Release of Vitamin B_{12} from Poly(*N*-vinyl-2-pyrrolidone)-Crosslinked Polyacrylamide Hydrogels: A Kinetic Study

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ABSTRACT: Hydrogels, composed of poly(*N*-vinyl-2-pyrrolidone) and crosslinked polyacrylamide, were synthesized and the release of vitamin B_{12} from these hydrogels was studied as a function of the degree of crosslinking and pH of the external swelling media. The three drug-loaded hydrogel samples synthesized with different crosslinking ratios of 0.3, 0.7, and 1.2 (in mol %) follow different drug-release mechanisms, that is, chain relaxation with zero-order, non-Fickian and Fickian, or diffusion-controlled mechanisms. To establish a correlation between their swelling behavior and drug-release mechanism, the former was studied by the weight-gain method and, at the same time, the concentration of the drug released was studied colorimetrically. Various swelling parameters such as the swelling exponent n, gel-characteristic constant k, penetration velocity v, and diffusion coefficient D were evaluated to reflect the quantitative aspect of the swelling behavior of these hydrogels. Finally, the drug-release behavior of the hydrogels was explained by proposing the swelling-dependent mechanism. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 76: 1706–1714, 2000

Key words: polyacrylamide; poly(*N*-vinyl-2-pyrrolidone); vitamin B_{12}; crosslinking ratio

INTRODUCTION

The incorporation of physiologically active compounds into hydrogels has led to a large range of applications such as in controlled drug-release systems,[1,2] release of agrochemicals,[3] wound dressings,[4] adsorption of metal ions,[5] and release of perfumes.[6] Most of the current research on drug-delivery systems involves the synthesis of hydrogels which release drugs for a considerable longer period, probably for some days or weeks or even months. However, these systems are not very suitable for drug delivery under certain circumstances, such as in short-term drug-delivery applications. One example is in the delivery of antibiotics and pain-relief medications after surgery or organ transplantation where fast relief is needed. In such a case, the basic requirement for a polymer matrix to be used as a drug-delivery device is that it must release the loaded drug within 12 to 36 h. It is also essential that the biological activity of the incorporated drug must be maintained during the process of incorporation.[7] If the synthesis of the gel requires a high temperature or use of an organic solvent, then it is not advisable to incorporate those biologically active compounds which may lose their activity or may become denatured.

The present study describes the use of poly(*N*-vinyl-2-pyrrolidone)-crosslinked polyacrylamide hydrogels (these will be denoted as PVP–PAamX)

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as short-term delivery devices to release vitamin B_{12} as a model drug. Vitamin B_{12}, an organometallic compound with the cobalt atom situated within a corrin ring, has been known to be a significant compound due to its involvement in various biochemical reactions. It has been suggested that it has an important role in the synthesis of nucleic acid, particularly that of the deoxyribose moiety. It also plays a role in the reduction of disulfide groups and in maintaining substances like glutathione and coenzyme A in the reduced state as well as the ratio NAD/NADH.[8] It has been shown to be involved in two mutase reactions involving the conversion of β-methyl aspartic acid to glutamic acid and of methylmalonyl coenzyme A to succinyl coenzyme A. It is also probably involved in the reduction of ribonucleotides to deoxyribonucleotides. The deficiency of vitamin B_{12} has caused diseases such as congenital pernicious anemia, gastrectomy, sprue, celiac syndrome, congenital malabsorption, inflammation, and ileal defects.[9] Vitamin B_{12} deficiency has also been reported in AIDS patients and patients suffering from spinal-cord diseases.[10]

Poly(*N*-vinyl-2-pyrrolidone) (PVP) has been known to be a good biocompatible material and is used in the manufacturing soft contact lenses along with 2-hydroxyethyl methacrylate (HEMA).[11] The nontoxic nature of PVP and its greater hydrophilicity promote its use for drug-delivery devices. One more significant point is that the synthesis of a gel does not require a high temperature or use of some organic solvent, so the probability of loss of activity during the drug incorporation is minimum. In brief, the object of the proposed study was to synthesize drug-delivery systems which can deliver a drug for the required duration and in the desired quantity by regulating the swelling behavior with the help of a crosslinker. Moreover, the study also aimed to explore the possibilities of using such drug-delivery devices in radiotherapy by incapsulating radiopharmaceuticals such as ^{57}Co-vitamin B_{12} and Tc-99m-DTPA into them. These devices may be in the form of discs, microparticles, nanoparticles, patches, or even cylindrical sections.

EXPERIMENTAL

Materials

PVP (Sigma, St. Louis, MO), potassium persulfate (KPS; Merck), acrylamide (Aam; Sigma), and *N,N′*-methylene bisacrylamide (MBA; Sigma) were of analytical grade and used as received. However, the monomer Aam was recrystallized in methanol to remove the inhibitor. Vitamin B_{12} injections of "Neurobion" (E. Merck, Mumbai, India, Batch Nos. G 5221498, G5215798, and G 5220698) were used as the model drug.

Synthesis of Drug-loaded Gels

A definite amount of PVP was dissolved in 15 mL of doubly distilled water, and to this solution, a definite quantity of the monomer Aam was added and the system was well stirred to give a homogeneous solution. Two injections of vitamin B_{12} (each 2 mL containing 1000 mg of B_{12}) were mixed into the above solution. To this, a calculated quantity of the crosslinker MBA was added and well mixed to give a homogeneous solution. Finally, KPS was added in a fixed quantity and the mixture was well stirred and poured on a Petri dish and kept in an oven at 50°C for a period of 6 h. The resulting, semitransparent pink-colored gels were cut into circular discs using a cork borer of diameter 3.2 cm. The gels were washed with distilled water to remove any loosely bound drug on the surface and then dried at 30°C for a period of 24 h in a dust-free chamber and were now ready to be used.

All three types of the drug-loaded samples were prepared with a different crosslinking ratio *X*. The crosslinking ratio *X* is given as mol MBA/mol Aam. The three hydrogel samples will be denoted as PVP–PAamX (0.3), PVP–PAamX (0.7), and PVP–PAamX (1.2), where the number in the parentheses describes the crosslinking ratio in mol %.

Swelling Study

To correlate the swelling tendency of these hydrogels with their drug-releasing capacity, the swelling behavior of drug-loaded gels was studied in a buffer solution of pH 7.4 at a physiological temperature of 37°C. The degree of swelling at different time intervals was calculated by the following expression[12]:

$$\text{Degree of swelling} = \frac{W_t - W_0}{W_0} \times 100\%$$

where W_t and W_0 are the sample weight at time t and in the dried state, respectively. The penetra-

tion velocity (v) of the buffer in each polymer was determined by the weight-gain method as reported elsewhere.[13] The penetration velocity was calculated from the slope of the initial portion of the penetrant uptake curve using the equation

$$v = \frac{1}{2dA}\frac{dW_g}{dt} \quad (1)$$

where dW_g/dt denotes the slope of the weight gain versus the time curves; d, the density of water at 37°C; and A, the area of one face of the disc.

The diffusion coefficient D of the solvent was calculated using the following equation[14]:

$$D^n = k/4(\pi r^2)^n \quad (2)$$

where r is the radius of the gel disc and n is the swelling exponent, which is denoted by the following equation:

$$\frac{Q_t}{Q_\infty} = kt^n \quad (3)$$

where Q_t and Q_∞ are the mass uptake of the solvent at time t and the equilibrium, respectively. On taking the natural log of eq. (3),

$$\ln \frac{Q_t}{Q_\infty} = \ln k + n \ln t$$

The values of n and k were calculated from the slope and the intercept of the plot of ln Q_t/Q_∞ against ln t, respectively.

pH Effect

To study the effect of pH on the swelling behavior of these hydrogels, preweighed samples were placed in various buffer solutions in the range of pH 4–9. After attainment of equilibrium, the gels were reweighed.

In Vitro Drug-release Study

To gain knowledge about the possible mode of action of the proposed drug-delivery device in the human body, it is often more convenient to perform the same study in an atmosphere almost similar to that in the body. Hence, for the study of *in vitro* drug release, the hydrogel discs were placed in 20 mL of phosphate buffer solutions at pH 7.4 at the physiological temperature of 37°C. The amount of drug released at different time intervals was studied colorimetrically (Systronics, India). After each time interval, the gels were removed from the solutions and put into 20 mL of fresh phosphate buffer. Concentrations of the released drug were then computed by comparing the absorbance with the standard curve prepared for the pure drug in the buffer in the appropriate concentration region.

RESULTS AND DISCUSSION

The swelling behavior of the PVP–PAamX hydrogel samples at pH 7.4 at 37°C is shown in the Figure 1. It is clear from the figure that the sample PVP–PAamX (0.3) swells more than do the samples PVP–PAamX (0.7) and PVP–PAamX (1.2), which may contribute to the fact that with increase in the crosslinking ratio the flexibility of the polymeric chains in the system is reduced, which results in decrease in the extent of the chain-relaxation process and, hence, swelling. Moreover, due to increase in the crosslinking ratio, the number of efficient crosslinks per unit volume increases; therefore, the crosslink density and network chain density increase, but by increasing the later parameter, there is less free volume or room to accommodate water and, hence, the degree of swelling decreases.

Various models have been proposed for the kinetics of penetrant sorption in glassy polymers.[15] For polymers which only absorb a small amount of penetrant with a little swelling, Fickian behavior is generally observed (i.e., weight gain is initially proportional to time$^{1/2}$). However, for systems which swell significantly in a solvent, deviations from Fickian behavior have been observed. A critical analysis of the swelling process reveals that two underlying molecular processes act: penetration of the solvent molecules into the void spaces in the network and subsequent relaxation of the network segments. The fundamental equation[16]

$$F = kt^n \quad (4)$$

where F denotes the amount of the solvent fraction at time t and where k and n are constants, defines three situations:

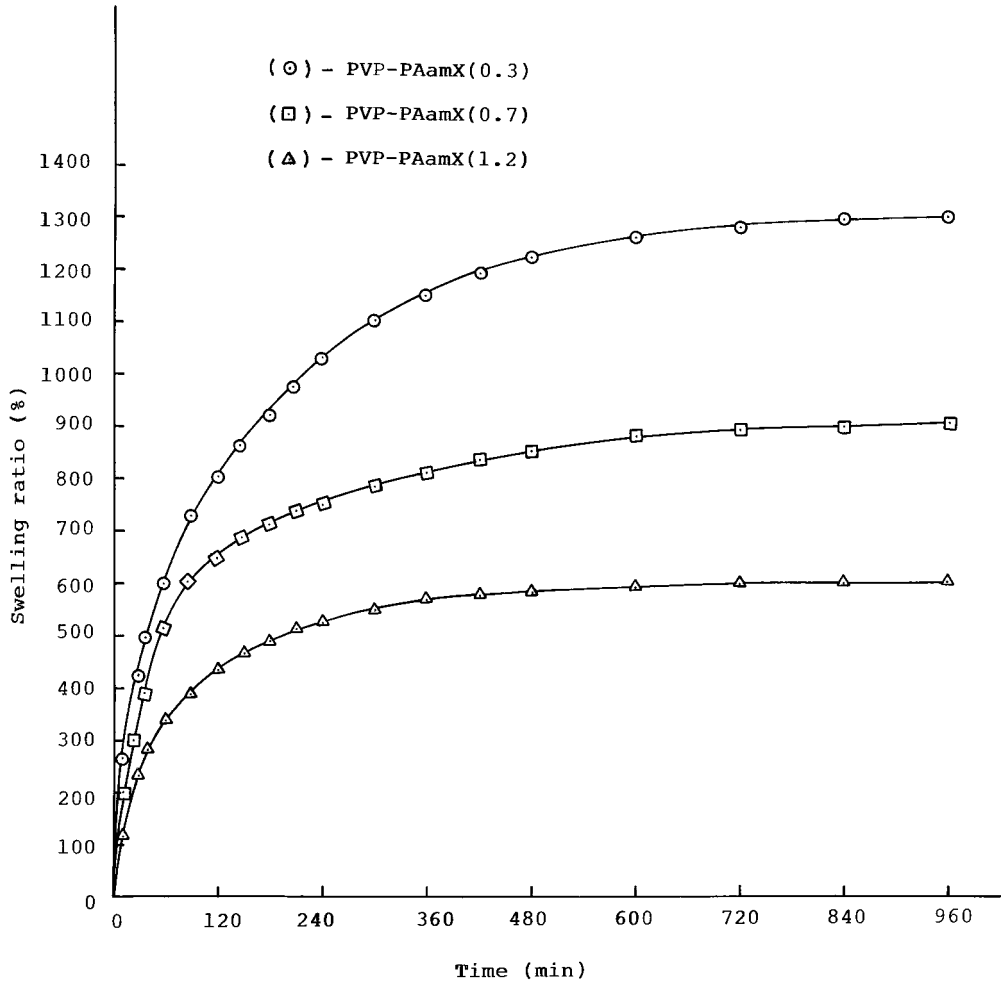

Figure 1 Swelling versus time curves for the three hydrogel samples; pH 7.4, temperature = 37°C.

1. For a perfectly Fickian process where the rate of solvent penetration is the slowest and, hence, is the rate-limiting step, the value of $n = 0.50$.
2. When the penetrant velocity is far greater than the chain relaxation rate, then the solvent uptake is proportional to time, that is, $n = 1$.
3. When the two rates are comparable, the value of n falls between 0.5 and 1.

However, from a study of a number of models in the literature, it appears that sample size is an important factor in influencing the observed behavior.[17] A rather large sample dimension may mask any anomalous effect occurring in the system. The large sample size does contribute to the accuracy in the measurement since removal of samples for weighing should not introduce much error. Equation (1) is applied to the initial stages of swelling and plots of ln F versus ln t are depicted in Figure 2.

The swelling exponents n for the three samples were found to be 0.95, 0.74, and 0.51, respectively. These values clearly indicate that sample PVP–PAamX (0.3) shows almost a relaxation-controlled case III transport,[18] while PVP–PAamX (0.7) and PVP–PAamX (1.2) show non-Fickian and Fickian swelling behavior, respectively. Clearly, this analysis indicates that these hydrogel samples can be used as swelling-controlled drug-release devices to allow the active ingradient to be released by classical diffusion as well as chain-relaxation-controlled

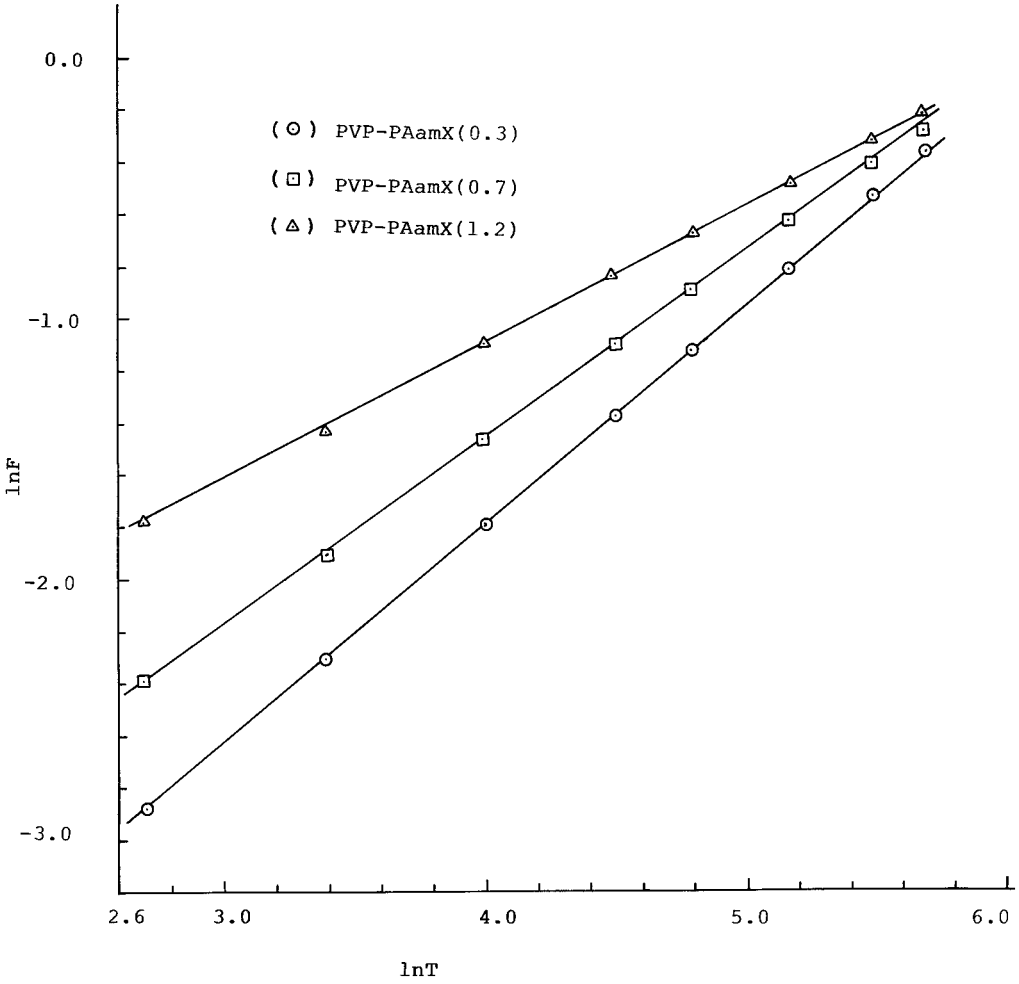

Figure 2 Plots of ln F versus ln t for the three hydrogel samples; pH 7.4, temperature = 37°C.

mechanisms. All the swelling parameters are described in the Table I.

pH-dependent Swelling Behavior

The equilibrium swelling capacity data as shown in Table II clearly suggest that the pH of the external media does not influence the swelling capacity of the hydrogels. The observed experimental findings have already been reported by the authors of this article in their previous work.[19] The reason for the pH-independent swelling behavior is that the proposed hydrogel is composed of two nonionic polymers, namely, PVP and PAam, which do not contain any ionizable groups within the gel network. As a result, these gels do

Table I Swelling Parameters of PVP–PAamX Hydrogels at 37°C at pH 7.4

Sample	Equilibrium Swelling Capacity (g H_2O/g gel)	Penetration Velocity ($v \times 10^3$) cm/s	n	$k \times 10^2$	Diffusion Coefficient ($D \times 10^4$) cm^2/s
PVP–PAamX (0.3)	13.04	7.03	0.95	5.07	7.21
PVP–PAamX (0.7)	9.26	4.87	0.74	8.71	4.23
PVP–PAamX (1.2)	6.12	3.06	0.52	16.52	2.89

Table II Effect of pH of External Media on the Equilibrium Capacity of the Hydrogels at 37°C

	Equilibrium Capacity at pH		
Sample	4.0	7.4	9.0
PVP–PAamX (0.3)	13.08	13.04	13.11
PVP–PAamX (0.7)	9.21	9.26	9.30
PVP–PAamX (1.2)	6.08	6.12	6.15

not respond to any change in pH of the external swelling media. Some authors have mentioned the partial hydrolysis of the amide groups of PAam into carboxylic groups at higher pH values.[20] However, no such hydrolysis was observed in the present case. There may be a possibility of such hydrolysis of amide groups at higher temperatures such as 50°C and onward, but since the experiments in the present study were carried out at the physiological temperature of 37°C, the feasibility of such a reaction is almost nil. The authors have not gone further into studying the same effect at higher temperatures, because if the proposed drug-delivery system is to be used in human body, it will deliver the drug at body temperature and, hence, it is not very fruitful to study the effect at higher temperatures that are never attained by the body.

In Vitro Drug Release

The study of *in vitro* drug release from a matrix gives an idea about its ability to function as a sustained and controlled drug-delivery system and this in fact forms the basis of its study of *in vivo* performance. Figure 3 describes the *in vitro* drug-release profile of the three hydrogel samples at 37°C in a phosphate buffer of pH 7.4. It is clear from the figure that the rate of drug release is much faster in the initial phase, which may contribute to the fact that when the drug-loaded gel is placed in the buffer solution its outer surface immediately comes into contact with the solvent with the result of diffusion of the solvent into the matrix, followed by a fast drug release. Similar observations have also reported by other workers.[21,22] The initial release rate from the three samples was 8.4×10^{-3}, 5.1×10^{-3}, and 2.1×10^{-3} g drug/g polymer min. The rates were calculated by fitting the initial dynamic swelling data to the equation

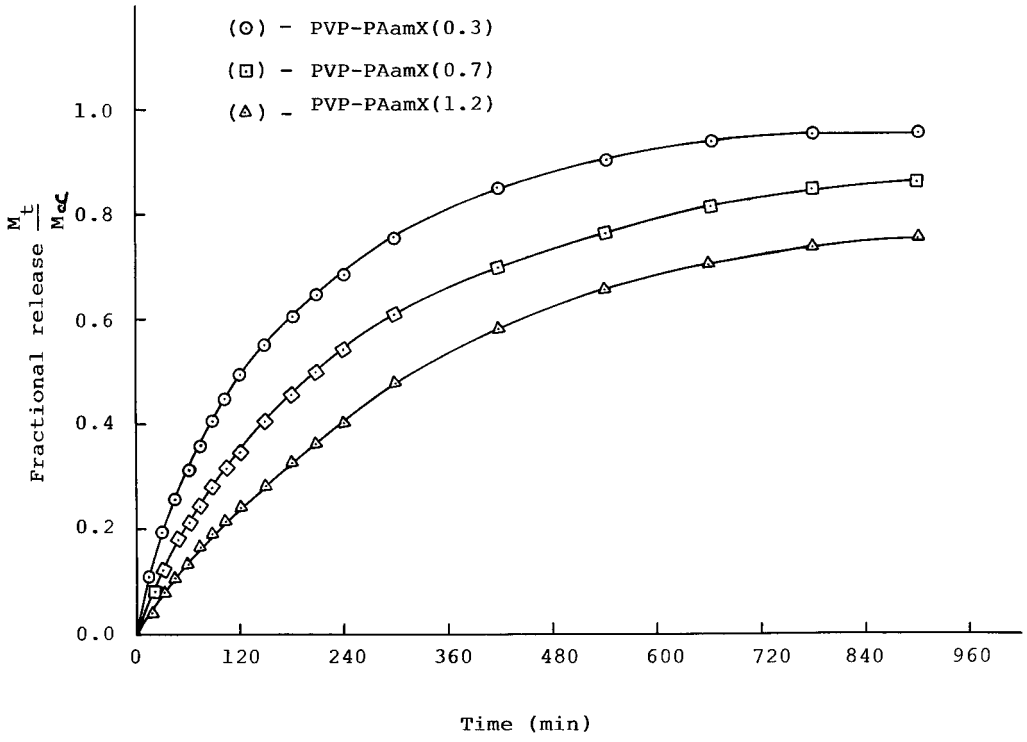

Figure 3 Fractional release of vitamin B_{12} from the three samples; pH 7.4, temperature = 37°C.

$$\frac{M_t}{M_\infty} = kt^n \quad (5)$$

and taking the derivates as time approaches zero. Here, M_t/M_∞ is the fractional release; t, the release time; and n, the diffusional exponent. From the same analysis, the diffusional exponents n were calculated as 0.95, 0.76, and 0.54. These values suggest that the drug-release mechanism can be governed by the crosslinking ratio of the drug-loaded polymer matrix. Now, for sample PVP–PAamX (0.3), which is the most loosely crosslinked polymer matrix, the diffusional exponent n is found to be 0.95, thus providing the right relaxational characteristics for a zero-order release behavior, whereas for the sample PVP–PAamX (0.7), the value of n is 0.76, which suggests that the hydrogel follows the diffusional mechanism with a chain-relaxation contribution (i.e., non-Fickian behavior). Finally, the value 0.54 for sample PVP–PAamX (1.2) corresponds to the Fickian or diffusion-controlled release behavior. In this way, the drug-loaded hydrogels follow different types of release mechanisms depending on their crosslinking ratios. Since these hydrogel samples follow a diffusion-controlled drug-release mechanism, it appears that the release of the drug should continue so long as the hydrogels swell by absorbing the solvent. But it was observed that these gels continue to release the drug (although at a very slow rate) even after the attainment of equilibrium swelling. However, the release was so slow that it took almost 24–36 h more to release the drug completely after attaining maximum swelling. Similar results were also reported by Yaung and Kwei[23] in the case of swelling and controlled release from a hydrogel composed of PVP and poly(acrylic acid). The release of the drug from the devices after attaining maximum swelling may be due to that since the size of the B_{12} molecules is very large as compared to that of penetrant molecules the drug molecules left in the interior part of the hydrogel disc continue to diffuse out slowly through the gel pores even after the maximum swelling has been attained by the gel. Here, it is worth mentioning that since the presence of the drug inside the hydrogel creates an osmotic swelling pressure between the gel and the solution phase the swelling is more pronounced in the case of drug-loaded hydrogels, rather than in the plain polymer matrix. When the drug-loaded gel attains maximum swelling, the osmotic swelling pressure becomes almost zero and, hence, the entrance of the solvent into the gel is discouraged. Now, the drug, still inside the hydrogel, diffuses out with an extremely small rate due to the absence of any driving force. This may be accounted for by the slow release of the remaining drug after attainment of maximum swelling.

Release Mechanism

From the above discussion, it is clear that these drug-loaded hydrogels release the drug via a swelling-dependent mechanism which consists of the diffusion of water into the polymer matrix accompanied by relaxation in the polymeric segments and then, ultimately, diffusion of the incorporated drug through the water-filled pores. Figure 4 represents a simplified view of the overall release process. Figure 4(a) shows the completely dried drug-loaded hydrogel disc in which the drug B_{12} molecules are incorporated inside the crosslinked network. When the gel is placed in the solvent, it diffuses into the polymer matrix, thus causing the polymeric chains to relax. As a result, the molecules of B_{12} diffuse out through the water-filled pores from the other surface of the hydrogel disc, whereas the drug molecules in the bulk of the matrix remain in the network [Fig. 4(b)].

The solvent front continues to move slowly toward the bulk with a simultaneous release of drug molecules from the matrix until all the drug comes out of the device [Fig. 4(c)]. Now, since the size of the B_{12} molecules is sufficiently large as compared to the solvent molecules (i.e., H_2O), the drug continues to be released even after the attainment of equilibrium swelling. Here, it is significant to mention that in the case of a highly crosslinked network structure the relaxation of polymeric segments is restricted or almost negligible, whereas for a hydrogel with a loosely crosslinked structure, the relaxation of the polymer chain contributes significantly toward the total swelling of the matrix and drug release from it. In this way, the crosslinking ratio plays a significant role in governing the drug-release behavior of the hydrogel samples.

CONCLUSIONS

The swelling and drug-release behavior of hydrogels composed of PVP and crosslinked PAam were studied at the physiological temperature of 37°C at pH 7.4. The hydrogels show pH-independent

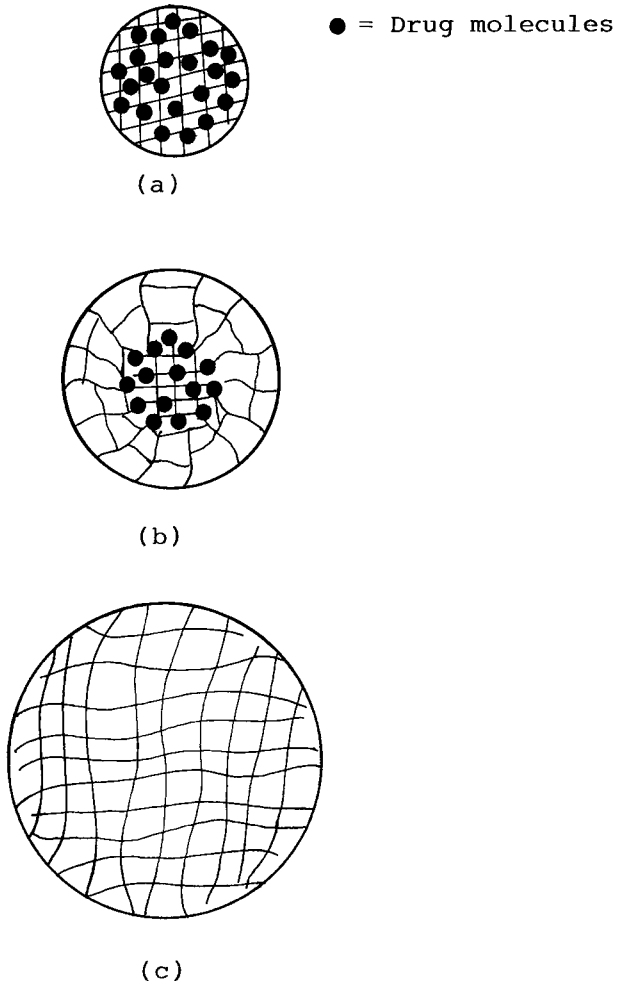

Figure 4 (a) Completely dried drug-loaded hydrogel, with the drug molecules inside the crosslinked network. (b) The hydrogel with the drug released from its outer portion in the initial stage of release process. (c) The hydrogel from which all the drug incorporated has been released, due to swelling.

swelling behavior due to presence of nonionic components. The hydrogel sample with a crosslinking ratio of 0.3% (in mol) is found to follow a relaxation-controlled drug-release mechanism with zero order, while the samples with the crosslinking ratios of 0.7 and 1.2% show non-Fickian and Fickian release behavior, respectively.

The advantage of the proposed drug-delivery system is that the release kinetics of the drug from the system can be tailored by just adjusting the concentration of the crosslinker and, hence, these hydrogels, apart from their possible use in short-term drug delivery, may also be used to release a drug for a longer period by using the sufficient amount of crosslinkers in the case where a prolonged release is required. The great biocompatibility of PVP, plus the mild conditions for gel synthesis and drug encapsulation, makes the proposed device a potential system for protein and peptide drug delivery. Moreover, since radiopharmaceuticals like ^{57}Co-vitamin B_{12} have widely been used for a differential diagnosis of anemia[24] and other gastrointestinal diseases, the proposed system may put some more emphasis on radiotherapy in investigating the sustained release and elucidating the metabolic pathways of different types of drugs such as antimicrobial drugs, colonic drugs, and anticancer drugs in the human body.

Gastrointestinal (GI) disorders such as gastric ulcers, inflammatory bowel disorders (IBD), or even colon cancers require site-specific drug-delivery systems for effective drug action. But since there is variation in pH along the GI tract, the devices sensitive to pH can not be used as they will not be able to maintain the constant therapeutic drug level at the target site for the desired period, which is the basic criteria for an optimal delivery system, for example, the Alzas Oros® osmotic pump, which releases its contents at independent zero-order kinetics.[25] The system studied is not only pH-independent but also follows zero-order kinetics at the crosslinking ratio 0.3 mol % and, hence, it may be developed in the near future as a site-specific drug-delivery system for the GI tract. One more advantage with this system is that it can function at any site along the whole GI tract irrespective of the pH at that particular site. Hence, the system studied will function uniformally as site-specific drug-delivery device at any desired site along the gastrointestinal tract. Finally, the system can be made pH-responsive by incorporating anionic or cationic monomers into the gel matrix and, therefore, may be used as a glucose-sensitive drug-delivery system for the treatment of diabetes.

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REFERENCES

1. Krishnaiah, Y. S. R.; Satyanarayana, S.; Rama Prasad, Y. V.; Narasimha Rao, S. J Control Rel 1998, 55, 245.
2. Kushwaha, V.; Bhowmick, A.; Behera, B. K.; Ray, A. R. Artif Cell Blood Substit Immobil Biotechnol 1998, 26, 159.
3. Donempudi, S.; Yaseen, M. J Polym Mater 1994, 11, 73.
4. Self, C. H.; Thompson, S. Nature Med 1996, 2, 817.
5. Snowden, M. J.; Thomas, D.; Vincent, B. Analyst 1993, 118, 1367.
6. Peppas, N. A.; Am Ende, D. J. J Appl Polym Sci 1997, 66, 509.
7. Nakamae, K.; Nizuka, T.; Miyata, T.; Furukawa, M.; Nishino, T.; Kato, K.; Inoue, T.; Hoffman, A. S.; Kanzaki, Y. J Biomater Sci Polym Ed 1997, 9, 43.
8. Hebert, V. Am J Clin Nutr 1988, 48, 882.
9. Antia, F. P.; Abraham, P. In Clinical Dietetics and Nutrition, 4th ed.; Oxford University: New Delhi, India, 1998; p 69.
10. Fauci, A. S.; Lango, D. L. In Principles of Internal Medicine, 14th ed.; Fauci, A. S.; Longo, D. L., Eds.; McGraw-Hill: New York, 1998; Vol. 2.
11. Sariri, R.; Tighe, B. Iran Polym J 1996, 5, 256.
12. Peng, T.; De Yao, K.; Yuan, C.; Goosen, M. F. A. J Polym Sci Part A Polym Chem 1994, 32, 591.
13. Peppas, N. A.; Fvanson, N. M. J Polym Phys Ed 1983, 21, 983.
14. Peppas, N. A. Pharm Acta Helv 1985, 60, 110.
15. Tighe, B. J. In Hydrogels in Medicine and Pharmacy; Peppas, N. A., Ed.; CRC: Boca Roton, FL, 1987; Vol. III, p 53.
16. Robert, C. C. R.; Buri, P. A.; Peppas, N. A. J Appl Polym Sci 1985, 30, 301.
17. Hopfenberg, H. B. J Membr Sci 1987, 3, 215.
18. Ritger, P. L.; Peppas, N. A. J Control Rel 1987, 5, 37.
19. Bajpai, S. K. submitted for publication in Iran Polym J.
20. Ramraj, B.; Radhakrishnan, G. J Appl Polym Sci 1994, 52, 837.
21. Chandy, T.; Sharma, C. P. Biomaterials 1993, 14, 939.
22. Thacharodi, D.; Panduranga Rao, K. Biomaterials 1995, 16, 145.
23. Yaung, J.-F.; Kwei, T. K. J Appl Polym Sci 1998, 69, 921.
24. IANCAS Bulletin on Radiopharmaceutical and Nuclear Medicine; 1995; Vol. 11; p 18.
25. Hui, H. W.; Robinson, R.; Lee, V. H. L. In Controlled Drug Delivery: Fundamentals and Application, 2nd ed.; Robinson, J. R.; Lee, V. H. L., Eds.; Marcel Dekker: New York, 1987; p 373.