

# Mechanistic analysis of protein delivery from porous poly(vinyl alcohol) systems

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*We report on the preparation of both porous and non-porous poly(vinyl alcohol) (PVA) hydrogels that can be used as carriers to release proteins. Non-porous gels were prepared by freezing and thawing of aqueous PVA solutions, while porous gels were formed by adding porosigens such as NaCl or ammonium carbamate into aqueous PVA solutions. Bovine serum albumin, a model protein, was loaded into non-porous prior to freezing and thawing, and release under various conditions was performed. The number of freezing/thawing cycles affected the initial rate of release, the amount of protein released, and the transport mechanism of BSA.*

*Key words: Hydrogels – Protein release – Bovine serum albumin – Poly(vinyl alcohol).*

Controlled drug or protein release from biocompatible polymers is an increasingly important area of research in pharmaceutical engineering. Polymer hydrogels are cross-linked networks of hydrophilic polymers that are used as versatile release materials. Specifically, hydrogels of poly(vinyl alcohol) (PVA) show great potential because of the unique biomedical properties of PVA and its ability to be prepared by benign preparation techniques [1, 2].

We stress that the success of a release system is most dependent on the carrier of the drug delivery system. Many preparation techniques require the use of toxic agents such as cross-linking agents, catalysts, and stabilizers. These chemicals can leach out of the carriers and make them unsuitable for biological applications. Therefore, there is a high demand for carriers made by benign manufacturing techniques.

One such benign process is the formation of PVA hydrogels by freezing and thawing aqueous PVA solutions. Upon freezing and thawing, crystalline regions form in the ensuing gels [1-6]. PVA is one of the few polymers that exhibit this phenomenon. Its crystalline regions serve as cross-linking sites in the gel. Thus, after freezing and thawing the gel behaves as a matrix of chemically cross-linked polymer chains. PVA itself is a non-toxic and non-carcinogenic material and has been approved for use in several devices such as contact lenses and artificial organs [7, 8].

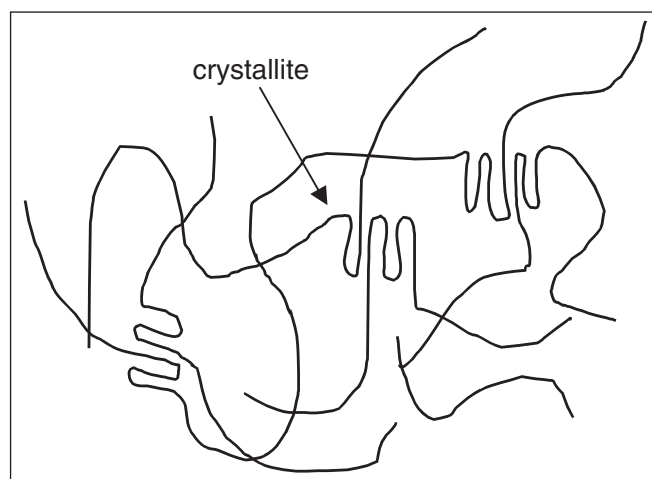
Drug or protein molecules can be loaded into PVA gels, but drug release can be slow due of the time required for water diffusion into the hydrogel. Many applications require faster release [9]. By creating channels or pores in the PVA hydrogels, water can diffuse much more rapidly into the gel and the release time can be greatly reduced.

The specific goals of this work were to create porous and non-porous hydrogels and to study the protein release properties of these materials.

## I. POLY(VINYL ALCOHOL) HYDROGELS

Hydrogels are polymer networks held together by physical or chemical cross-links. Although the polymer is hydrophilic, the cross-links make the gel insoluble in water. These networks are stable at room temperature and swell when immersed in water. The extent of the swelling is determined by the density of cross-links and the intrinsic properties of the polymer [1].

Chemical cross-linking agents have been used to successfully form cross-links, but these materials tend to be toxic even in small quantities. Harmful agents hinder the use of polymer films in pharmaceutical and biomedical applications. A possible solution to the cross-linking dilemma is the utilization of a unique property of PVA: when an aqueous solution of PVA is exposed to repeated cycles of freezing and thawing, crystallites form and act as physical cross-links (see *Figure 1*), and the final polymer behaves as if it were chemically cross-linked [1, 10-19].



**Figure 1** - Structure of amorphous and crystalline PVA regions after freezing and thawing.

The properties of PVA hydrogels are affected by the process of freezing and thawing. Since 1975, our group [3] has been working with PVA systems synthesized by freezing and thawing. The crystallization of PVA was first verified using turbidity measurements of PVA solutions as they were subjected to various freezing and thawing periods [3, 20-24]. The properties of water-swollen PVA systems were determined by their temperature-time histories. It was also concluded that crystallite formation was a function of thawing time, freezing time and the polymer concentration of the PVA solution. During thawing the size of the crystallites first increased and then decreased probably because of the breakdown of crystallite structures. Higher weight percent solutions produced larger crystallites while longer freezing time resulted in increased crystallinity [3].

Hyon *et al.* [4] suggested a mechanism for the formation of crystallites in PVA gels. As the temperature of the PVA solution decrease below room temperature, the intermolecular interactions among the chains may produce crystallites. This phenomenon is facilitated by the formation of hydrogen bonds. As the cooling time increases, the crystallization proceeds further. The end result is a material of high mechanical strength prepared without chemical cross-links.

Urushizaki *et al.* [5] determined that the number of freezing and thawing cycles affected the degree of physical cross-linking. They concluded that as the number of freezing and thawing cycles increased the gels became stiffer and firmer. Stauffer and Peppas [6] further investigated the mechanical integrity and swelling ratio of PVA gels. An increase in freezing time, number of freezing and thawing cycles, or PVA concentration resulted in increased mechanical strength and decreased swelling ratios. Their work also determined that stable hydrogels were formed after only one hour of freezing. The rate of equilibrium water uptake decreased with increased number of cycles, suggesting that the additional cycles caused substantial densification of the gel structure. The weight-swelling ratio of the hydrogels indicated that hydrogels underwent major structural changes during the early freezing and thawing cycles.

Drugs, proteins, and other therapeutic substances can be released from chemically cross-linked films. Fujisato *et al.* [7] released several important compounds such as glucose, heparin and albumin from chemically cross-linked PVA films.

Our group [8, 25-35] investigated the release of a model protein, bovine serum albumin (BSA), from physically cross-linked PVA systems. BSA was incorporated into the PVA solution prior to freezing and thawing. First, the integrity of the protein was analyzed by freezing a dilute BSA solution for seven days. Both UV spectrometry and ELISA tests confirmed that the protein conformation was unaffected by the freezing and subsequent thawing. The release of BSA exhibited Fickian diffusion (the diffusional coefficient,  $n$ , was between 0.49 and 0.56). The crystallinity had little effect on the BSA release.

Mongia *et al.* [11, 24] studied the mucoadhesive properties of PVA. The best candidates for mucoadhesive applications were samples prepared from 20 wt% aqueous PVA solutions that were exposed to two freezing and thawing cycles. These gels demonstrated high values of work of adhesion in contact with mucin and high mechanical strength. Drug release from PVA samples was also investigated. PVA disks were prepared

by freezing and thawing of 20 wt% aqueous PVA solutions. The gels were exposed to two, three, or four cycles of freezing for twelve hours followed by thawing for two hours. The disks were then soaked in theophylline or oxprenolol hydrochloride solutions for 24 h and the drugs were incorporated prior to the cyclical process. The drug release was monitored over time by using a UV/Vis spectrophotometer.

The mode of drug incorporation greatly affected the release behavior. The release of theophylline was much faster for systems prepared by immersing a hydrogel in a drug solution than for the samples with the drug incorporated prior to freezing and thawing.

Certain release applications require faster release than can be achieved by hydrogels prepared by freezing and thawing of an aqueous PVA solution. The delivery of therapeutic agents to the stomach requires faster release. One way to achieve a faster swelling time is to prepare highly porous hydrogels [9].

The focus of the present contribution was to synthesize non-porous and porous PVA hydrogels for drug delivery applications and to characterize the release of model protein from these carriers.

## II. EXPERIMENTAL PART

### 1. Synthesis of porous and non-porous hydrogels

Freezing and thawing cycles were employed to synthesize PVA gels. Aqueous solutions of 10 and 15% by weight of PVA were subjected to two, three and four freezing and thawing cycles at -20 and 25°C, respectively. Each cycle consisted of 4 h of freezing and 8 h of thawing at room temperature.

PVA (Elvanol HV, E.I. DuPont de Nemours, Wilmington, DE, United States,  $M_n = 64,000$ , polydispersity index = 2.02, degree of hydrolysis = 99.0) and deionized water were mixed and placed in an oven for 6 h at 90°C. The resulting solution was cooled and poured between two flat glass plates separated by Teflon spacers and secured with clips. After the gel underwent freezing and thawing, the polymer film was cut into thin disks, approximately 12 mm in diameter.

Porous hydrogels were synthesized by the incorporation of two different salts into a PVA solution. Sodium chloride and ammonium carbamate were incorporated in a 1:4 and 1:1 ratio of salt to PVA. Ammonium carbamate decomposes at 40°C so it was added to the PVA solution after it had cooled. After the salt was incorporated into the PVA solution, the mixture underwent freezing and thawing. After the gels have been synthesized, the gels were heated to 40°C to cause the ammonium carbamate to decompose. The gels were then dried in the hood.

Sodium chloride was mixed with the dry PVA prior to the addition of water. The procedure outlined above for the synthesis of hydrogels was followed. After the gels have been synthesized, the gels were subjected to several washings over 36 h to remove the sodium chloride. The films were cut into thin disks and dried.

### 2. Protein loading into non-porous and porous gels

Bovine serum albumin (Fraction V Powder, Sigma Chemical Company, St. Louis, MO, United States) was incorporated

into the hydrogels. After the PVA was dissolved in water, the solution was cooled for 2 h at room temperature. A concentrated drug solution (7.6% by weight of BSA) was mixed with the PVA and water. BSA was also incorporated by imbibition. A concentrated drug solution was prepared (5% drug in deionized water). The dried disks were saturated with drug by placing two disks in 20 ml of the BSA solution. The gels remained in the drug solution for approximately 24 h.

### 3. Protein release studies of non-porous and porous hydrogels

BSA release studies from PVA gels were conducted under USP II conditions but at 80 rpm and  $37 \pm 0.2^\circ\text{C}$ . Each gel was immersed in 500 ml of deionized water. Three milliliters of release medium was removed at hours 1, 3, 6, 9, 12, 24 and 48, and 50 fresh milliliters replaced the older release medium. The BSA solutions were analyzed using a UV/Vis spectrometer (Perkin-Elmer Corporation, Norwalk, CT, United States) at 279 nm.

## III. RESULTS AND DISCUSSION

### 1. Synthesis of non-porous and porous gels

Non-porous gels were synthesized by freezing and thawing 10 and 15 wt% aqueous PVA solutions. Care was taken when casting solutions to avoid incorporating bubbles into the sample. Both solutions produced stable gels after two cycles. The gels were transparent and flexible.

Diffusion of water is slow in non-porous gels because water must diffuse through the glassy matrix of the gels; however, a dried gel with an open pore structure allows for much faster diffusion and swells much faster due to capillary wetting.

Porous hydrogels could be prepared in a variety of ways including the addition of porosigens. Porosigens, such as sucrose, sodium chloride and PEG were incorporated into the polymer solution prior to preparation, and their extraction from the polymer network created a porous network. The size of the porosigen determined the pore size.

Park *et al.* [9] prepared porous hydrogels by the gas blowing or foaming technique. Vinyl monomers were polymerized in the presence of gas bubbles created by the reaction of acid and  $\text{NaHCO}_3$  to create a porous material. The reaction between acid and  $\text{NaHCO}_3$  also served as a control point for the rate of gelation and foam formation. When the samples were dehydrated with ethanol and then dried overnight at  $60^\circ\text{C}$ , the dried samples reached equilibrium swelling after only a few minutes.

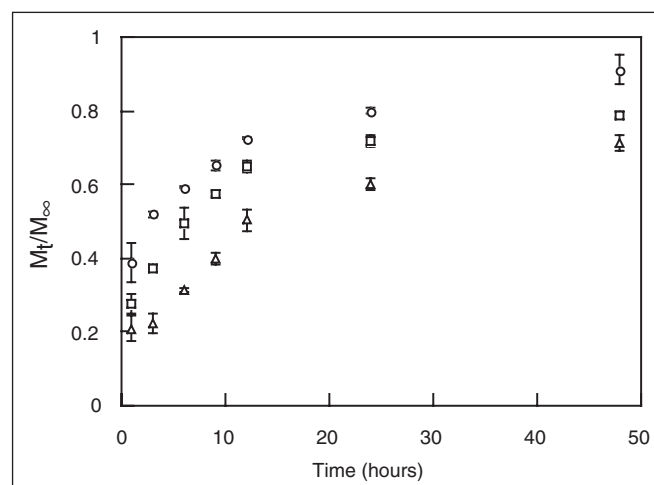
Our group [12] has synthesized porous materials of PVA using the porosigen technique. Sodium chloride was incorporated into a PVA solution prior to freezing and thawing. Gels that were dried immediately after freezing and thawing exhibited a dual-sorption affect during swelling. Subjecting gels to a 10-day period of washing with deionized water effectively removed all of the NaCl and PVA that did not participate in the crystallization process [36, 38-45]. Here, porous gels were synthesized by addition of porosigens. Ammonium carbamate was incorporated as a solid. The salt was slowly added to a 15 wt% aqueous PVA solution under stirring. The particles were well dispersed and dissolved throughout the polymer solution.

Gels were also synthesized by mixing dry PVA with NaCl prior to adding water and heating. The ratio of PVA to salt was 1:4. A 10 wt% solution was prepared and stable, slightly cloudy gels formed after two, three and four freezing and thawing cycles.

### 2. Drug release studies of non-porous PVA systems

BSA was incorporated into PVA solutions prior to freezing and thawing. The ratio of PVA to BSA was 1:10. After two, three, and four freezing and thawing cycles, stable hydrogels were formed and good gelation was observed.

BSA release from non-porous hydrogel samples produced from freezing and thawing of 15 wt% aqueous PVA solutions. Typical release curves (see *Figure 2*) showed that the number of freezing/thawing cycles affected the BSA release behavior. After 48 h, 92% of the BSA was released from the 2-cycle gel, 78% was released from the 3-cycle gel and only 71% was released from the 4-cycle gel. More freezing and thawing lead to a denser polymer network which caused a reduction in the protein released as observed before in preliminary data by Stewart *et al.* [40].



**Figure 2** - BSA released from non-porous PVA hydrogels as a function of time. Gels were prepared from a 15 wt% solution and exposed to two (O), three (□), and four (Δ) freezing and thawing cycles.

The mechanical strength, ability to absorb large quantities of water, and biocompatibility of PVA gels made them excellent materials for release systems. PVA gels released bioactive compounds through a diffusional mechanism. Diffusion of a drug or protein out of a hydrogel could be characterized as:

$$M_t/M_\infty = kt^n \quad \text{Eq. 1}$$

where  $M_t$  is the amount released at any time,  $M_\infty$  is the total amount of drug released,  $t$  is time, and  $k$  and  $n$  are constants [13]. For Fickian diffusion from planar systems  $n=0.5$ , for non-Fickian diffusion  $n > 0.5$ , and for Case II diffusion  $n = 1$ .

In these studies, BSA release reached constant levels from 3 to 12 h. After two days,  $2 \pm 0.2$  mg was released from the 2-cycle, 3-cycle, and 4-cycle gels. The amount of drug released was analyzed by *Equation 2*. Therefore, an intercept represented by the term  $\alpha$  was added to the right hand side of *Equation 1*

to give:

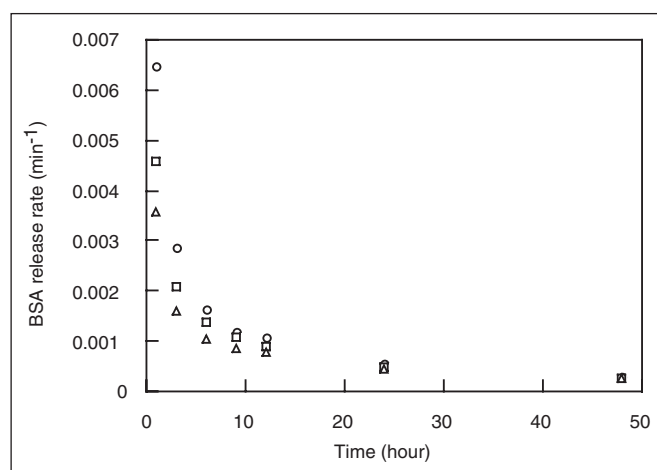
$$M_t/M_\infty = \alpha + kt^n \quad \text{Eq. 2}$$

where  $\alpha$  is the amount of drug released as soon as the release study begins. The value of  $n$  was calculated as 0.80, 0.90 and 0.94, respectively, for release from gels prepared after 2, 3 or 4 cycles. Thus, the transport mechanism was non-Fickian diffusion leading to zero order-release behavior as the number of freezing/thawing cycles increased.

The BSA released from the gel was analyzed with UV spectroscopy to study possible conformational changes. Therefore, the preparation of the gels and conditions of the release study did not affect the structure of the protein incorporated.

### 3. Drug release studies of porous PVA systems

Porous gels synthesized in the presence of NaCl were immersed in a 5% BSA solution for 24 h in order to incorporate the protein into the gels. A series of control gels underwent the same procedure. The BSA release rate was affected by the freezing and thawing cycles (see Figure 3). However, after 6 h the release rates for all three gels were the same. The porous gels showed a significantly faster rate of BSA release with respect to the non-porous ones. The preparation of the porous gels using sodium chloride did not affect the structure of the protein incorporated.



**Figure 3** - BSA release rate from porous PVA hydrogels as a function of time. Gels were prepared from a 15 wt% solution and exposed to two (O), three (□), and four (Δ) freezing and thawing cycles.

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Both porous and non-porous PVA hydrogels were synthesized by freezing and thawing aqueous PVA solutions. Porous gels were prepared by incorporating a salt into a PVA solution and then extracting it from the polymer network. Both ammonium carbamate and NaCl acted as porogens but did not prevent the formation of crystalline regions in the PVA during the freezing/thawing process.

Non-porous systems were loaded with BSA by incorporating the protein into a solution prior to freezing and thawing. The number of freezing-thawing cycles affected the initial rate of

release and the amount of protein released. BSA release was non-Fickian with a tendency towards zero-order release as the number of cycles increased.

### REFERENCES

- HASSAN C.M., PEPPAS N.A. - Structure and applications of poly(vinyl alcohol) hydrogels produced by conventional cross-linking or by freezing/thawing methods. - *Adv. Polym. Sci.*, **153**, 37-65, 2000.
- PEPPAS N.A., SCOTT J.E. - Controlled release from PVA gels prepared by freezing-thawing processes. - *J. Controlled Release*, **18**, 95-100, 1992.
- PEPPAS N.A. - Turbidimetric studies of aqueous PVA solutions. - *Makromol. Chem.*, **176**, 3433-3440, 1975.
- HYON S., IKADA Y. - US Patent 4,663,358, 1987.
- URUSHIZAKI F., YAMAGUCHI H., NAKAMURA K., NAMAJIRI S., SUGIBAYASHI K., MORIMOTO T. - Swelling and mechanical properties of poly(vinyl alcohol) hydrogels. - *Int. J. Pharm.*, **58**, 135-142, 1990.
- STAUFFER S., PEPPAS N.A. - Poly(vinyl alcohol) hydrogels prepared by freezing-thawing cyclic processing. - *Polymer*, **33**, 3932-3936, 1992.
- FUJISATO T., OKADA T., TABATA Y., IKADA Y. - PVA gels by freeze-thawing - *Polym. Prepr.*, **39**, 1069-1071, 1990.
- FICEK B., PEPPAS N.A. - Novel preparation of poly(vinyl alcohol) microparticles without cross-linking agent for controlled drug delivery of proteins. - *J. Controlled Rel.*, **27**, 259-264, 1993.
- CHEN J., PARK H., PARK K. - Synthesis of superporous hydrogels: Hydrogels with fast swelling and superabsorbent properties- *J. Biomed. Mater. Res.*, **44**, 53-58, 1999.
- LI J., WANG N., WU X. - Poly(vinyl alcohol) nanoparticles prepared by freezing-thawing process for protein/peptide drug delivery- *J. Controlled Release*, **56**, 117-126, 1998.
- PEPPAS N.A., MONGIA N. - Ultrapure poly(vinyl alcohol) hydrogels with mucoadhesive drug delivery characteristics. - *Eur. J. Pharm. Biopharm.*, **43**, 51-58, 1997.
- HASSAN C.M., PEPPAS N.A. - Drug dissolution and binding in ionizable interpenetrating networks from poly(vinyl alcohol) and poly(acrylic acid). - *Polym. Mater. Sci. Eng.*, **79**, 473-474, 1998.
- RITGER P., PEPPAS N.A. - A simple equation for description of solute release. I. Fickian and non-Fickian release from non-swelling devices in the form of slabs, spheres, cylinders or discs. - *J. Controlled Release*, **5**, 23-36, 1987.
- WITMAN J.E., HASSAN C.M., PEPPAS N.A. - Multilaminate PVA Hydrogel Systems as Protein Controlled Release Devices. - *AAPS Pharm. Sci.*, S-4050, 2000.
- FICEK B.J., PEPPAS N.A. - Novel preparation of poly(vinyl alcohol) microparticles without cross-linking agent for controlled drug delivery of proteins. - *J. Controlled Release*, **27**, 259-264, 1993.
- FICEK B.J., PEPPAS N.A. - Novel preparation of poly(vinyl alcohol) microparticles without cross-linking Agent for controlled drug delivery of proteins. - In: *Biomaterials for Drug and Cell Delivery*, A.G. Mikos, R. Murphy, H. Bernstein, N.A. Peppas (Eds.), MRS Pittsburgh, PA, 1994, pp. 223-226.
- GUDEMAN L.F., PEPPAS N.A. - Preparation and characterization of pH-sensitive, interpenetrating networks of poly(vinyl alcohol) and poly(acrylic acid). - *J. Appl. Polym. Sci.*, **55**, 919-928, 1995.
- PEPPAS N.A., MONGIA N., LUTTRELL A.S. - Bioadhesive poly(vinyl alcohol) as a carrier for controlled release of growth factors and proteins. - *Proceed. World Meeting APGI/APV*, **1**, 817-818, 1995.
- MALLAPRAGADA S.K., PEPPAS N.A. - Effect of dissolution on lamellar thickness distribution of semi-crystalline poly(vinyl alcohol). - *Polym. Mater. Sci. Eng.*, **73**, 22-23, 1995.
- GUDEMAN L.F., PEPPAS N.A. - pH-sensitive membranes from

- poly(vinyl alcohol)/poly(acrylic acid) interpenetrating networks. - J. Membr. Sci., **107**, 239-248, 1995.
21. HICKEY A.S., PEPPAS N.A. - Mesh size and diffusive characteristics of semicrystalline poly(vinyl alcohol) membranes prepared by freezing/thawing techniques. - J. Membr. Sci., **107**, 229-237, 1995.
  22. MALLAPRAGADA S.K., PEPPAS N.A., COLOMBO P. - Modification of drug release profiles and swelling behavior in poly(vinyl alcohol) due to the presence of a crystalline phase. - Polym. Mater. Sci. Engin., **74**, 416-417, 1996.
  23. PEPPAS N.A., MALLAPRAGADA S. - Crystal dissolution-controlled release systems: A novel technology for facile programmed release of drugs. - Adv. Contr. Deliv., **1**, 89-90, 1996.
  24. MONGIA N.K., ANSETH, K.S., PEPPAS, N.A. - Mucoadhesive poly(vinyl alcohol) hydrogels produced by freezing/thawing processes: Applications in the development of wound healing systems. - J. Biomat. Sci., Polym., **7**, 1055-1064, 1996.
  25. STRINGER J.L., PEPPAS N.A. - Diffusion of small molecular weight drugs in radiation-cross-linked poly(ethylene oxide) hydrogels. - J. Controlled Release, **42**, 195-202, 1996.
  26. PEPPAS N.A., WRIGHT S.L. - Characterization, morphology and diffusional behavior of poly(vinyl alcohol)-based hydrogels - Report POVAL Committee, **108**, 96-127, 1996.
  27. PEPPAS N.A., WRIGHT S.L. - Solute diffusion in PVA/PAA interpenetrating networks. - Macromolecules, **29**, 8798-8804, 1996.
  28. PEPPAS N.A., ANSETH K.S., MONGIA N.K. - Mucoadhesive PVA hydrogels for release of wound healing drugs. - Trans. World Biomat., **5**, 643, 1996.
  29. PEPPAS N.A., MONGIA N.K., BUGERT C.A. - Mucoadhesive poly(vinyl alcohol) films produced by freezing/thawing processes for the release of small molecular Weight solutes and for wound healing systems. - Proceed. Intern. Symp. Control. Rel. Bioact. Mater., **23**, 157-158, 1996.
  30. MALLAPRAGADA S.K., PEPPAS N.A. - Crystal dissolution-controlled release systems: I. Physical characteristics and modeling analysis. - J. Controlled Release, **45**, 87-94, 1997.
  31. HASSAN C.M., PEPPAS N.A. - Structure and Morphology of Freeze/Thawed PVA Hydrogels. - Macromolecules, **33**, 2472-2479, 2000.
  32. MALLAPRAGADA S.K., PEPPAS N.A. - Crystal unfolding and chain disentanglement during semicrystalline polymer dissolution. - AIChE J., **43**, 870-876, 1997.
  33. MALLAPRAGADA S.K., PEPPAS N.A., COLOMBO P. - Crystal dissolution-controlled release systems. II. Metronidazole release from semicrystalline poly(vinyl alcohol) systems prepared by annealing. - J. Biomed. Mater. Res., **36**, 125-130, 1997.
  34. HICKEY A.S., PEPPAS N.A. - Solute diffusion in poly(vinyl alcohol)/poly(acrylic acid) composite membranes prepared by freezing/thawing techniques. - Polymer, **38**, 5931-5936, 1997.
  35. PEPPAS N.A., HICKEY A.S. - Protein and drug transport in PVA/PAA composite membranes prepared by freezing/thawing techniques. - In : Biomaterials Carriers for Drug Delivery, and Scaffolds for Tissue Engineering, N.A. Peppas, D.J. Mooney, A.G. Mikos and L. Brannon-Peppas Eds., AIChE, New York, 1997, pp. 328-330.
  36. PEPPAS N.A., WRIGHT S.L. - Drug dissolution and binding in ionizable interpenetrating networks from poly(vinyl alcohol) and poly(acrylic acid). - Europ. J. Pharmac. Biopharmac., **46**, 15-29, 1998.
  37. ARGADE A.B., PEPPAS N.A. - Poly(acrylic acid)/poly(vinyl alcohol) copolymers with superabsorbent properties. - J. Appl. Polym. Sci., **70**, 817-829, 1998.
  38. HASSAN C.M., TRAKARNPAN P., PEPPAS N.A. - Solubility characteristics of poly(vinyl alcohol) and gels prepared by freezing/thawing processes. - In: Water Soluble Polymers: Solution Properties and Applications, Z. Amjad Ed., 1998, pp. 31-40.
  39. HASSAN C.M., PEPPAS N.A. - Pure PVA hydrogels using freezing/thawing techniques as carriers for drug delivery. - Proceed. Intern. Symp. Control. Rel. Bioact. Mater., **25**, 50-51, 1998.
  40. STEWART J.E., HASSAN C.M., PEPPAS N.A. - Protein release from PVA gels prepared by freezing and thawing techniques. - Proceed. Int. Symp. Control. Rel. Bioact. Mater., **26**, 1004-1005, 1999.
  41. HASSAN C.D., PEPPAS N.A. - CD analysis of diffusional and structural characteristics and PVA hydrogels prepared by freezing-thawing techniques. - AAPS Pharm. Sci., S-92, 1999.
  42. HASSAN C.M., STEWART J.E., PEPPAS N.A. - Diffusional characteristics of freeze/thawed PVA hydrogels: applications to protein controlled release from multilaminar devices. - Europ. J. Pharm. Biopharm., **49**, 161-166, 2000.
  43. HASSAN C.M., PEPPAS N.A. - Cellular freeze/thawed PVA hydrogels. - J. Appl. Polym. Sci., **76**, 2075-2079, 2000.
  44. HASSAN C.M., WARD J.H., PEPPAS N.A. - Modeling of crystal dissolution of poly(vinyl alcohol) gels produced by freezing/thawing processes. - Polymer, **41**, 6729-6739, 2000.
  45. PEPPAS N.A., TENNENHOUSE D. - Semicrystalline poly(vinyl alcohol) films and their blends with poly(acrylic acid) and poly(ethylene glycol) for biomedical and drug delivery applications. - J. Drug Deliv. Sci. Techn., **14** (4), 291-297, 2004.

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