

Adsorption of vitamin B₁₂ in aqueous solutions by poly(N-isopropylacrylamide/itaconic acid) copolymeric hydrogels

Poli(N-izopropilakrilamid/itakonik asit) kopolimerik hidrojelleri tarafından sulu çözeltilerde vitamin B₁₂ adsorpsiyonu

Research Article

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ABSTRACT

N-isopropylacrylamide/itaconic acid copolymeric hydrogels (NIPAAm/IA) containing different quantities of itaconic acid have been irradiated with γ radiation. The hydrogels were used in an experiment concerning the uptake and release behavior of Vitamin B₁₂. In the experiment of the adsorption of Vitamin B₁₂, type S adsorption isotherm was found. The effect of comonomer concentration and irradiation dose on the uptake and release behavior of the hydrogels was studied.

Key Words

Hydrogel, N-isopropylacrylamide, itaconic acid, Vitamin B₁₂.

ÖZET

Farklı miktarda itakonik asit içereb N-izopropilakrilamid/itakonik asit kopolimerik hidrojelleri (NIPAAm/IA) γ -radyasyonu ile sentezlendi. Sentezlenen hidrojeller Vitamin B₁₂ tutulumu ve salımını incelemekte kullanıldı. Vitamin B₁₂ adsorpsiyon deneylerinde S tipi adsorpsiyon izotermi bulundu. Hidrojellerin ilaç tutulumu ve salımı davranışları üzerinde komonomer konsantrasyonu ve ışınlama dozunun etkileri araştırıldı.

Anahtar Kelimeler

Hidrojel, N-izopropilakrilamid, itakonik asit, Vitamin B₁₂

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INTRODUCTION

Hydrogels are of interest in biomedical applications because of their tunable chemical and three-dimensional physical network structures, high water content in an aqueous medium without dissolution, good mechanical properties, and biocompatibility [1-4]. High-energy radiation has been successfully applied for more than a few decades for synthesis, modification and sterilization of hydrogels. Intelligent or smart hydrogels have been developed as stimuli-responsive materials, which can undergo volume changes in response to changes in temperature, pH and antigen concentration. These unique characteristics are of great interest in drug release, cell encapsulation and tissue engineering [5-9]. Poly(N-isopropylacrylamide) (P(NIPAAm)) is a well-known thermo-responsive polymer which exhibits a lower critical solution temperature (LCST) of around 32°C in an aqueous medium. P(NIPAAm)-based hydrogels absorb water and exist in swollen states below the LCST. They undergo an abrupt and drastic shrinkage in volume as the medium temperature is raised above the LCST. These unique characteristics make the P(NIPAAm) based hydrogels specially useful in biomedical applications, such as the controlled release of drugs and tissue engineering [10].

There are three methods for synthesis of the hydrogels involving N-isopropylacrylamide (NIPAAm): chemical initiation polymerization, ultraviolet irradiation polymerization, and radiation polymerization. In chemical initiation polymerization, the initiators commonly used are redox systems, ammonium persulphate or hydrogen peroxide as oxidizer, ferrous salt or tetramethylethylenediamine as reducers, and methylenebisacrylamide or glycol dimethylacrylate as crosslinkers. This method is studied more thoroughly. In ultraviolet irradiation polymerization, mercury-arc lamp with high voltage is often adopted. Photosensitizer is often added to improve photosensitivity. As irradiation time increases, the system temperature increases as well. Radiation sources for radiation

polymerization could be ^{60}Co and electron beam accelerator. γ -ray produced by ^{60}Co has high penetrating power into the objective substance. However, electron beam brought out from the accelerator is only applied to the surface of the carrier. Syntheses of relative hydrogels involving NIPAAm by ^{60}Co radiation polymerization were less studied due to the restricted equipment [11]. In this simple method, crosslink degree may be controlled by feed composition, dose rate, and total dose of radiation without any additive. The product obtained is pure, not contaminated. The polymerization has nothing to do with the temperature and may be conducted at lower temperature. Thus, the containers, especially the enzyme and protein harbored, are not easy to lose their activities. Research on these hydrogels and their use are very important in both theory and application.

In more recent years, a series of papers has been published by Güven and co-workers who synthesized pH-sensitive hydrogels from the copolymers of acrylamide and diprotic itaconic and maleic acid and showed that the use of even very small quantities of diprotic acid proved to impart remarkable properties to the hydrogels of starting monomers and/or homopolymers [12-15]. In our previous studies, we have reported P(NIPAAm/IA) and P(NIPAAm/MA) hydrogels synthesized by radiation induced polymerization technique. The hydrogels thus prepared were characterized with respect to their swelling properties and their applications on drug delivery systems were investigated [16-18]. In this study, we report drug adsorption capacity and drug release behavior of these hydrogels. Vitamin B₁₂ was used as a model drug. Vitamin B₁₂ is an essential nutritional compound required by all mammals. Vitamin B₁₂, also known as cobalamin, is necessary for the synthesis of red blood cells and maintenance of a healthy nervous system [19].

Materials and Methods

Materials

N-isopropylacrylamide (NIPAAm) was obtained from Aldrich Chemical Company. Itaconic acid (IA) was purchased from Fluka Chemical Company. Vitamin B₁₂ used was the purest commercially available (Sigma Co).

Preparation of Hydrogels

The hydrophilic NIPAAm monomer was used as the base monomer in the synthesis of hydrogels. The comonomer was itaconic acid (IA) carrying diprotic acid groups. Aqueous solutions of NIPAAm (10% w/w) were prepared in distilled water. Different amounts of IA were added to 1 ml of NIPAAm 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 air at 25°C with a PX-30 Iskladovatelj type gamma irradiator in Ankara Nuclear Research and Training Centre. The absorbed dose was 48-104 kGy at a dose rate of 3 kGy/hour [16].

Drug Loading and Release Experiments

The drug loading experiments was carried out in the concentration range of 75 to 200 ppm (mg/L) of Vitamin B₁₂ solutions prepared in phosphate buffer at pH 7.4 and the dry hydrogels were transferred into 50 mL of aqueous solutions Vitamin B₁₂ and allowed to equilibrate 4°C for one week. After incubation the polymer rods were removed from the solution and rinsed in cold buffer. To gain knowledge about the possible mode of action of the proposed drug-delivery device in 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 50 mL of phosphate buffer solutions 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 Vitamin B₁₂ release and placed again into the same vessel so that the liquid volume was kept constant. Vitamin B₁₂ release was determined spectrophotometrically using a Shimadzu Model UV-160A spectrophotometer at 360 nm [20].

The release of adsorbed Vitamin B₁₂ was followed at pH 7.4. The amount of the percentage release of Vitamin B₁₂ at pH 7.4 was calculated from the following equation:

$$\text{Fractional release} = M_t / M_\infty \quad (1)$$

where M_t is the amount of the released drug in water at any time and M_∞ is the equilibrium release at infinitely long time.

Results and Discussion

Drug Uptake

Vitamin B₁₂ was used as the model drug for the investigation of drug uptake and release behaviour of PNIPAAm and P(NIPAAm/IA) hydrogels. In a batch adsorption system at equilibrium, total solute concentration (C_t , mol/l) is

$$C_t = C_B + C \quad (2)$$

Where C_B is the equilibrium concentration of the solute on the adsorbent in mol/l (bound solute concentration) and C is the equilibrium concentrations of the solute in the solution in mol/l (free concentration). The value of bound concentration may be obtained by difference by using equation 2.

For a fixed free solute concentration, C_B is proportional to the polymer concentration on the binding system; the amount bound can therefore be conveniently expressed as the binding ratio, r , defined by

$$r = C_B / P \quad (3)$$

Thus with C_B in mol/l and P in base mol (moles of monomer units) per liter, r then represents the average number of molecules of solute bound to each monomer unit at that free solute concentration [21,22].

P values of the hydrogels were calculated by the following equation:

$$P = m/M \quad (4)$$

Where m is the mass of the hydrogel and M is the average molar mass of repeating units of polymer.

Plot of the binding ratio (r) against the free concentrations of the drug solution is shown in Figure 1. Figure show that adsorption of Vitamin B₁₂ within NIPAAm/IA hydrogels corresponds to type S adsorption isotherm in the Giles classification system for adsorption of a solute from its solution.

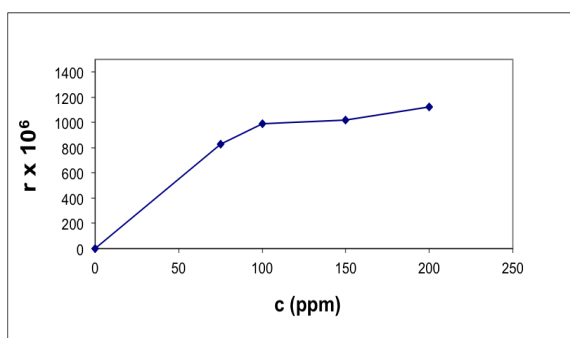


Figure 1. Binding isotherms of P(NIPAAm/IA)-Vitamin B₁₂ systems.

In the S curves in the Giles classification system, the initial direction of curvature shows that adsorption become stronger as concentration rises. In practice, the S curve usually appears when three conditions are fulfilled: the solute molecule (a) is monofunctional, (b) has moderate intermolecular attraction, causing it to pack vertically in regular array in the adsorbate layer, and (c) meet strong competition, for substrate sites, from molecules of the solvent or of another adsorbed species [23,24].

The weakness of the adsorbent-adsorbate forces will cause the uptake at low concentrations to be small, but once a molecule has become adsorbed, the adsorbate-adsorbate forces will promote the adsorption of further molecules- a cooperative process- so that the isotherm will become convex to concentration axis.

Table 1a. Variation of Vitamin B₁₂ uptake with itaconic acid (IA) content in the gel structure. Total dose given was 48 kGy.

IA %	0	1	2	3
Total Vitamin B ₁₂ uptake (mg/g dry gel)	1.25	1.79	2.48	2.62

Type S isotherms may originate through the adsorption of either nonpolar molecules or polar molecules, always provided that the adsorbent-adsorbate force is relatively weak. A polar adsorbate of particular interest in this context is water, because the dispersion contribution to its overall interaction energy is unusually small compared with the polar contribution. Not surprisingly, water provided many examples of the type S isotherms. Once the hydrogel is covered with a layer of adsorbed water, however, the adsorbent-adsorbate interaction would be virtually reduced to the weak dispersion energy of water with Vitamin B₁₂.

In later experiments, the drug uptake into PNIPAAm and P(NIPAAm/IA) hydrogels were measured as effects of the contents of IA and irradiation doses. As can be seen from this table, Vitamin B₁₂ uptake into the hydrogels increases with increase in IA content. Drug loading efficiency increases for the drug, due to the some specific interactions of carboxyl groups of itaconic acid with the drug. The drug 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.

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 in vivo performance. Figure 2 describes the in vitro drug-release profile of the hydrogel samples at 37°C in phosphate buffer of pH 7.4. For all gels, the Vitamin B₁₂ release increases rapidly at first and then gradually reaches the equilibrium value in approximately 24 hr. 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 comes into contact with solvent into the matrix, followed fast drug release.

Table 1b. Variation of Vitamin B₁₂ uptake (mg/g dry gel) with irradiation dose (kGy)

Irradiation dose (kGy)	48	82	104
Total Vitamin B ₁₂ uptake (mg/g dry gel)	2.62	1.59	1.45

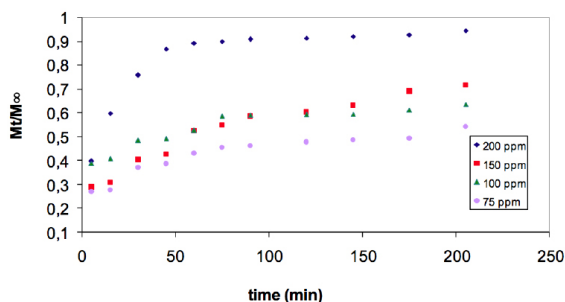


Figure 2. Fractional drug-release profile of the hydrogel samples at 37°C in phosphate buffer of pH 7.4.

Conclusion

The obtained results revealed that P(NIPAAm/IA) hydrogels have high adsorption capacities for Vitamin B₁₂. It has been found that the drug uptake capacity of the hydrogels both increases with increasing IA content in the gel structure. This has been explained due to the incorporation of more specific acidic groups into the network and consequent higher swelling capacity of the gels. Furthermore, the drug 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. In the experiments of the adsorption, type-S adsorption isotherm in Giles' classification system was also found. It is concluded that P(NIPAAm/IA) hydrogels can be used as a water retainer for carrying some substances in aquatic fields in pharmacy, agriculture, environmental and biomedical applications.

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