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RESEARCH ARTICLE

Physical and chemical properties of PVA-CMC based hydrogel carrier loaded with herbal hemostatic agent for application as wound dressings

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ABSTRACT

Background: Polyvinyl alcohol (PVA) -based hydrogels are promising biomaterials and suitable candidates for application as wound dressings due to their excellent biocompatibility and favorable properties. **Aims and Objectives:** The present research focuses on the studying physical and chemical properties of PVA- chemistry manufacturing and controls (CMC) -based hydrogel carrier loaded with herbal hemostatic agent for application as wound dressings. **Materials and Methods:** An aqueous solution of carboxymethyl cellulose was mixed in a certain ratio with PVA, to avoid the risks of chemical reagents and cross-linkers, for structure formation, the physical method of freezing-thawing cross-linking was used instead of using traditional chemical cross-linking. **Results:** The physical and chemical properties of the obtained hydrogels, such as the gel fraction (GF), water uptake capacity, and protein adsorption, as well as viscoelastic properties, have been studied. The increased content of CMC reduced the GF, elasticity, and strength. However, this led to an increase in the degree of swelling and protein adsorption. **Conclusions:** Hydrogels were developed using a physical method for application as wound dressings based on the PVA and CMC containing Inebrin derived from the medicinal plant *Lagochílus inebrians* as a model of topical hemostatic agent for capillary bleeding.

KEY WORDS: Hydrogel; Polyvinyl Alcohol; Sodium Carboxymethyl Cellulose; Inebrin; Freeze-thawing Method

INTRODUCTION

Hydrogels are three-dimensional cross-linked hydrophilic polymers with very high internal water content that can provide a moist wound environment and absorb exudates. Based on this principle, hydrogels have been selected as a good candidate for wound dressings. Many hydrogels are made by physical or chemical cross-linking methods,[1] while physical cross-linking, such as repeated freeze-thaw cycles, is considered the most suitable cross-linking method for biomedical and pharmaceutical applications due to its non-toxicity, lack of solvents, and biocompatibility.[2] Polyvinyl alcohol (PVA), widely used in the manufacture of hydrogels, has desirable properties such as non-toxicity, biocompatibility, high hydrophilicity, relatively easy film-forming ability, chemical, and mechanical resistance.[3]

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Chemistry manufacturing and controls (CMC) is a modified natural polymer with excellent water absorption capacity. CMC is physiologically non-toxic and compatible with mucous membranes, bones, and skin. CMC can be used as a matrix for wound healing and skin regeneration.[4,5] The advantage of CMC is the ability to mix with other polymers, such as PVA, which is biocompatible, less toxic, and hydrophilic.[6]

The importance of mixing polymers with PVA lies in the fact that the resulting new materials have the desired properties. Accordingly, polymers such as alginate,[7] dextran,[8] and chitosan[9] were mixed with PVA to improve the clinical properties of wound dressing hydrogels. Meanwhile, hydrogels made from mixing two polymers combine the advantages of both.

At present, various medicinal additives are used to expand the properties of biomedical polymer materials, such as antibiotics, metal nanoparticles, honey, and plant extracts (*Aloe vera*, chamomile). In folk medicine of Central Asian countries, *Lagochilus inebrians* is widely used as a hemostatic agent, from which a substance (extract) Inebrin is isolated, which shows hemostatic activity.[10]

In this study, physically cross-linked PVA-CMC hydrogels containing Inebrin as a model of a local hemostatic agent were obtained using the freeze-thaw cycles. PVA-CMC hydrogels formed a matrix of physically cross-linked polymer chains containing uncross-linked polymers, water, and Inebrin. The properties of the hydrogel, such as the gel fraction (GF), viscosity, elasticity, degree of swelling, and protein adsorption on the surface, were investigated.

MATERIALS AND METHODS

Materials

PVA (average mol. mass 72,000 g/mol; 98.9% hydrolyzed) was purchased from Huahaifi, China. CMC (medium viscosity, mol. weight ~130,000 g/mol) purchased from Chibio Biotech, China, hemostatic agent "Inebrin" was obtained from the Institute of Bioorganic chemistry of the Uzbek Academy of Sciences on a free basis, albumin from bovine serum was purchased from LLC "Biolot," Russia. Distilled water was used in the study. All other chemicals were used without any additional cleaning.

Preparation of PVA-CMC Hydrogel

PVA-CMC hydrogel was prepared by several freeze-thaw cycles. Aqueous solutions containing 10% (weight/volume) PVA and 2% (weight/volume) CMC, and CMC and PVA solutions were mixed in different proportions (0%, 25%, 35%, 45%, 55%, 65%, and 75%), adding 30 mg of Inebrin. Appropriate amounts of the resulting mixture were poured into Petri dishes, then frozen at −20°C for 20 h and thawed for 6 h at room temperature for three continuous cycles.

Determination of the GF

The resulting PVA-CMC hydrogels were dried at 50°C for 24 h, weighed and labeled as W_0, then kept in distilled water for 24 h until the swelling mass (W_S) to remove the soluble (unbound) CMC from the hydrogel. Then, the hydrogel was dried again at 50°C and again weighted (M_1). The GF% was calculated using the following equation (1):

$$\text{Gel fraction (GF\%)} = (W_1/W_0) \times 100.$$

Determination of the Swelling Behavior

The samples of PVA-CMC hydrogel were cut into 2 × 2 cm pieces, dried at 50°C for 5 h, the mass of the dried samples was designated as W_D. The dried samples were immersed in distilled water and incubated at 37°C, then weighed (W_S) at specific time intervals. Water uptake of PVA-CMC hydrogels was determined using the following equation (2).

The ratio of water uptake or swell ratio (SR %)

$$= (W_S - W_D/W_D) \times 100.$$

Protein Adsorption on the Hydrogel Surface

The amount of bovine serum albumin (BSA) adsorbed on the hydrogel surface was determined using an ultraviolet (UV)-visible spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan). A calibration curve was constructed for a BSA standard solution in the range from 0.5 to 5 mg/ml to establish the relationship between the apparent absorption of BSA at 630 nm and its concentration. Distilled water was used to prepare standard solutions. From the calibration curve, a study was conducted using equation (3), A = acL, where A is optical density, c is concentration, a is proportionality constant, and L is constant path-length. To adsorb BSA, first pieces of PVA-CMC hydrogel, 1 cm to 1 cm in size, were immersed in 10 ml sodium phosphate buffer (pH7.4) and incubated at 37°C for 24 h until an equilibrium swelling mass was reached, then the swollen pieces of hydrogel were immersed into a buffer solution containing BSA (10 mg/ml) and shaken for 4 h at 37°C. After protein adsorption, the hydrogel pieces were carefully removed. The protein adsorption in each sample was calculated from the difference between the protein concentrations before and after immersion of the hydrogel pieces in a protein-phosphate buffer solution using an albumin reagent kit (absorption range at 630 nm).

In Vitro Release of Inebrin from PVA-CMC Hydrogel

The release of Inebrin from the hydrogel was studied at 37°C. To measure the rate of release, a 4 g piece of hydrogel containing a certain amount of Inebrin was immersed in a 0.01 M PBS solution (pH 7.4). The PBS solution was periodically replaced with a fresh solution. Then, the concentration of released Inebrin in PBS was determined by UV-visible spectra (Shimadzu 1800) at a wavelength of 290 nm and using a prepared standard absorption curve. The

test was repeated 3 times, and the data obtained are based on the standard deviation.

RESULTS

The part of the polymer blend polymerized and converted to gel form, while the unreacted part dissolves in the solution and indicates as sol. The analysis of gel and sol quantity is used for the determination of GF. For the determination of GF, pre-weighed pieces of hydrogel were dried first at room temperature and then in the oven at 50°C till their weight becomes constant. It was found that GF of hydrogels decreased along with an increased concentration of CMC, as shown in Figure 1.

The swelling behavior can be described as the water uptake of the hydrogel. Dried hydrogel pieces were weighed, then immersed in PBS solution, and left to swell at temperature of 37°C. To determine the swelling dynamic, hydrogel pieces were periodically taken out of the solution and placed in the same solution after weighing. The maximum swelling degree increases along with increasing content of CMC in the hydrogel from 500% to 3200% which is shown in Figure 2.

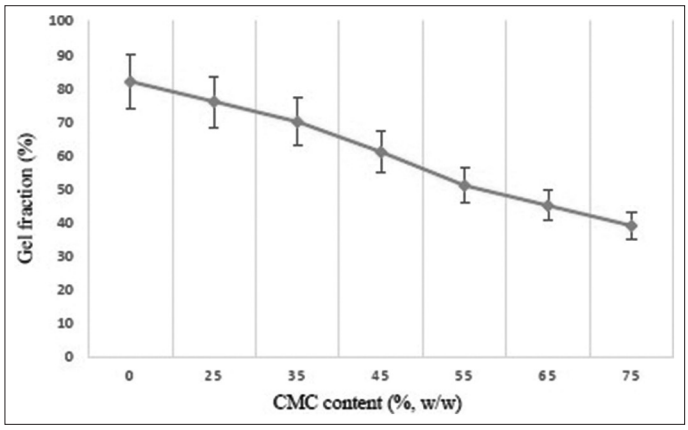

Figure 1: Effect of chemistry manufacturing and controls concentration on the gel fraction. The data are given taking into account the standard deviation (n = 3)

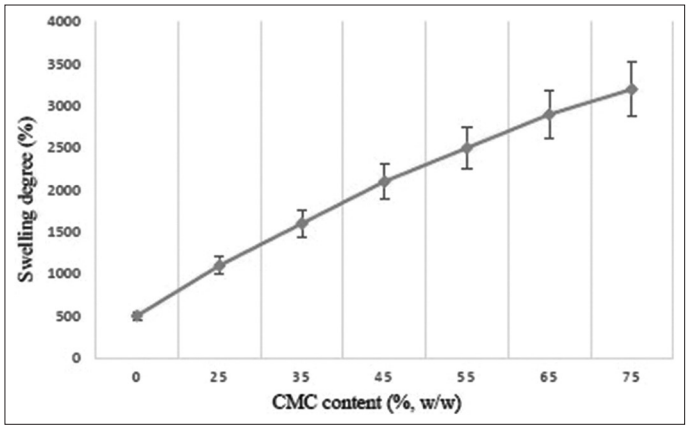

Figure 2: Effect of chemistry manufacturing and controls concentration on the swelling behavior. The data are given taking into account the standard deviation (n = 3)

The data in Figure 3 show the protein adsorption degree. As demonstrated in figure, the protein adsorption level increases in direct proportion to the content of CMC from 0.5 to approximately 1.4 mg/cm^2.

Inebrin release dynamics presented in Figures 4 and 5. To measure the rate of released drug, a piece of hydrogels were

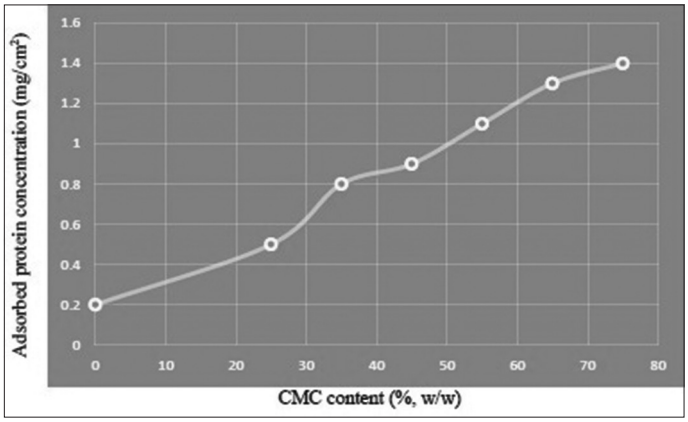

Figure 3: Effect of chemistry manufacturing and controls concentration on the protein adsorption amount. The data are given taking into account the standard deviation (n = 3)

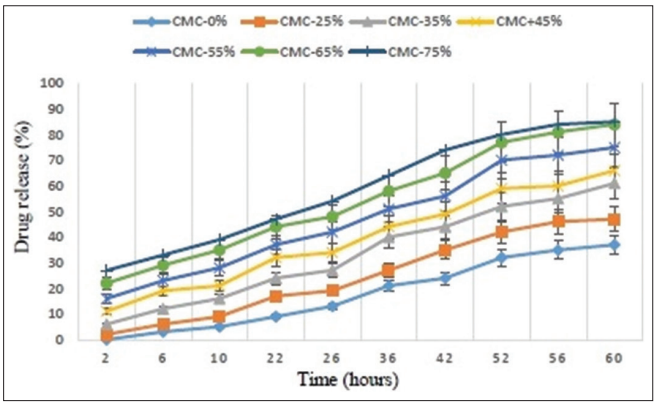

Figure 4: The release dynamic in Inebrin from polyvinyl alcohol-chemistry manufacturing and controls hydrogels at 25°C. The data are given taking into account the standard deviation (n = 3)

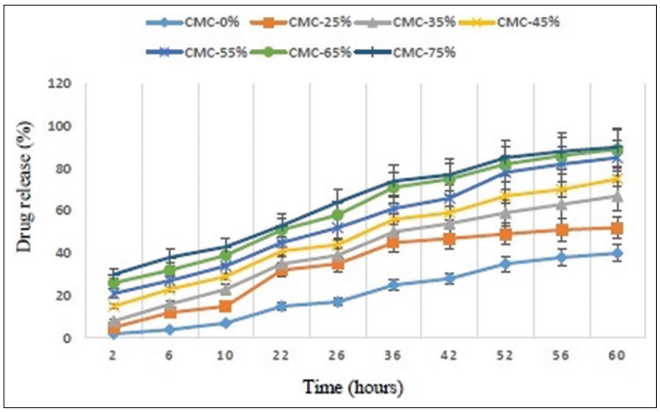

Figure 5: The release dynamic in Inebrin from polyvinyl alcohol-chemistry manufacturing and controls hydrogels at 37°C. The data are given taking into account the standard deviation (n = 3)

immersed in PBS which was replaced by the fresh solution at certain times. The concentration of released Inebrin was determined by UV–visible spectra (Shimadzu 1800) at a wavelength of 290 nm and by the help of a prepared standard absorption curve. The highest drug release rate was discovered between 52nd and 60th h of incubation: About 70–85% of Inebrin was released from hydrogels which content 55 to 75% CMC respectively at 25°C and 78 to 90% at 37°C.

DISCUSSION

Polymer blend hydrogels, which possess the properties of biocompatibility, complexation, biodegradation, and the presence of their own antimicrobial activity, are one of the most promising materials in the treatment of wounds.[11]

Successive freeze-thaw cycles resulted in cross-linking of the polymers to form the PVA-CMC hydrogel. The effect of mixing different CMC content in the solution is calculated (0%, 25%, 35%, 45%, 55%, 65%, and 75%) and the inclusion of Inebrin as a model of local hemostatic agent for the percentage of the GF% according to the formula (1), [Figure 1]. As a rule, the lower GF was observed and was accompanied by less elasticity of the resulting gel. In the absence of CMC and the drug substance (the content of CMC is 0% and without the drug substance), the GF seems to have increased to a maximum value of about 80%, which suggests that the PVA almost crystallized to the highest degree and, consequently, cross-linking occurred. At the same time, GF% gradually decreased with an increase in the concentration of CMC in the hydrogel or the addition of Inebrin to <40% (at 75% CMC content). This behavior may be due to the content of CMC and Inebrin in the hydrogel, which can apparently reduce the cross-linking reaction, and therefore the gelation process is clearly reduced. The results obtained provided the principle of operation of wound dressing materials in terms of preserving the wet local environment by mixing CMC in various ratios with PVA to form a hydrogel.

In our study of swelling, when the PVA-CMC hydrogel was immersed in distilled water for 30 min, a small amount of mixed CMC dissolved in the swelling medium. The amount of dissolved CMC depends on the initial mixed CMC in the hydrogel. The maximum absorption capacity increases with increasing content of CMC in the hydrogel to a certain swelling limit. While in the absence of CMC (0%), a structure with a high degree of cross-linking was obtained, however, this structure is not able to hold a large amount of water, which reduces the ability to swell, which is about 500%. After increasing the CMC content to 75%, the percentage of water absorption gradually increases to about 3200%. This is due to the fact that a high CMC content increases the hydrophilicity of the hydrogel,[12] which sometimes leads to the partial or complete destruction of a hydrogel with a much higher CMC content [Figure 2].

Protein adsorption on the surface of the PVA-CMC hydrogel was performed in *in vitro* experiments and calculated using the equation (3). The results of protein adsorption are shown in Figure 3. As shown in the figure, BSA adsorption increased from 0.5 mg/cm^2 to 1.4 mg/cm^2, with an increase in the amount of CMC in hydrogels from 0 to 75 (%, w/w). Interestingly, an Inebrin-filled hydrogel demonstrated the possibility of BSA adsorption compared to an Inebrin-free hydrogel, indicating that the high CMC content negatively affected protein adsorption on the hydrogel surface. Although the hydrophilicity of the hydrogel increased with increasing CMC content, which showed a decrease in non-specific protein adsorption, which forms an adsorbed layer,[13] the results can be explained by the inclusion of CMC molecules in the formed pores in the structure of the hydrogel.

The diffusion of the drug and the dissolution of the hydrogel occurs simultaneously and causes the release of the drug from the matrices of hydrogels. PVA and CMC are water-soluble substances and make the hydrogel water-soluble. The drug can be released faster, destroying the hydrogel network. The release profile was studied at two temperatures (24 and 37°C). It is shown that the rate of release of Inebrin depends on the temperature and composition of the hydrogel, which affects its rheological properties. When the CMC content increases from 0 to 75% and the temperature increases from 25 to 37°C, the release rate of the drug becomes higher [Figures 4 and 5]. It should be noted that the maximum amount of the loaded drug was released at the 56th and 60th h of incubation, depending on the temperature and composition of the hydrogel, insignificant release of drug from hydrogels is occurred in the rest of the period. Thus, it is assumed that drug release from hydrogels occurs due to drug diffusion and destruction of the hydrogel structure which is compatible with the previous findings.[14]

Strength and Limitations

The study was conducted by blending only two types of polymers. Better results could be achieved by adding other polymers and components such as polyvinyl pyrrolidone, starch, and glycerol.

CONCLUSIONS

We can say that the PVA-CMC hydrogel with Inebrin was developed using the method of physical cross-linking. The results showed that the content of CMC in the hydrogel cross-linked by physical method significantly affected its molecular structure and morphological properties. PVA-CMC hydrogel demonstrates high water absorption capacity, necessary flexibility, elastic, and perforated surface structure. The drug release rate has been occurred through diffusion of Inebrin through the hydrogel matrix and then its release inside the PBS media, followed by erosion of hydrogel matrix at later stages.

Based on the above, PVA-CMC hydrogel containing Inebrin can be proposed as a candidate for use as a potential dressing material in the process of wound healing, in particular for capillary bleeding.

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