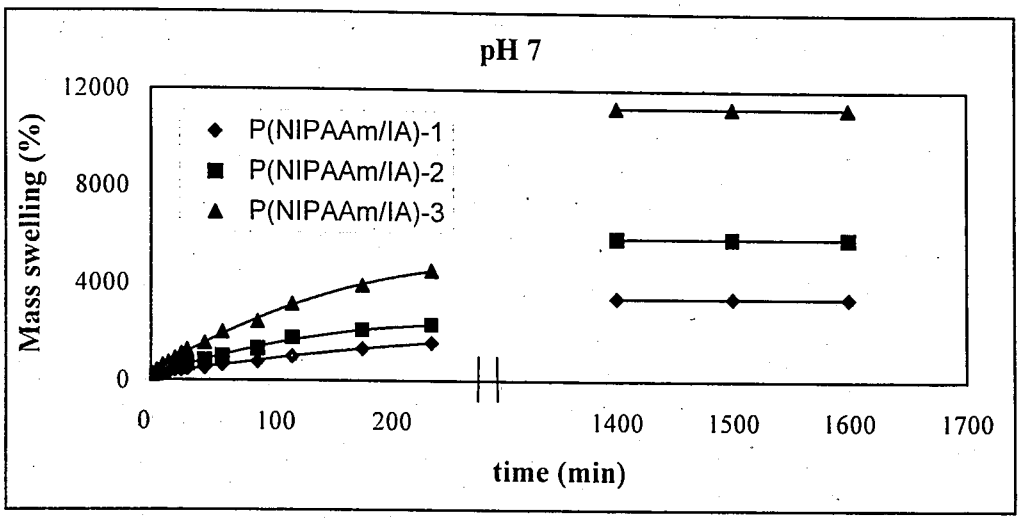
4.1.3. Swelling Equilibria and Phase Transition of Hydrogels

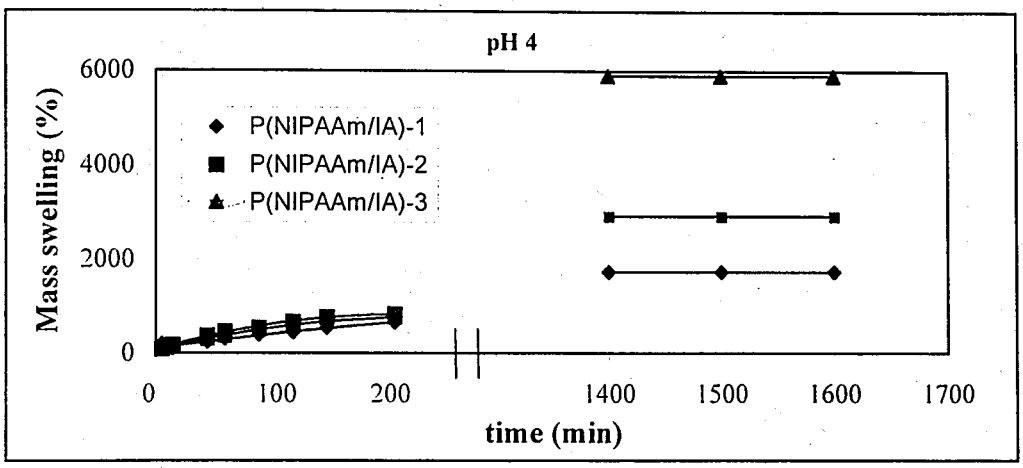
In Figure 4.6., temperature dependence of the equilibrium swelling ratios of a 10 per cent w/w aqueous solution of NIPAAm obtained by irradiating to different doses ranging between 6 and 104 kGy. Although the swelling of the lightly cross-linked gels (6 kGy) is larger than that of the highly cross-linked one (104 kGy), above LCST, all gels shrink to similar volume, independently from the crosslinking percentage.

The most important property of PNIPAAm hydrogels is their temperature-induced volume phase transition in aqueous solution. The volume phase transition of gels is similar to the coil-globule transition of the polymer chains [35]. It has been suggested that the LCST is caused by a critical balance of hydrophobic and hydrophilic groups in the polymer side chain [76-77].

At low temperature, the strong hydrogen bonding between such hydrophilic group as NH and C=O and surrounding water will cause the formation of highly organized layer around the polymer chains [78]. Thermodynamically, the formation of this structured water contributes to the enthalpy of mixing which outweighs the unfavourable free energy related to the exposure of hydrophobic isopropyl groups of the side chain to water. The hydrogel will be in a highly swollen state.

With increasing temperature, the hydrogen bonding weakens, which leads to a reduction in the structuring of water around hydrophobic groups. As this structured water is released, the interactions between hydrophobic side groups of the polymer increase.





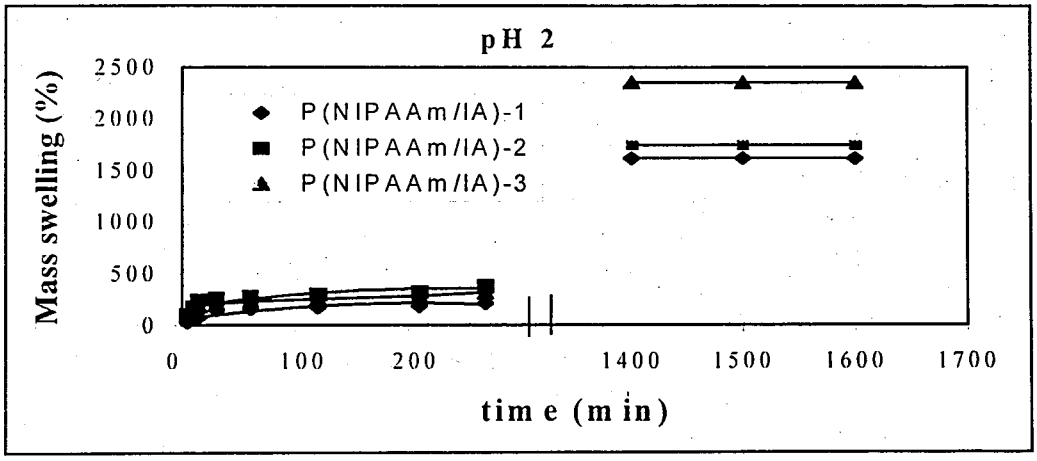
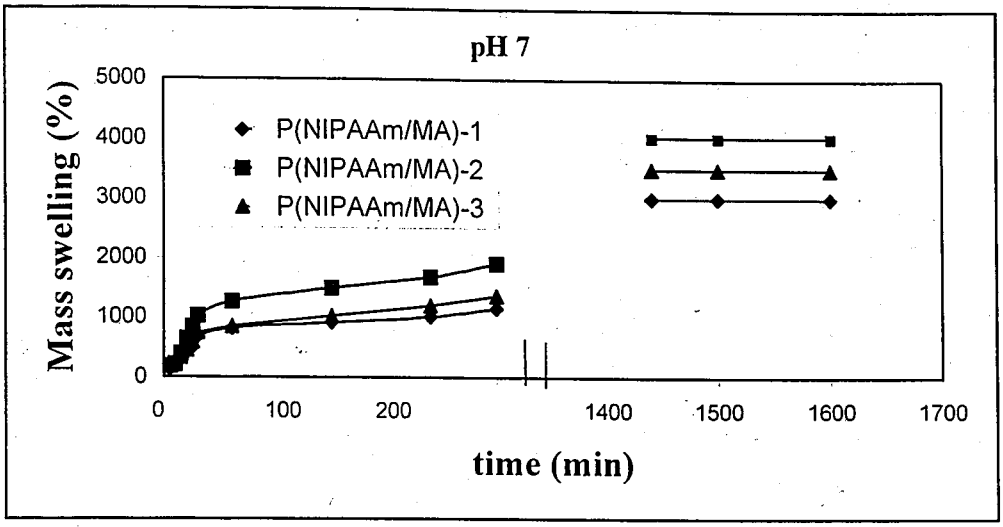
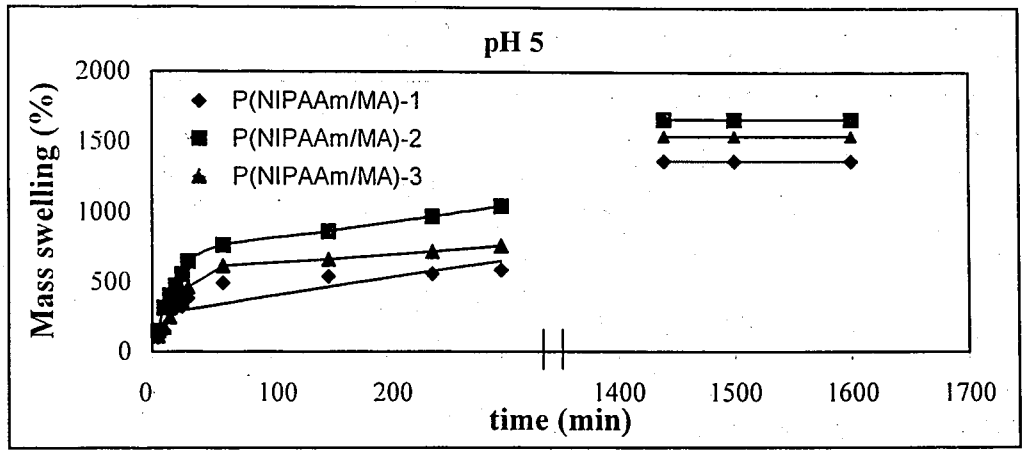


Figure 4.4. Percentage mass swelling as a function of time for the series of NIPAAm/IA copolymeric hydrogels at 25°C at different pH





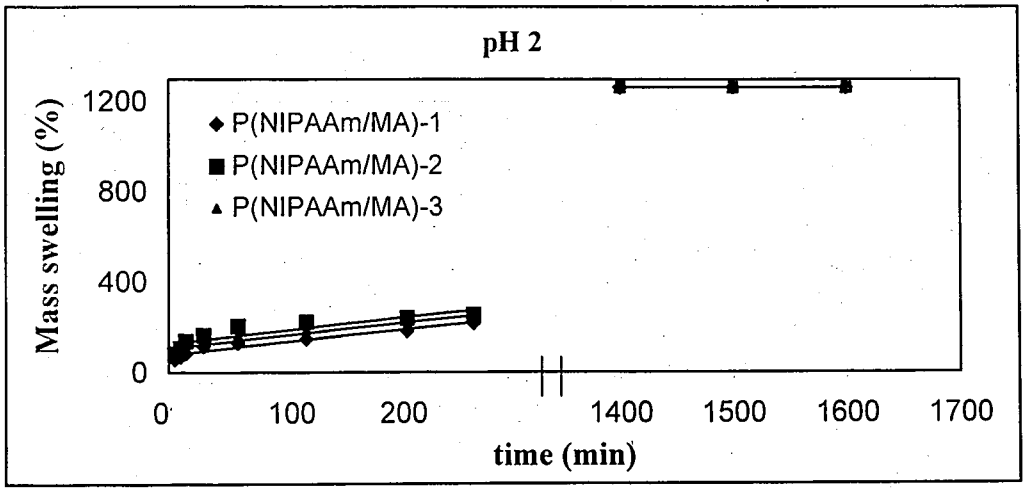


Figure 4.5. Percentage mass swelling as a function of time for the series of NIPAAm/MA copolymeric hydrogels at 25°C at different pH

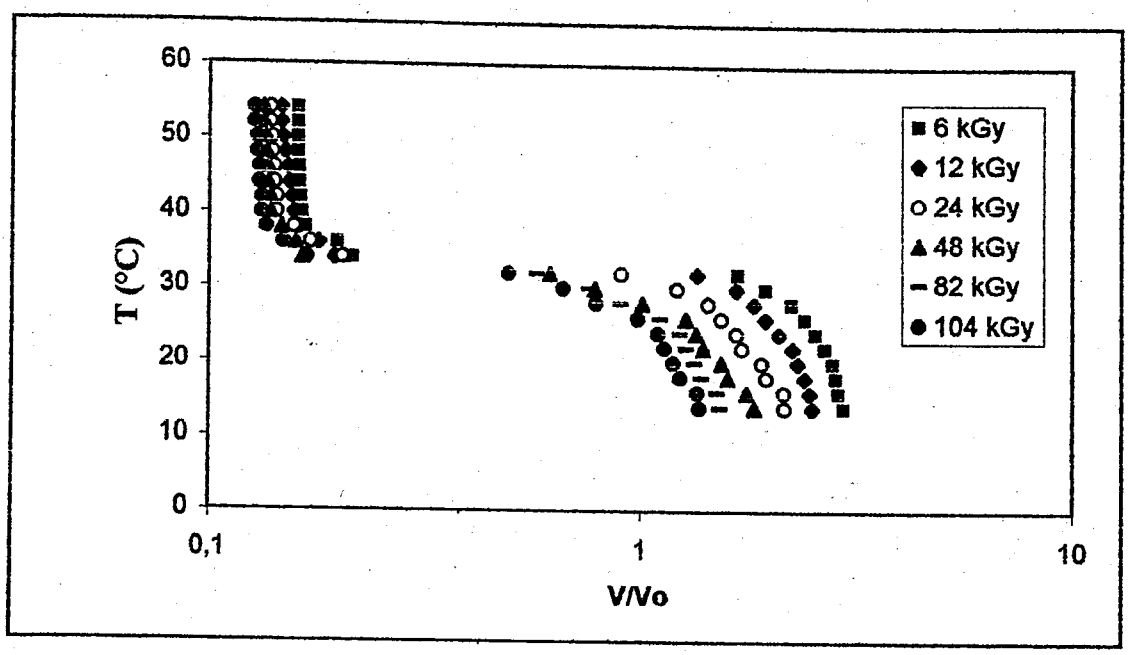


Figure 4.6. Temperature dependence of the equilibrium swelling ratios of a 10 per cent w/w aqueous solution of NIPAAm obtained by irradiating to different dose

As shown in Figure 4.6, LCST is around 33°C for PNIPAAm. The results indicate that the higher irradiation dose causes the narrower phase transition. At LCST, these hydrophobic interactions become dominant, which from the thermodynamic point of view means that the entropic term becomes dominant and the free energy of mixing takes a positive value. This will lead to a collapse of the PNIPAAm gel.

As can be seen from Figure 4.7, equilibrium percentage mass swelling of PNIPAAm hydrogel decreases as the irradiation dose increases because of increasing crosslinking percentage in the hydrogel. The percentage equilibrium mass swelling was calculated from the following equation:

$$\text{Percentage equilibrium mass swelling} = [(m_\infty - m_o)/m_o] \times 100 \qquad (4.4)$$

where m_0 is the mass of the dry gel and m_∞ is the mass of swollen gel at equilibrium.

4.1.3.1. Effect of Comonomer Concentration. The incorporation of an ionic group into thermally reversible hydrogel may be used as a model of affinity binding by and release from such gels where the counter ion is the binding partner to the immobilized ligand. Also, when weak acidic or basic groups are incorporated, then the gels should exhibit both reversible temperature and pH swelling and deswelling. Furthermore, the incorporation of immobilized ionic groups should greatly enhance the swelling of the gels when these groups are ionized [79-80].

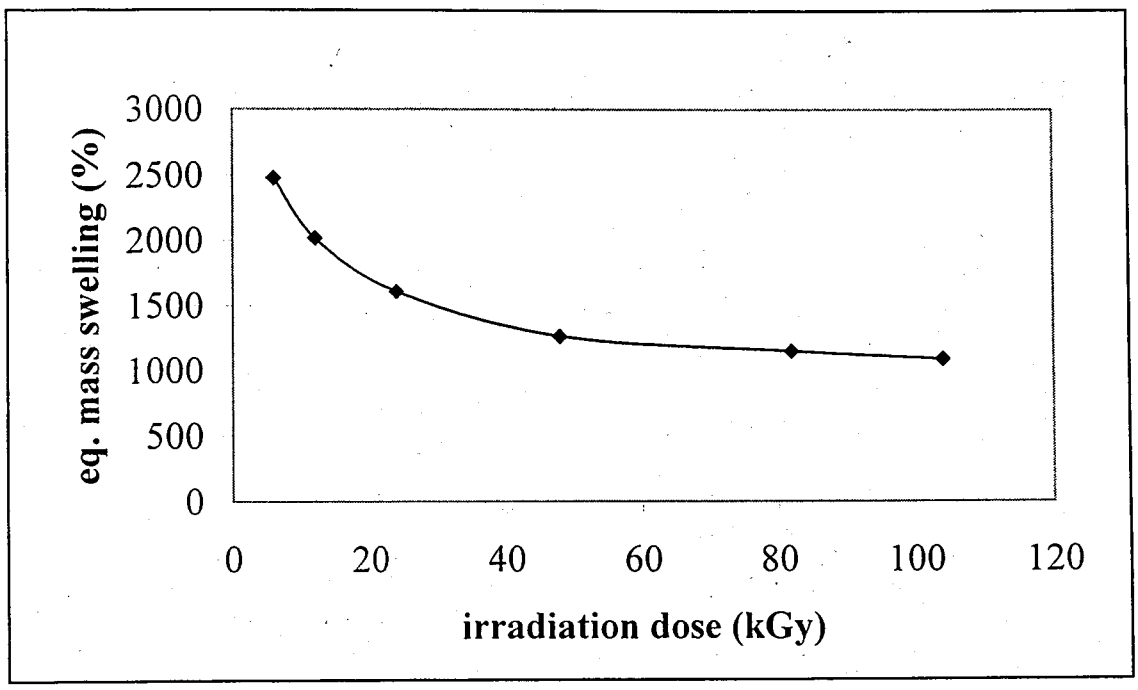


Figure 4.7. Equilibrium percentage mass swelling as a function of irradiation dose for PNIPAAm hydrogels in distilled water at 25°C

As shown in Figures 4.8 and 4.9, equilibrium percentage mass swelling of NIPAAm/IA and that of NIPAAm/MA copolymeric hydrogels (at fixed irradiation dose) increase as the comonomer concentration increases because of increasing the electrostatic interactions of the neighbouring carboxylate groups in IA or MA in the hydrogels. The swelling curves as a function of time for NIPAAM/IA and NIPAAM/MA copolymeric gels at 25°C in deionised water are shown in Figure 4.10 and 4.11, respectively. It can be seen that increase in diprotic acid content (IA or MA)

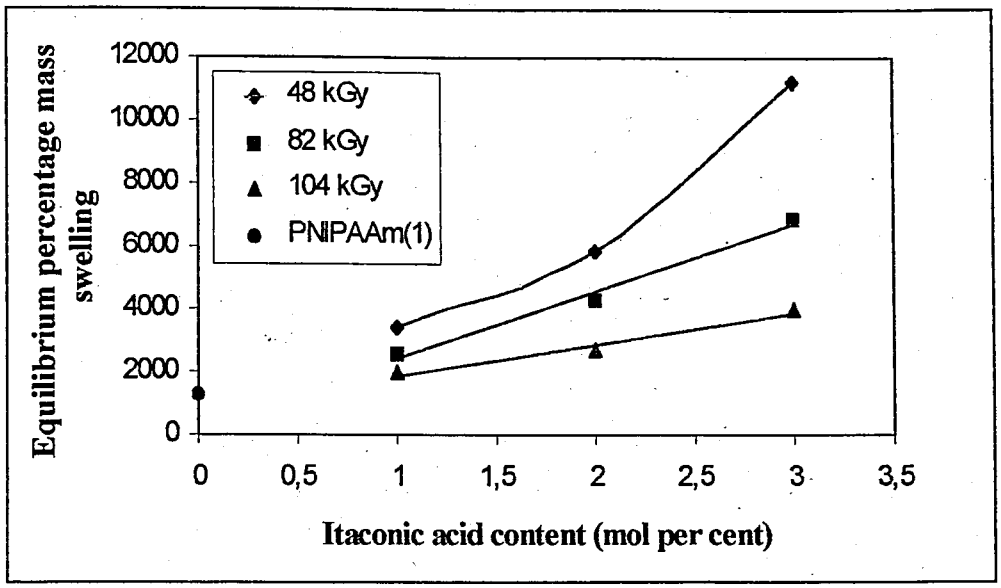


Figure 4.8. Equilibrium percentage mass swelling as a function of itaconic acid content (mole per cent) for NIPAAm/IA copolymeric hydrogels

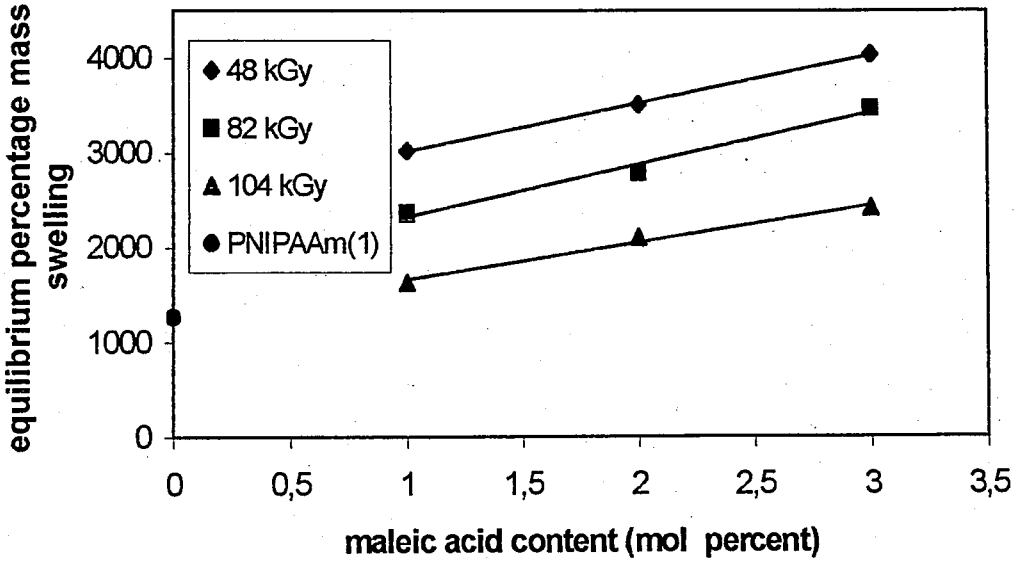


Figure 4.9. Equilibrium percentage mass swelling as a function of itaconic acid content (mole per cent) for NIPAAm/MA copolymeric hydrogels

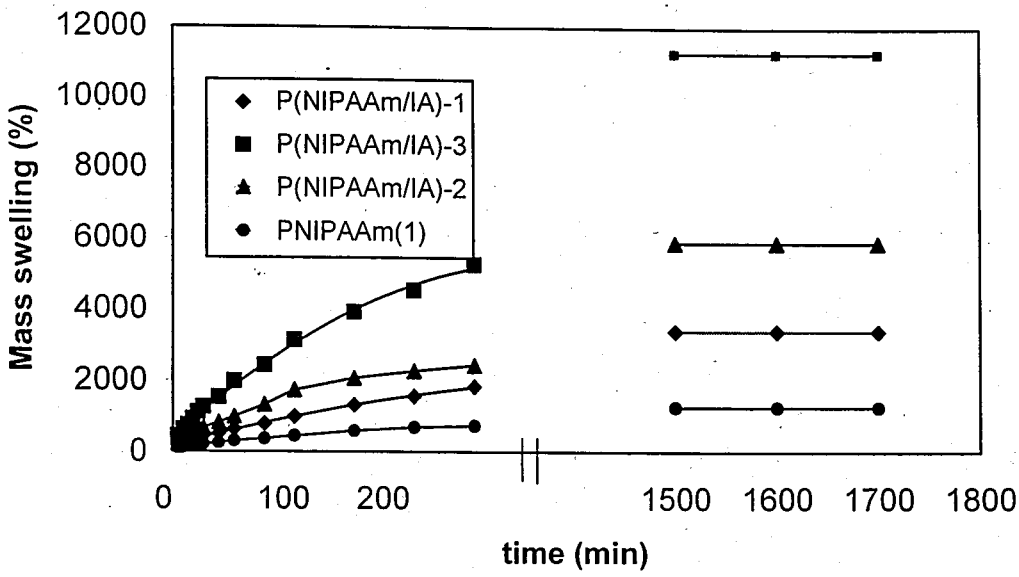


Figure 4.10. Percentage mass swelling as a function of time for the series of NIPAAM/IA copolymeric hydrogels at 25°C in distilled water

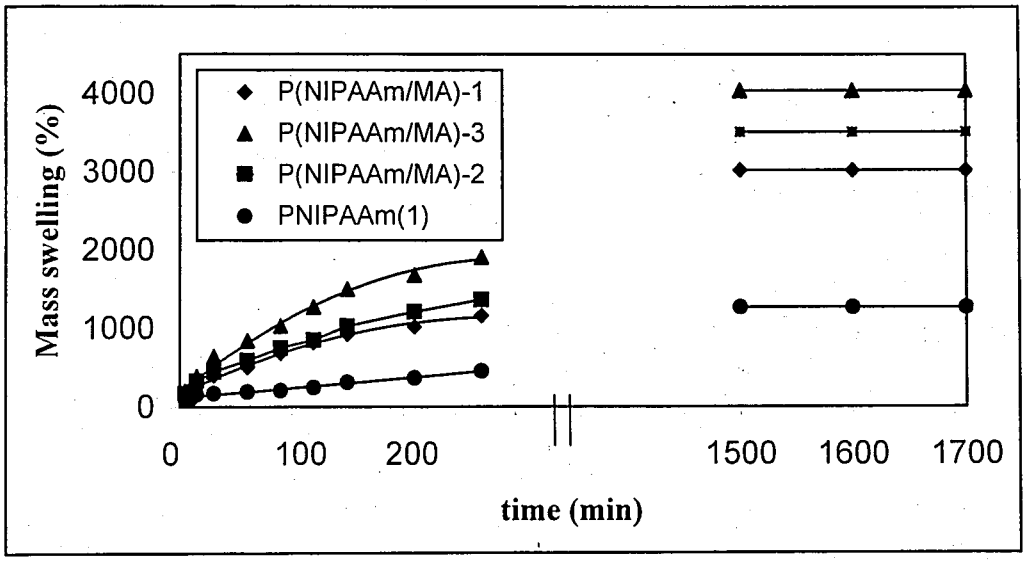


Figure 4.11. Percentage mass swelling as a function of time for the series of NIPAAm/MA copolymeric hydrogels at 25°C in distilled water

from 0 to 3 per cent causes immense increases in water uptake in deionised water. The results indicate that the mass swelling of the hydrogels increases with diprotic acid content.

According to Equation 2.47, the equibrium swelling ratio has a relation to ionic concentration, crosslinked density, and affinity of the hydrogel for water. In our work, the crosslinking density was fixed in Figure 4.10 and 4.11 (same irradiation dose, 48 kGy), so the factors that affected the swelling ratio of hydrogels are the affinity of hydrogel for water and total charges inside the gel. Because the IA or MA is a hydrophilic anionic monomers, the greater the diprotic acid content, the larger the affinity of gel for water and the higher the swelling ratio of the hydrogel. In other words, the electrostatic interactions and mutual repulsions of the neighbouring carboxylate groups in IA or MA cause expansion of the polymeric chain. This occurrence leads to a higher swelling ratio of the hydrogel having more diprotic acid content. Figures 4.12 and 4.13 show the plots of mass swelling versus square roots of time. The initial slopes, namely, swelling rate constants of the hydrogels were calculated from the following relation [22]:

$$\text{Percentage mass swelling} = k_s t^{0.5} \tag{4.5}$$

where k_s is the swelling rate constant.

The results show that the initial slopes of the curves increase sharply with the increase of the comonomer concentration: this is a result of the electrostatic interactions of the neighbouring carboxylate groups in IA or MA. The initial slopes (k_s) were presented in Table 4.4. Figures 4.14-4.15 show the temperature dependence of the equilibrium swelling ratio for series NIPAAm/IA and NIPAAm/MA copolymeric gels at different comonomer concentration at the same irradiation dose. The results clearly show that as the comonomer concentration increases, the swelling ratio increases. The higher diprotic acid content (IA or MA) leads to the broader phase transition and a shift of the LCST to a higher temperature.

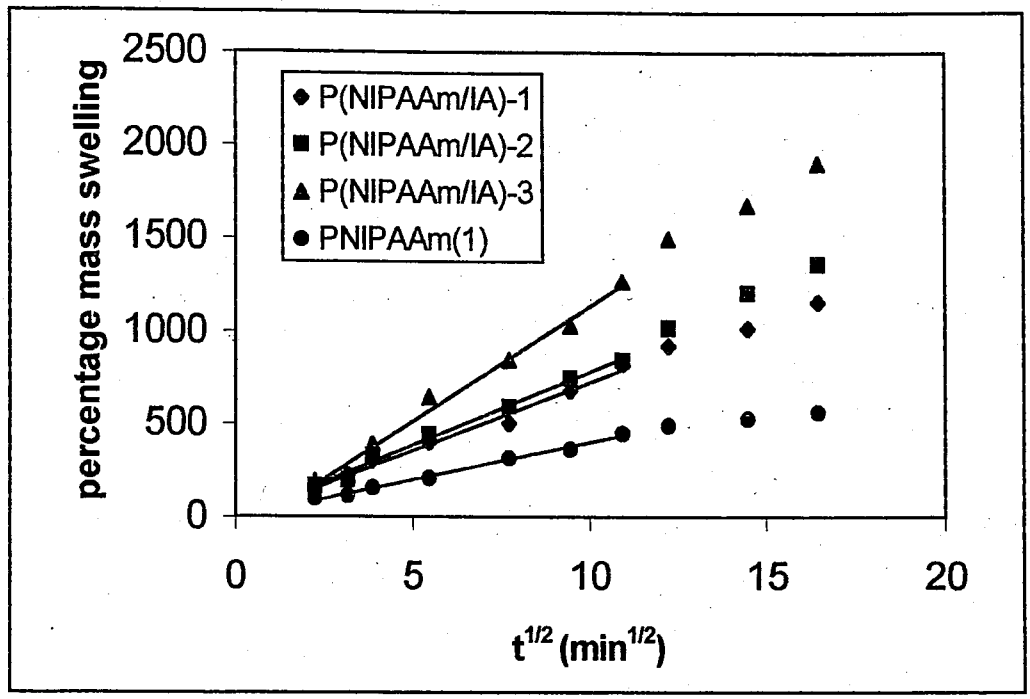


Figure 4.12. The plot of percentage mass swelling versus square root of time for NIPAAM/IA copolymeric hydrogels at 25°C in distilled water

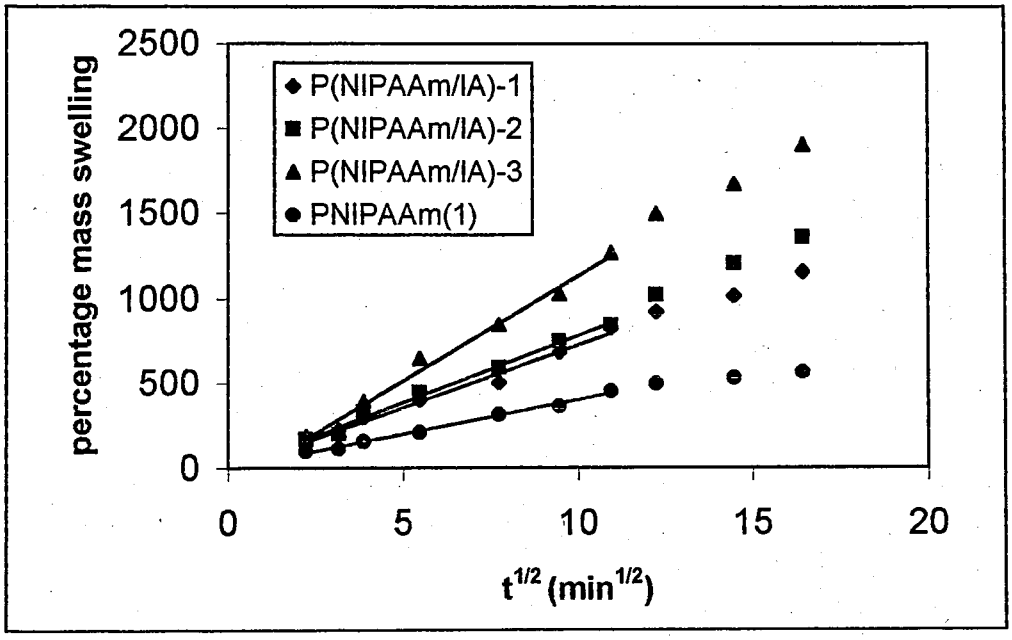


Figure 4.13. The plot of percentage mass swelling versus square root of time for NIPAAM/MA copolymeric hydrogels at 25°C in distilled water

Table 4.4. Initial slope (k_s) values of NIPAAm/IA and NIPAAm/MA copolymeric hydrogels at 25°C in distilled water

Gel name	Initial Slope (k_s)
PNIPAAm-1	40
P(NIPAAm/IA)-1	84
P(NIPAAm/IA)-2	136
P(NIPAAm/IA)-3	251
P(NIPAAm/MA)-1	72
P(NIPAAm/MA)-2	78
P(NIPAAm/MA)-3	110

Table 4.5 presents LCST of the NIPAAm/IA and NIPAAm/MA copolymeric gels. The LCST of these hydrogels disappear when mole percentage of IA or MA exceeds 3 per cent. It has been shown that the LCST of PNIPAAm copolymers is strongly influenced by the nature of the comonomer [81]. Hydrophobic compounds lower the LCST and hydrophilic compounds raise it. The LCST disappears when a hydrophilic compound contains more than a certain amount of comonomer.

4.1.3.2. Effect of Irradiation Dose. As shown in Figures 4.16 and 4.17, equilibrium percentage mass swelling of PNIPAAm/IA and that of PNIPAAm/MA copolymeric hydrogels (at fixed comonomer concentration) decrease as the irradiation dose increase because of increasing crosslinking percentage in the hydrogels.

The effect of irradiation dose on the temperature dependence of equilibrium swelling ratios of NIPAAm/IA and NIPAAm/MA copolymeric hydrogels at the same comonomer concentration are shown in Figures 4.18-4.19. The results clearly show that as the irradiation dose increases, the swelling ratio decreases. The results also indicate that the higher irradiation dose leads to the narrower phase transition and a shift of the LCST to a lower temperature.

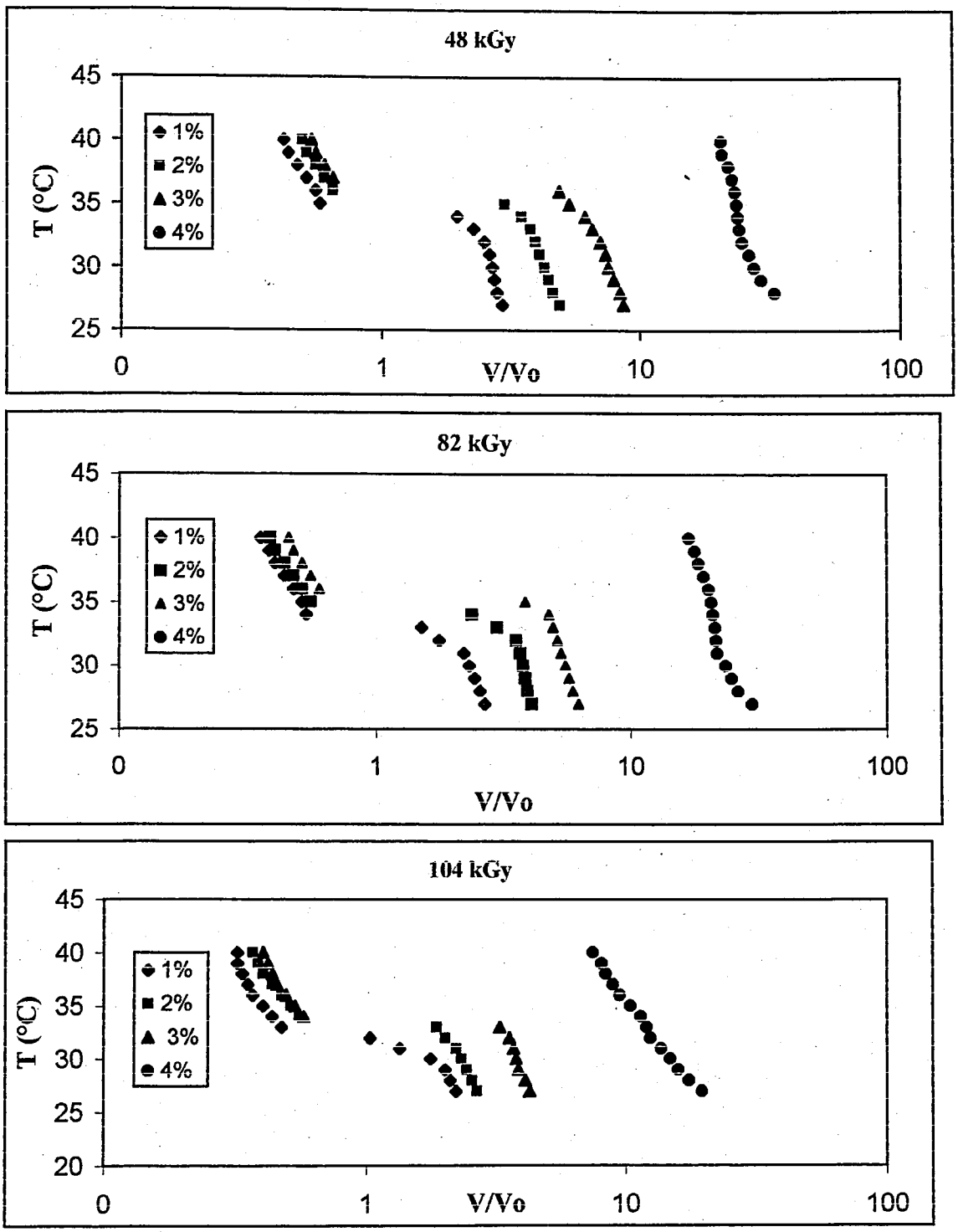


Figure 4.14. Variation of temperature dependence of the equilibrium swelling ratios of NIPAAm-IA with itaconic acid content (mole per cent)

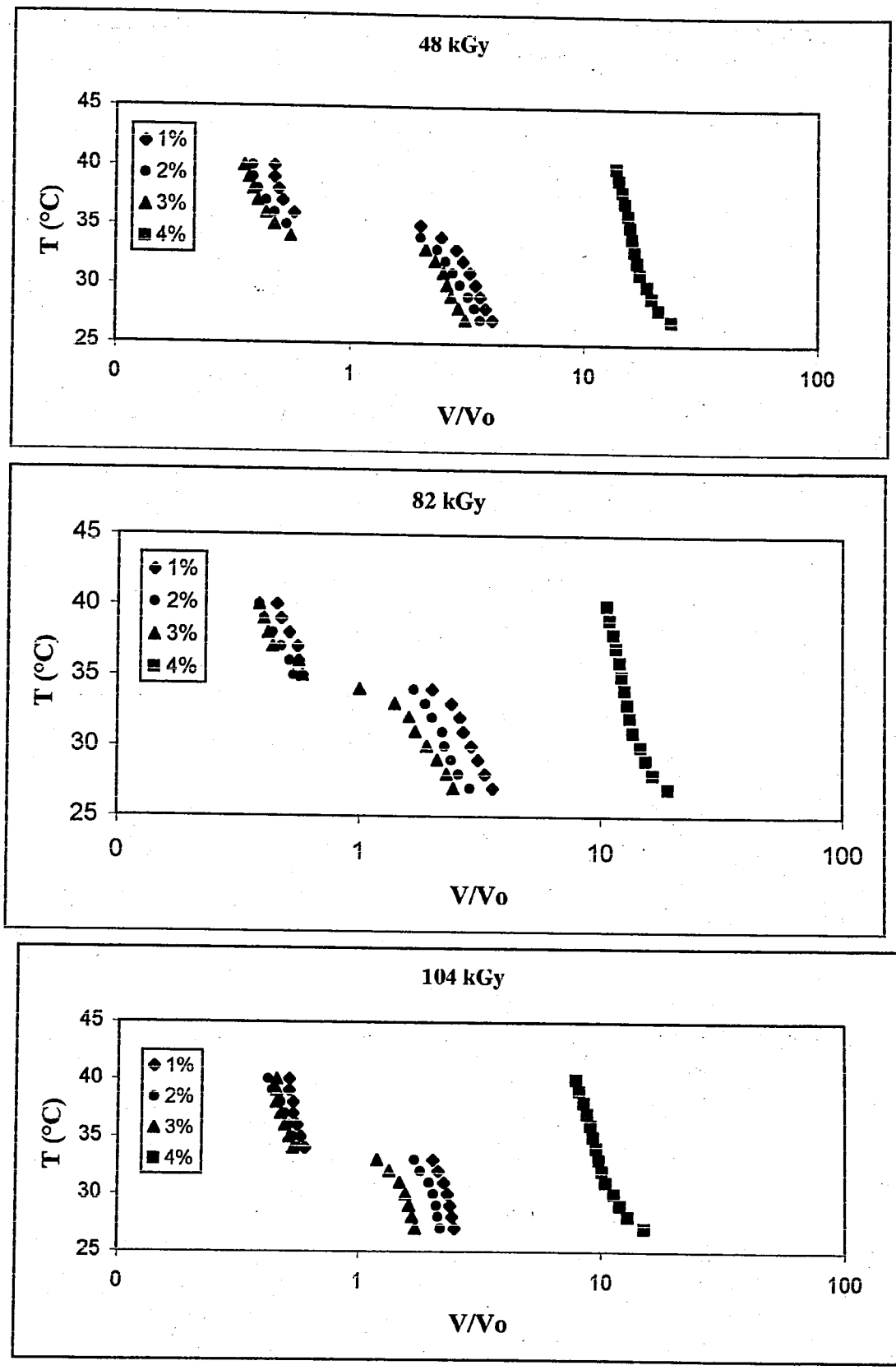


Figure 4.15. Variation of temperature dependence of the equilibrium swelling ratios of NIPAAm-MA with maleic acid content (mole per cent)

Table 4.5. The LCST values of P(NIPAAm/IA) and P(NIPAAm/MA) hydrogels

Gel name	LCST of P(NIPAAM/IA)	LCST of P(NIPAAM/MA)
PNIPAAm-1	33	33
P(NIPAAm/diprotic acid)-1	35	34
P(NIPAAm/diprotic acid)-2	36	35
P(NIPAAm/diprotic acid)-3	37	36
P(NIPAAm/diprotic acid)-4	34	34
P(NIPAAm/diprotic acid)-5	35	34
P(NIPAAm/diprotic acid)-6	36	35
P(NIPAAm/diprotic acid)-7	33	34
P(NIPAAm/diprotic acid)-8	34	34
P(NIPAAm/diprotic acid)-9	35	34

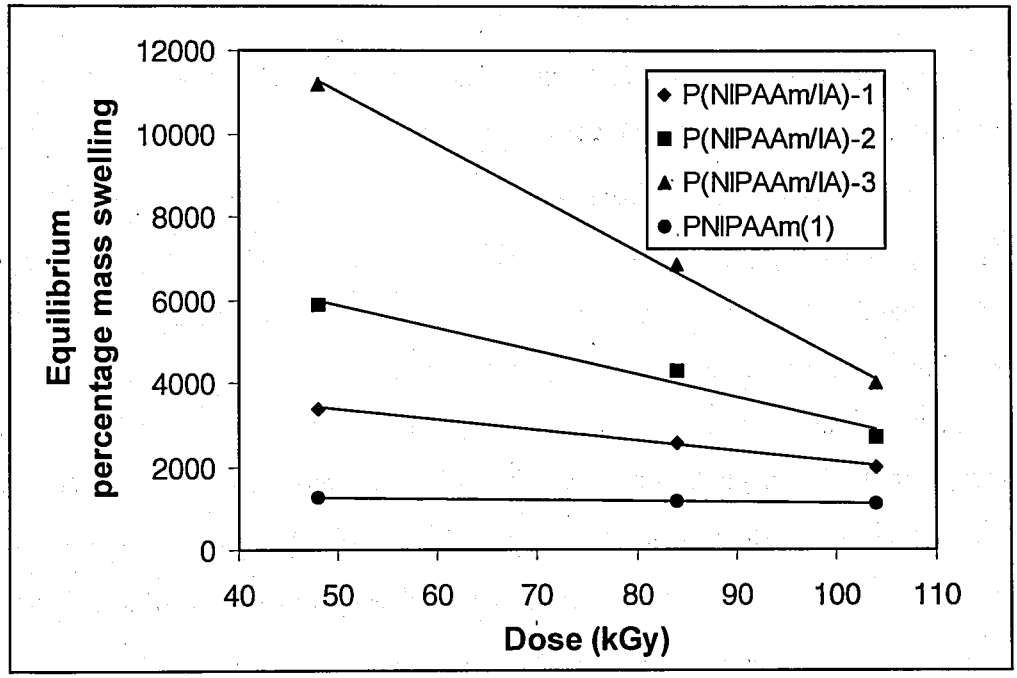


Figure 4.16. Variation of equilibrium percentage mass swelling with irradiation dose

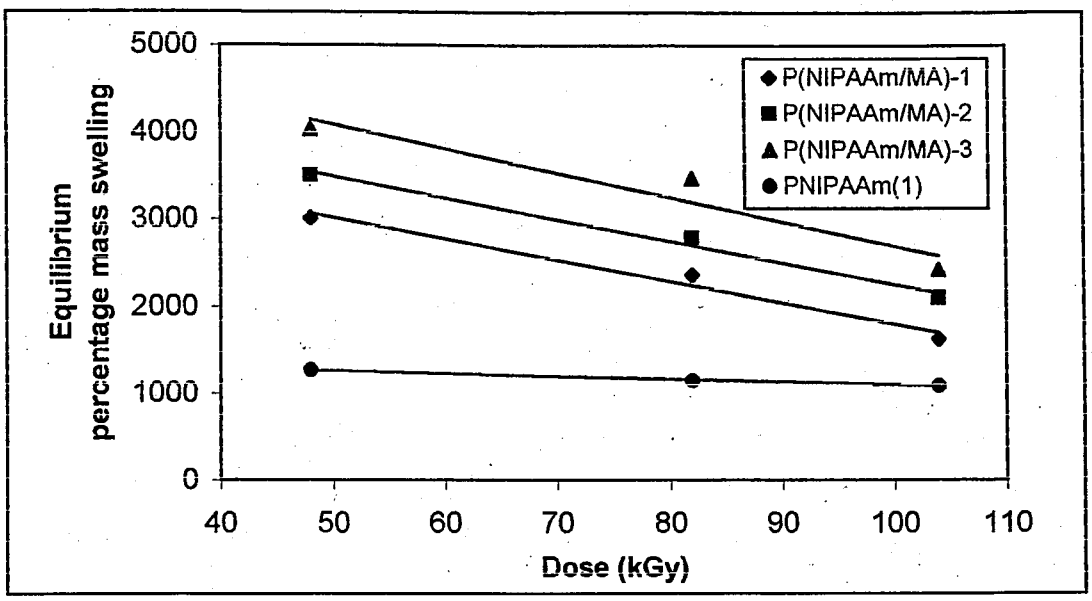


Figure 4.17. Variation of equilibrium mass swelling per cent with irradiation dose

4.1.4. Determination of M_c and ν_e Values

One of the basic parameters that describe the structure of an electrolyte and non-electrolyte hydrogels is the number average molecular weight between cross-links ($\overline{M_c}$). This describes the average molecular weight of polymer chains between two consecutive junctions. These junctions may be chemical cross-links, physical entanglements, crystalline regions, or even polymer complexes. Several theories have been proposed to calculate the molecular weight between cross-links in polymeric networks. The earliest theory to describe the equilibrium swelling characteristics of networks was developed by Flory and Rehner for a cross-linked polymer system where the polymer chains are reacted in the solid state, and the macromolecular chains exhibit a Gaussian distribution. The Flory-Rehner theory is used to determine $\overline{M_c}$, effective crosslinking densities of polymer networks (ν_e) and polymer-solvent interaction parameter (χ). The Flory-Rehner theory consists of the elastic, mixing, and ion contributions [28,50]. The analysis of the terms in the Flory-Rehner Equation shows that the influence of χ becomes minor for charged hydrogels at high degrees of swelling [82].

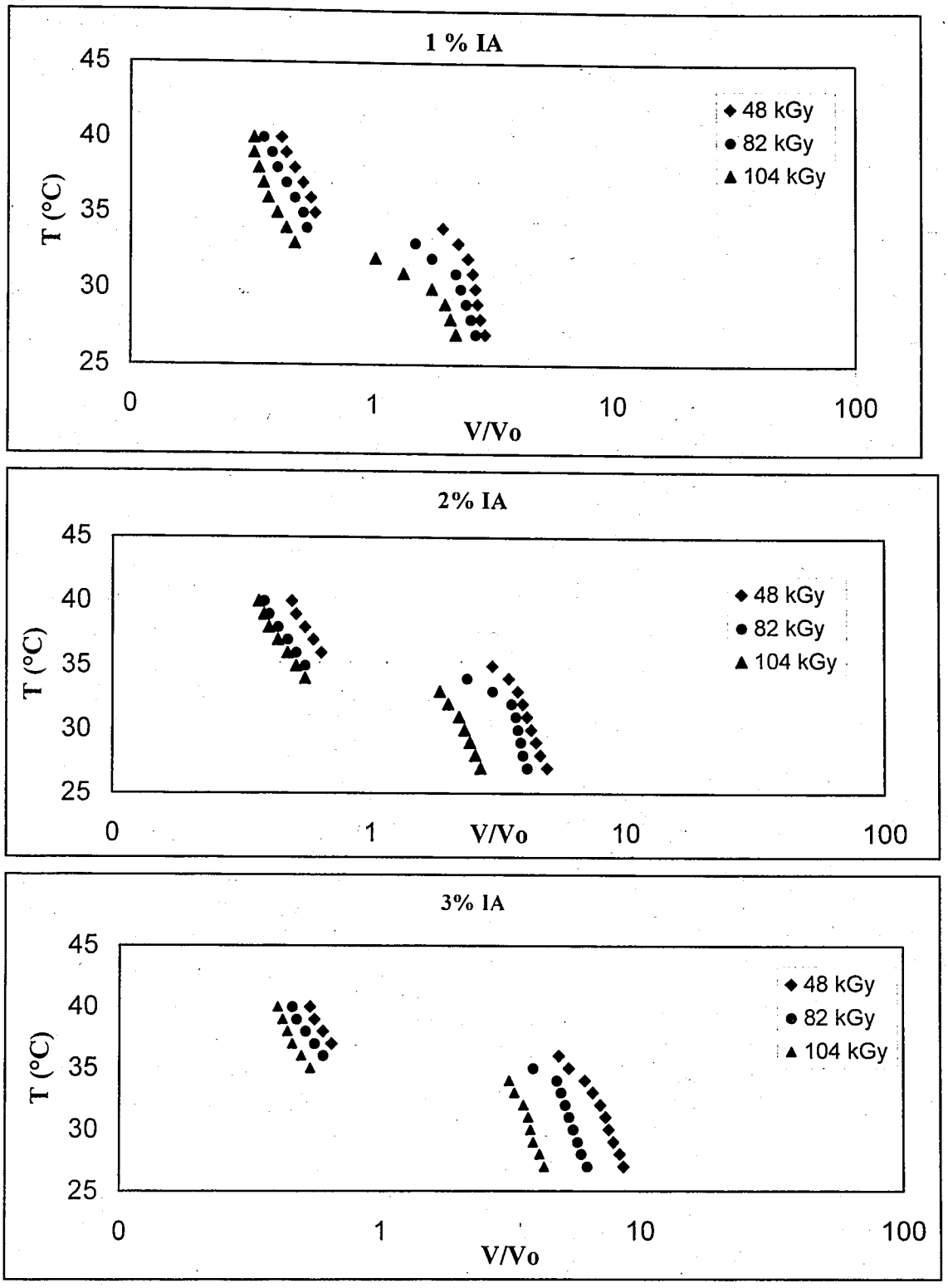


Figure 4.18. Variation of temperature dependence of the equilibrium swelling ratios of P(NIPAAm-IA) hydrogels with irradiation dose

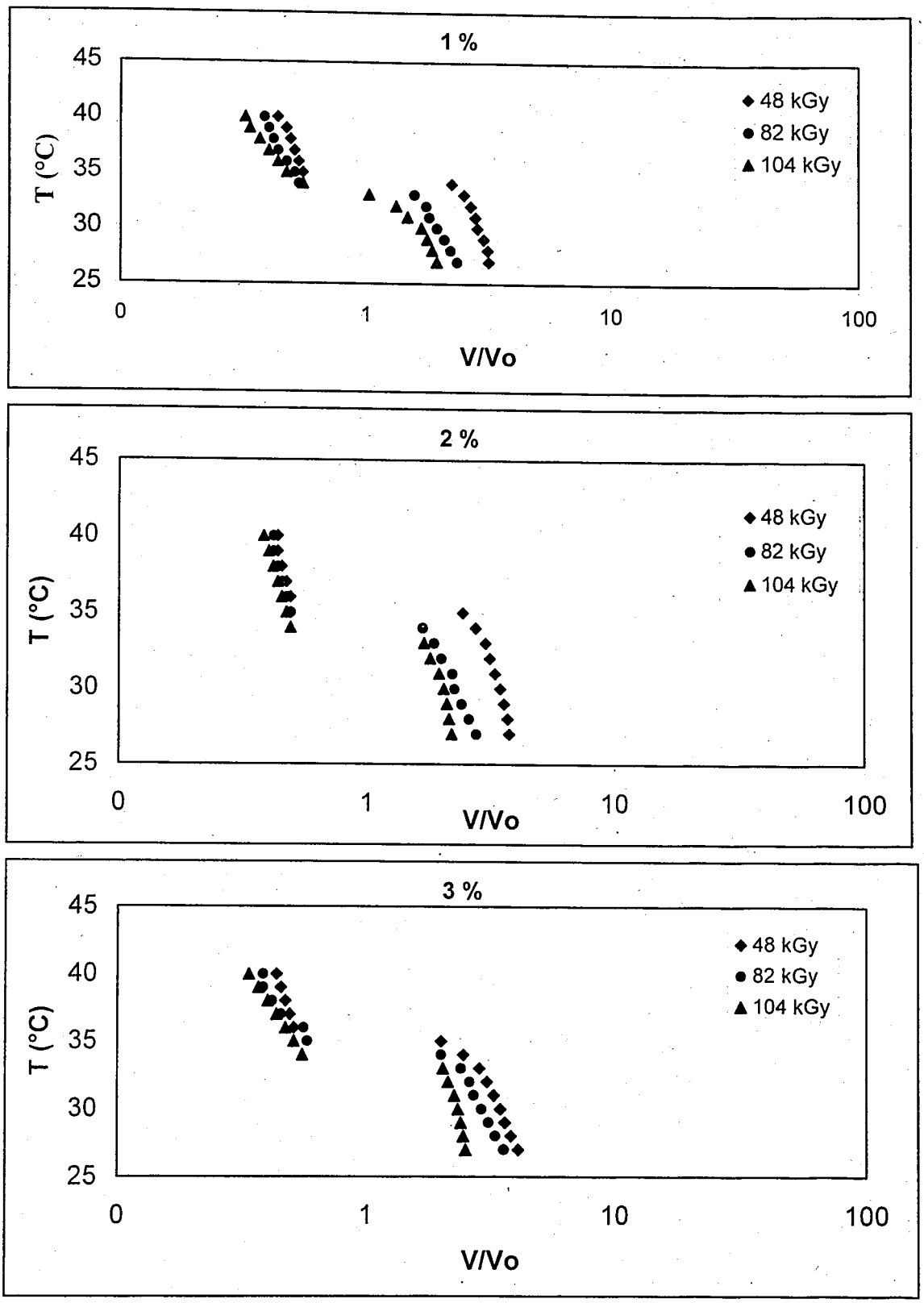


Figure 4.19. Variation of temperature dependence of the equilibrium swelling ratios of NIPAAm-MA with irradiation dose

In this study, it was assumed that the fraction of charged structural units, i.e., weakly ionised IA and MA in the networks sufficiently low to have a negligible effect on the mixing term; so, to obtain a reasonable value of χ , the Flory-Huggins theory with a Flory χ parameter fitted to network swelling data was used [28].

$$\chi = [\ln(1-v_{2m}) + v_{2m}] / v_{2m}^2 \qquad (4.6)$$

where v_{2m} is the volume fraction of the swollen gel in the equilibrium state. By applying the Flory-Rehner Equation to PNIPAAm gels, χ was calculated as a constant 0.53 in water [83]. The χ values were obtained by using Equation 4.6.

In this work, χ values of homopolymer and copolymer gels of NIPAAm were found to be 0.53 and 0.51, respectively; χ was held constant at 0.52 (see Appendix D). Equilibrium swelling values were used to calculate the effective crosslinking densities of polymer networks (v_e) by the following Equation [83]:

$$v_e = \frac{\ln(1-v_{2m}) + v_{2m} + \chi \quad v_{2m}^2}{V_1 v_2^0 \left[\left(\frac{v_{2m}}{v_2^0} \right)^{1/3} - 0.5 \left(\frac{v_{2m}}{v_2^0} \right) \right]} \qquad (4.7)$$

where v_{2m} is the volume fraction of the swollen gel in the equilibrium state and V_1 is the molar volume of the solvent (see Appendix E).

The effect of presence of diprotic acid on the network properties of polymer-solvent (water) interaction parameter is obvious from Tables 4.6-4.7. With increasing amount of ionizable constituent (IA or MA) in copolymer structure the average $\overline{M_c}$ values increase, whereas effective crosslinking densities (v_e) decrease. This indicates that IA does not act as a cross-linking agent.

Table 4.6. $\overline{M_c}$ and ν_e values of NIPAAm/IA copolymeric hydrogels

Gel name	$\overline{M_c}$ x 10^{-3} (g/mol)	ν_e x 10^5(mol/cm^3)
PNIPAAm (1)	37	3.0
P(NIPAAm/IA)-1	123	0.9
P(NIPAAm/IA)-2	245	0.5
P(NIPAAm/IA)-3	652	0.2
P(NIPAAm/IA)-4	99	1.1
P(NIPAAm/IA)-5	160	0.7
P(NIPAAm/IA)-6	303	0.4
P(NIPAAm/IA)-7	104	1.1
P(NIPAAm/IA)-8	102	1.1
P(NIPAAm/IA)-9	132	0.8

Table 4.7. $\overline{M_c}$ and ν_e values of NIPAAm/MA copolymeric hydrogels

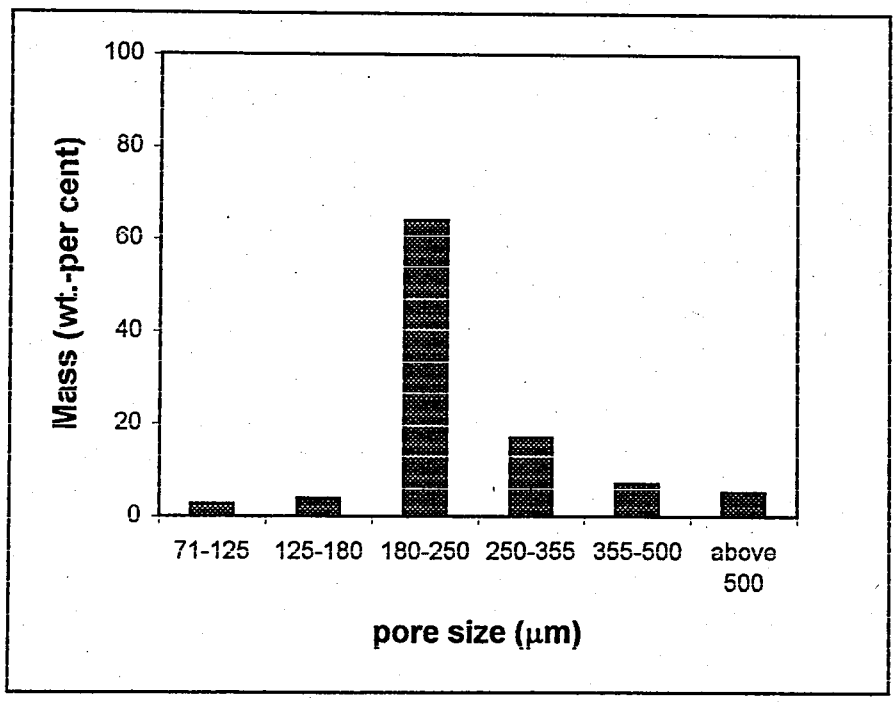
Gel name	$\overline{M_c}$ x 10^{-3} (g/mol)	ν_e x 10^5(mol/cm^3)
PNIPAAm(1)	37	3.0
P(NIPAAm/MA)-1	110	1.0
P(NIPAAm/MA)-2	127	0.7
P(NIPAAm/MA)-3	149	0.2
P(NIPAAm/MA)-4	97	1.1
P(NIPAAm/MA)-5	105	1.1
P(NIPAAm/MA)-6	126	0.8
P(NIPAAm/MA)-7	90	0.5
P(NIPAAm/MA)-8	97	1.1
P(NIPAAm/MA)-9	99	1.1

4.2. Preparation of Poly(N-isopropylacrylamide) Microspheres by Inverse-Suspension Polymerization

PNIPAAm gel beads prepared by inverse suspension polymerization were used for swelling, drug adsorption and release experiments. Rigid, transparent and spherical beads were obtained without using an external emulsifier and therefore, they are free from impurities affecting the drug delivery process.

The polymerization reaction was carried out at 100 rpm stirring rate and the beads obtained were in the size range of 71-500μm in the swollen states. The particles were sieved using ASTM sieves and those of selected fractions were used for further experiments.

Figure 4.20 shows a typical size distribution of PNIPAAm microspheres. The bar graph indicates that about 64 per cent of the microspheres were in the range of 180-250μm. A representative optical micrograph of the microspheres in this range is shown in Figure 4.21.



4.20. Size distribution of PNIPAAm microspheres

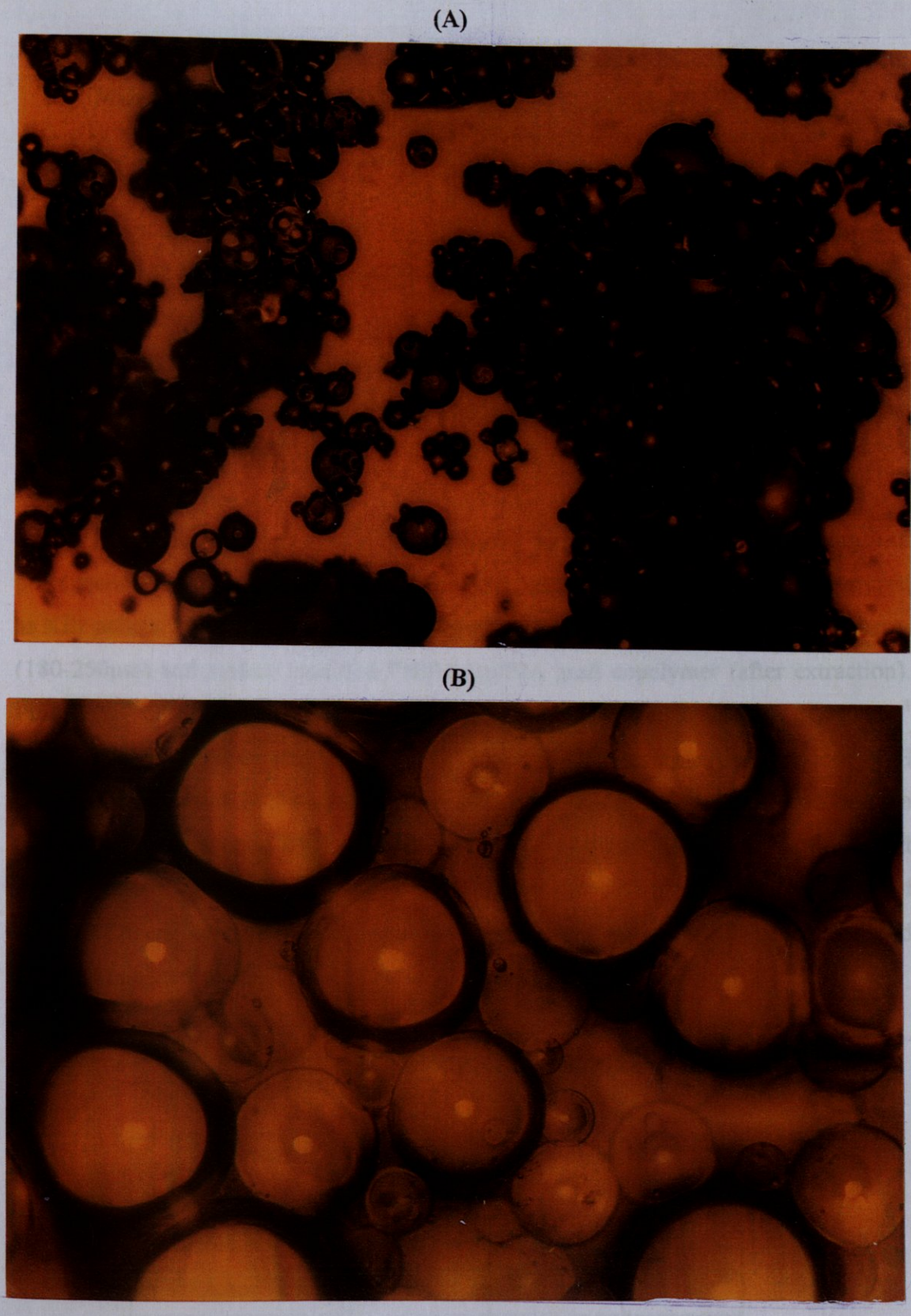


Figure 4.21. A representative optical micrograph of PNIPAAm microsphere obtained by inverse suspension polymerization technique: (A)in dry state (B) in swollen state

4.2.1. Preparation of Radiation-Induced Surface Modified PNIPAAm Microspheres with Itaconic Acid as a Graft Copolymer

The selected fraction (180-250μm) of PNIPAAm microspheres prepared by the inverse suspension polymerization technique was used for radiation-induced surface modified with IA as a PNIPAAm/PIA graft copolymer. The amount of IA in the copolymer was determined by titration of extract against NaOH (0.1 N) to phenolphthalein end point. The mole per cent of IA in the copolymeric hydrogels are summarized in Table 4.8. As the irradiation dose increases, IA content in the copolymeric gels increases.

FT-IR spectroscopy was also used to investigate the grafting of poly(itaconic acid) onto the surface of PNIPAAm microspheres. Samples were analyzed as KBr pellets. Figures 4.22 shows FT-IR spectrum of the pure PNIPAAm microspheres (180-250μm) and surface modified PNIPAAm/PIA graft copolymer (after extraction). Pure PNIPAAm microspheres showed characteristic amide I band at 1640 cm^{-1} (C=O streching) and amide II band at 1564 cm^{-1} (N-H bending). Also only one N-H streching band which is characteristics for secondary amides at 3435 cm^{-1} can be seen in the FT-IR spectrum of PNIPAAm microspheres. In the FT-IR spectrum of the graft copolymer with 9.8 per cent itaconic acid content, carbonyl (C=O) streching band of acid at 1720 cm^{-1} appeared which indicates the successful formation of surface modified PNIPAAm microspheres.

Table 4.8. Mole per cent of IA in the gel system

Gel Name	Irradiation Dose (kGy)	Mole % IA in the gel
P(NIPAAm/IA)-1	5	6.0
P(NIPAAm/IA)-2	24	8.7
P(NIPAAm/IA)-3	48	9.8

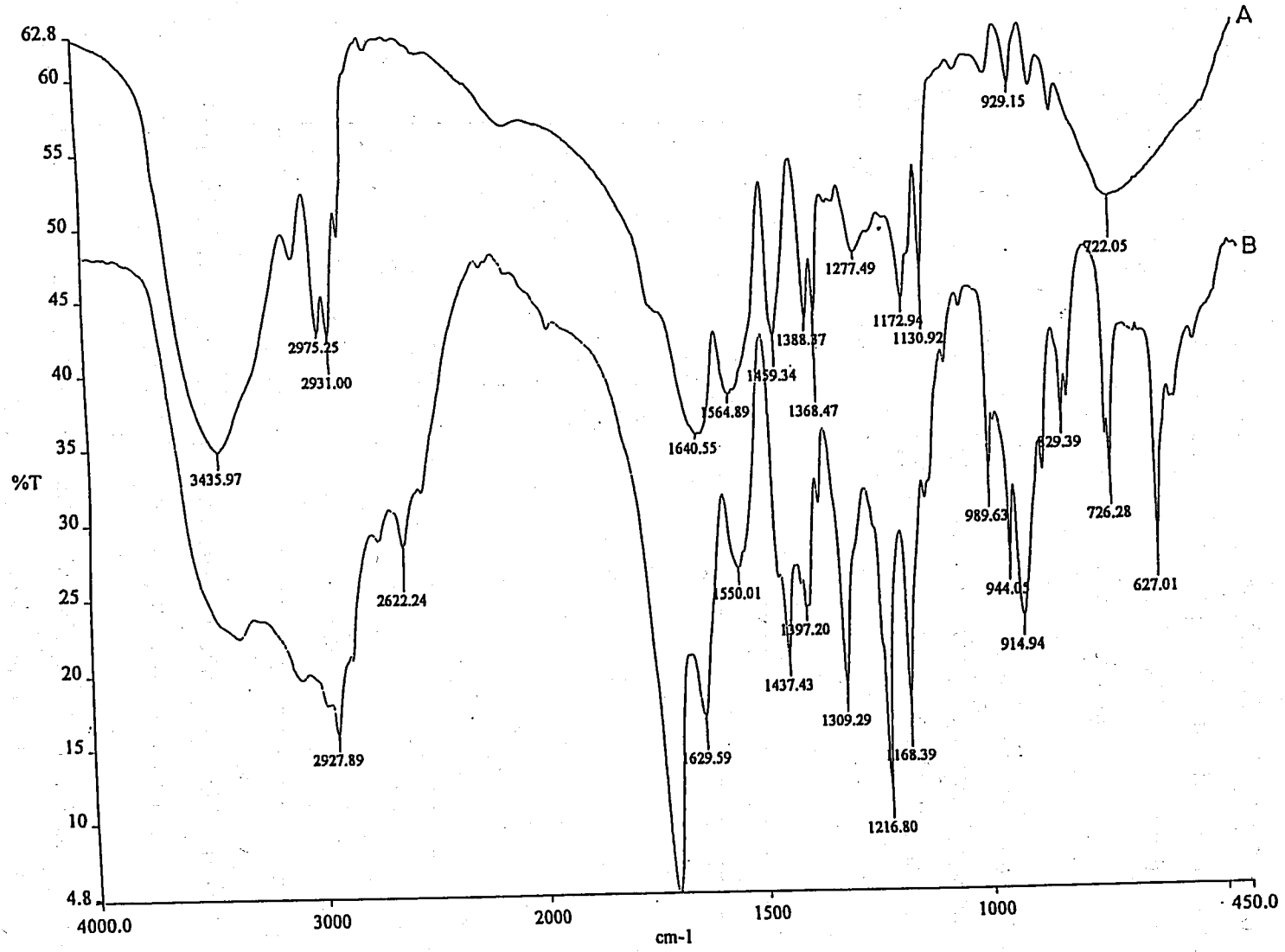


Figure 4.22. FT-IR spectrum of the pure PNIPAAm microspheres (180-250 μm) (A) and surface modified PNIPAAm/PIA graft copolymer (B) (after extraction)

4.3. Preparation of Poly(N-isopropylacrylamide) Gels by Solution Polymerization

In this work, the poly(N-isopropylacrylamide) gels (PNIPAAm) were also prepared by the solution polymerization technique. This involves the free-radical cross-linking copolymerisation of NIPAAm with a small amount of N,N'methylenebisacrylamide (MBA) in aqueous solution.

Ammonium peroxodisulfate (APS) and N,N,N',N'-tetramethylethylenediamine (TEMED) were, respectively, the initiator and the accelerator. Figure 4.23 shows the effect of temperature on the equilibrium swelling ratio for PNIPAAm gels prepared by solution polymerization

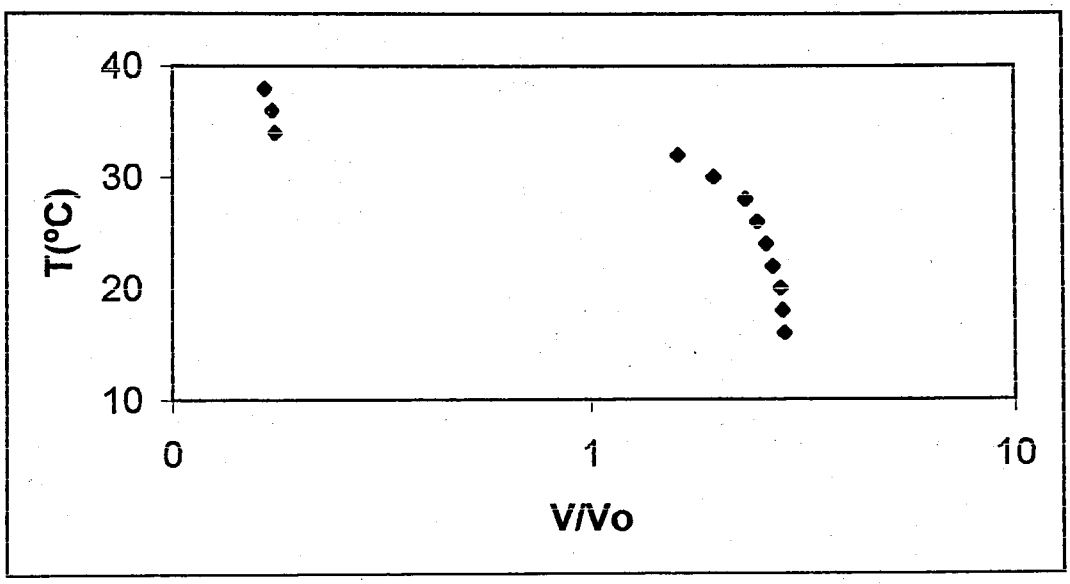


Figure 4.23. The effect of temperature on the equilibrium swelling ratio for PNIPAAm prepared by the solution polymerization technique

4.4. The Influence of the Preparation Techniques of Poly (N-isopropylacrylamide) Gels on the Swelling Properties

The influence of the preparation technique of PNIPA gels on their swelling properties is shown in Table 4.9. The results clearly indicate that the gels A, which were prepared by inverse suspension polymerization technique, swell in water much more

than both gels B which were obtained by solution polymerization and the gels C, which were obtained by radiation induced polymerization technique. The water absorption capacities of gels B and gels C are almost same.

When the swelling rates of the gels A, B and C were compared, polymer gels in the form of beads absorb water more rapidly than the gels in the form of rods (Gel B and C).

For example, 1 g of gel beads in the dried state absorbs 10 ml of water in 10 min, whereas the same amount of gel in the form of rods absorbs only 1 ml of water in the same interval. The higher water absorption capacity and rapid swelling feature of PNIPAAm gel beads make them the first choice in the drug delivery process.

Table 4.9. The influence of the preparation technique of PNIPAAm gels on their swelling properties

Swelling T(°C)	Water absorption capacity (ml of water in swollen gel/ g of dry gel)		
	A	B	C
4	32	27	28
25	26	23	23

4.5. Controlled Release Behaviours of Gels

4.5.1. Controlled Release Behaviours of P(NIPAAm/IA) Hydrogels Prepared by Radiation Induced Polymerization

4.5.1.1. Release of Methylene Blue from the Hydrogels. Methylene Blue (MB) was used as the model drug for the investigation of drug release behaviour of PNIPAAm and P(NIPAAm/IA) hydrogels. MB is a positively charged dye molecule. It is a molecular weight of 319.86.

The released MB was analyzed at 664 nm by ultraviolet spectrophotometer. As shown in Figure 4.24, the maximum absorbance of MB was measured at 664 nm. Figure 4.25 shows calibration line of MB. Itaconic acid has two carboxyl groups as shown in Figure 3.3. The interactions between cationic groups of MB and the carboxyl groups of itaconic acid leads to specific MB adsorption. The non-specific MB adsrorption is due to physically bonding of the positively charged MB to completely ionized hydrogel at pH 7.4. The amounts of total (specific and non-specific) MB uptake into one gram of dry PNIPAAm and P(NIPAAm/IA) hydrogels are given in Figure 4.26. As can be seen from the figure, the MB uptake into the hydrogels increases with increase in IA content. Since PNIPAAm is non-ionic hydrogel, ionizable groups on the polymer were increased by addition of itaconic acid groups to the hydrogel. These hydrogels have many carboxyl groups which can increase interactions between cationic groups of cationic MB and carboxyl groups of hydrogel. As shown in Figure 4.27, the MB uptake into the hydrogels decreases with increase in the irradiation dose. The pores (molecular mesh) in this hydrogel can become smaller as the irradiation dose increases.

In order to determine the amount of non-specific adsorbed MB, hydrogels are placed in pH 7.4 phosphate buffer solution. The release profiles of MB in NIPAAm/IA copolymeric gels in phosphate buffer solution of pH 7.4 at 37°C were shown in Figure 4.28 and Figure 4.29. The amount of the percentage release of MB at pH 7.4 was calculated from the following equation :

$$\text{The release per cent of non-specific adsorbed MB} = (w_t/w_{total})*100 \quad (4.8)$$

where, w_t is the weight of released MB at time t and w_{total} is the total weight of specific and non-specific adsorbed MB in the gel system.

Figure 4.28 shows that the amount of release percent for non-specific adsorbed MB was higher for pure PNIPAAm hydrogel than those for P(NIPAAm/IA) hydrogels. The amount of release percent decreases with the increase of IA content in the gel system. This can be explained by the increase in the diffusional path due to the high swelling of P(NIPAAm/IA) hydrogels. While 98 per cent of MB was released from

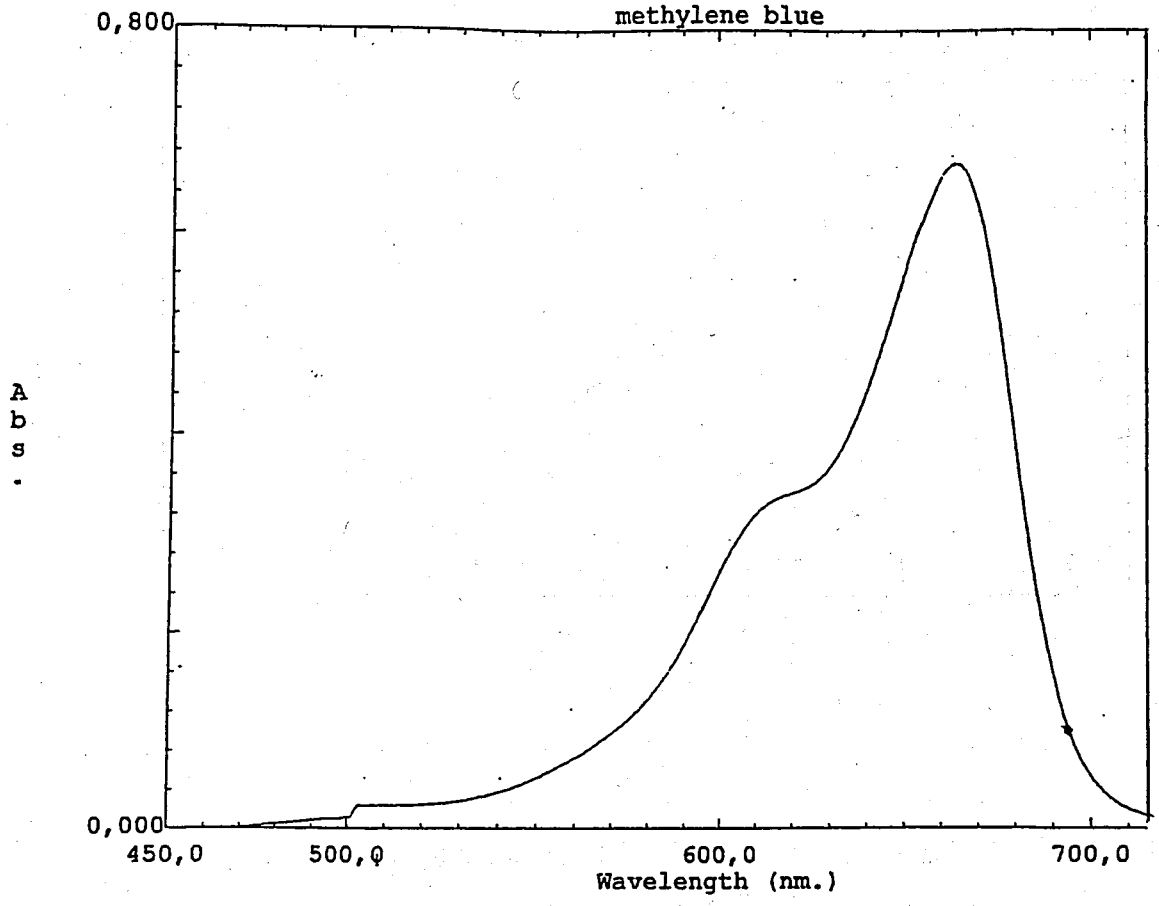


Figure 4.24. UV-Visible spectrum of methylene blue

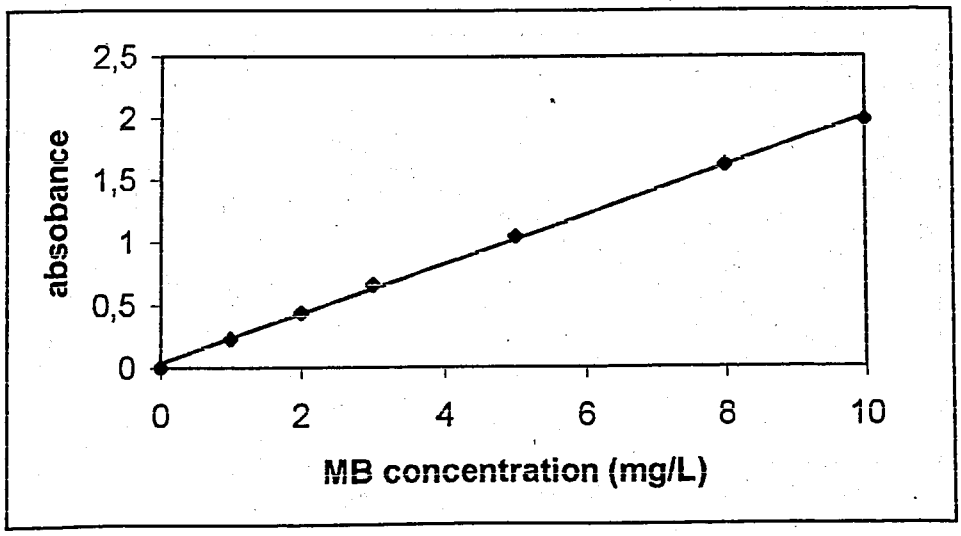


Figure 4.25. Calibration line of MB

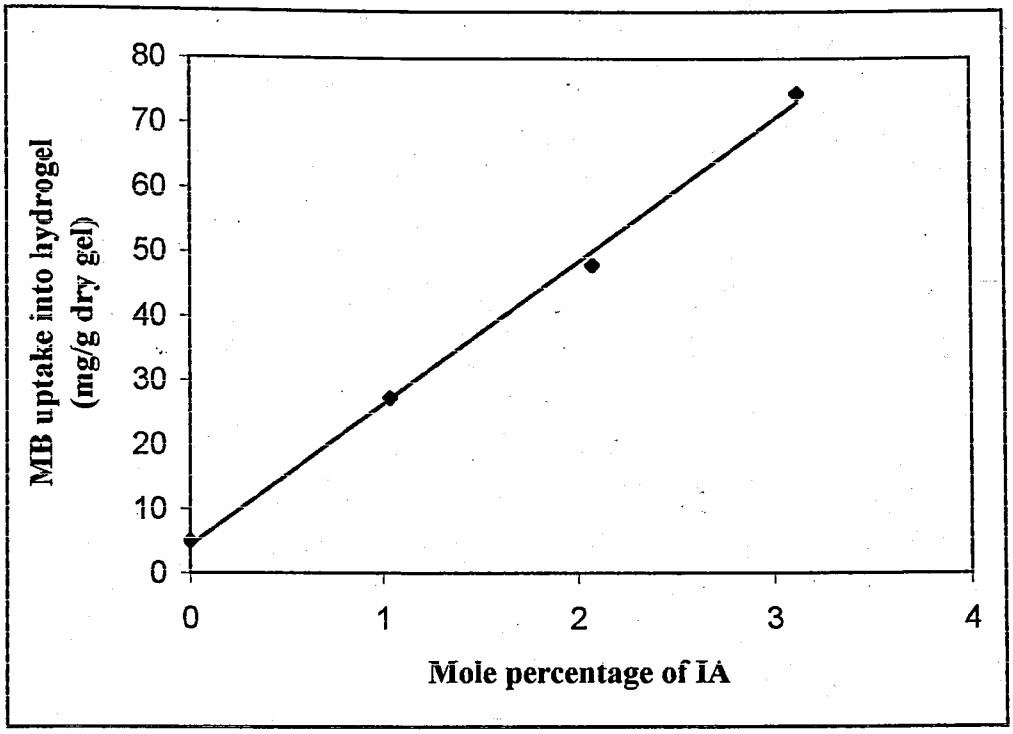


Figure 4.26. Variation of total Methylene Blue (MB) with itaconic acid (IA) content in the gel system prepared at the irradiation dose of 48 kGy

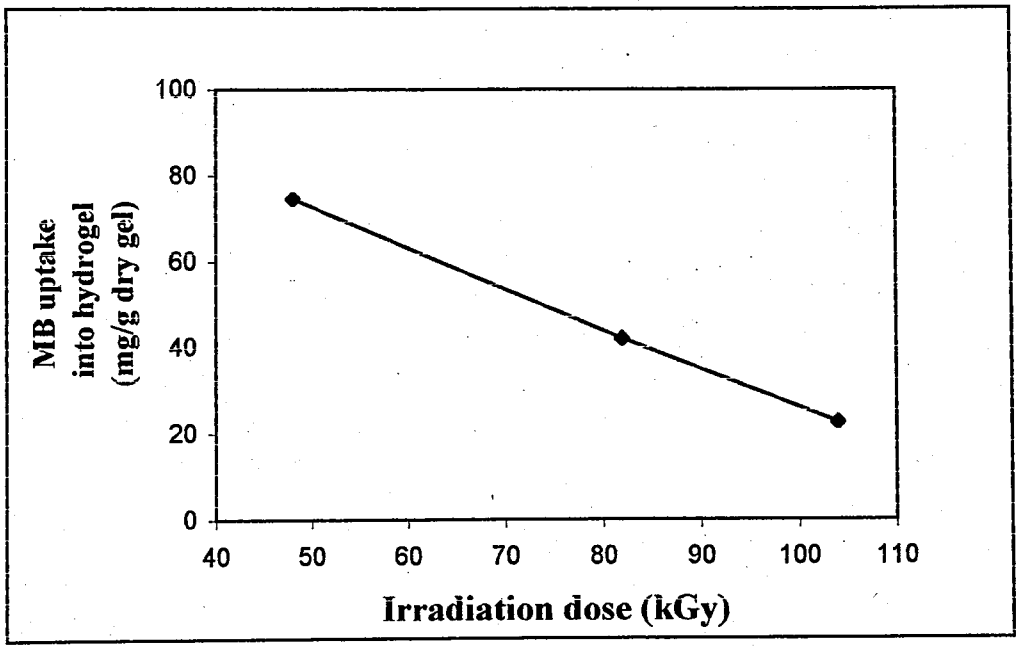


Figure 4.27. Variation of total Methylene Blue (MB) with irradiation dose (kGy), mole per cent of itaconic acid is 3.0

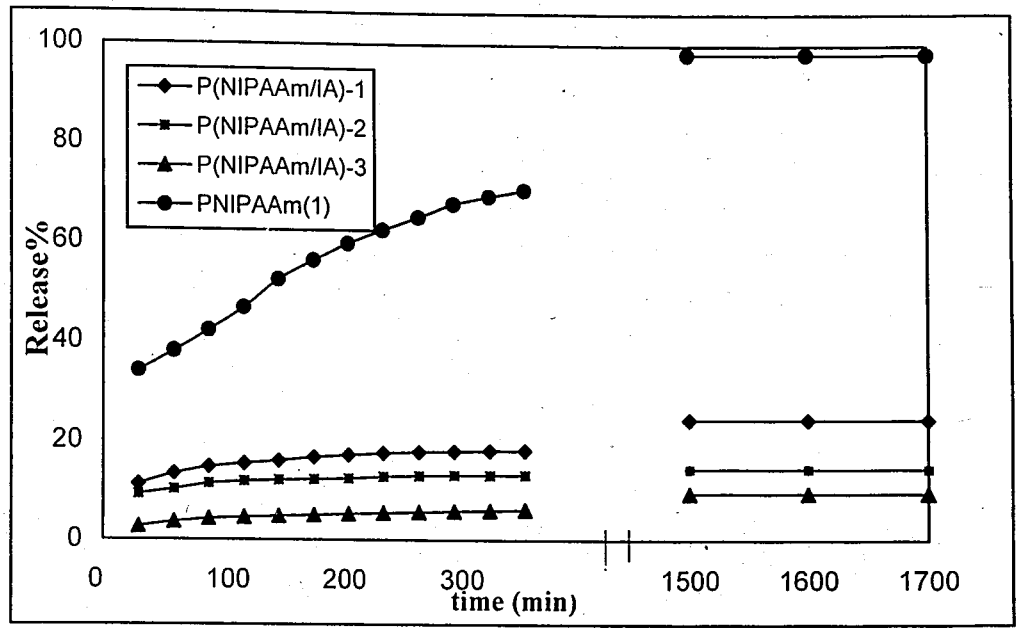


Figure 4.28. Release percent of non-specific adsorbed MB from P(NIPAAm/IA) hydrogels prepared at the irradiation dose of 48 kGy in phosphate buffer solution of pH 7.4 at 37°C

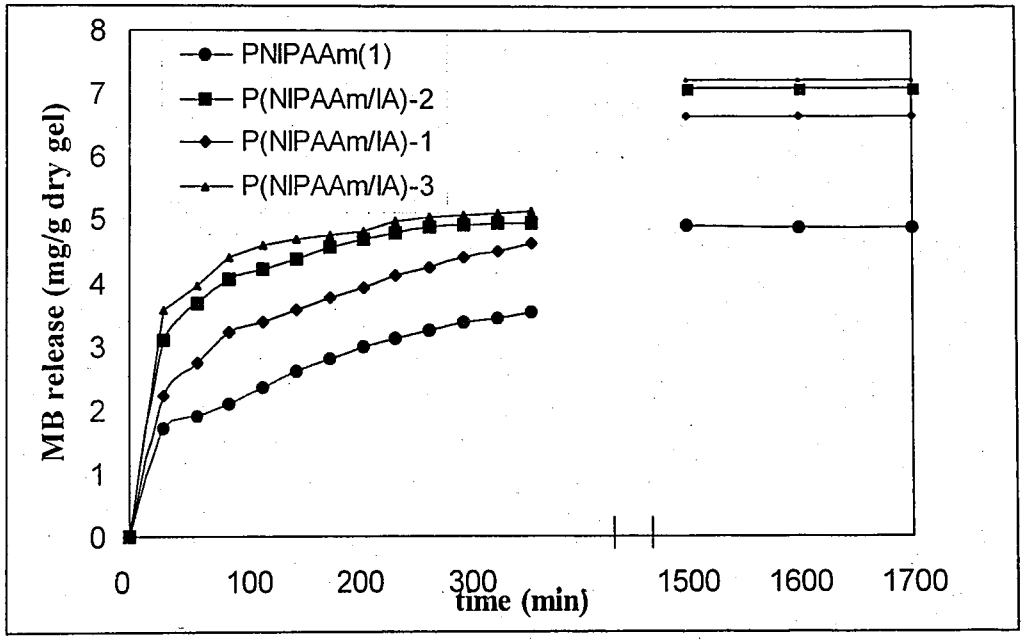


Figure 4.29. The release amount of the non-specific drug per unit mass of dry P(NIPAAm/IA) hydrogels prepared at 48 kGy (mg/g dry gel) in phosphate buffer solution of pH 7.4 at 37°C

PNIPAAm hydrogels, this value decreased to 9.53 per cent with increasing IA content to 3 per cent in the gel system.

However, as illustrated in Figure 4.29, the amount of the released drug per unit mass of dry P(NIPAAm/IA) hydrogels was higher than pure PNIPAAm hydrogel. At the constant temperature of 37°C, the amount of the equilibrium drug released increased with increase in IA content, because the equilibrium swelling ratio of NIPAAm/IA copolymeric hydrogels increased with increasing IA content in the gel system.

The incomplete release of MB from P(NIPAAm/IA) hydrogels at pH 7.4 was expected to be due to binding of the cationic MB to the polymer. The difference between the total and non-specific MB uptake is therefore taken to be equal to the amount of specific adsorbed MB in the hydrogel. The controlled release of specific adsorbed MB from P(NIPAAm/IA) hydrogels was investigated primarily at pH 5.5 since the second dissociation constants (pK_{a2}) of IA is around 5.44. The drug release was followed until equilibrium and then the hydrogel was transferred into MB free buffer at pH 4 (pK_{a1}= 3.85). Finally, the hydrogels were placed into pH 2 buffer for the complete release of drug. The percentage release of MB with time at each hydrogel system given in Figure 4.30-4.32. The percentage release of specific adsorbed MB at pH 5.5, 4.0 and 2.0 were calculated from the following equation:

$$\text{The amount of release per cent of specific adsorbed MB} = \frac{w_t}{w_{sp}} x100 \qquad (4.9)$$

where, w_t is the weight of released MB at time t and w_{sp} is the total weight of specific adsorbed MB in the gel system.

Figure 4.33 shows the variation of the total, non-specific and specific MB uptake with itaconic acid content in the gel system. The results obtained from Table 4.10 and Figures 4.30-4.33 illustrate that the release amount of the specific adsorbed drug from the hydrogels increases with the increasing IA content in the gel system in all buffer solutions and the maximum equilibrium release amount was realized at pH=2.

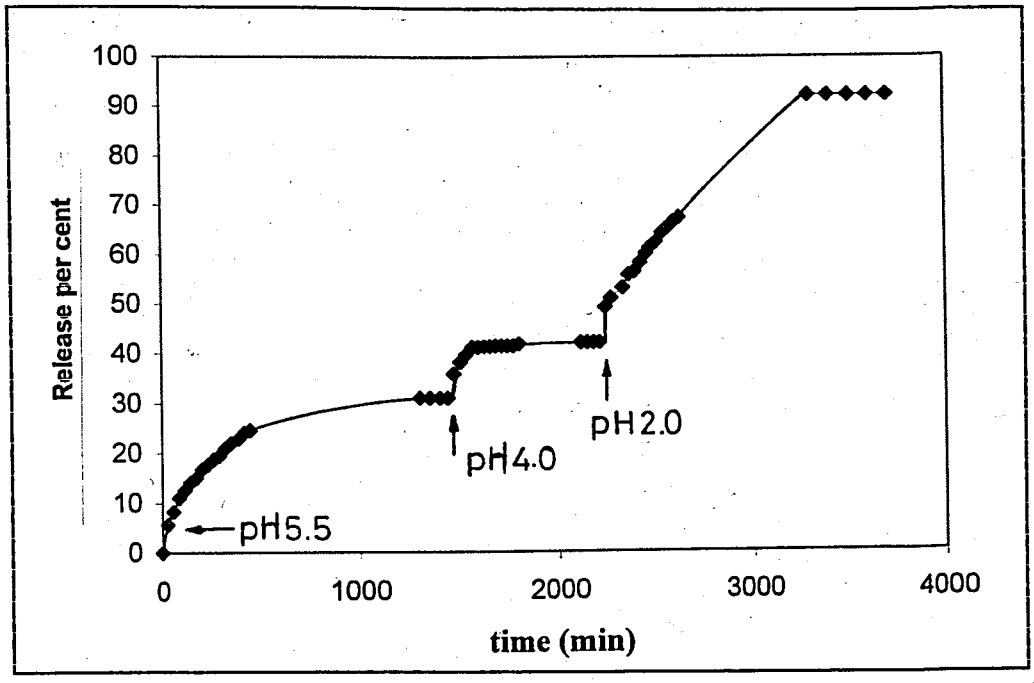


Figure 4.30. Release per cent of specific adsorbed MB from P(NIPAAm/IA)-1

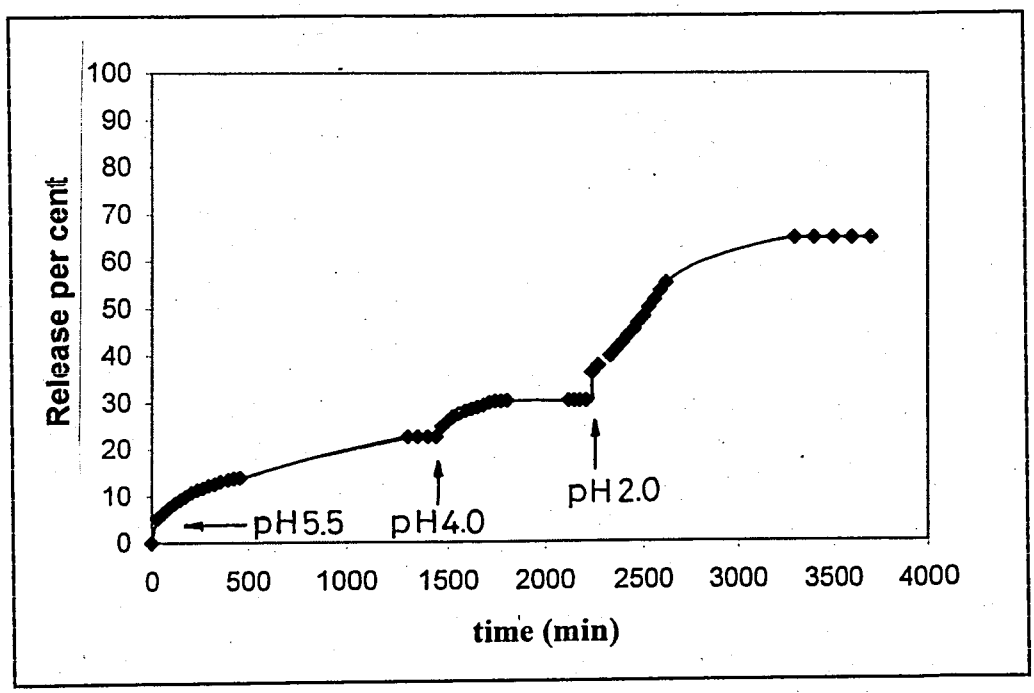


Figure 4.31. Release per cent of specific adsorbed MB from P(NIPAAm/IA)-2

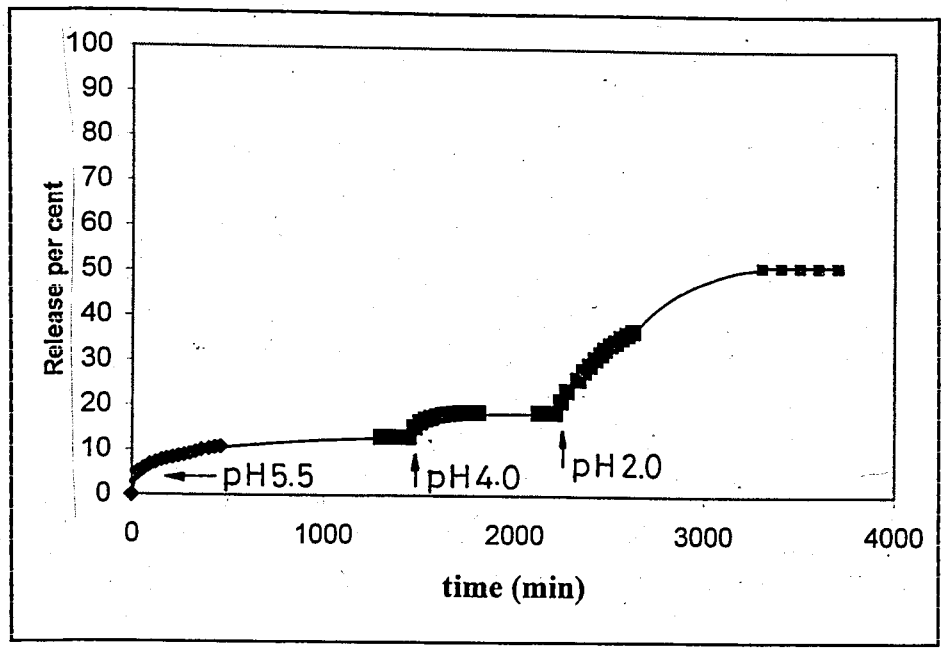


Figure 4.32. Release of specific adsorbed MB from P(NIPAAm/IA)-3

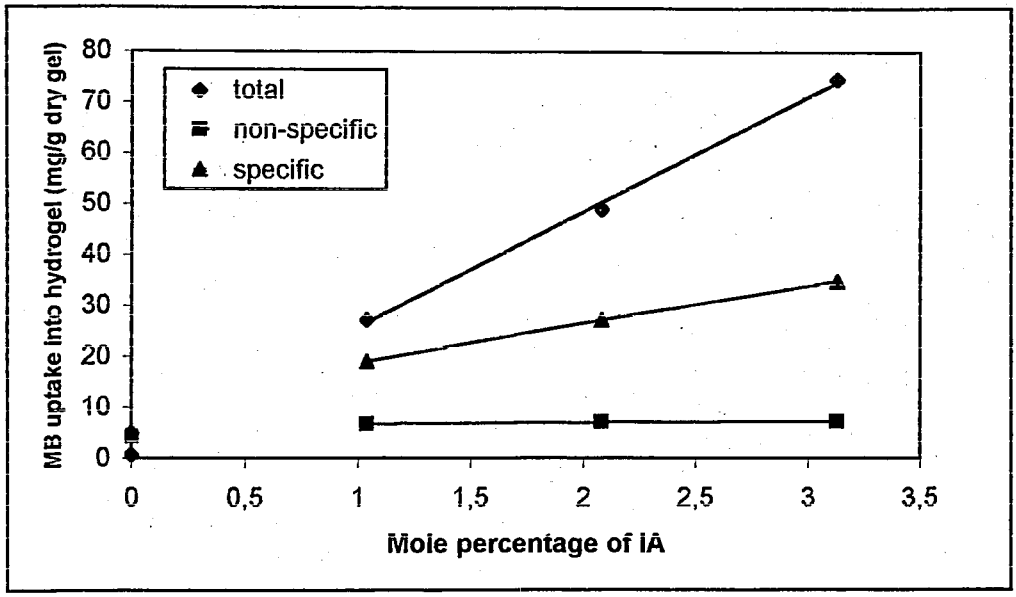


Figure 4.33. Variation of total, non-specific and specific adsorbed MB with itaconic acid content in the gel system prepared at the irradiation dose of 48 kGy

However, MB is not completely released and some portions are entrapped within the gel. In other words, the more IA content in the gel system, the more MB bound to the polymer. Even at pH 2, the MB is not completely released from the gel. In Table 4.10. $w_{non\text{-}released}$ shows the amount of MB bound to polymer after completing the release of MB at pH 2. $w_{non\text{-}released}$ was calculated from the following equation:

$$w_{non\text{-}released} = w_{total} - w_{non\text{-}specific} - w_{specific} \quad (4.10)$$

In the release of specific adsorbed MB from the hydrogel, one anionic carboxylate group was protonated at pH 5.5. Then the second carboxylate group was protonated at pH 4 and the copolymeric network was in a more collapsed state. The complete release of MB was observed at pH 1. While 92 per cent of MB was released from P(NIPAAm/IA)-1 at pH 2, this value decreased to 52 per cent with increasing IA content to 3 (mole per cent) in the gel structure.

Table 4.10. Variation of total, non-specific and specific adsorbed MB with itaconic acid content in the gel system prepared at the irradiation dose of 48 kGy.

Gel name	$m_{non\text{-}specific}$ (mg/g dry gel)	$m_{specific}$ (mg/g dry gel)			m_{total} (mg/g dry gel)	$m_{non\text{-}released}$ (mg/g dry gel)
		pH 5.5	pH 4	pH 2		
PNIPAAm-1	4.3	-			4.98	-
P(NIPAAm/IA)-1	6.6	5.9	2.1	11.0	27.3	1.6
P(NIPAAm/IA)-2	7.1	6.1	2.3	18.8	49.0	14.7
P(NIPAAm/IA)-3	7.2	4.9	1.9	27.9	74.6	32.6

4.5.1.2. Release of Sildenafil Citrate (Viagra) from the Hydrogels. Sildenafil citrate (Viagra), an oral therapy for erectile dysfunction, is the citrate salt of sildenafil, a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). Sildenafil citrate is a molecular weight of 666.7 [84]. The novality of this compound its oral route of delivery and eficacy across a broad

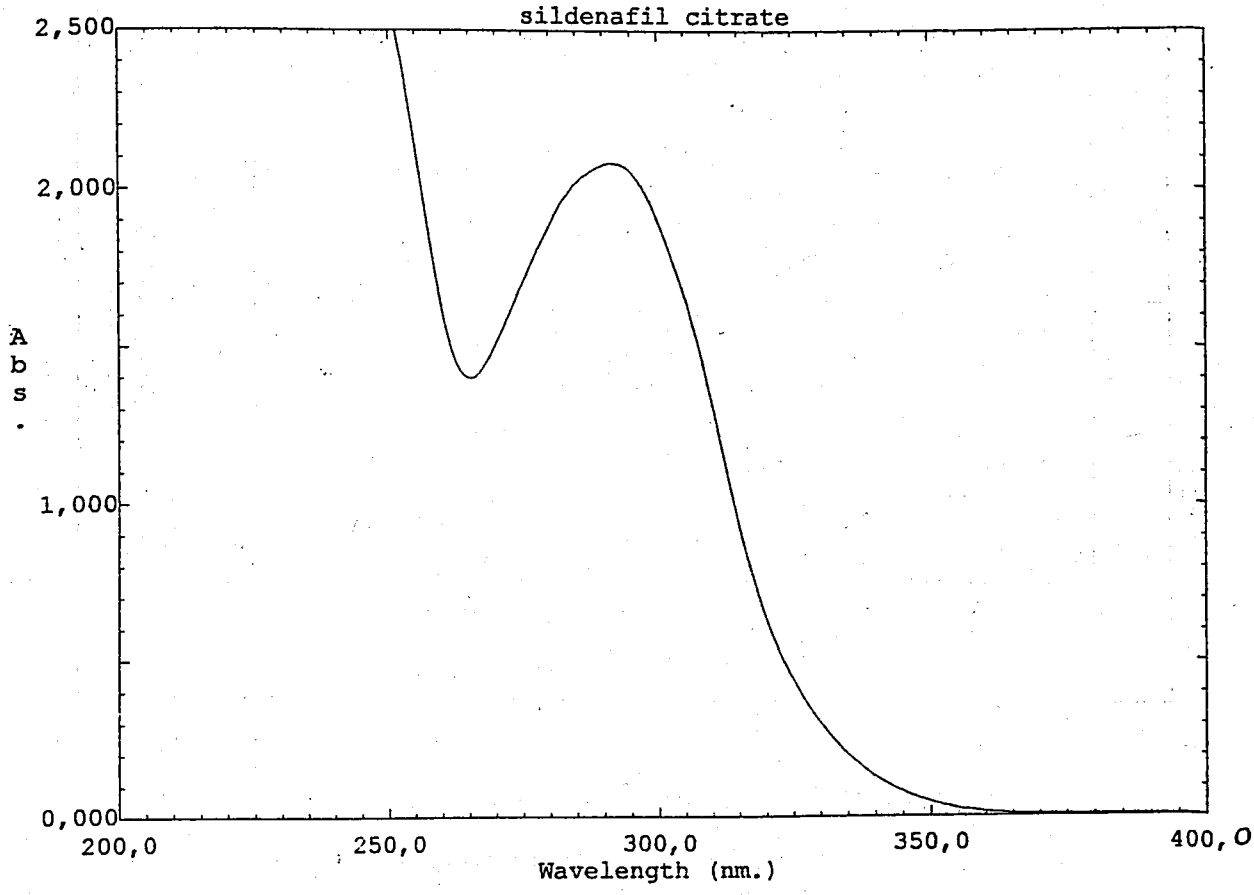
range of etiologies. Sildenafil citrate (Viagra) was used as a model drug for the investigation of drug release behaviour of PNIPAAm and P(NIPAAm/IA) hydrogels.

The released viagra (VG) was analyzed at 291 nm by ultraviolet spectrophotometer. As shown in Figure 4.34, the maximum absorbance of VG was measured at 291 nm. Figure 4.35 shows calibration line of VG. The amounts of total (specific and non-specific) VG uptake into one gram of dry PNIPAAm and P(NIPAAm/IA) hydrogels are given in Figure 4.36. As can be seen from the figure, the VG uptake into the hydrogels increases with increase in IA content. Since PNIPAAm is non-ionic hydrogel, ionizable groups on the polymer were increased by addition of groups on the NIPAAm monomer. These hydrogels have many carboxyl groups which can be result in an increase interactions between VG and carboxyl groups of hydrogel.

As shown in Figure 4.37, the VG uptake into the hydrogels decreases with increase in the irradiation dose. The pores (molecular mesh) in this hydrogel can become smaller as the irradiation dose increase.

The release profiles of VG in NIPAAm/IA copolymeric gels in phosphate buffer solution of pH 7.4 at 37°C were shown in Figure 4.38 and Figure 4.39. The amount of the percentage release of VG at pH 7.4 was calculated from the Equation 4.8. Figure 4.38 shows that the amount of release percent for non-specific adsorbed VG was higher for pure PNIPAAm hydrogel than those P(NIPAAm/IA) hydrogels. The amount of release percent decreases with the increase of IA content in the gel system. This can be explained by the increase in the diffusional path due to the high swelling of P(NIPAAm/IA) hydrogels. While 76 per cent of VG was released from PNIPAAm hydrogels this value decreased to 13 per cent with increasing IA content to 3 mole per cent in the gel system.

However, as illustrated in Figure 4.39, the amount of the released drug per unit mass of dry P(NIPAAm/IA) hydrogels was higher than pure PNIPAAm hydrogel. At the constant temperature of 37°C, the amount of the equilibrium drug released increased increased with increase in IA content, because the equilibrium swelling ratio of



4.34. UV-Visible spectrum of sildenafil citrate (VG)

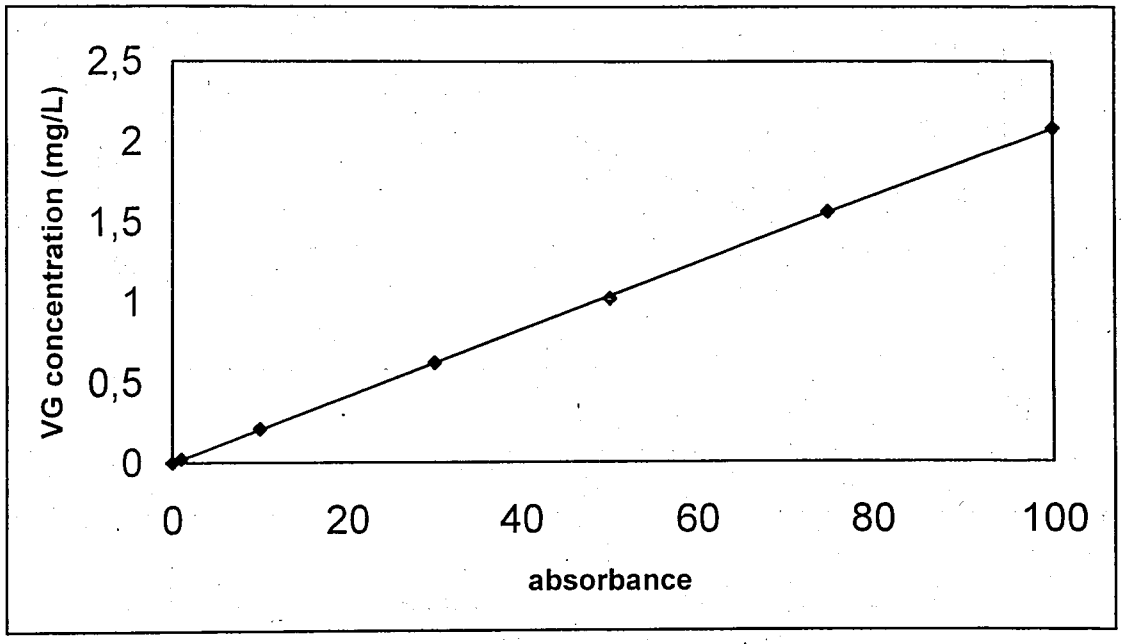


Figure 4.35. Calibration line of VG

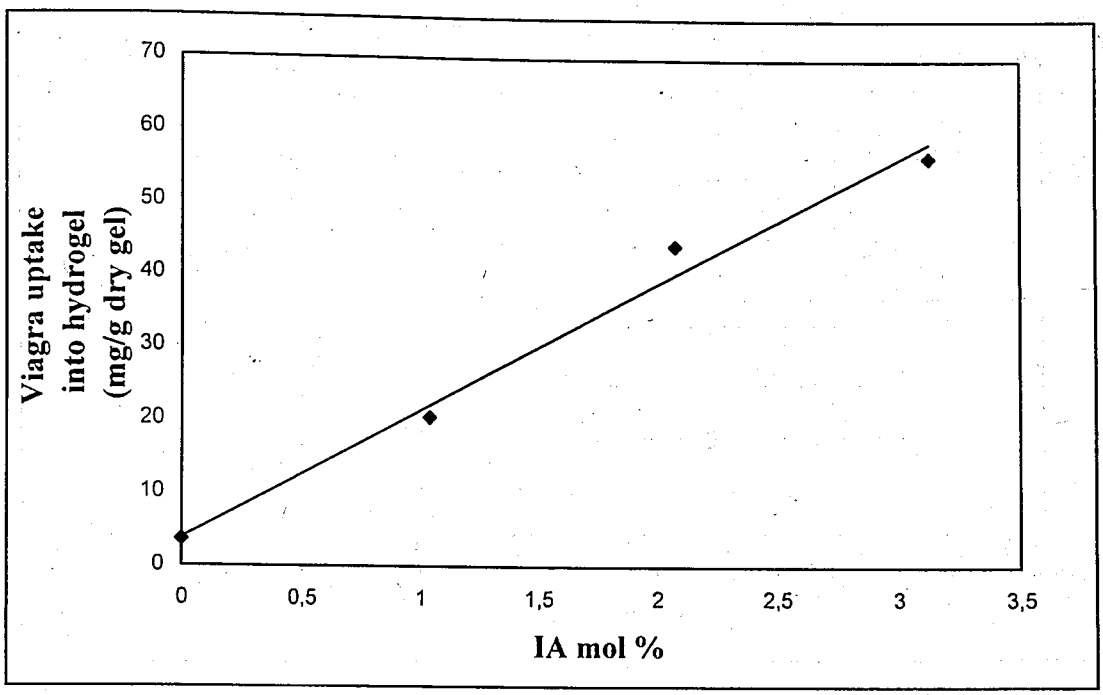


Figure 4.36. Variation of total Viagra (VG) with itaconic acid (IA) content in the gel system prepared at the irradiation dose of 48 kGy

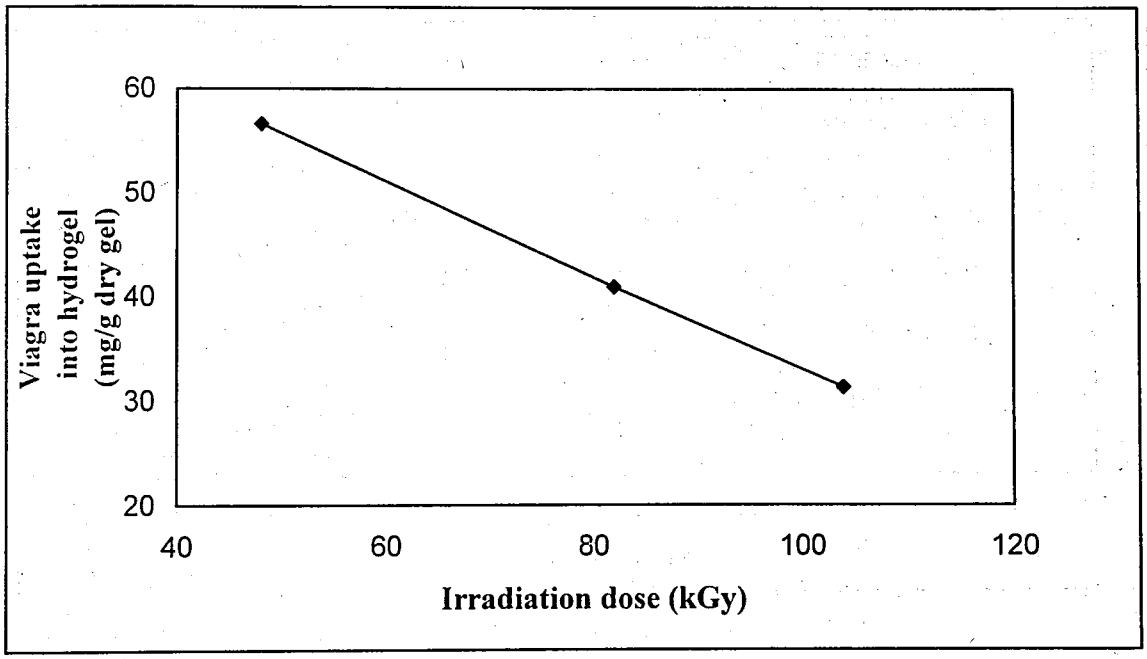


Figure 4.37. Variation of total Viagra (VG) with irradiation dose (kGy)

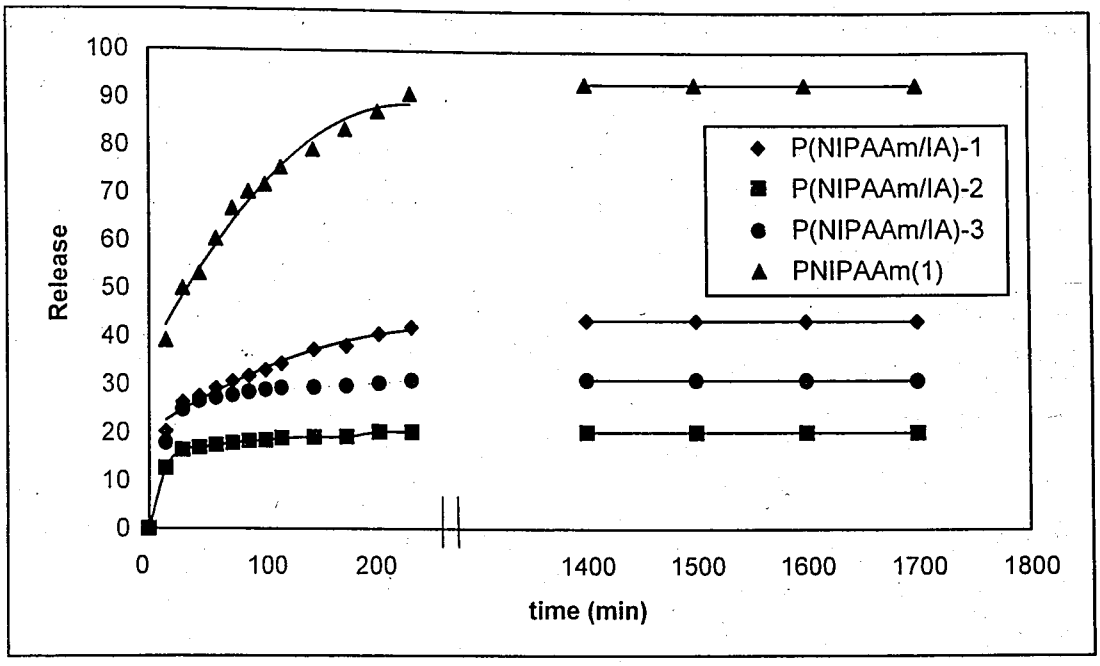


Figure 4.38. Release percent of non-specific adsorbed VG from P(NIPAAM/IA) hydrogels prepared at the irradiation dose of 48 kGy in phosphate buffer solution of pH 7.4 at 37°C

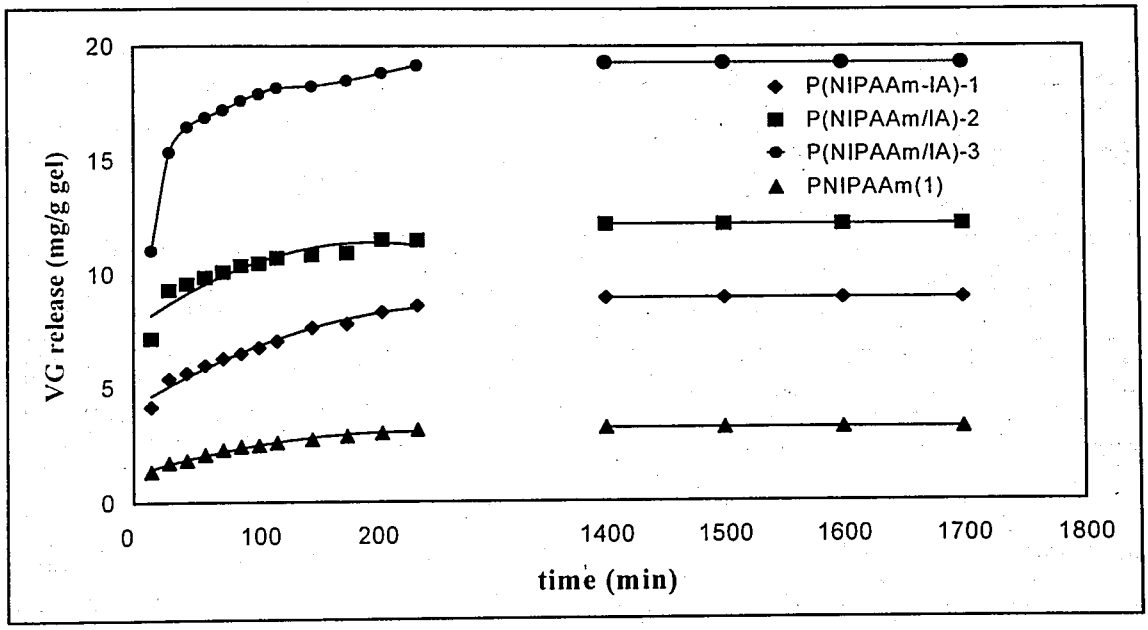


Figure 4.39. The release amount of the non-specific drug per unit mass of dry hydrogel prepared at the irradiation dose of 48 kGy (mg/g gel) in phosphate buffer solution of pH 7.4 at 37°C

NIPAAm/IA copolymeric hydrogels increased with increasing IA content in the gel system.

The percentage release of VG with time at each hydrogel system given in Figure 4.40-4.42. The percentage releases of specific adsorbed VG at pH 5.5, 4.0 and 2.0 were calculated from the equation 4.8. Figure 4.43 shows the variation of the total (uptake), non-specific and specific adsorbed VG with itaconic acid content in the gel system. Table 4.11 and Figures 4.40-4.43 show that the release amount of the specific adsorbed drug from the hydrogels increases as the amount of IA content in the hydrogel system increases. In a similar manner with MB, the maximum equilibrium release IA content in the gel system was occurred at pH 2. As the amount of IA content in the hydrogel system increases, non-released amount of drug increases. While 89.5 per cent of VG was released from P(NIPAAm/IA)-1, this value decreased to 57.5 per cent with increasing IA content to 3.0 mole per cent in the gel system.

Table 4.11. Variation of total, non-specific and specific adsorbed VG with itaconic acid content in the gel system prepared at the irradiation dose of 48 kGy

Gel name	$m_{non\text{-}specific}$ (mg/g dry gel)	$m_{specific}$ (mg/g dry gel)			m_{total} (mg/g dry gel)	$m_{non\text{-}released}$ (mg/g dry gel)
		pH 5.5	pH 4	pH 2		
PNIPAAm-1	3.2	-			3.6	-
P(NIPAAm/IA)-1	8.9	2.1	0.9	7.3	20.5	1.2
P(NIPAAm/IA)-2	12.2	2.9	1.4	17.9	44.2	9.8
P(NIPAAm/IA)-3	19.3	3.1	2.5	15.9	56.5	15.9

4.5.1.3. Release of Lidocaine from the Hydrogels. Lidocaine (LD) is a local anesthetic drug and widely used IV for short-term management of life-threatening ventricular arrhythmias. It is quickly metabolized and excreted. LD acts preferentially on the ischemic tissue to decrease the rate of rise of the action potential and to decrease the slow but spontaneous diastolic depolarization in Purkinje fibers. It is molecular weight of 234 [85].

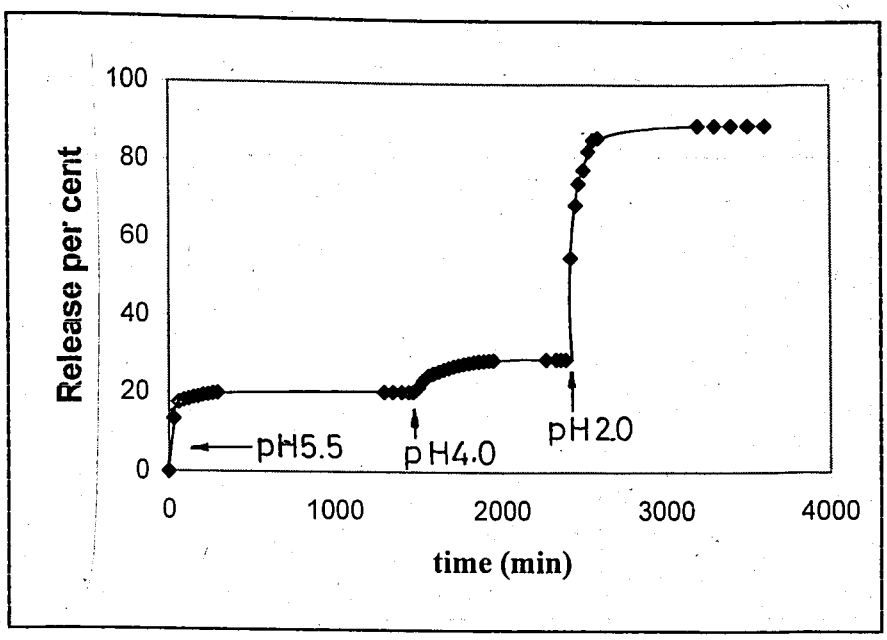


Figure 4.40. Release of specific adsorbed VG from P(NIPAAm/IA)-1 microspheres prepared at the irradiation dose of 48 kGy

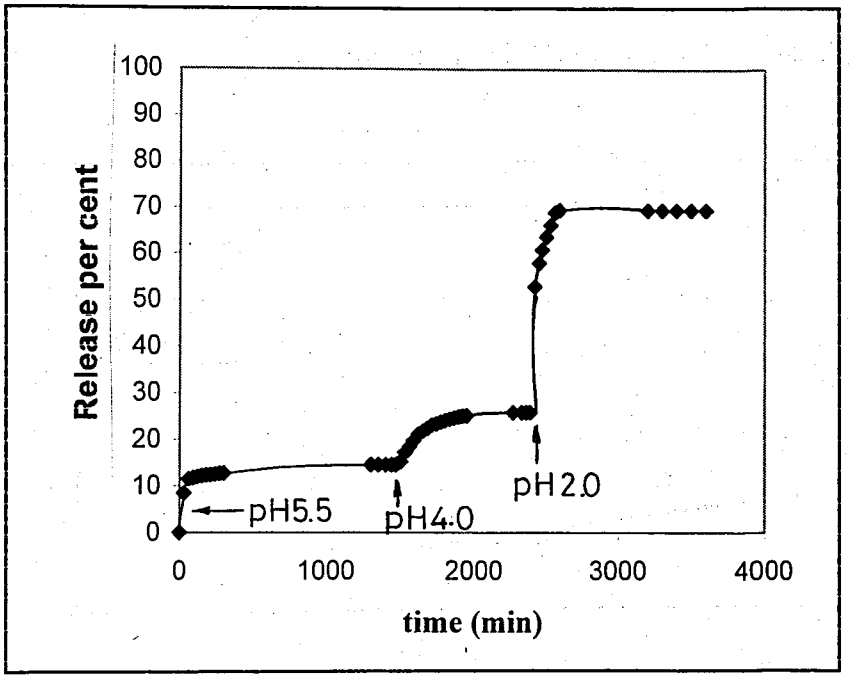


Figure 4.41. Release of specific adsorbed VG from P(NIPAAm/IA)-2 microspheres prepared at the irradiation dose of 48 kGy

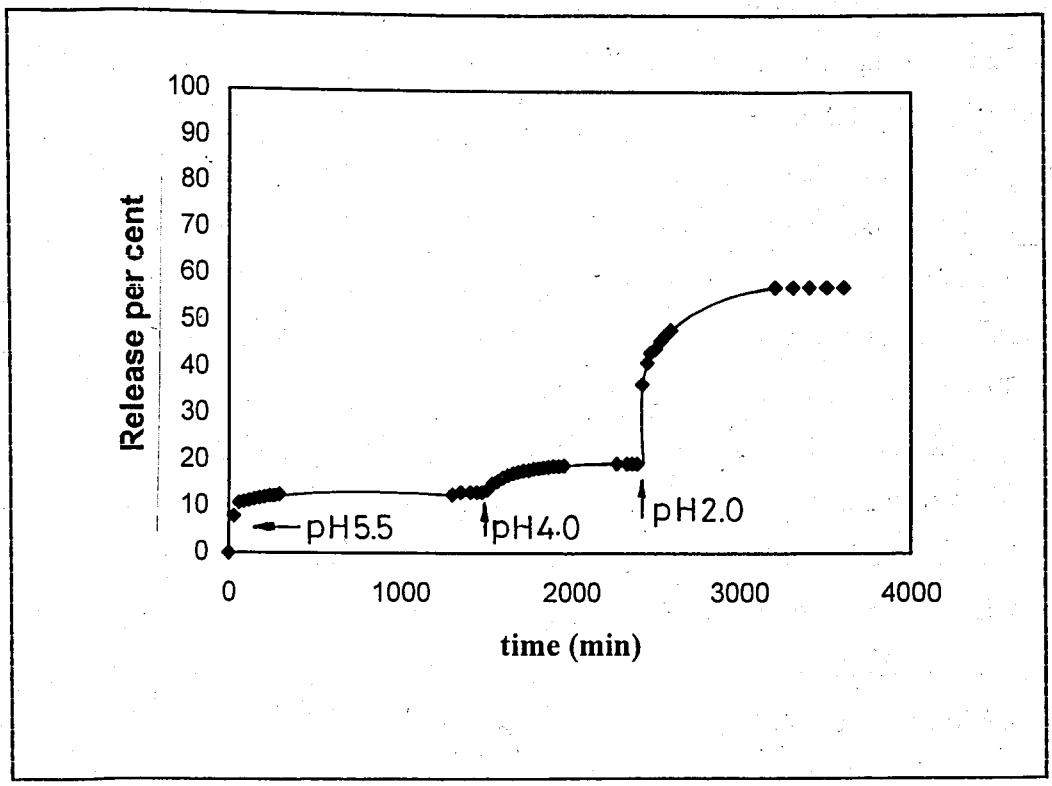


Figure 4.42. Release of specific adsorbed VG from P(NIPAAm/IA)-3

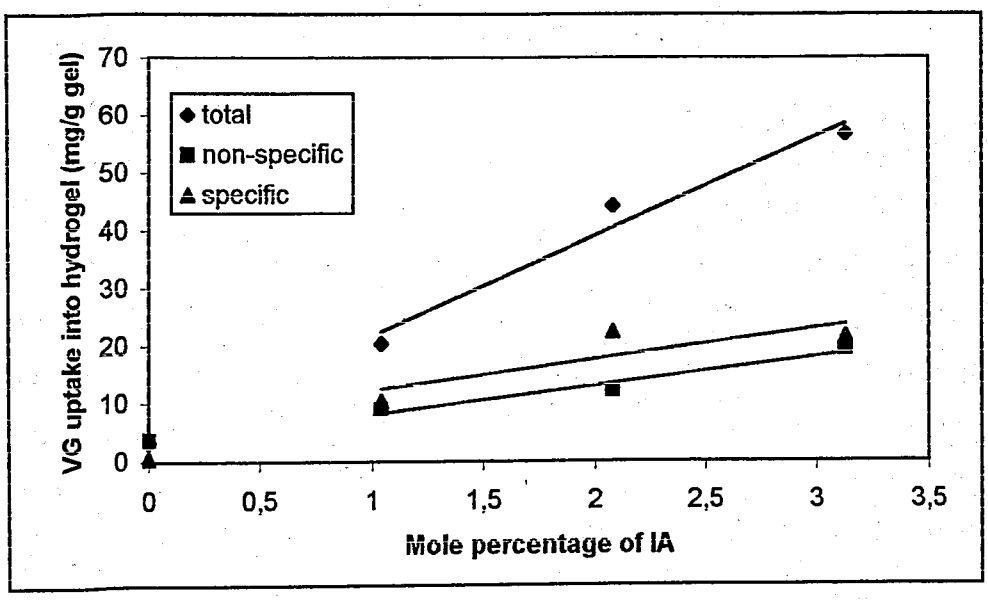


Figure 4.43. Variation of total, non-specific and specific adsorbed VG with itaconic acid content in the gel system prepared at the irradiation dose of 48 kGy.

Lidocaine (LD) was also used as a model drug for the investigation of drug release behaviour of PNIPAAm and P(NIPAAm/IA) hydrogels. The released LD was analyzed at 262 nm by ultraviolet spectrophotometer. As shown in Figure 4.44, the maximum absorbance of LD was measured at 262 nm. Figure 4.45 shows calibration line of LD. The amounts of total (specific and non-specific) LD uptake into one gram of dry PNIPAAm and P(NIPAAm/IA) hydrogels are given in Figure 4.46. As can be seen from this figure, the LD uptake into the hydrogels increases with increase in IA content. When the LD uptake was compared with the uptake of the other drugs (MB and VG) , it was found that the LD uptake is much greater than the uptake of the other drugs. Since VG contains COO^- functional counterions. This prevents the interaction of this drug with our ionic hydrogels. Moreover, the molecules of VG and MB are larger than the molecules of LD. Therefore, the molecules of LD can diffuse into gel pores easily than the other drug molecules. This leads to high LD uptake into P(NIPAAm/IA) hydrogels. Table 4.12 shows the variation of total (uptake) drug capacities of PNIPAAm and P(NIPAAm/IA) hydrogels.

As shown in Figure 4.47, the LD uptake into the hydrogels decreases with increase in the irradiation dose. The pores (molecular mesh) in this hydrogel can become smaller as the irradiation dose increase.

The release profiles of LD in NIPAAm/IA copolymeric gels in phosphate buffer solution of pH 7.4 at 37°C were shown in Figure 4.48 and Figure 4.49. The amount of the percentage release of LD at pH 7.4 was calculated from the Equation 4.8. Figure 4.48 shows that the amount of release percent for non-specific adsorbed LD was higher for pure PNIPAAm hydrogel than those for P(NIPAAm/IA) hydrogels. The amount of release percent decreases with the increase of IA content in the gel system. This can be explained by the increase in the diffusional path due to the high swelling of P(NIPAAm/IA) hydrogels. While 76 per cent of LD was released from PNIPAAm hydrogels this value decreased to 13 per cent with increasing IA content to 3 mole per cent in the gel structure.

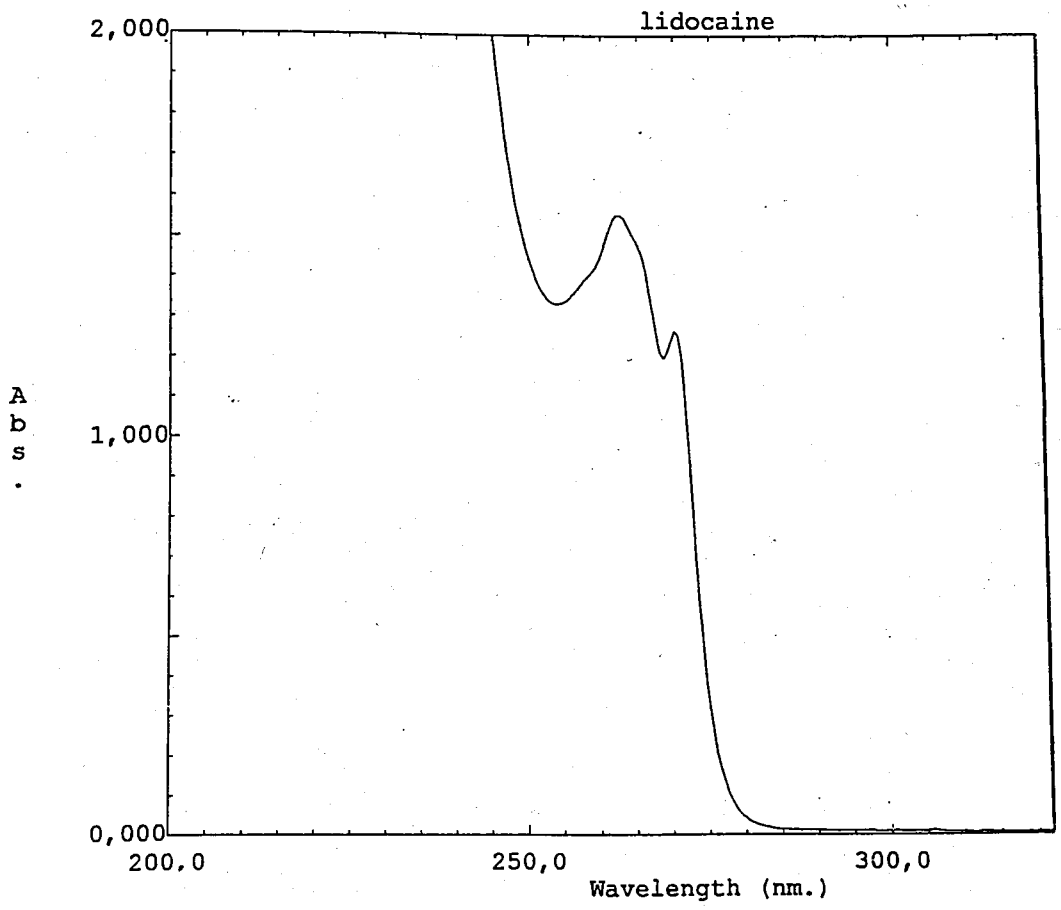


Figure 4.44. UV-Vis Spectrum of Lidocaine

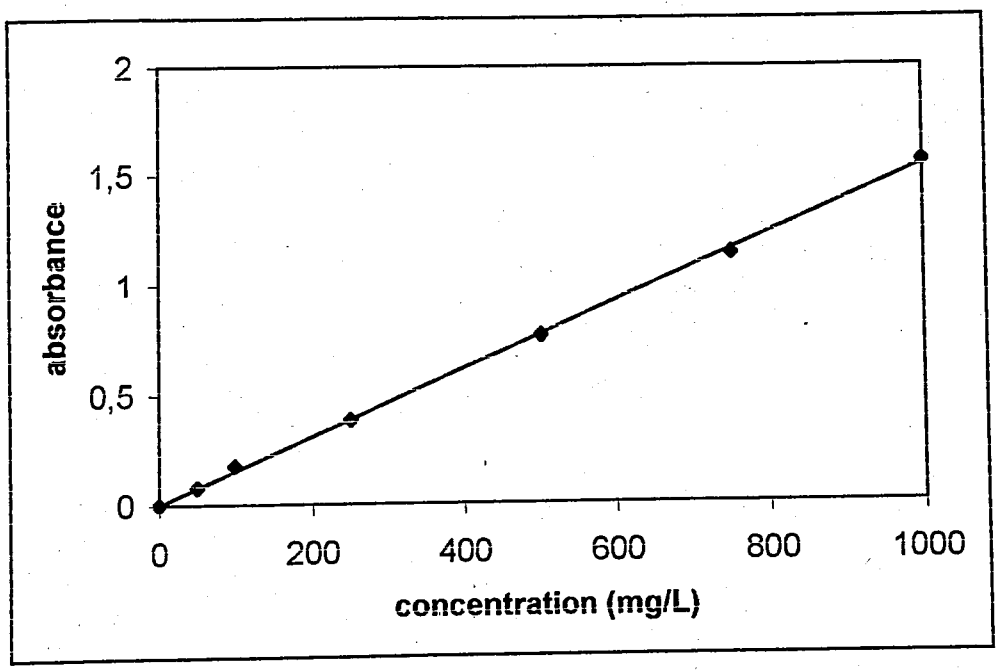


Figure 4.45. Calibration line of LD

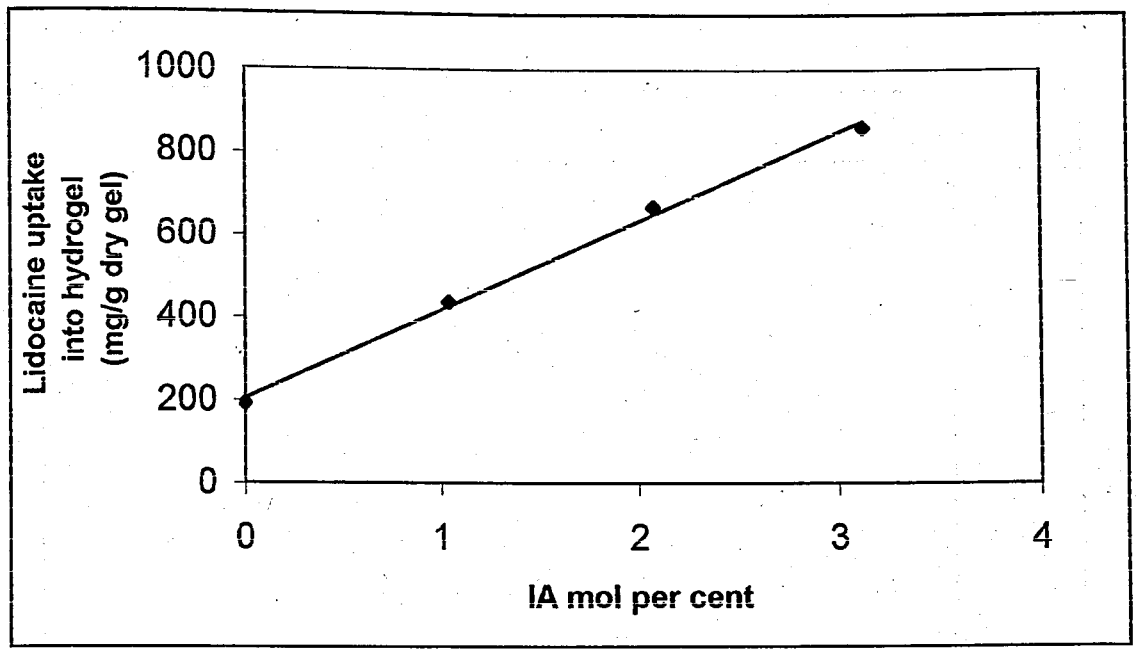


Figure 4.46. Variation of total Lidocaine (LD) with itaconic acid (IA) content in the gel system prepared at the irradiation dose of 48 kGy

Table 4.12. Variation of total drug uptake capacity of P(NIPAAm/IA) hydrogels with itaconic acid

Drug name	Total MB uptake (mg/ g dry gel)	Total VG uptake (mg/ g dry gel)	Total LD uptake (mg/ g dry gel)
PNIPAAm(1)	5.0	3.6	192.3
P(NIPAAm/IA)-1	27.3	20.5	437.3
P(NIPAAm/IA)-2	49.0	44.2	669.0
P(NIPAAm/IA)-3	74.6	56.5	862.1

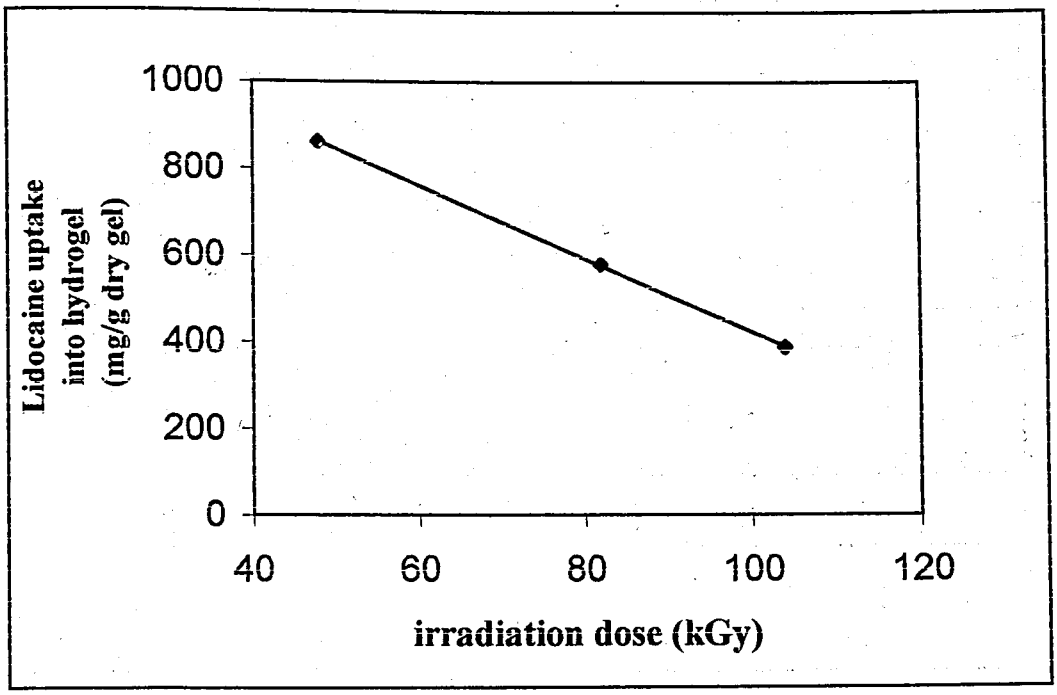


Figure 4.47. Variation of total Lidocaine (LD) with itaconic acid (IA) content in the gel in the irradiation dose (mole per cent of itaconic acid is 3.0)

The percentage release of LD with time at each hydrogel system given in Figure 4.50-4.52. The percentage releases of specific adsorbed LD at pH 5.5, 4.0 and 2.0 were calculated from the Equation 4.9. Figure 4.53 and Table 4.13 shows the variation of the total, non-specific and specific adsorbed LD with itaconic acid content in the gel system. The results illustrate that the release amount of the specific adsorbed drug from the hydrogels increases with the increasing IA content in the gel system in all buffer solutions.

In a similar manner with the other drugs (MB and VG), maximum equilibrium release amount occurred at pH 2. After completing release at pH 2, it was noticed that the LD is not completely released and some portions are entrapped within the gel. As the amount of IA content in the hydrogel system increases, non-released amount of drug increases (Table 4.13). The complete release of LD was observed at pH 1.

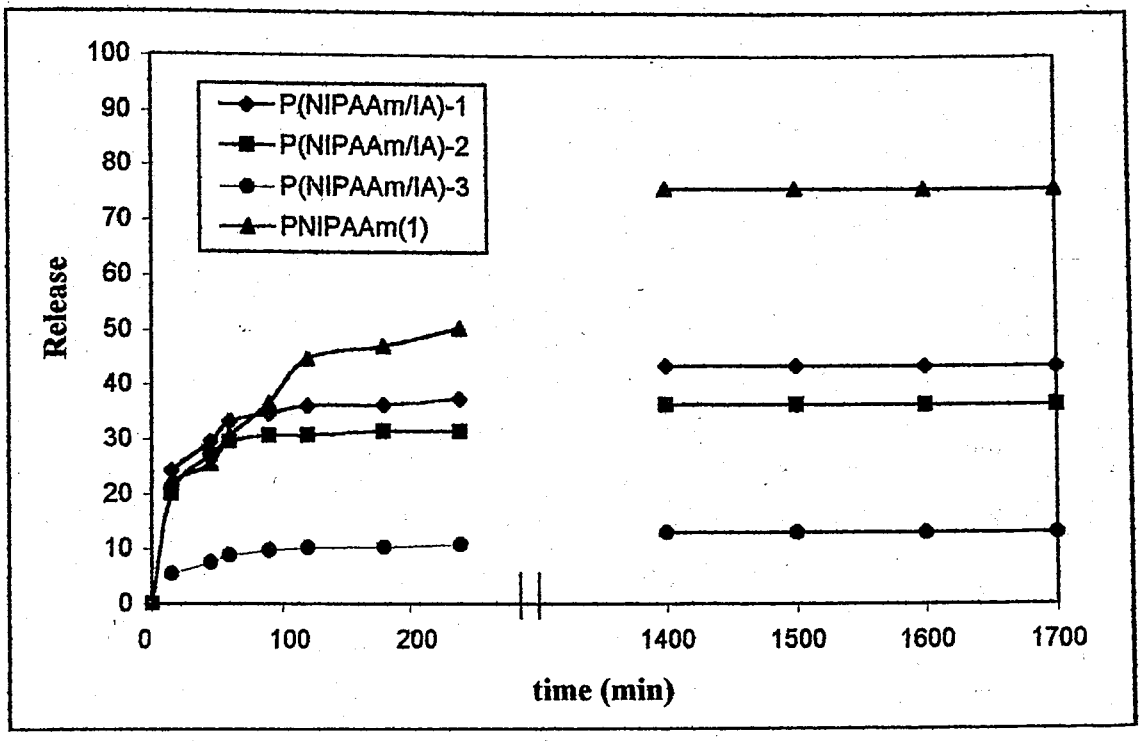


Figure 4.48. Release percent of non-specific adsorbed LD from P(NIPAAm/IA) hydrogels prepared at the irradiation dose of 48 kGy at pH 7.4 (37°C)

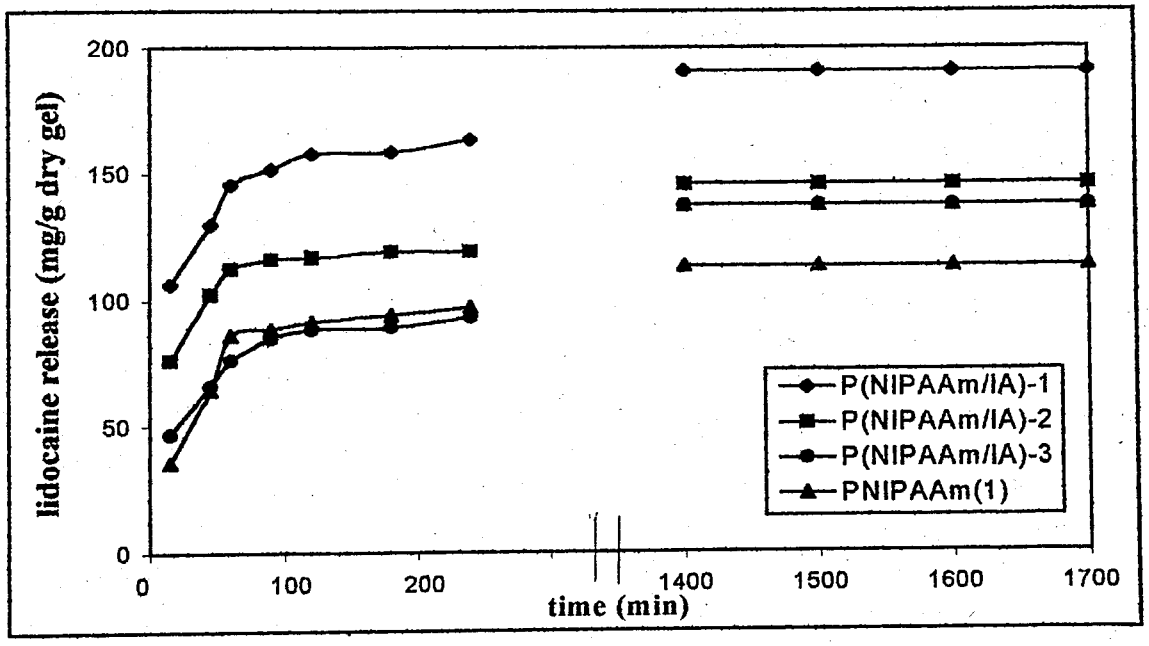


Figure 4.49. The release amount of the non-specific drug per unit mass of dry hydrogels prepared at 48 kGy at pH 7.4 buffer solution at 37°C (mg/g dry gel)

Figure 4.49 shows that the amount of the released drug per unit mass of dry P(NIPAAm/IA) hydrogels was higher than pure PNIPAAm hydrogel. At the constant temperature of 37°C, the amount of the equilibrium drug released increased increased with increase in IA content, because the equilibrium swelling ratio of NIPAAm/IA copolymeric hydrogels increased with increasing IA content in the gel system. The percentage release of LD with time at each hydrogel system given in Figure 4.50-4.52. The percentage releases of specific adsorbed LD at pH 5.5, 4.0 and 2.0 were calculated from the Equation 4.9.

Figure 4.53 and Table 4.13 shows the variation of the total, non-specific and specific adsorbed LD with itaconic acid content in the gel system. The results illustrate that the release amount of the specific adsorbed drug from the hydrogels increases with the increasing IA content in the gel system in all buffer solutions.

In a similar manner with the other drugs (MB and VG), maximum equilibrium release amount occurred at pH 2. After completing release at pH 2, it was noticed that the LD is completely released and some portions are entrapped within the gel. As the amount of IA content in the hydrogel system increases, non-released amount of drug increases (Table 4.13). The complete release of LD was observed at pH 1.

4.5.1.4. Effect of Temperature on Controlled Release Behaviours of Hydrogels. LCST hydrogels may be used to deliver or release drugs or other biologically active agent in at least two different ways:

Process 1: Release of an active agent from a swollen LCST gel at T<LCST when it is placed in aqueous medium T<LCST.

Process 2: Release of an active agent from a swollen LCST gel at T<LCST when it is placed in aqueous medium T>LCST.

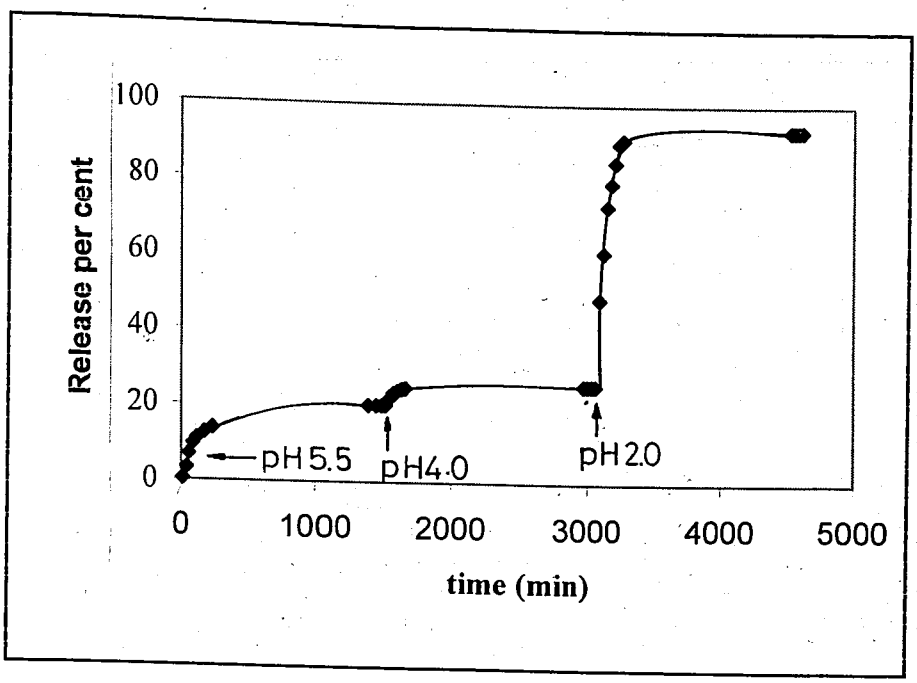


Figure 4.50. Release percent of specific adsorbed LD from P(NIPAAm/IA)-1

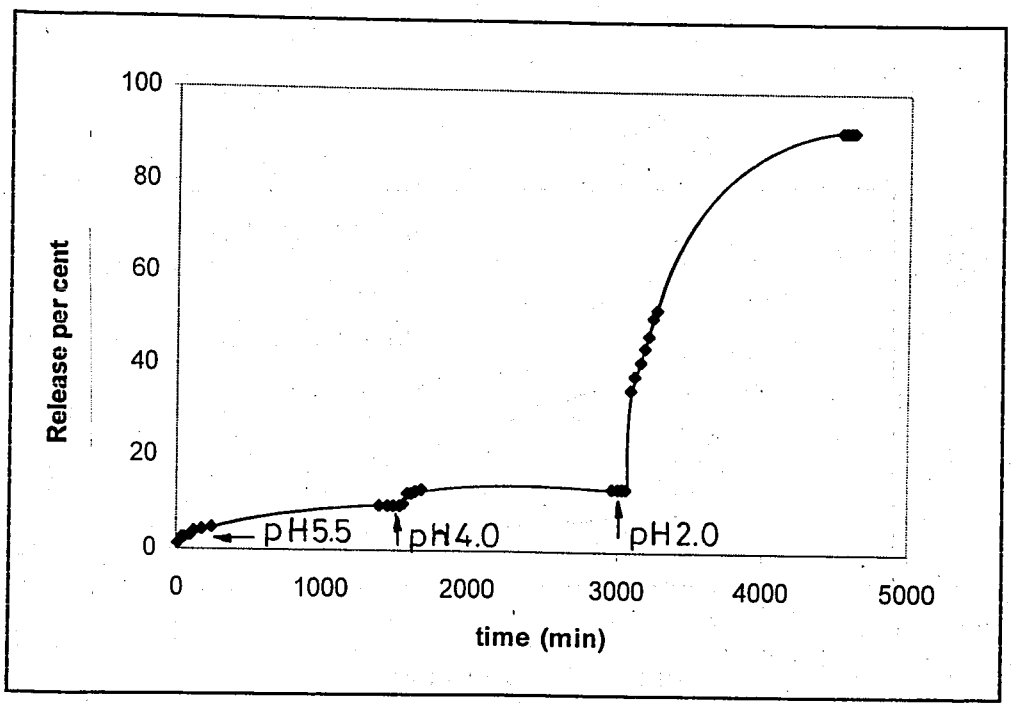


Figure 4.51. Release percent of specific adsorbed LD from P(NIPAAm/IA)-1

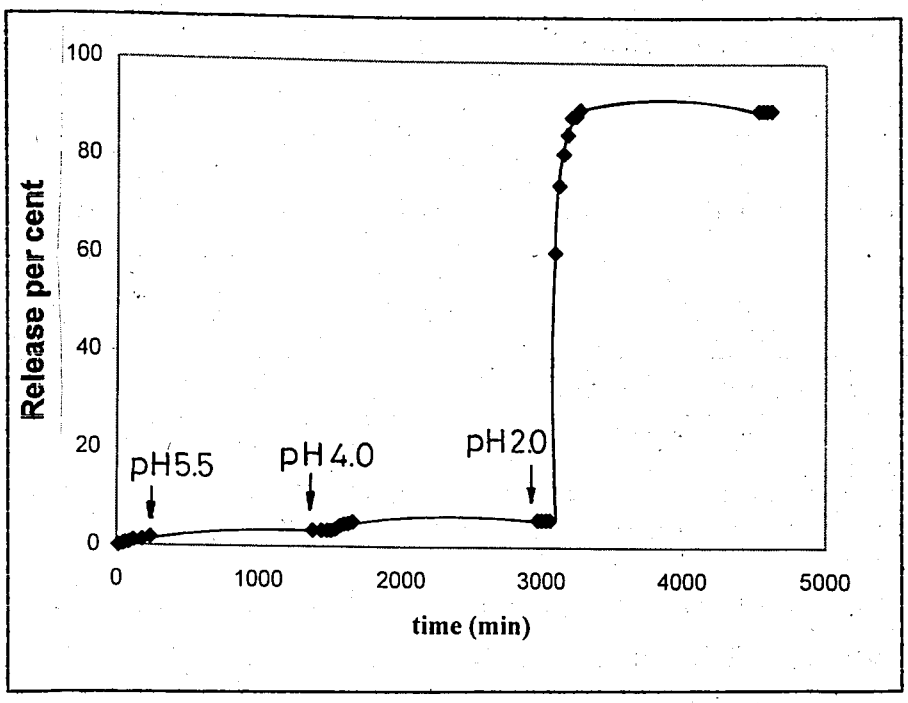


Figure 4.52. Release percent of non-specific adsorbed LD from P(NIPAAm/IA)-3

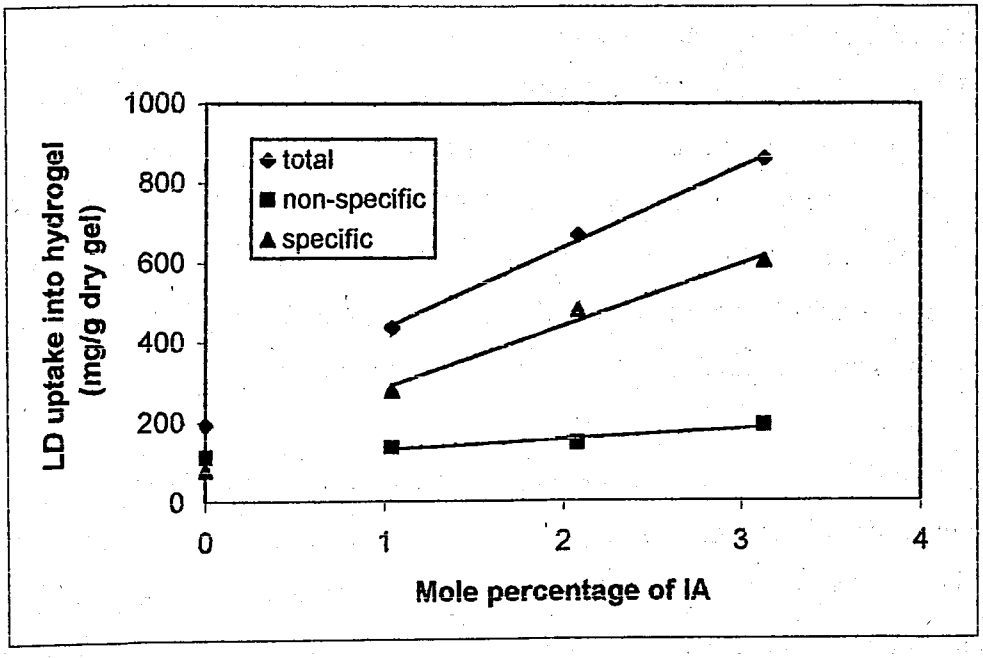


Figure 4.53. Variation of total, non-specific and specific adsorbed LD with itaconic acid content in the gel system prepared at the irradiation dose of 48 kGy

Table 4.13. Variation of total, non-specific and specific adsorbed LD with itaconic acid content in the gel system prepared at the irradiation dose of 48 kGy.

Gel name	$m_{non\text{-}specific}$ (mg/g dry gel)	$m_{specific}$ (mg/g dry gel)			m_{total} (mg/g dry gel)	$m_{non\text{-}released}$ (mg/g dry gel)
		pH 5.5	pH 4	pH 2		
PNIPAAm-1	113.0	-			192.3	-
P(NIPAAm/IA)-1	137.5	55.8	16.7	206.3	437.3	21.0
P(NIPAAm/IA)-2	145.8	57.8	19.3	404.0	669.0	41.9
P(NIPAAm/IA)-3	190.8	60.4	24.2	519.6	862.1	67.1

In process 1, the agent should be released by a normal Fickian type diffusion mechanism, since the gel does not pass through its LCST. In according to Equation 2.70, the amount of drug release was proportional to initial square root of time (n=0.5) when the solvent diffusion or drug release follows the well-known Fickian diffusion mechanism. In our work, the pure PNIPAAm and P(NIPAAm/IA) copolymeric hydrogels were initially placed into in phosphate buffer solution of pH 7.4 at 4°C and waited one week. Then the drug release behaviour of these hydrogels were investigated in phosphate buffer solution of pH 7.4 at 25°C (below their LCSTs). It was f ound that the initial release amounts are square root time dependent for all drugs (MB, VG and LD) from pure PNIPAAm-1 and NIPAAm/IA copolymeric gels (Figures 4.54-4.56). Since this process does not take advantage of the special thermally reversible shrinking or swelling properties of the gels, it is not particularly advantageous when compared to other, "normal" hydrogels, for drug delivery applications [10-11].

In process 2, on the other hand, the gel is caused to rapidly deliver the imbibed drug along with its aqueous solvent as the gel collapses on warming through its LCST. This is a very complex process, and the release kinetics shows two regions: initially there is rapid release, followed by a slower release. The first region is probably due to release of the pore water near the surface along with drug dissolved in it. As the surface "skins over" it represents an increasing resistance to the transport of drug out of the gel.

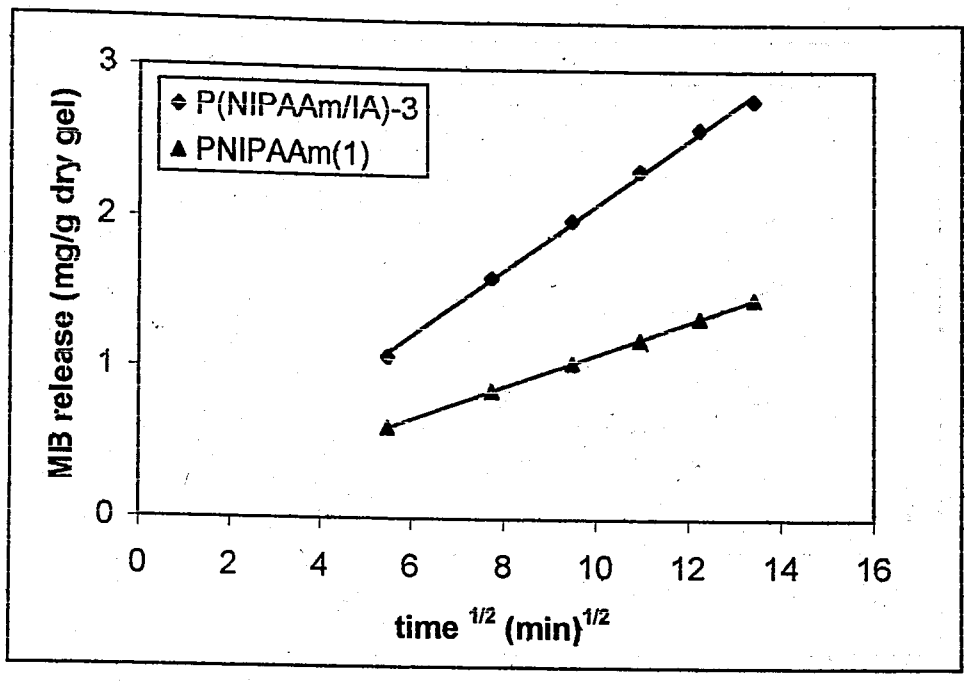


Figure 4.54. First order, square root of time release kinetics of MB from P(NIPAAm/IA)-3 and PNIPAAm(1) hydrogels raised from 4°C to 25°C

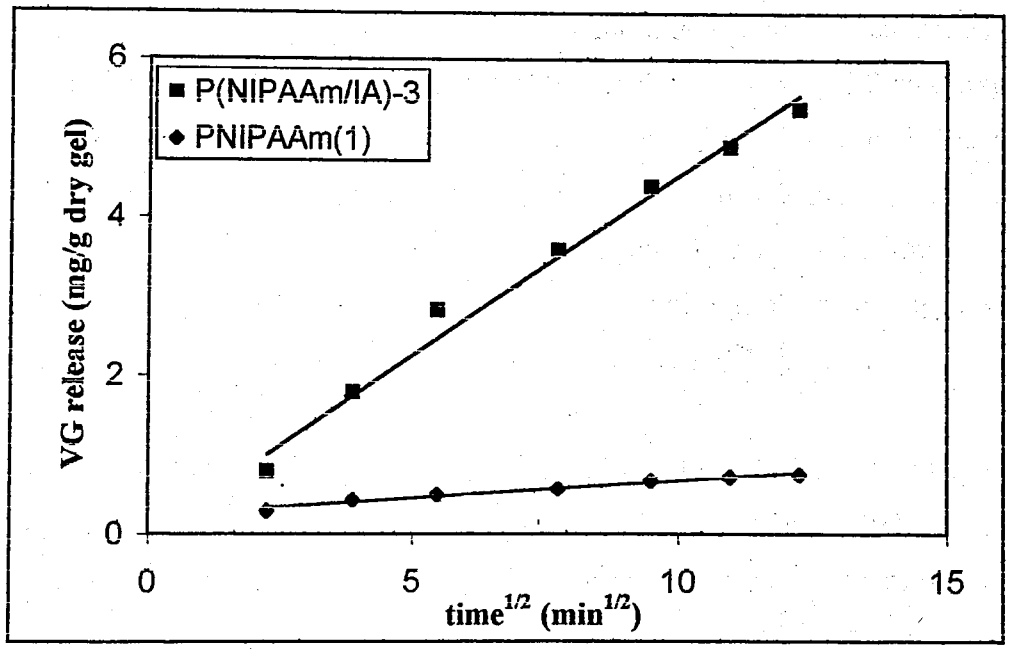


Figure 4.55 First order, square root of time release kinetics of VG from P(NIPAAm/IA)-3 and PNIPAAm-1 hydrogels raised from 4°C to 25°C

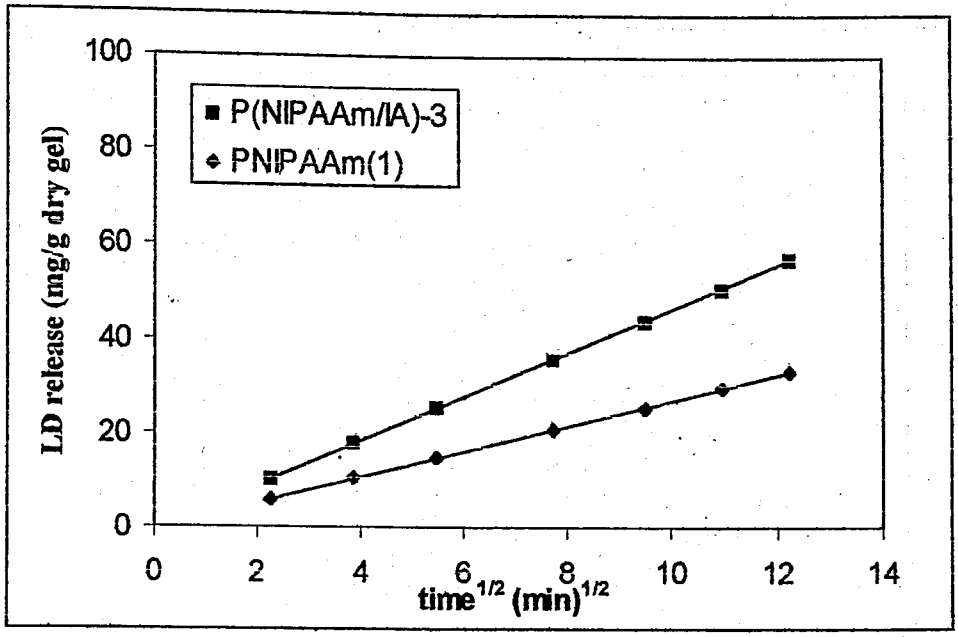


Figure 4.56. First order, square root of time release of LD from P(NIPAAm/IA)-3 and PNIPAAm(1) hydrogels raised from 4 to 25°C below their LCST

Meanwhile, the gel molecules inside this skinned gel are attempting to phase separate, which must apply an increasing hydrostatic pressure on the remaining water and its drug solute. This will enhance the hydraulic as well as the diffusive transport rates of each out of the gel. At the same time, the gel continues to collapse, which represents anincreasing resistance to both diffusion and hydrostatic pressure flow across the thickening "skin" membrane. This leads to an apparently constant but lower -release rate after the initial minutes. Eventually the system will equilibrate, with pockets of water trapped inside a collapsed, sponge-like gel structure [10-11].

Figures 4.57-4.59 show release kinetics of all the drugs (MB, VD and LD) from pure PNIPAAm-1 and NIPAAm/IA copolymeric gels raised from 4°C to 37°C in phosphate buffer solution of pH 7.4. The release kinetics show two square root of time dependent regions: initially there is a rapid release, followed by a slower release. Clearly, "normal" Fickian diffusion controlled release in this complex delivery system was not expected in the initial minutes. However, it is possible that slow Fickian release through the collapsed polymer network is the mechanism for delivery at long times.

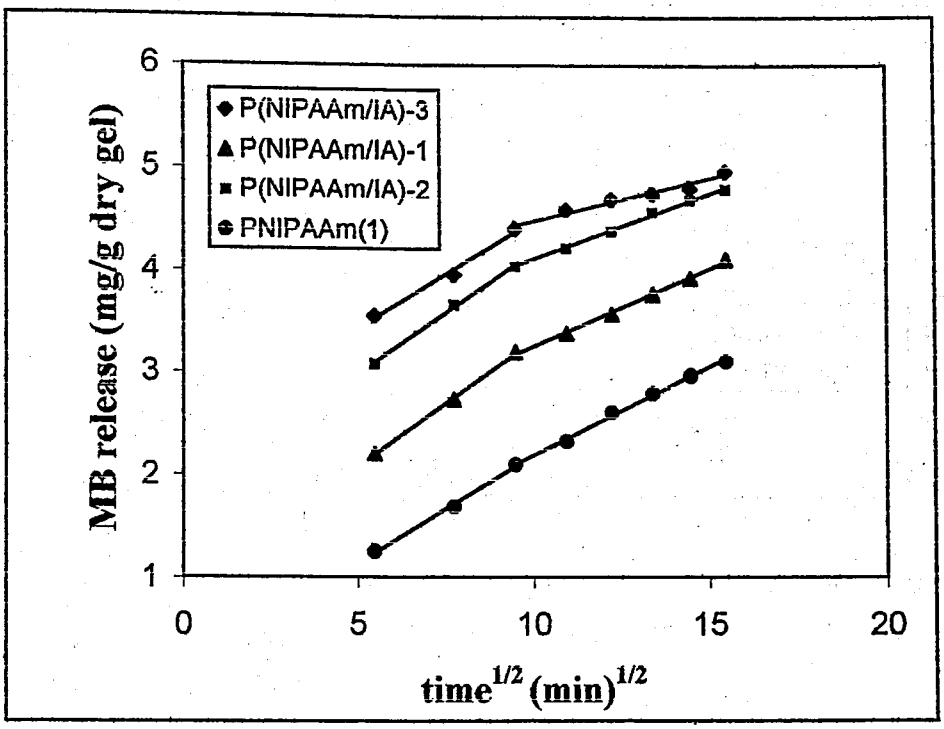


Figure 4.57. Unusual release kinetics of MB from P(NIPAAM/IA) hydrogels (mg/g gel) raised from 4°C to 37°C

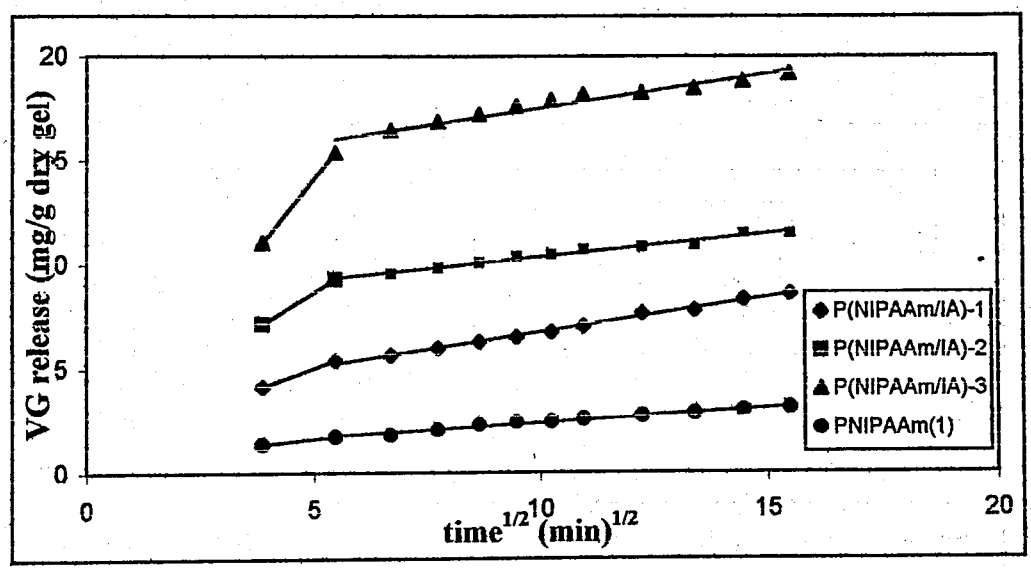


Figure 4.58. Unusual release kinetics of VG from P(NIPAAm/IA) hydrogels (mg/g gel) raised from 4°C to 37°C

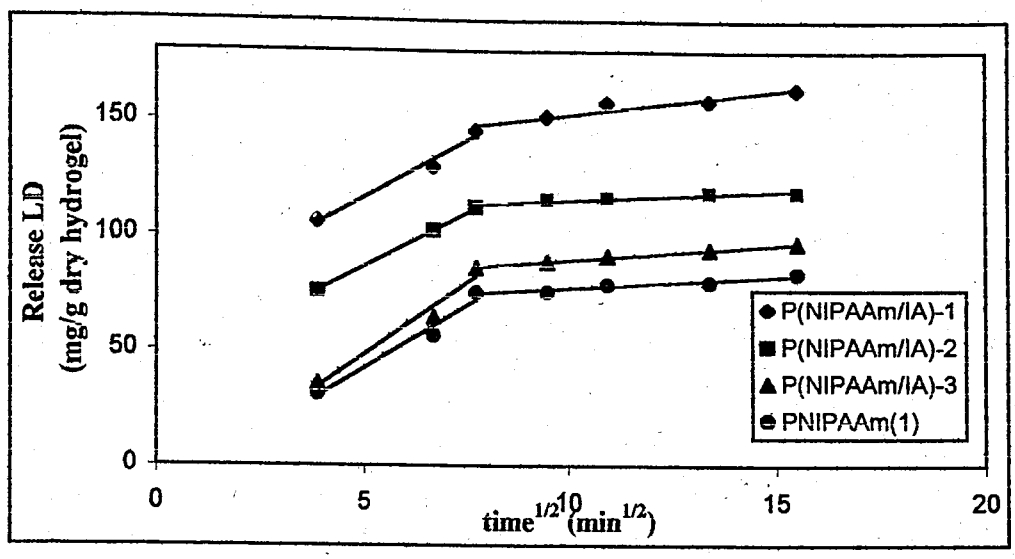


Figure 4.59. Unusual release kinetics of LD from P(NIPAAM/IA) hydrogels (mg/g gel) raised from 4°C to 37°C

Figures 4.60-4.65 and Tables 4.14-4.15 show comparison of release kinetics of all drugs (MB, VG and LD) from pure PNIPAAm and NIPAAm/IA copolymeric gels raised from 4°C to 25°C with the gels raised from 4°C to 37°C. The results indicated that the higher temperature (T>LCST) induced volume shrinkage of PNIPAAm networks leading to higher the drug release.

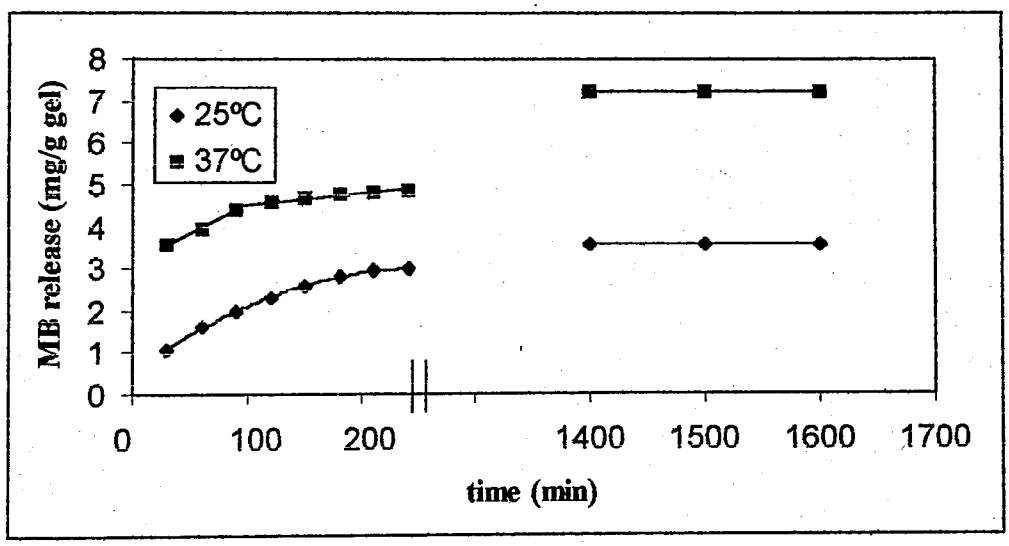


Figure 4.60. Release kinetics of MB from P(NIPAAm/IA)-3 hydrogels (mg/g gel) raised from 4°C to 25°C and 37°C

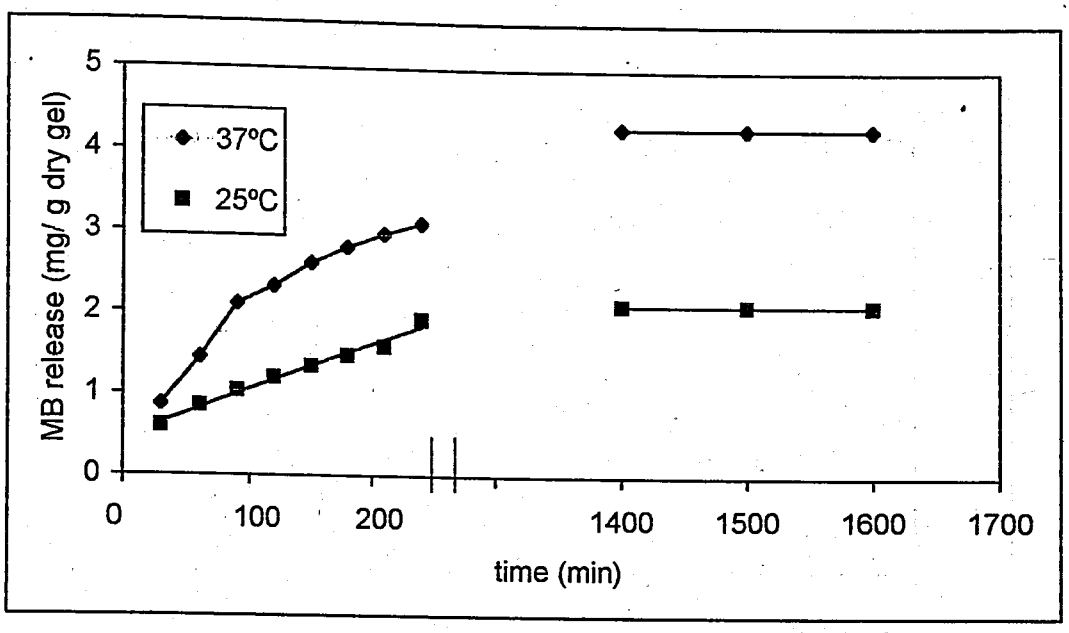


Figure 4.61. Release kinetics of MB from PNIPAAm(1) hydrogels (mg/g dry gel) raised from 4°C to 25°C and 37°C

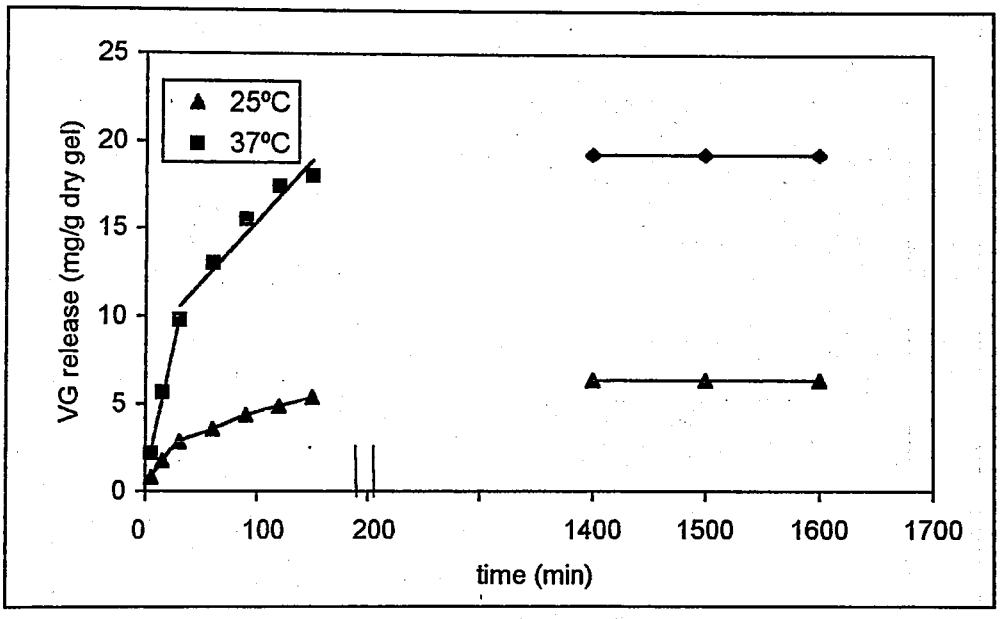


Figure 4.62. Release kinetics of VG from P(NIPAAm/IA)-3 hydrogels (mg/g gel) raised from 4°C to 25°C, 37°C

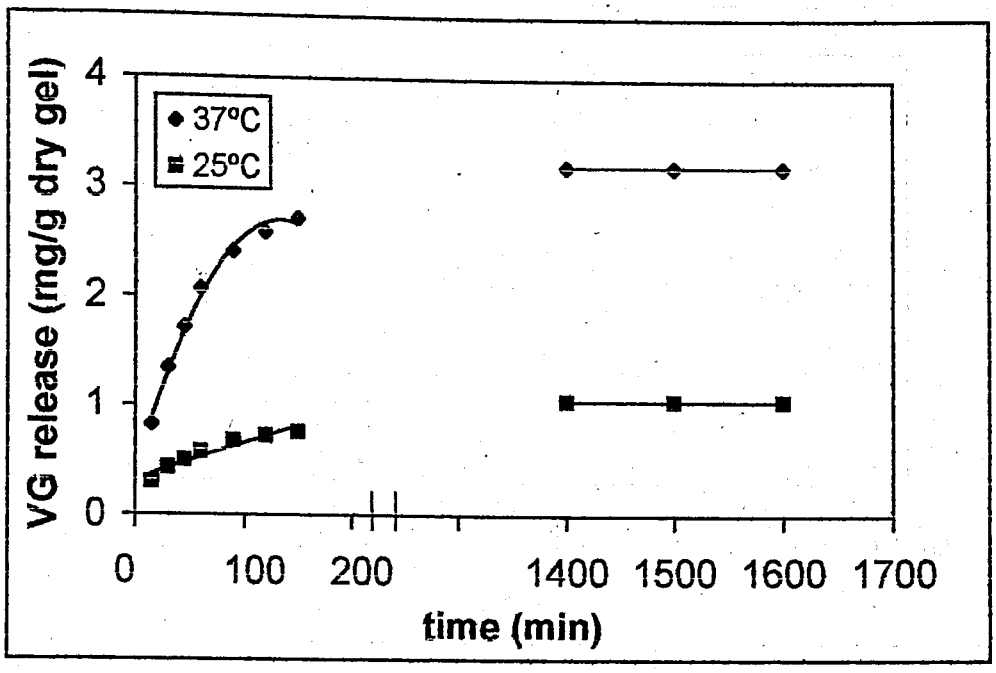


Figure 4.63. Release kinetics of VG from PNIPAAm(1) hydrogels (mg/g gel) raised from 4°C to 25°C and 37°C

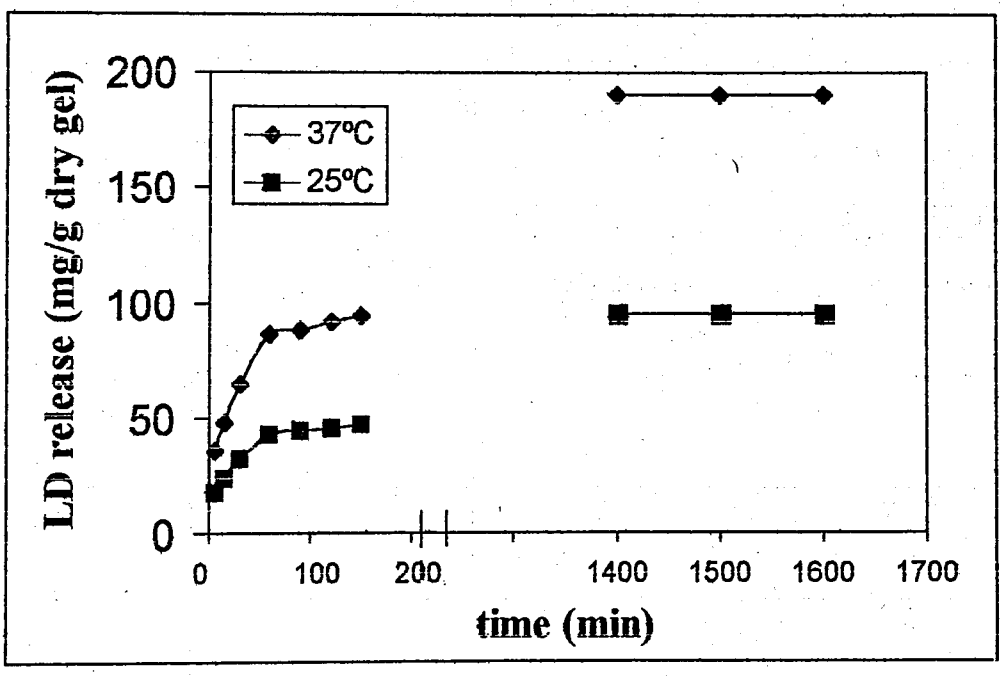


Figure 4.64. Release kinetics of LD from P(NIPAAM/IA)-3 hydrogels (mg/g gel) raised from 4°C to 25°C and 37°C

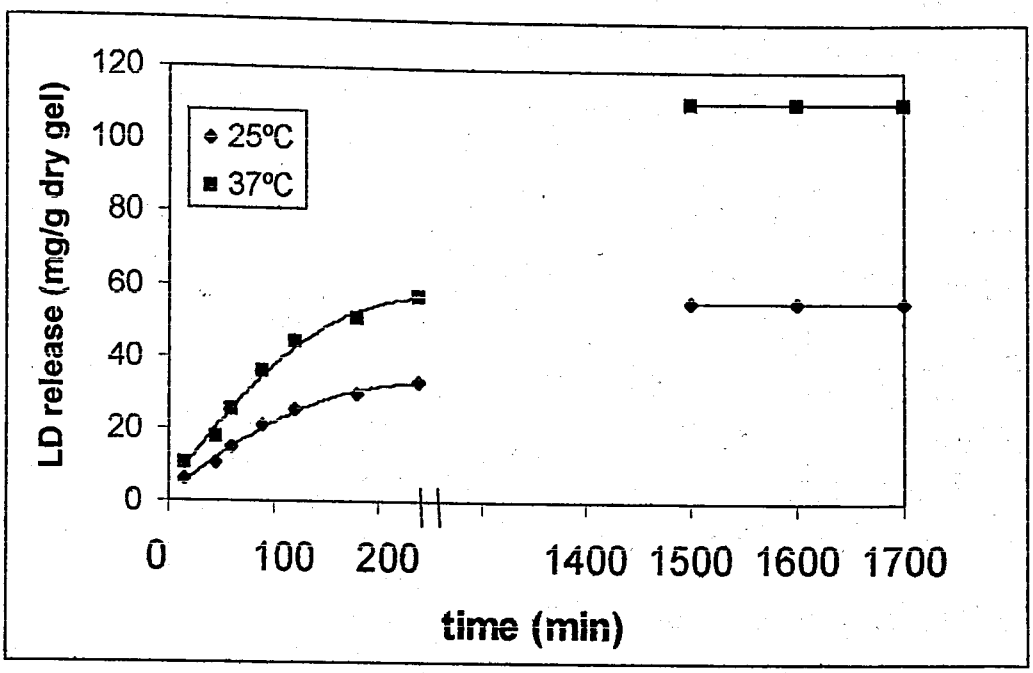


Figure 4.65. Release kinetics of LD from PNIPAAm(1) hydrogels (mg/g gel) raised from 4°C to 25°C and 37°C

Table 4.14. Total drug uptake capacity of PNIPAAm(1) and their equilibrium release amounts in the buffer solution of pH 7.4 with respect to different temperature of swelling medium

Drug name	m_{total} (mg/g dry gel)	$m_{non\text{-}specific}$ (37°C) (mg/g dry gel)	$m_{non\text{-}specific}$ (25°C) (mg/g dry gel)
MB	5.0	4.3	2.0
VG	3.6	3.2	0.9
LD	192.3	111.3	55.6

Table 4.15. Total drug uptake capacity of pure PNIPAAm(1) and their equilibrium release amounts in the buffer solution of pH 7.4 with respect to different temperature of swelling medium

Drug name	m_{total} (mg/g dry gel)	$m_{non\text{-}specific}$ (37°C) (mg/g dry gel)	$m_{non\text{-}specific}$ (25°C) (mg/g dry gel)
MB	5.0	4.3	2.0
VG	3.6	3.2	0.9
LD	192.3	111.3	55.6

4.5.1.5. Effect of Drug Loading Amount on the Release of P(NIPAAm/IA) Gels. The effect of the loading level on the release of the drugs (MB, VG and LD) from unit mass of the P(NIPAAm/IA) gels is shown in Figure 4.66-4.68. As expected, the equilibrium drug release content increases with time. In other words, the release is highest from the sample with the highest drug content.

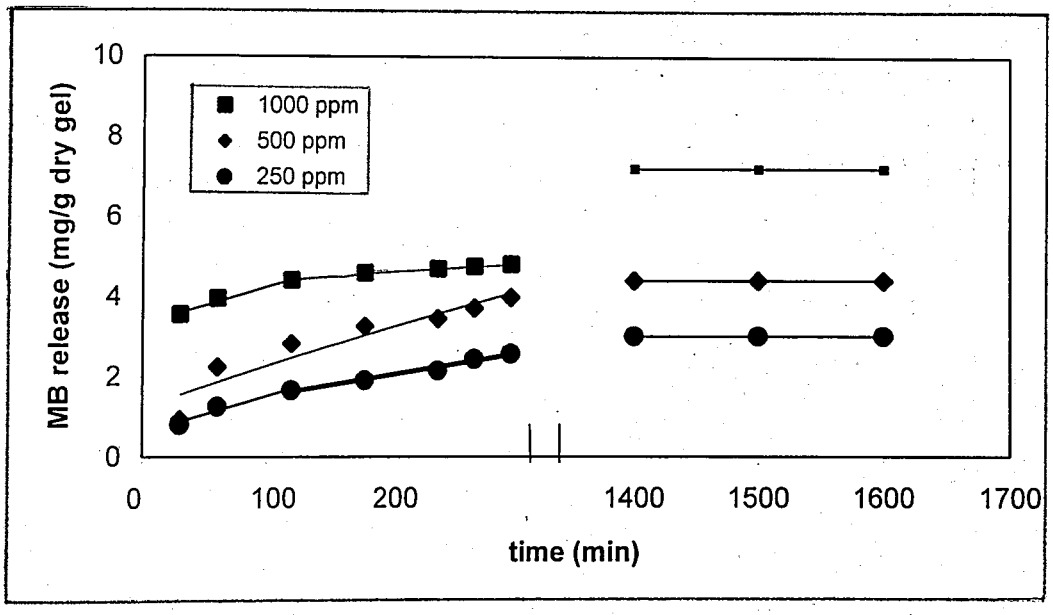


Figure 4.66. Effect of drug loading on release of MB from P(NIPAAM/IA)-3 hydrogels (mg/g gel) raised from 4°C to 37°C

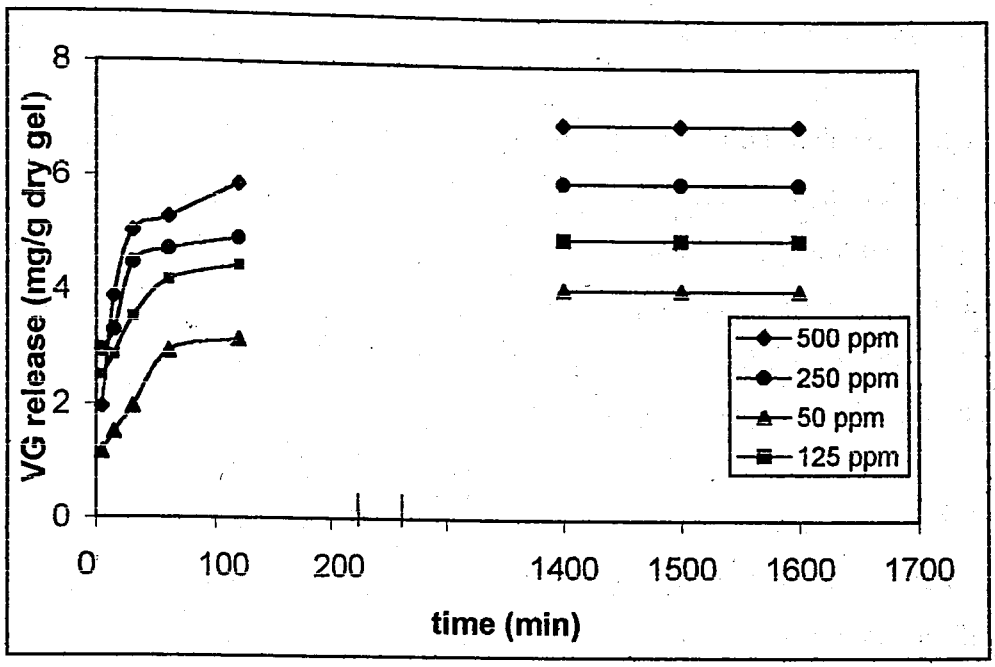


Figure 4.67. Effect of drug loading on release of VG from P(NIPAAm/IA)-4 hydrogels (mg/g gel) raised from 4°C to 37°C

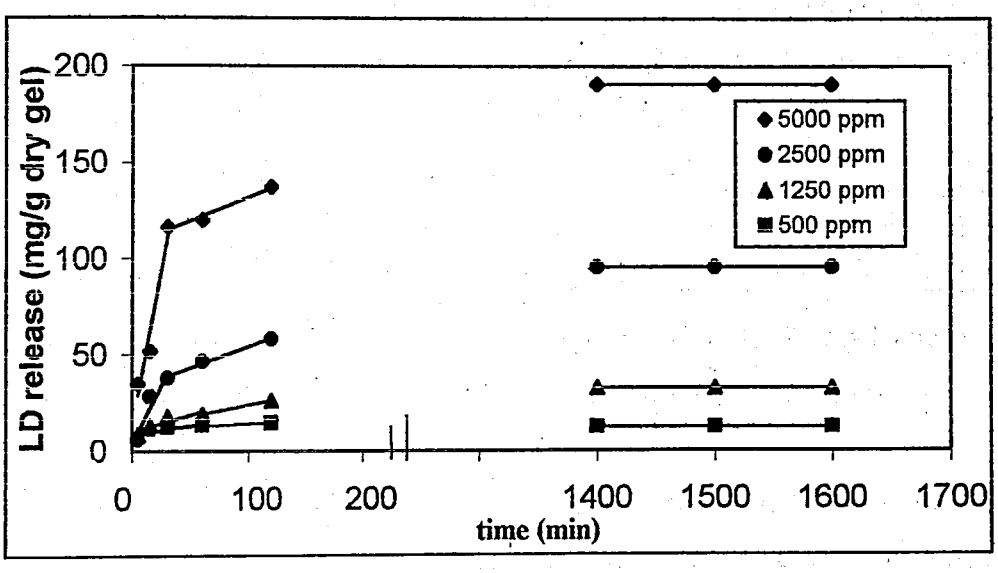


Figure 4.68. Effect of drug loading on release of LD from P(NIPAAm/IA)-3 hydrogels (mg/g gel) raised from 4°C to 37°C

4.5.2. Controlled Release Behaviours of P(NIPAAm/IA) Microspheres

PNIPAAm hydrogel rods prepared by the radiation induced polymerization process are useful only in topical and implant application. To be useful for oral dosage forms, hydrogels would have to be in granules prepared by cutting or granulating hydrogel sheets or rods followed by sieving into proper particle sizes. Such hydrogel granules are generally irregular in shape which may be objectionable not only from the standpoint of product aesthetic, but also from the standpoint of reproducibility in controlling the drug release [3, 74, 86-87].

For this reason, PNIPAAm microspheres (71-500 μm) were prepared by inverse suspension polymerization and sieved using ASTM sieves as explained in Section 4.2. PNIPAAm(1) shows the selected fraction of pure PNIPAAm microspheres obtained by inverse suspension polymerization technique. PNIPAAm(1) microspheres were in the range of 180-250 μm (most abundant one).

P(NIPAAm/IA) microspheres were prepared by the radiation induced surface modification techniques as explained in Section 4.2.1. P(NIPAAm/IA)-1 shows the microspheres prepared at the irradiation dose of 48 kGy .

The detailed experimental procedures for the drug loading and the release experiments were explained in Section 3.4.3 and Section 3.4.4., respectively. The release profiles of the drugs (MB, VG and LD) from P(NIPAAm/IA)-1 microspheres in phosphate buffer solution of pH 7.4 at 37°C were shown in Figure 4.69-4.74.

Table 4.16 shows comparison of equilibrum drug release per cent for P(NIPAAm/IA) microspheres with those for PNIPAAm microspheres in phosphate buffer solution of pH 7.4 at 37°C . The results show that the amount of the release percent from non-specific adsorbed drug was higher for pure PNIPAAm microspheres than P(NIPAAm/IA) microspheres This can be explained by the increase in the diffusional path due to the high swelling of P(NIPAAm/IA) microspheres.

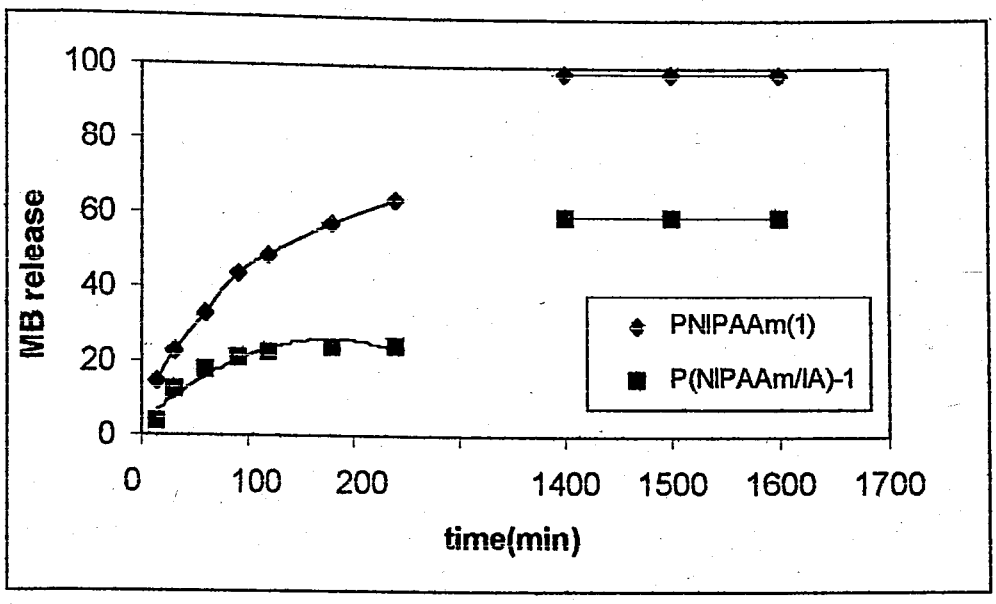


Figure 4.69. Release percent of non-specific adsorbed MB from the microspheres in buffer solution of pH 7.4 at 37°C

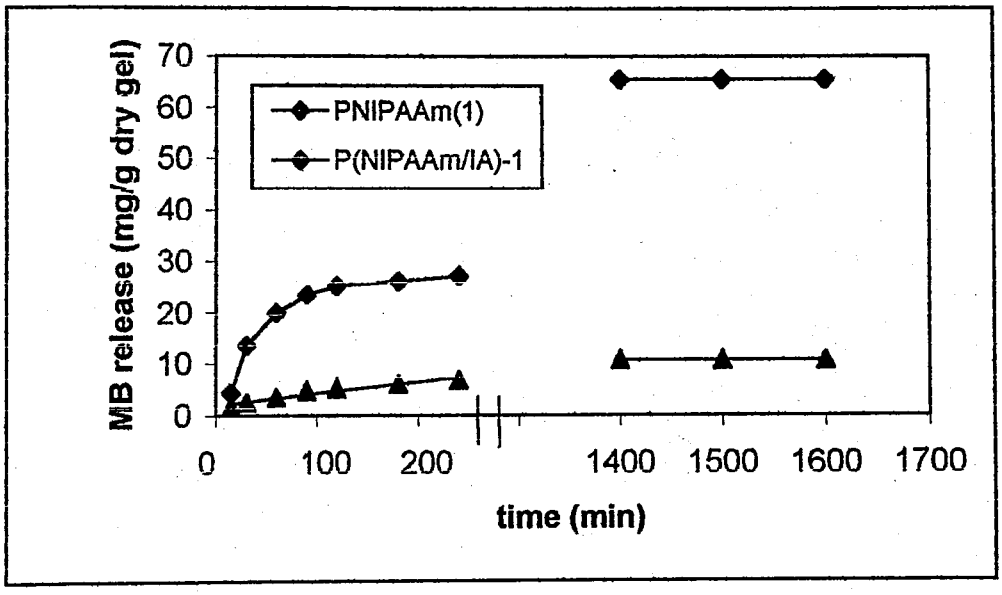


Figure 4.70. Release of non-specific adsorbed MB (mg/g dry gel) in buffer solution of pH 7.4 at 37 C from microspheres

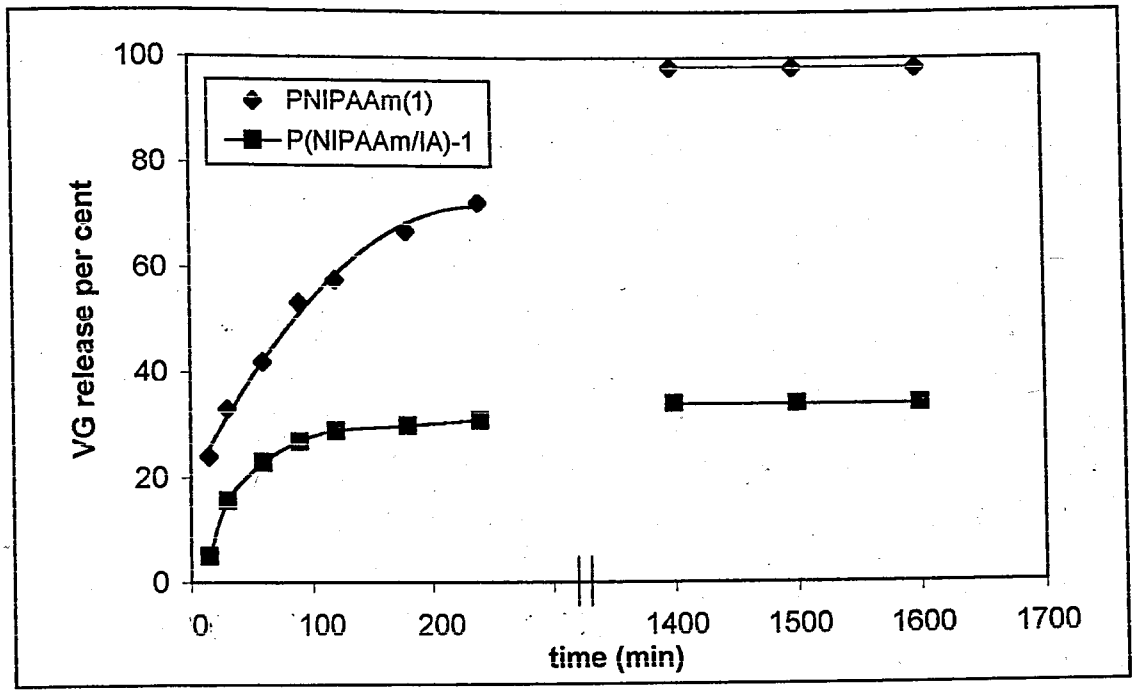


Figure 4.71. Release percent of non-specific adsorbed VG from the microspheres in buffer solution of pH 7.4 at 37°C

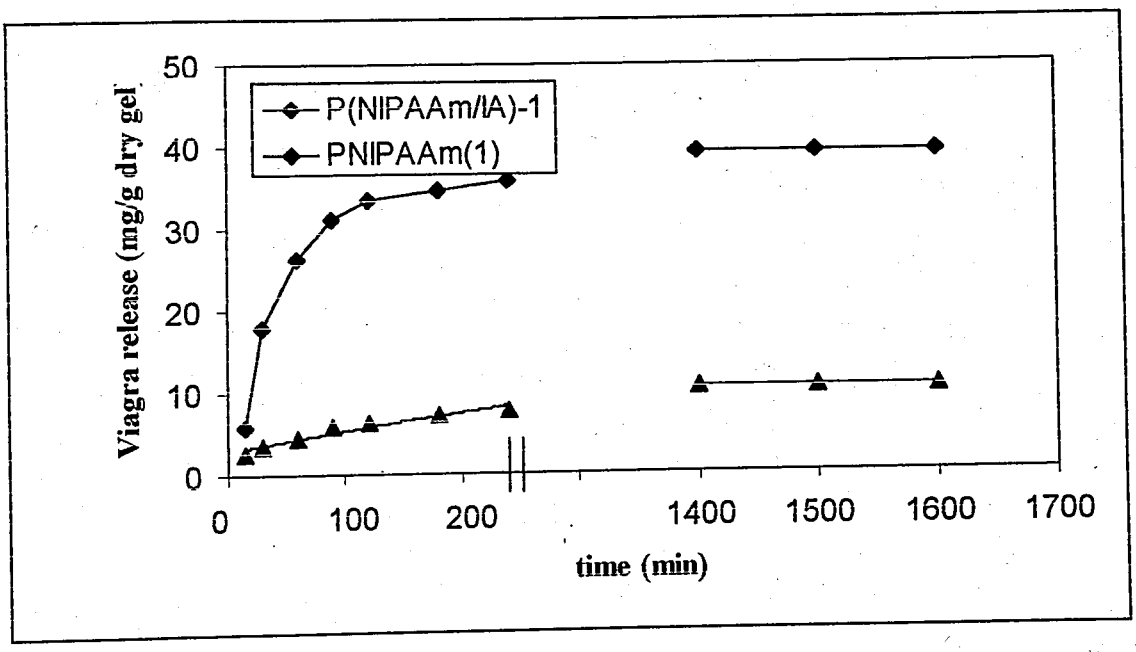


Figure 4.72. Release of non-specific adsorbed VG (mg/g dry gel) in buffer solution of pH 7.4 at 37°C from microspheres

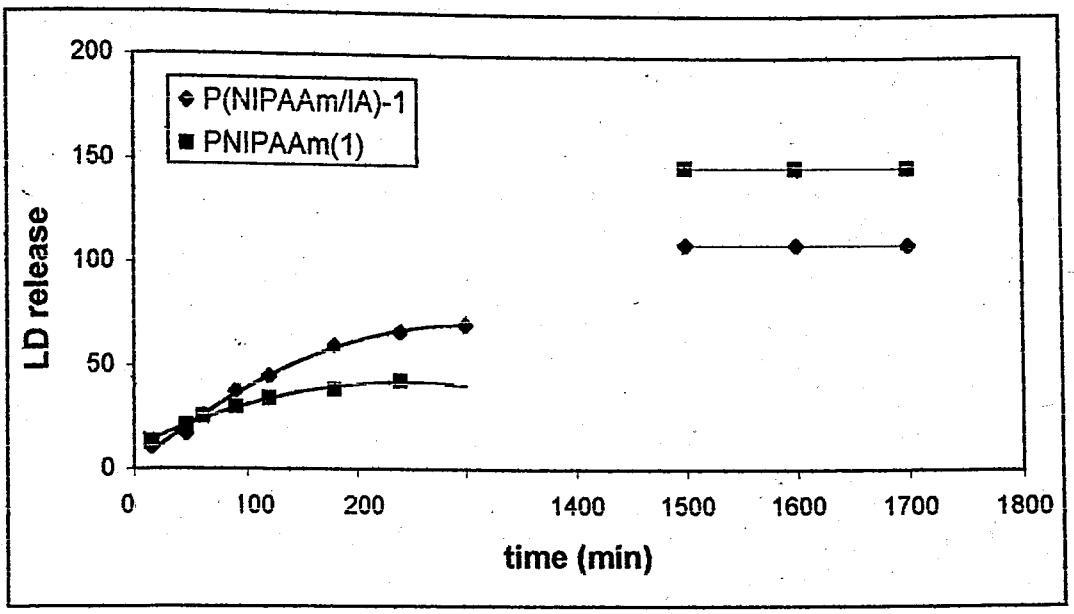


Figure 4.73. Release percent of non-specific adsorbed LD (mg/g gel) from the microspheres in buffer solution of pH 7.4 at 37°C

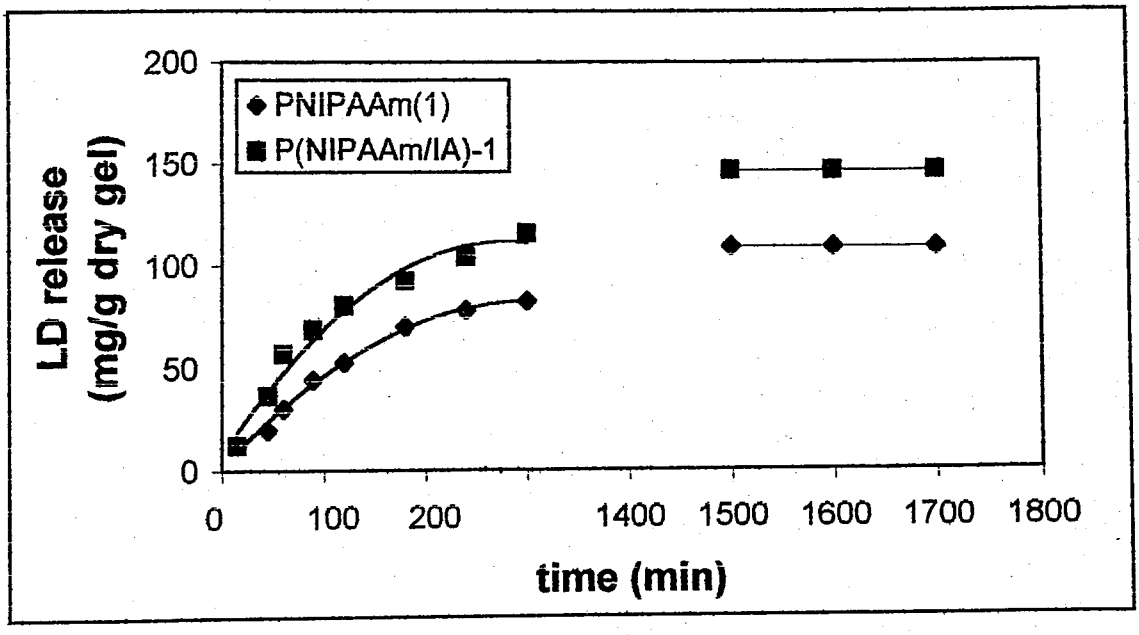


Figure 4.74. Release of non-specific adsorbed VG (mg/g dry gel) in buffer solution of pH 7.4 at 37 C from microspheres

Table 4.16. Comparison of equilibrium drug release per cent of non-specific adsorbed from NIPAAm/IA copolymeric microspheres with PNIPAAm microspheres.

MB Release %		VG Release %		LD Release %	
PNIPAAm	PNIPAAm/IA	PNIPAAm	PNIPAAm/IA	PNIPAAm	PNIPAAm/IA
98	60	98	38	93	54

The controlled release of specific adsorbed drugs (MB, VG and LD) from P(NIPAAm/IA) microspheres was investigated primarily at pH 5.5. The drug release was followed until equilibrium and then the microspheres were transferred into LD free buffer at pH 4 and after reaching new equilibrium to pH 2. The percentage release of all the drugs with time were given in Figure 4.75-4.77.

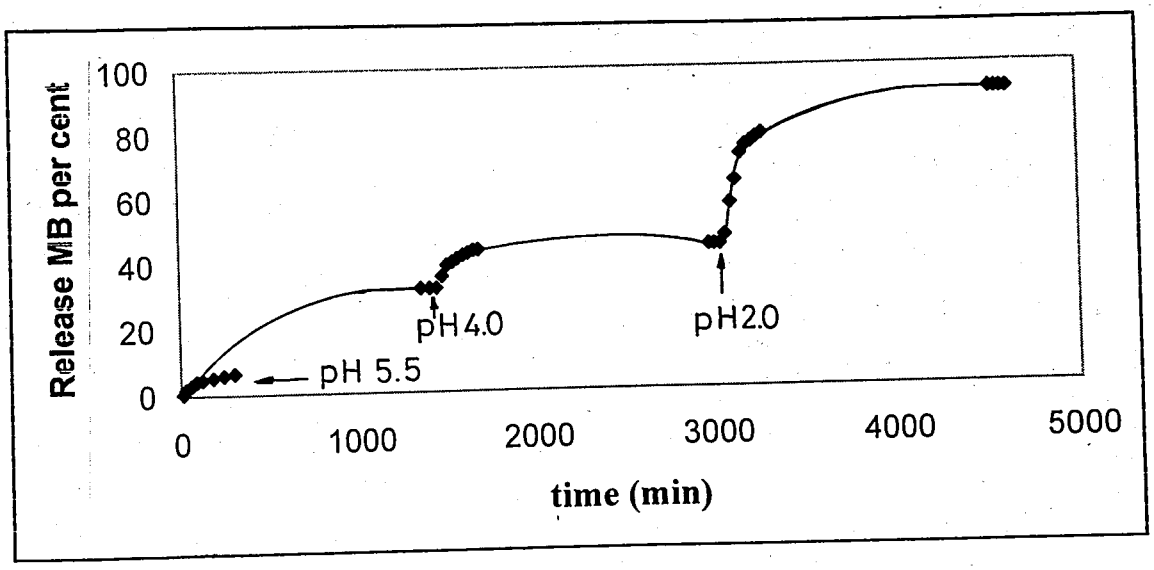


Figure 4.75. Release of specific adsorbed MB from P(NIPAAm/IA)-1 microspheres prepared at the irradiation dose of 48 kGy.

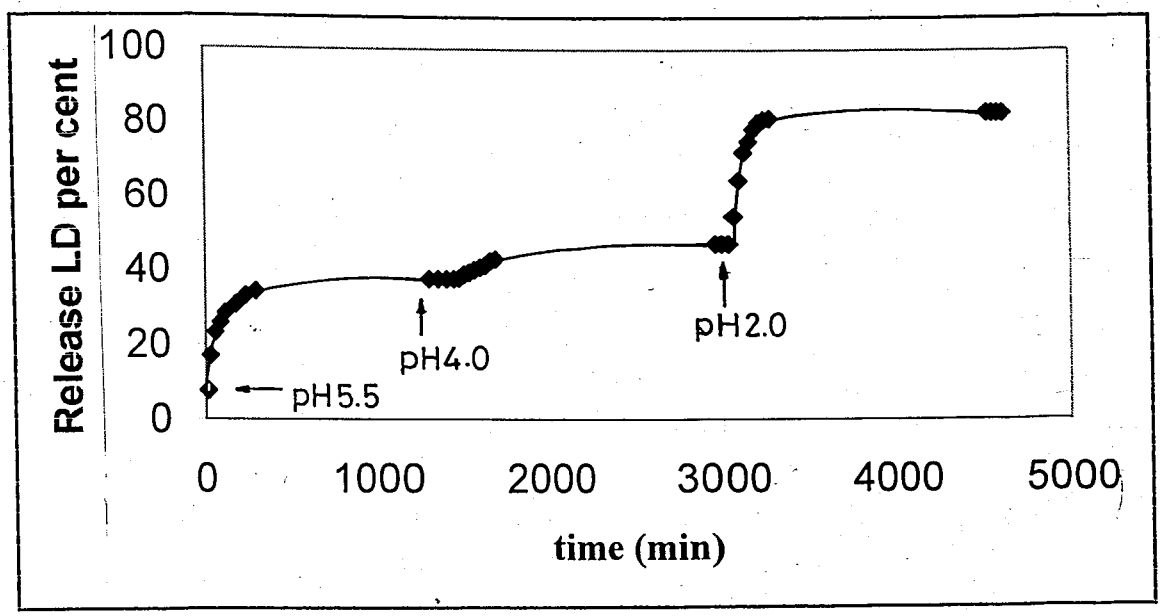


Figure 4.76. Release of specific adsorbed VG from P(NIPAAm/IA)-1 microspheres prepared at the irradiation dose of 48 kGy

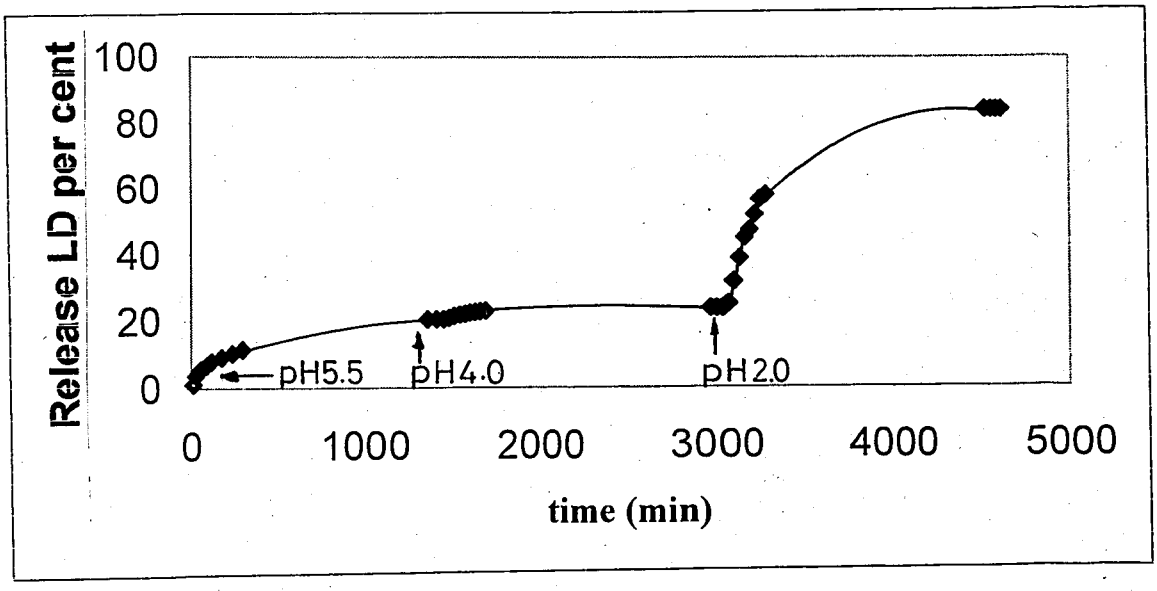


Figure 4.77. Release of specific adsorbed LD from P(NIPAAm/IA)-1 microspheres prepared at the irradiation dose of 48 kGy.

The results in Figures 4.75-4.77 and Table 4.17 illustrate that all drugs are not completely released and some portions ($m_{non\text{-}released}$) are entrapped within the gel. Even at pH 2, the drugs are not completely released from the gel. The complete release was

observed at pH 1. Table 4.17 shows that the maximum uptake into P(NIPAAm/IA)-1 microspheres was realized for the aqueous solution of lidocaine (LD). The molecules of viagra and methylene blue are larger than the molecules of LD, hence, the molecules of LD can diffuse into gel pores more easily than the other drug molecules.

The diffusion concept is explained in Section 4.6 in a more detail. In all pH buffer solutions, the controlled release amount of the adsorbed LD from P(NIPAAm/IA)-1 microspheres was higher than the controlled release of the other drugs (MB and LD).

Table 4.17. Variation of total, non-specific and specific adsorbed capacity of P(NIPAAm/IA)-1 microspheres in the different drug solutions

Drug name	$m_{non\text{-}specific}$ (mg/g dry gel)	$m_{specific}$ (mg/g dry gel)			m_{total} (mg/g dry gel)	$m_{non\text{-}released}$ (mg/g dry gel)
		pH 5.5	pH 4	pH 2		
MB	65.5	12.8	5.0	22.3	109.2	3.64
VG	39.4	20.2	5.1	27.9	103.6	11.0
LD	146.6	21.5	3.4	80.0	271.5	20.0

4.5.2.1. Effect of Microsphere Size on Controlled Release Behaviours of P(NIPAAm/IA) Microspheres. Figures 4.76-4.77 show the effect of microsphere size on the release profiles of the drugs (VG and LD) from PNIPAAm microspheres in phosphate buffer solution of pH 7.4 at 37°C. The results clearly illustrate that the release increases as the pore size increases because of higher swelling.

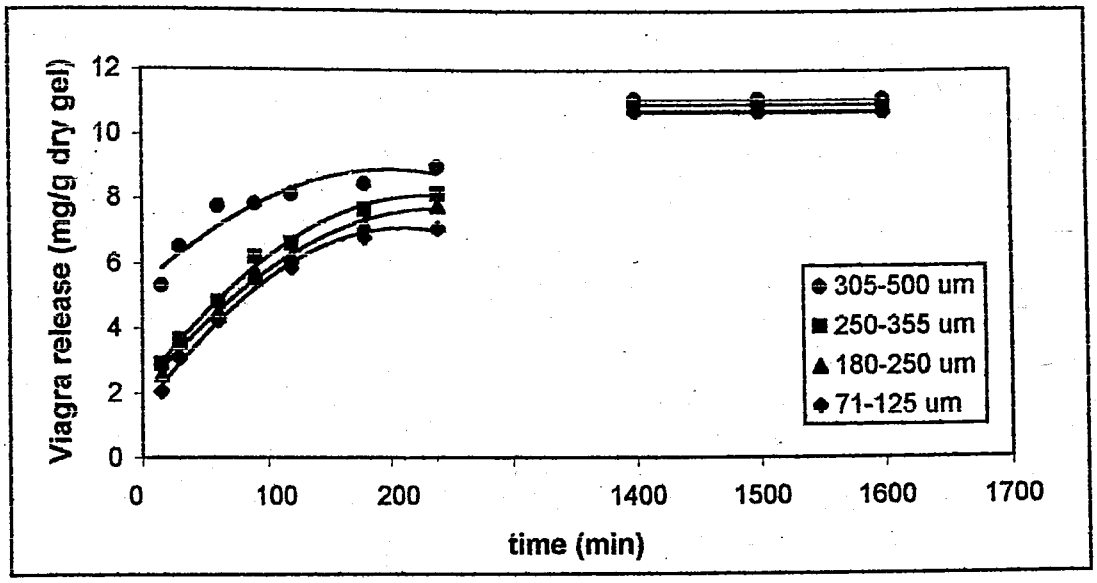


Figure 4.78. The effect of microsphere size on the release profiles of VG from pure PNIPAAm microspheres in phosphate buffer solution of pH 7.4 at 37°C

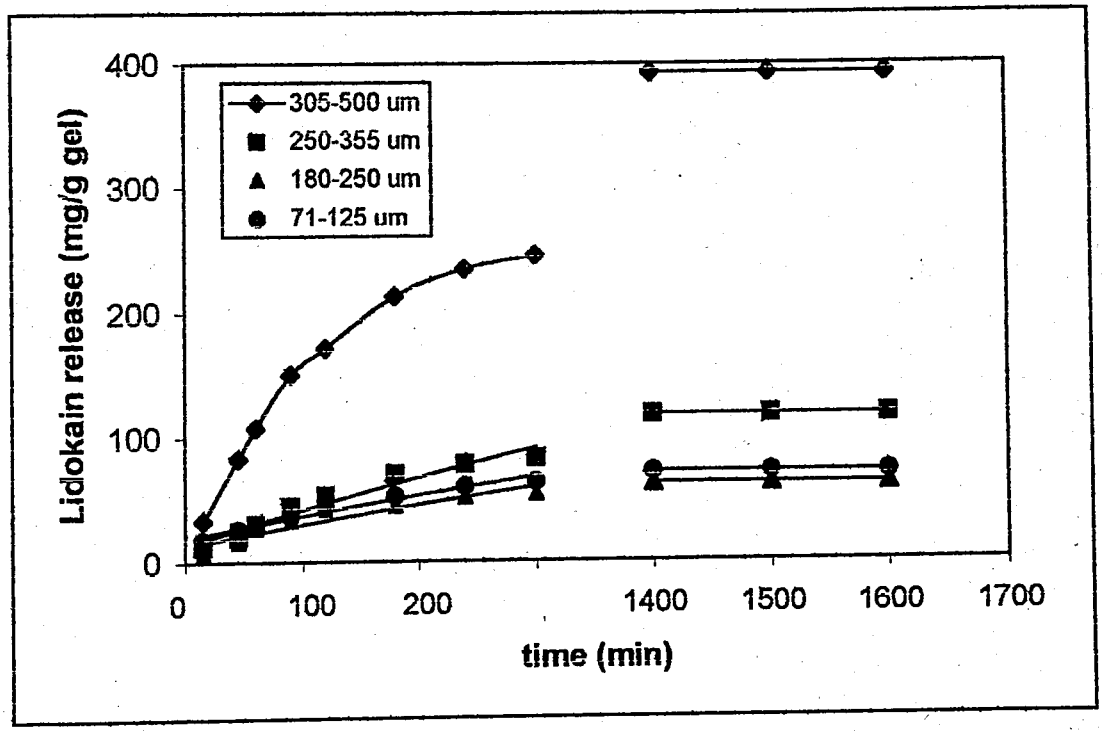


Figure 4.79. The effect of microsphere size on the release profiles of LD from pure PNIPAAm microspheres in phosphate buffer solution of pH 7.4 at 37°C

4.5.3. Morphology

The scanning electron microscopy (SEM) was used for the investigation of drug adsorption and release experiments how affects the structure of PNIPAAm microspheres (180-250 μm). Figure 4.80 shows the scanning electron micrograph of pure PNIPAAm microspheres in dry form. The dry pure PNIPAAm microspheres were equilibrated in 5000 ppm (mg/L) of lidocaine (LD) prepared in phosphate buffer at pH 7.4 at 4°C for one week. After incubation the microspheres were removed from the solution and dried in vacuum at ambient temperature. Figure 4.81 shows the scanning electron micrograph of the PNIPAAm microspheres after the lidocaine loading.

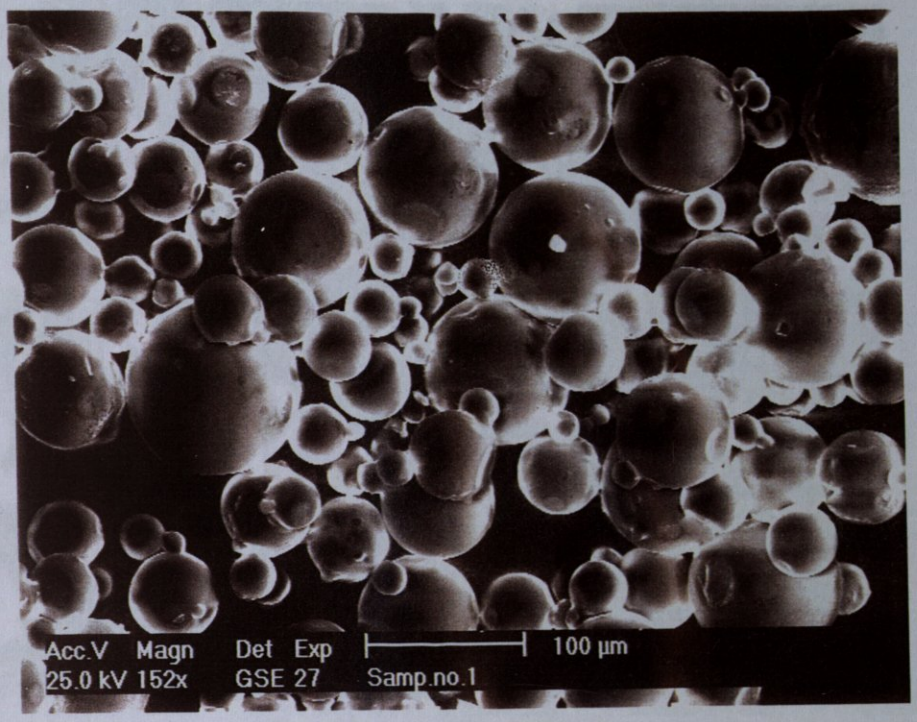
Figure 4.82 illustrates the more detailed surface morphology of the PNIPAAm microspheres after the lidocaine loading. Figure 4.83 shows the scanning electron micrograph of the PNIPAAm microspheres after loading LD and completing release experiments in all buffer solutions (pH 7.4, pH 5.5, pH 4.0 and pH 2.0) and dried in vacuum. As shown from the Figures 4.80, 4.81 and 4.83, there is no noticable difference between them.

4.6. Diffusion

Analysis of the mechanism of solute diffusion in swellable polymeric systems has received considerable attention in recent years because of important applications of swellable polymers in biomedical, pharmaceutical, environmental and agricultural engineering [63].

To elucidate the nature of the diffusion of water, Equation 2.70 was used. This equation is applied to the initial stages of swelling. From the plots of ln (M_t/M_∞) versus ln t , the exponents n and k values were calculated from the slope and intercept of the lines, respectively.

$$F = M_t/M_\infty \qquad (4.11)$$



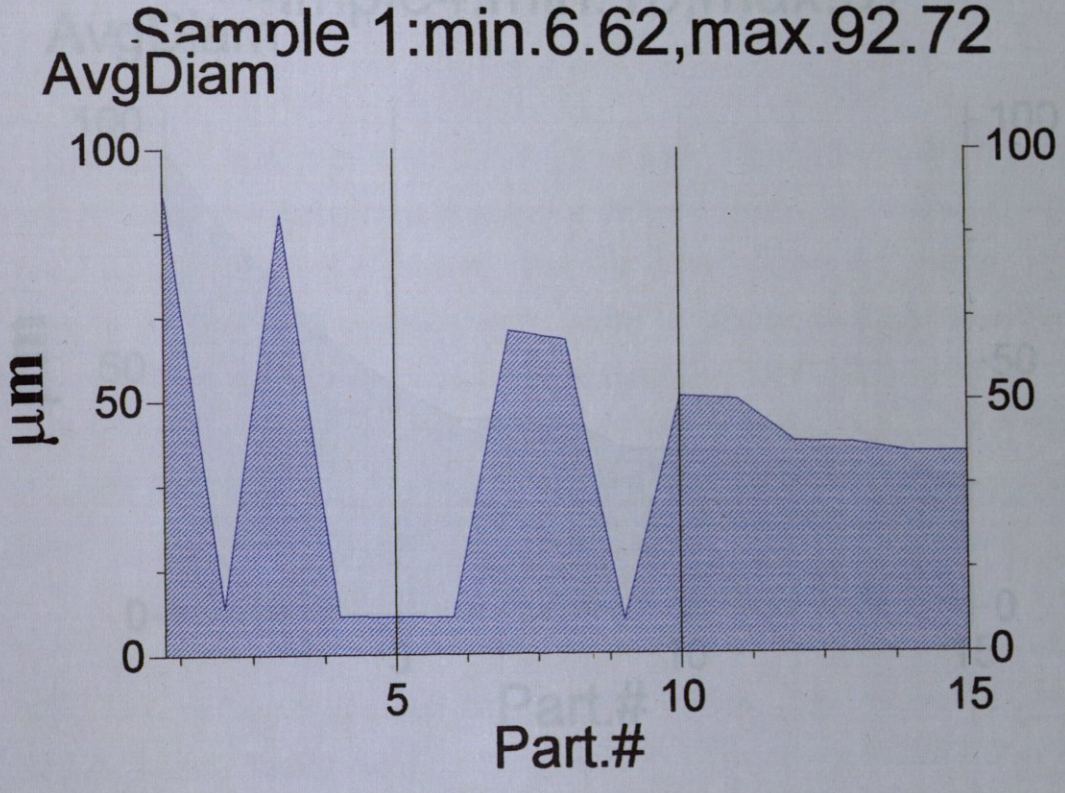
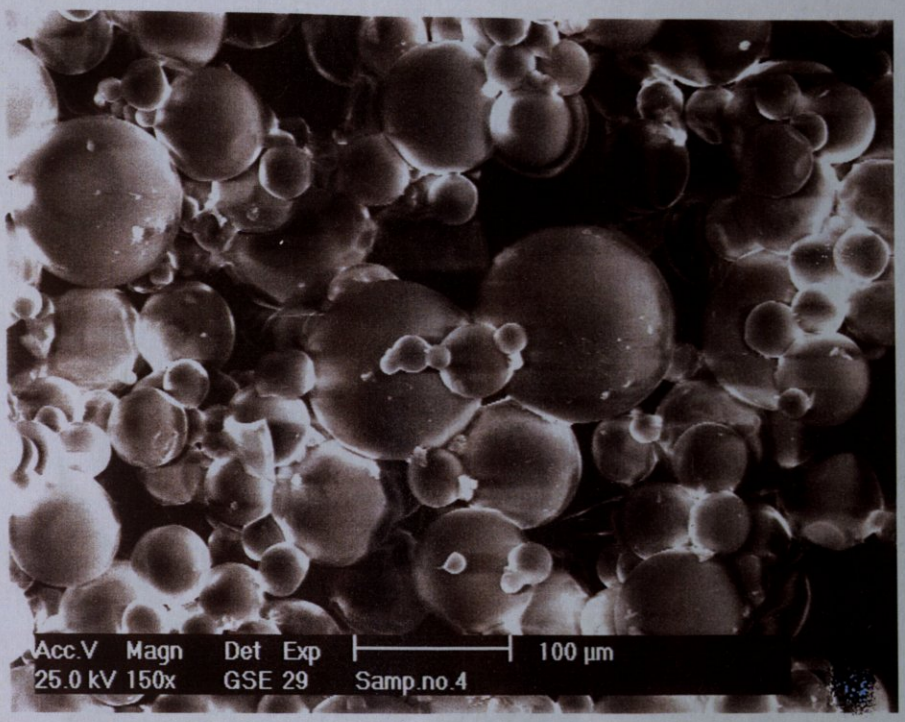


Figure 4.80. The scanning electron micrograph of pure PNIPAAm microspheres and its size distribution



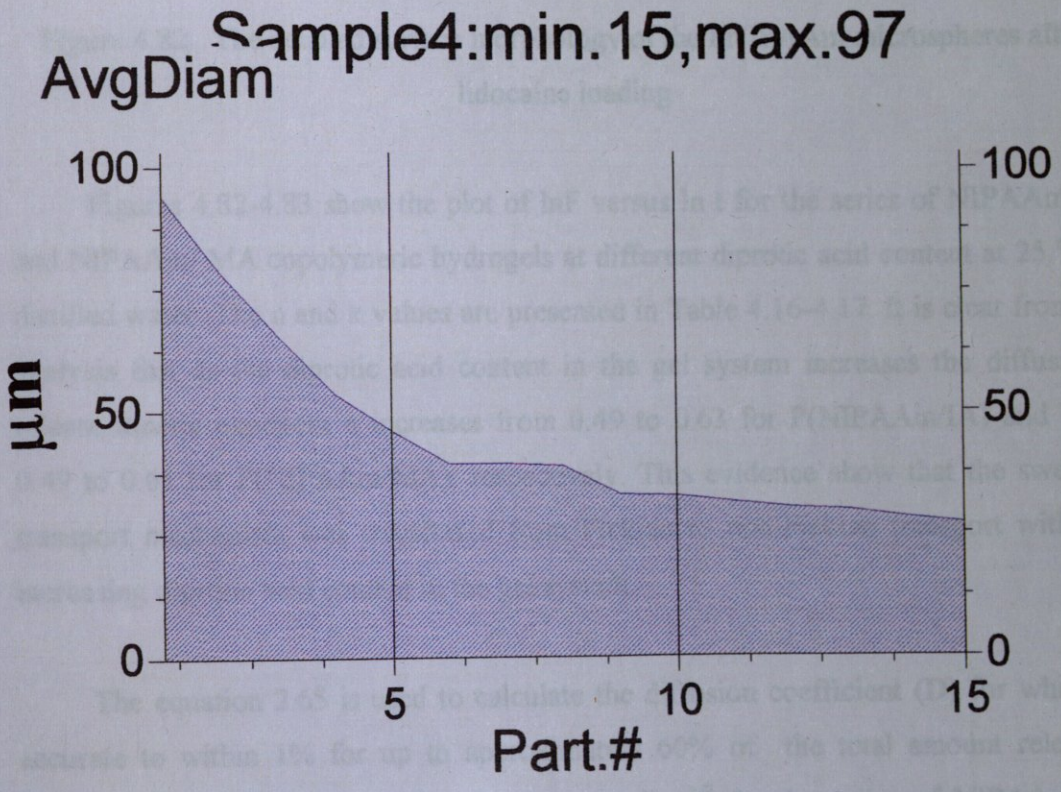


Figure 4.81. The scanning electron micrograph of the PNIPAAm microspheres after the lidocaine loading and its size distribution

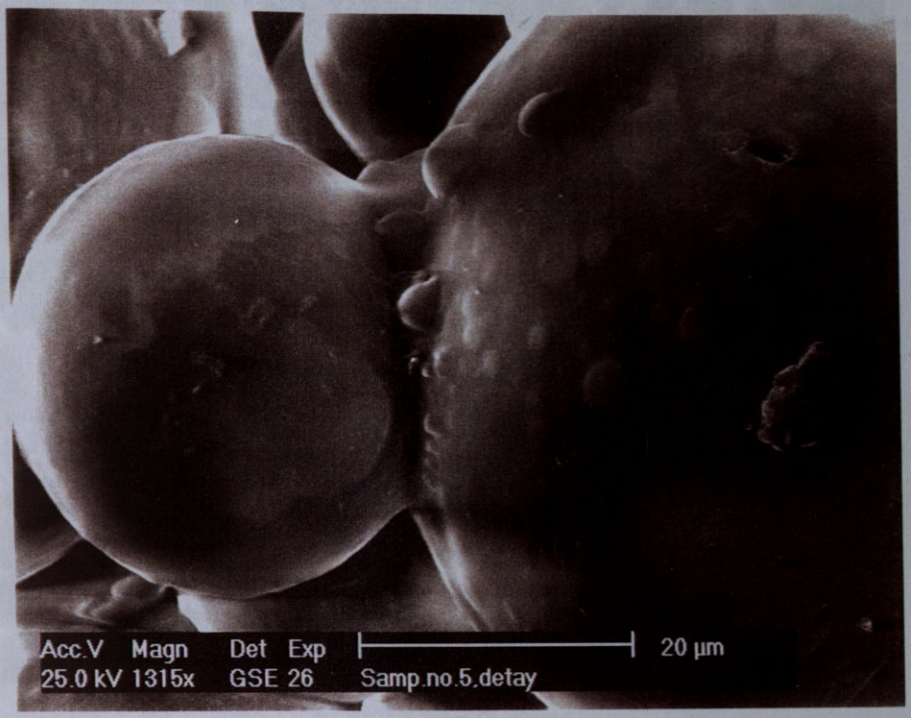
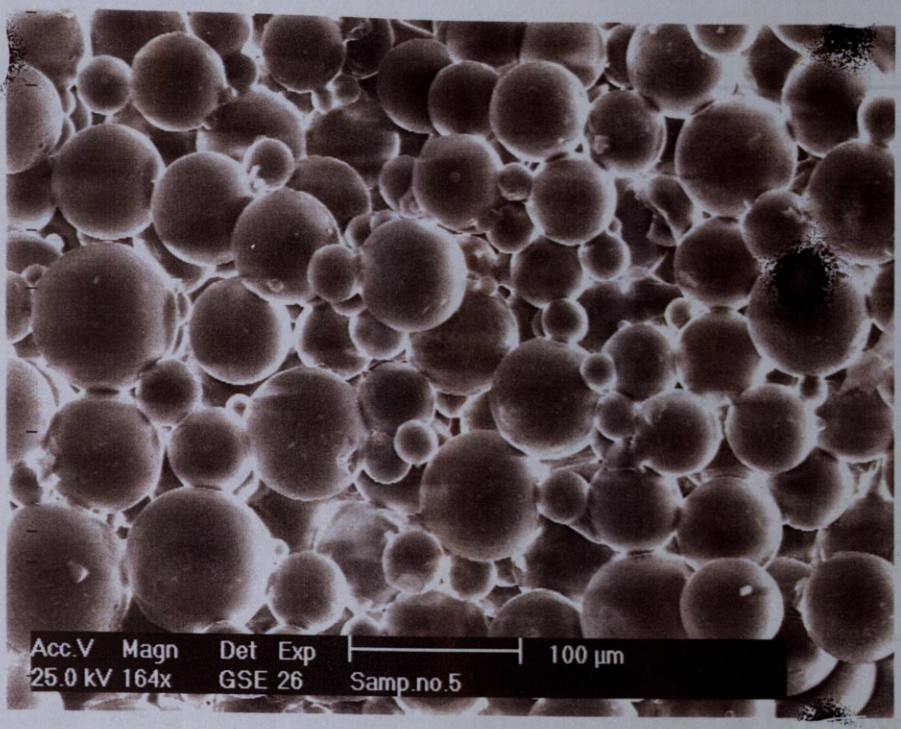


Figure 4.82. The detailed surface morphology of the PNIPAAm microspheres after lidocaine loading

Figures 4.82-4.83 show the plot of lnF versus ln t for the series of NIPAAm/ IA and NIPAAm/ MA copolymeric hydrogels at different diprotic acid content at 25 °C in distilled water. The n and k values are presented in Table 4.16-4.17. It is clear from the analysis that as the diprotic acid content in the gel system increases the diffusional release kinetic exponent n increases from 0.49 to 0.63 for P(NIPAAm/IA) and from 0.49 to 0.61 for P(NIPAAm/MA), respectively. This evidence show that the swelling transport mechanism was transferred from Fickian to non-Fickian transport with the increasing diprotic acid content in the gel system.

The equation 2.65 is used to calculate the diffusion coefficient (D) for which is accurate to within 1% for up to approximately 60% of the total amount released. Figures 4.84-4.85 show the plot of lnF versus ln $t^{1/2}$ for the series of NIPAAm/ IA copolymeric hydrogels at different itaconic acid concentration at 25°C in distilled water. The D values are presented in Table 4.16-4.17. It is clear from the results that as the diprotic acid content in the gel system increases the diffusion coefficient decreases.



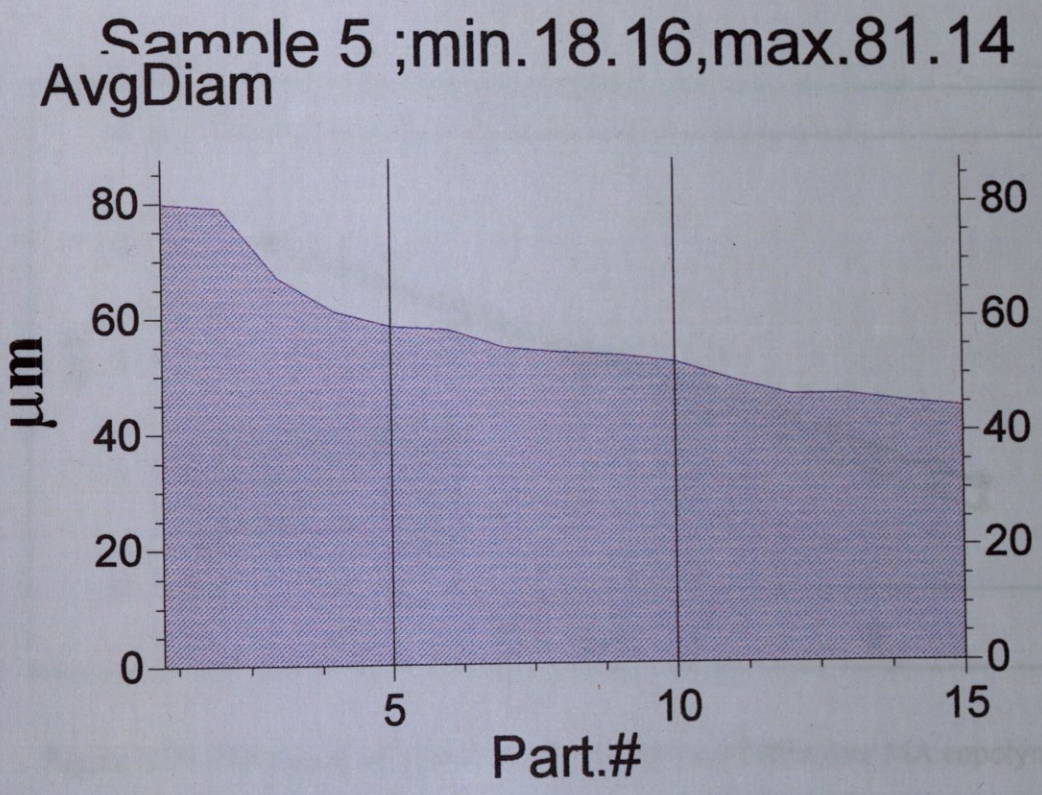


Figure 4.83. The scanning electron micrograph of the PNIPAAm microspheres after loading LD and completing release experiments in all buffer solutions (pH 7.4, pH 5.5, pH 4.0 and pH 2.0) and its size distribution

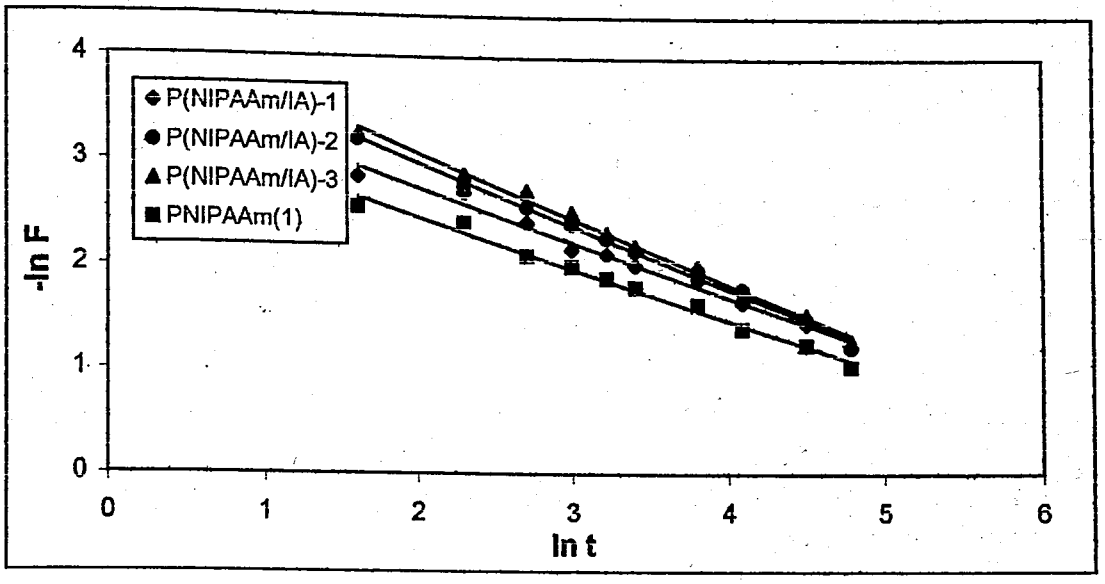


Figure 4.84. The plot of lnF versus ln t for the series of NIPAAm/ IA copolymeric hydrogels at different itaconic acid concentration at 25 °C in distilled water

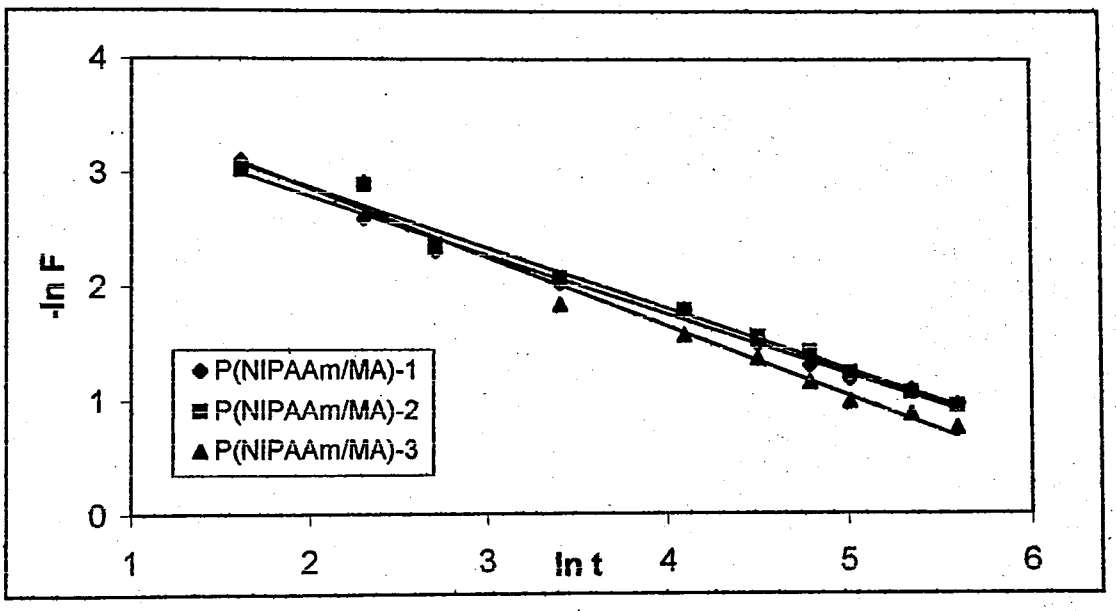


Figure 4.85. The plot of lnF versus ln t for the series of NIPAAm/ MA copolymeric hydrogels at different maleic acid concentration at 25 °C in distilled water

Table 4.18. The parameters of diffusion of water into the P(NIPAAm/ IA) hydrogels

Gel name	k	n	D x 10^6 /cm^2sec^{-1}
P(NIPAAm)-1	3.45	0.49	1.32
P(NIPAAm/IA)-1	0.26	0.52	0.67
P(NIPAAm/IA)-2	0.11	0.60	0.30
P(NIPAAm/IA)-3	3.44	0.63	0.23

Table 4.19. The parameters of diffusion of water into the P(NIPAAm/ MA) hydrogels

Gel name	k	n	D x 10^6 /cm^2sec^{-1}
P(NIPAAm)-1	3.45	0.49	1.32
P(NIPAAm/MA)-1	0.26	0.52	0.62
P(NIPAAm/MA)-2	0.11	0.53	0.50
P(NIPAAm/MA)-3	3.44	0.61	0.16

As can be seen from Figure 4.88, the equilibrium swelling of P(NIPAAm/IA)-9 hydrogel in water is greater (2684) than the equilibrium swelling of P(NIPAAm/IA)-9 in the aqueous solution of all drugs (1911-447) The molecules of the drugs are larger than the molecules of water; hence, molecules of water can diffuse into gel pores more easily than molecules of all drugs. Because the molecular weight of

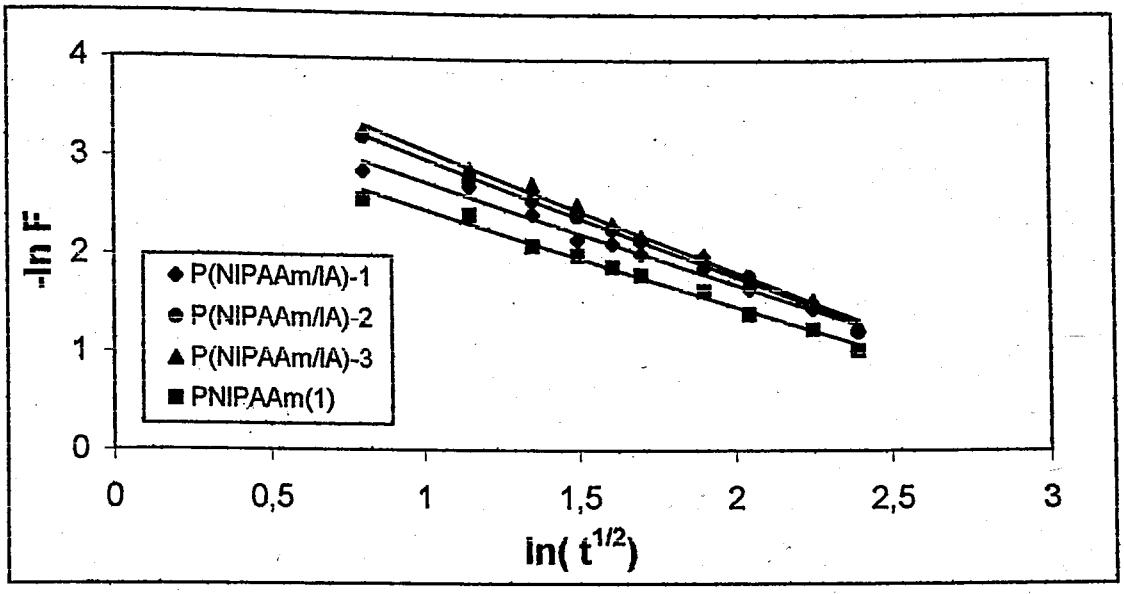


Figure 4.86. The plot of lnF versus ln $t^{1/2}$ for the series of NIPAAm/ IA copolymeric hydrogels at different itaconic acid concentrations at 25 °C in distilled water

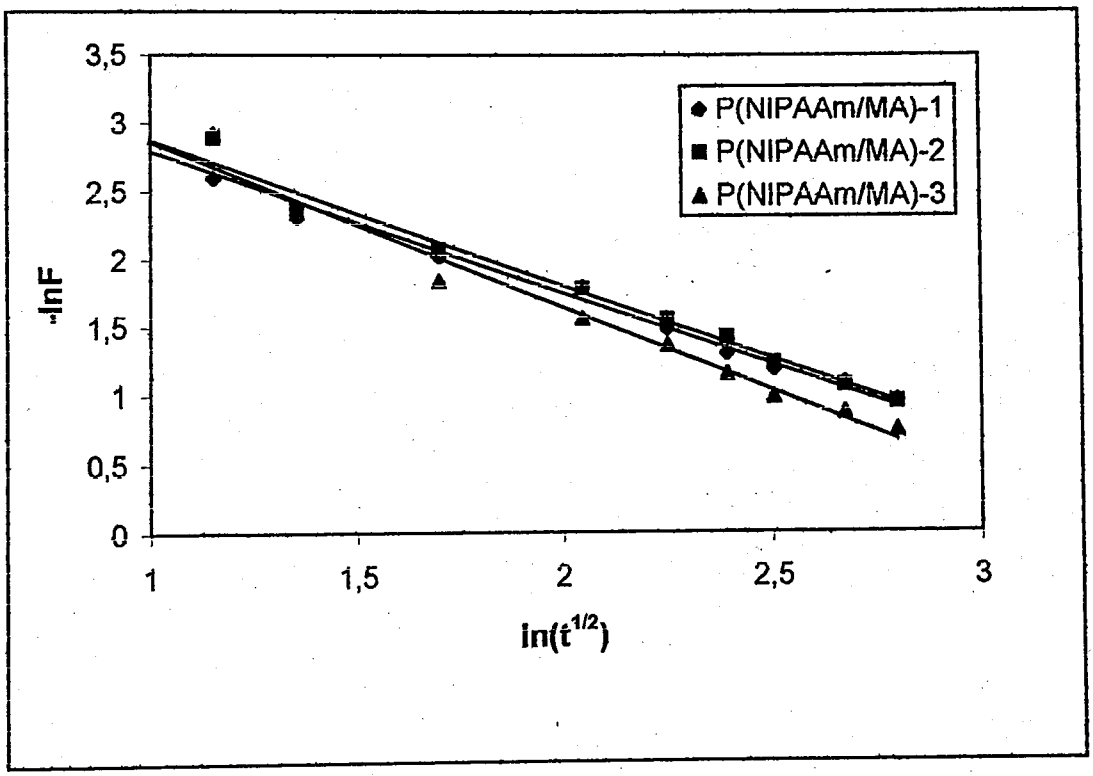


Figure 4.87. The plot of lnF versus ln $t^{1/2}$ for the series of NIPAAm/ MA copolymeric hydrogels at different maleic acid concentrations at 25 °C in distilled water

the drugs in the following order: water < lidocaine < methylene blue < viagra, the swelling of P(NIPAAm/IA)-9 hydrogel in the aqueous solution of drugs follows the reverse order.

In the experiments, the number to determine the type of diffusion (n) was found to be over 0.50 (Table 4.20). Hence the diffusion of the drugs into P(NIPAAm/IA) hydrogel was taken to be of non-Fickian character.

This is generally explained as being a consequence of the slow relaxation rate of the hydrogel. The swelling parameters of diffusion coefficients of the hydrogel in the aqueous solution of all drugs are listed in Table 4.20.

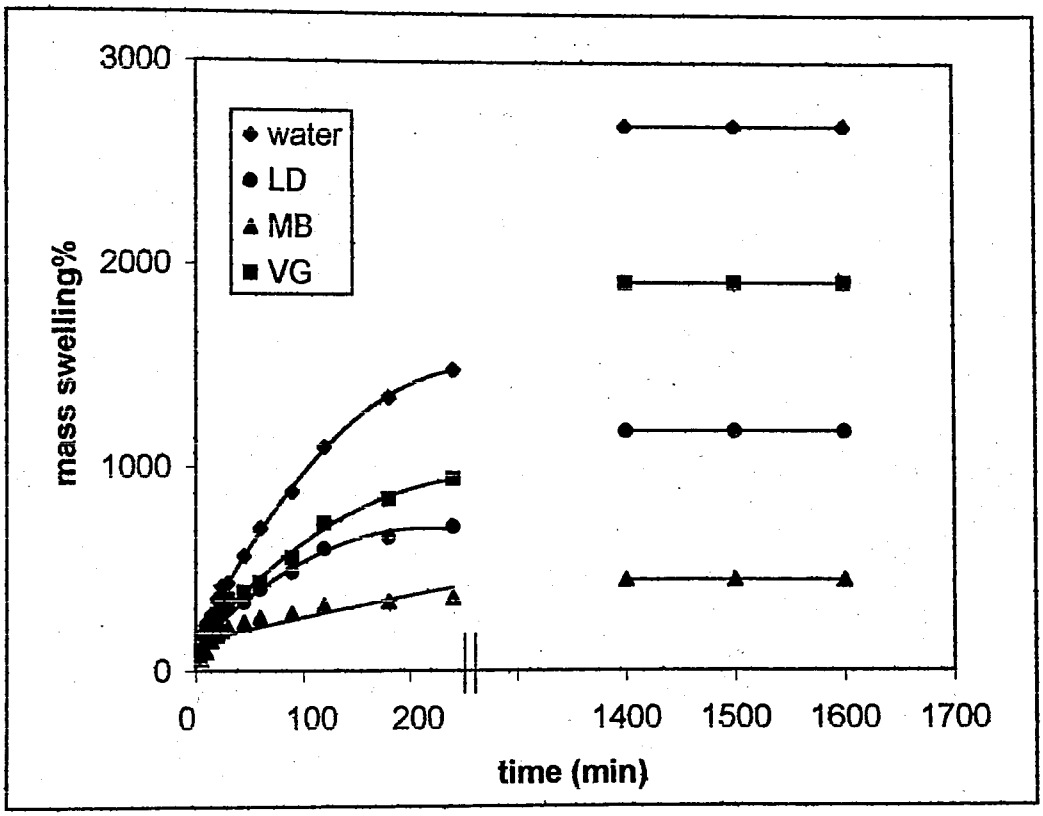


Figure 4.88. The mass swelling curves of P(NIPAAm/IA)(9) in the different drug solutions:

Table 4.20. The parameters of diffusion of water and the aqueous solution of drugs into P(NIPAAm/ IA)(9) hydrogels

Solution	k	n	D x 10^7 /cm^2sec^{-1}
Water	3.78	0.59	0.17
Lidocaine	3.68	0.56	0.21
Methylene Blue	3.22	0.53	0.52
Viagra	2.65	0.52	1.62

5. CONCLUSIONS AND RECOMMENDATIONS

The following conclusions which can be drawn from the experimental studies are summarized below; together with the recommendations for future work.

5.1. CONCLUSIONS

The hydrogels containing different amounts of diprotic acid moieties (itaconic acid or maleic acid) and cross-link densities were prepared from the ternary mixture of N-isopropylacrylamide/diprotic acid/ water by γ-rays at ambient temperature. The dependence of swelling properties and phase transitions on the comonomer concentration, irradiation dose and temperature were investigated.

The value of the equilibrium degree of swelling of an ionic network very much depends on the concentration of ionizable groups in the network. Increase in the diprotic acid content (itaconic acid or maleic acid) from 0 to 3 mole per cent causes immense increases in water uptake in deionised water. The equilibrium percentage mass swelling of NIPAAm/IA copolymeric hydrogel increased from 1264 to 11200 as the mole per cent of itaconic acid content increased from 0 to 3. Similarly, equilibrium percentage mass swelling of NIPAAm/MA copolymeric hydrogel increased from 1264 to 4039 as the mole per cent of maleic acid content increased from 0 to 3. The electrostatic interactions and mutual repulsions of the neighbouring carboxylate groups in itaconic acid or maleic acid cause expansion of the polymeric chain. This occurrence leads to higher swelling ratio of the hydrogel with more content of the diprotic acid content. This shows that the use of even very small quantities of diprotic acid-containing monomers proved to impart remarkable properties to the hydrogels of NIPAAm monomers.

Neither the value of the LCST nor the value of the degree of swelling is affected by varying the pH of the swelling medium. PNIPAAm is non-ionic hydrogel and does not have any group that could be ionised in aqueous solution. With the introduction of

the diprotic acid groups into the main chain, pH of the solution becomes an even more important factor determining swelling kinetics and equilibrium swelling value. Under acidic conditions, anionic carboxylate groups are protonated and the copolymeric network deswelled. At high pH, the acidic units are completely ionised one. This occurrence makes the swelling ratio of the gels drastically increase with an increase of ionisable constituent. The swelling shows sudden increase at pH values around corresponding pK_a values.

The higher diprotic acid content leads to the broader phase transition and a shift of the LCST to the a higher temperature. Based on these results, it can be concluded that the copolymerization of NIPAAm with the diprotic acid bearing a carboxylic groups makes it possible to change the hydrophilicity of the polymer by altering the pH-value, resulting in a change in the LCST.

In this work, the PNIPAAm hydrogels was used as a model system for thermally induced volume phase transition of gels. It exhibit discrete and reversible volume change in response to an infinitesimal change in temperature. At low temperatures, the PNIPAAm hydrogel swells and expands while at high temperatures the hydrogel collapses. At 33°C, both swollen and collapsed state were observed indicating a first-order phase transition. Although the swelling of the lightly cross-linked PNIPAAm hydrogels (6 kGy) is larger than that of the highly cross-linked one (84 kGy). Above LCST, all gels shrink to similar volume, independently from the cross-linking percentage.

Equilibrium percentage mass swelling of NIPAAm/IA and NIPAAm/MA copolymeric hydrogels decrease as the irradiation dose increases because of increasing crosslinking percentage in the hydrogels.

With increasing diprotic acid amount in the copolymer structure, the values of the number average molecular weight between cross-links increase, whereas effective crosslinking densities decrease.

PNIPAAm gel in the form of rods and beads were prepared by solution and inverse suspension polymerization techniques. It can be concluded that the polymerization techniques used significantly affect the properties of PNIPAAm gels. The swelling capacity of the gels further increases when they are prepared in the form of microspheres prepared by using inverse suspension polymerization technique.The selected fraction (180-250μm) was used for preparing NIPAAm/itaconic acid graft copolymer by radiation-induced surface modification technique. FT-IR spectrum proves successful formation of the P(NIPAAm/IA) graft copolymer by this technique.

Methylene blue, sildenafil citrate (viagra) and lidocaine were used as model drugs for the investigation of drug adsorption and controlled release behaviour for the gels in the form of the rods and microspheres. The adsorption capacity of the gels are found to increase with increasing amount of itaconic acid in the gel system. On the other hand, the adsorption of the drugs within the hydrogels decreases with increase in the irradiation dose.

The controlled release of the non-specifically adsorbed drugs from P(NIPAAm/IA) gels was followed at pH 7.4. pH 5.5, pH 4.0 and pH 2.0 buffer solutions were used for the controlled release of specifically bonded drug from the gels; since pK_a values of the itaconic acid are 5.45 and 3.85. Based on the experimental results, it can be concluded that all the drugs mentioned above are not completely released and some portions are bound to polymer.

The effect of the initial drug loading amount, temperature and microsphere size on the controlled release behaviour of the gels were investigated. The experimental results show that:

- The release is the highest from the sample with the highest drug content.
- The higher temperature (T>LCST) induced volume shrinkage of PNIPAAm networks leading to the higher drug release.
- The release increases as the pore size of microsphere increases because of higher swelling.

The geometry of the gels in the form of rods and beads significantly affects the adsorption capacities of PNIPAAm gels. For all drugs, the adsorption capacity of the microspheres is greater than that of the hydrogel rods prepared by the radiation induced polymerization.

Finally, P(NIPAAm/IA) hydrogels were used for swelling and diffusion studies in water and aqueous solution of the drugs. The swelling studies indicate that the swelling increased with the following order: water > lidocaine> methylene blue >sildenafil citrate. Diffusions of water and the drugs were found to be non-Fickian character.

5.2. RECOMMENDATIONS

For future study, the adsorption of Bovine Serum Albumine (BSA), blood proteins and enzymes onto the PNIPAAm/IA hydrogels may be investigated and the factors affecting their adsorption capacities like as pH, temperature may be determined.

The development of metal-chelating polymers continues to be a subject of paramount importance, undoubtedly because of their wide application for the separation and monitoring of metal ions. The uptake capacities for uranyl ions and some heavy metal ions may be investigated by NIPAAm/IA and NIPAAm/MA copolymeric hydrogels. The effect of external stimuli such as pH of the solution , ionic strength and temperature on the metal ion uptake capacity of these hydrogels may be studied.

The removal of colour from textile waste waters is a major environmental problem because of the difficulty of treating such waters by conventional methods. In future, a study on removing some cationic dyes from water by adsorption on a new polymeric adsorbent such as by NIPAAm/IA and NIPAAm/MA copolymeric hydrogels may be carried out.

APPENDIX A: THE CALCULATIONS OF SWELLING DATA FROM THE GRAVIMETRIC ANALYSIS

For a poly(N-isopropylacrylamide) gel, swelling data from the gravimetric analysis was the following:

Dry weight of the sample : 0.0244 g

Swollen weight of sample in water : 0.8525 g

q_w =swollen weight /dry weight = 0.8525/0.0244 = 34.94

$$V/V_o = \nu_2^0\left(1+\frac{(q_w-1)\rho}{d_1}\right) = 0.0909\ (1 + (34.94 - 1)\ 1.1) = 3.48$$

$$\nu_{2m} = 1 / [\ 1 + \rho / d_1\ (q_w - 1)\ ] = 1 / [\ 1 + 1.1/1.0(34.94-1)] = 0.026$$

$$Q = 1/0.026 = 38.83$$

APPENDIX B: THE CALCULATIONS OF SWELLING DATA FROM THE VOLUMETRIC METHOD

The swelling measurements were also carried out with the volumetric method. The data for poly(N-isopropylacrylamide) gel taken from digital caliper as follows:

D_o = initial diameter of the after synthesis

D = swelling equilibrium diameter of the gel

$$V/V_o = (D / D_o)^3 = (7.45/4.93)^3 = 3.46$$

APPENDIX C: THE CALCULATION OF THE VOLUME FRACTION OF THE NETWORK AFTER PREPARATION (ν_2^0)

The volume fraction of the network after preparation, ν_2^0, was given as follows :

$$\nu_2^0 = V_{dry} / V_{initial} = (m_{dry}/ \rho) / V_{initial} \quad (C.1)$$

For the PNIPAAm gel which were prepared according to the procedure described in section 3.3.1.1, ν_2^0 can be calculated as follows:

$$\nu_2^0 = (10 \text{ g} / 1.1 \text{ g/ml})/100 \text{ ml} = 0.0909$$

APPENDIX D : THE CALCULATION OF INTERACTION PARAMETER (χ)

Using Equation 4.6, χ was calculated as follows:

$$\chi = -[\ln (1-\nu_{2m}) + \nu_{2m}] / \nu_{2m}^2$$

$$\chi = -[\ln (1-0.026) + 0.026] / (0.026)^2 = 0.51$$

APPENDIX E : THE CALCULATIONS OF THE CROSSLINKING DENSITY (ν_e) AND THE AVERAGE MOLECULAR WEIGT BETWEEN CROSS-LINKS ($\overline{M_c}$)

Using Equation 4.7, ν_e was calculated as:

$$\nu_e = \frac{\ln(1-v_{2m}) + v_{2m} + \chi \quad v_{2m}^2}{V_1 v_2^0 \left[\left(\frac{v_{2m}}{v_2^0}\right)^{1/3} - 0.5\left(\frac{v_{2m}}{v_2^0}\right)\right]}$$

$$\nu_e = \frac{[\ln (1-0.026) + 0.026 + 0.52\ (0.026)^2]}{18^*\ 0.0909^*[(0.0216/0.0909)^{1/3} - 0.5(0.0216/0.0909]}$$

$$\nu_e = 8.97 \times 10^{-6}\ \text{mol/cm}^3$$

$$\overline{M_c} = \rho\ /\ \nu_e \qquad \text{(E.1)}$$

$$\overline{M_c} = 1.1\ /\ 8.97 \times 10^{-6} = 122631\ \text{g/mol}$$

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