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New superporous hydrogels composites based on aqueous Carbopol® solution (SPHCcs): synthesis, characterization and in vitro bioadhesive force studies

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Abstract

In this investigation new superporous hydrogels composites based on aqueous Carbopol® solution (SPHCcs) were prepared. SEM images indicated that the inner surface of SPHCcs contained a lot of pores connected each other and the outer surface of them was non-porous. The swelling ratio decreased with increasing the content of aqueous Carbopol® solution, and the final swelling ratio was similar to that of the SPH although the initial swelling ratio was lower. The density measurement revealed that the porosity increased when aqueous Carbopol® solution was incorporated. It was observed from in vitro bioadhesive force study that SPHCcs adhered to the intestinal mucosal more quickly and exhibited higher mucoadhesion as compared with SPH. It is evident that the hydrogels synthesized in this study could be a potential candidate for transmucosal drug delivery system.

Keywords: Superporous hydrogel composite; Carbopol®; Swelling; Mucoadhesion

1. Introduction

During the past few years there has been an increasing interest in the development of transmucosal drug delivery (TMD) for drug administration. The main reason for this interest is that it offers the prospect of prolonging the residence time of formulations at the site of drug absorption and a close contact on the absorption surface [1,2]. Various synthetic and natural polymers have been investigated for their applications in TMD system as mucoadhesive polymers including poly (acrylic acid) (PAA) [3], hyaluronic acid [4], chitosan [4], and collagen [5]. These polymers are usually produced to be conventional hydrogels for use in TMD. However, conventional hydrogels swell very slowly and the time of dried hydrogels reaching swelling equilibrium ranged from a few hours to several days because they have rigid crystalline structure and low elasticity in the polymer chains, which resulted in slower adhesion to the mucous membrane.

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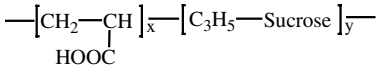
In the previous study [6,7], a new type of hydrogels, superporous hydrogels (SPH), with numerous pores connected together to form open channel structure have been developed and they can absorb water very rapidly and swell to equilibrium size in a short time regardless of the size of xerogel. While these SPH with fast swelling kinetics and high water content are generally more beneficial for increasing permeability and biocompatibility, the mechanical strength of the fully swollen SPH is too poor to be useful. In order to improve the mechanical strength of the fully swollen SPH, SPH composites were prepared by adding croscarmellose sodium (Ac-Di-Sol®) during their synthesis. The mechanisms of synthesis and the structure of SPH and SPH composites have been explained in detail by Dorkoosh et al. [8]. Furthermore, Dorkoosh et al. have designed novel drug delivery systems for oral administration of peptide and protein drugs using SPH and SPH composites as the carrier [9–11].

This work report the preparation of new superporous hydrogels composites based on aqueous Carbopol® solution (SPHCcs). Carbopol® is the commercial name of a series of highly hydrophilic polyacrylic acid polymer widely applied in drug delivery systems, especially in TMD. It can swell quickly in water due to the crosslinking band in the structure and adhere to the intestinal mucus due to the ability of functional groups (COOH) to form hydrogen bridges to interpenetrate the mucus layer. The Carbopol® gel has better strength and elasticity. Its introduction is expected to improve the properties of the SPH, in particular their mucoadhesive properties and swelling behavior. SPHCcs was characterized by scanning electron microscopy, swelling ratio, and density. In vitro bioadhesive forces were examined in conjunction with the amount and category of aqueous Carbopol® solution.

2. Experimental

2.1. Materials

Acrylic acid (AA), acrylamide (AM), *N*,*N*′-methylenebisacrylamide (Bis), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Sigma (St. Louis, USA). AA was distilled under reduced pressure before use. Pluronic® F127 (PF127) was a gift from BASF (Parsippany, NJ). Sodium bicarbonate was obtained from Shanghai Hongguang chemical plant (Shanghai, China). Carbopol 934P, 974P were gifted from BFGoodrich (Cleveland, USA). The degree of crosslinking of Carbopol® 974P is higher than that of Carbopol® 934P and the effect of the two Carbopol® used for the investigation on the drug release is different. The chemical structure of Carbopol® is as follows.



The water used was double distilled. All other compounds were of analytical grade and used as obtained.

2.2. Hydrogels synthesis

The preparation of SPH and SPHCcs were based on the method reported by Dorkoosh et al. [8].

The following components were added sequentially into a glass weighing bottle (25 mm × 40 mm) at ambient temperature: 300 μl of 50% AM; 200 μl of 50% AA; 70 μl of 2.5% Bis; 300 μl of water; 30 μl of 10% PF127; 25 μl of 20% APS; 25 μl of 16.7% TEMED. The glass weighing bottle was extensively vortexed after each ingredient was added. After 15 min, 100 mg of sodium bicarbonate were added to the mixture and the bottle was vortexed. The procedure of preparing SPHCcs was similar to SPH. However, aqueous Carbopol® solution were added to the mixture after adding APS and before adding TEMED. Polymerization was allowed to continue for approximately 10 min in all procedures.

2.3. Morphological examination

In order to prevent the morphology of porous structures from changing, the dried hydrogels were put in liquid nitrogen and then cut to expose their inner structure. The inner surface of the hydrogels were coated with a thin layer of gold alloy and imaged in a SEM (S-520, Hitachi).

2.4. Swelling ratio

The following equation was used to calculate the swelling ratio: $Q = (W_s - W_d)/W_d$, where Q is swelling ratio, W_s the mass in the swollen state and W_d the mass in the dried state. At the beginning of each experiment, the dried gel was measured gravimetrically to obtain W_d and then it was immersed in an excess of distilled water for swelling. At various time intervals, the hydrogel was removed from the water and weighed when excessive water on the surface was blotted to determine W_s [6].

2.5. Density measurements

The density (d) of the dried hydrogels was calculated by: $d = W_d/V_d$, where W_d is the weight of a dried hydrogel and V_d is the volume of the dried hydrogel. Since SPH and SPHCcs lost their regular shapes during the drying process, direct measurement of their volumes becomes difficult. Therefore, for measurement of their volumes, the solvent displacement method was applied. Briefly, the level of the hexane in a cylinder and the inner

diameter of the cylinder were determined by a vernier caliper in order to measure the volume of hexane. Then a piece of hydrogel was submerged in hexane, and the level of the hexane in the cylinder was measured again. The calculation of V_d was based on the difference between the level and the inner diameter of the cylinder.

2.6. Measurements of in vitro bioadhesive force

2.6.1. Substrate preparation

The rat (male, Sprague-Dawley, weighing 200–220 g) was fasted overnight before the experiment. The animal was killed by an overdose of diethyl ether vapor and its abdomen was opened by a mid-line incision. The jejunum was isolated and washed gently with ice-cold isotonic phosphate buffer (pH 7.4). The jejunum sections were used as the substrate.

2.6.2. Bioadhesive force measurements

The bioadhesion capacity was determined by using a modified precision torsion balance (Shanghai precision science instrument limited co., 2500 mg/5 mg). The xerogel was fixed to the upper support and a section of the jejunum tissue was attached, mucosal side up, to the lower rubber support. A plastic film with a small hole was placed over the mucosal surface to allow a fixed area of the test. The size of the hole was adjusted to make the detachment weight no more than 2500 mg. The lower rubber support was then laid on a motorized platform and the upper support was attached to the modified precision torsion balance. The motorized platform was lifted slowly until the intimate contact between the xerogel and the mucosal tissue occurred. After keeping the contact for 2 min, the reader knob of the modified precision torsion balance was turned slowly until total separation of the components was achieved. The maximum weight required for separation was measured with the balance. The force per unit area required to detach the test hydrogels from the biological substrates in the present study was calculated from the equation: $F = W \cdot G/A$, where F = bioadhesive force ($N\,m^{-2}$); W = maximum detachment weight recorded by the balance (kg); G = acceleration due to gravity ($m\,s^{-2}$); A = xerogel-tissue contact area (m^2).

A fresh piece of tissue was used for each determination and six replicates were obtained from each sample.

3. Results and discussion

3.1. Hydrogels synthesis

SPH and SPHCcs were synthesized by a solution polymerization technique using APS and TEMED as an initiator system. The role of each component in the synthesis of these hydrogels has been discussed [8]. The porous hydrogels can be prepared in the presence of gas bubbles. For instance, the monomers can be polymerized or water-soluble polymer chains can be cross-linked around gas bubbles generated by a blowing agent. In order to obtain SPH and SPHCcs with well-distributed pores, polymerization and foaming involved in the preparation of polymers must be carefully controlled. Namely, the gelling ought to occur when the foam takes place, so that the porous structure can be stabilized. The lifetime of the foam is relatively short, but stabilization is possible through proper adjustment of parameters such as the film-air interfacial tension, the film viscosity, the interbubble gas diffusion, and the surface charge [12,13].

One of the important factors that influence the synthesis of the polymers was the pH of the AA monomer solution. When the pH of the AA monomer solution was between 1.0 and 4.0, the gas bubbles formed quickly and were very unstable, resulting in the formation of polymers with ill-distributed pores. Moreover, it was observed that the amount of the gas bubbles retained in the polymers increased with the rising of the pH. As the pH reached 5.0, polymers with well-distributed pores were produced because of the stability and the proper formation rate of the foam. This is due to the influence of the pH of the foaming solution on the activity of the foam, and it could be anticipated that the foam films are easy to rupture when the pH of the foam solutions decreases.

In this experiment, Carbopol® plays an important role in stabilizing the foam during the synthesis. It was observed that the foam volume was increased in the presence of the Carbopol® and, when the addition of Carbopol® solution was not more than 400 μl, the foam generated was fine and uniform during the synthesis. In addition, the heat of polymerization is better dissipated in the presence of Carbopol® because the formation of Carbopol® gel is an endothermic process. As a rule, the bubble size increases when the temperature is higher. This results in the reduction of the thickness and strength of the foam film and the diffusion of the interbubble gas. The stability of the foam is lowered. The decrease of temperature during the synthesis is advantageous to stabilizing the foam.

Fig. 1 showed the scanning electron microscopic pictures of SPH and SPHCcs. The SPH (Fig. 1A and B) had many closed-pores and no interconnected capillary channels in its inner surface and some pores in its smooth outer surface. During the preparation, many of the gas bubbles escaped from the solution because the viscosity of the solution was not high enough in the absence of Carbopol®. In the structure of SPHCcs (Fig. 1C and D), the outer surface of the hydrogel was composed of the conventional hydrogel with high mechanical strength and their inner surface contained large numbers of the pores connected to each other. SPHCcs contained certain amount of Carbopol® and

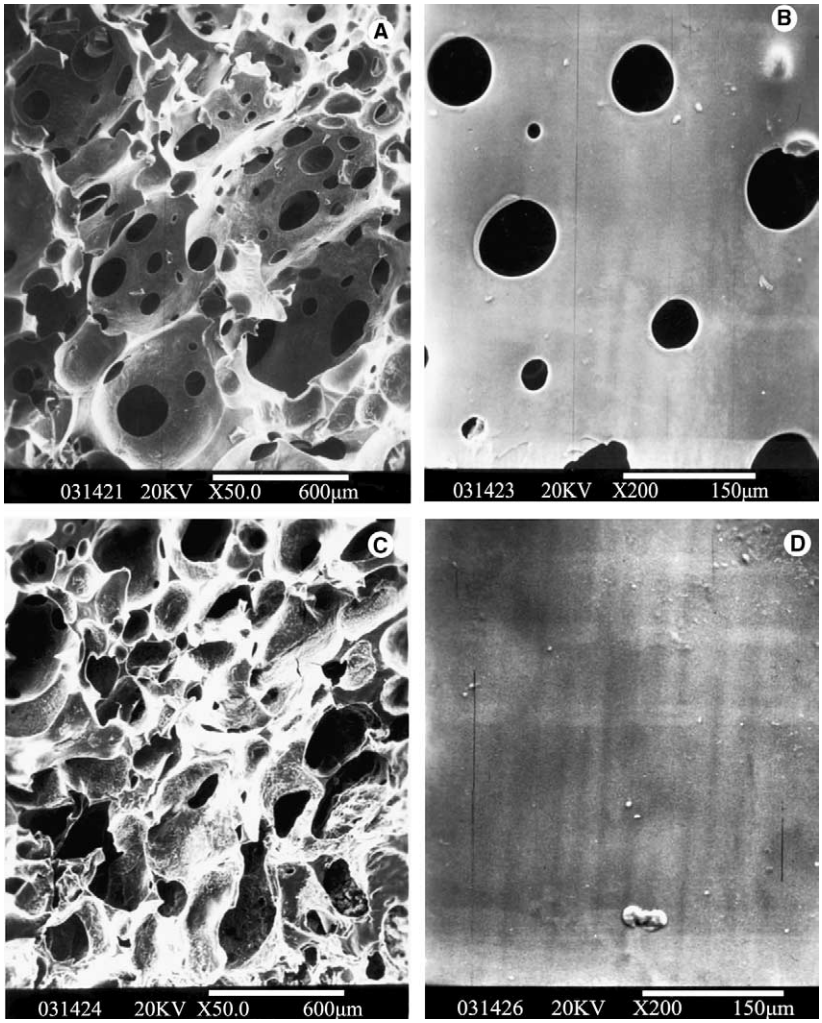


Fig. 1. SEM pictures of SPH and SPHCcs: (A) the inner surface of SPH; (B) the outer surface of SPH; (C) the inner surface of SPHCcs; (D) the outer surface of SPHCcs. SPH had no Carbopol® and the pH of the AA monomer solution was 5.0. SPHCcs contained 400 μl of 1.3% aqueous Carbopol® 934P solution.

PF127 which worked together to retain most of the bubbles, resulting in the production of superporous hydrogels composites with some of pores connected to each other. Carbopol® appeared to have multiple useful functions in preparing new SPHCcs.

The concentration of TEMED was reduced from the 20% level employed by Dorkoosh et al. [8] to 16.7%. This change significantly reduced the rate of polymerization and facilitated the formation of hydrogels with well-distributed pores and high porosity.

3.2. Swelling ratio studies

As shown in Figs. 2 and 3, at the beginning of swelling, SPHCcs cannot absorb water very rapidly compared with the SPH. However, as compared to SPH, the final swelling ratio of SPHCcs was about the same or more. These were probably due to the structure mentioned in Section 3.2. The outer conventional hydrogel layer can significantly retard the swelling of SPHCcs and this resulted in the sharp ascension of the swelling ratio after absorbing water very slowly, especially when the amount of aqueous Carbopol® solution was larger. The swelling ratio decreased with increasing Carbopol® content in the initial mixture. This may be due to the following facts: Carbopol® reduced the flexibility of the polymer chain and occupied some space of the pores inside the hydrogel. When comparing Fig. 2 with Fig. 3, the swelling ratio of the SPH composites containing aqueous Carbopol® 974P solution was lower than that of the SPH composites containing aqueous Carbopol® 934P solution. This may be possibly attributed to the

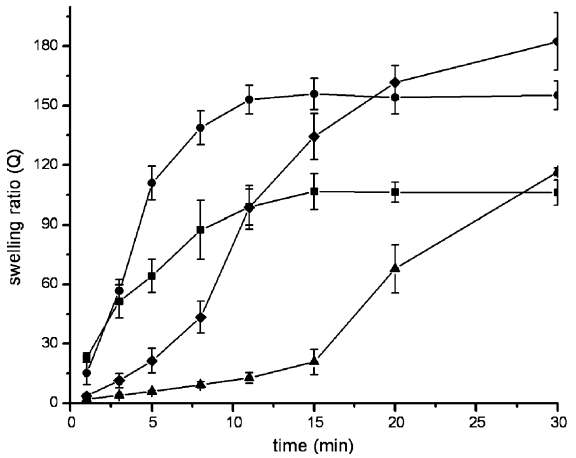


Fig. 2. Swelling ratio curves for SPH and SPHCcs with different amounts of 1.3% aqueous Carbopol® 934P solution: (■) 0 (SPH); (●) 200 μl; (♦) 400 μl; (▲) 600 μl. Data were depicted as the mean ± SD of three experiments.

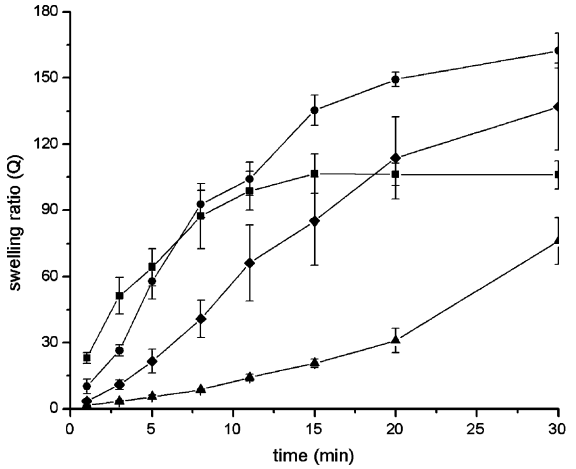


Fig. 3. Swelling ratio curves for SPH and SPHCcs with different amounts of 1.3% aqueous Carbopol® 974P solution: (■) 0 (SPH); (●) 200 μl; (♦) 400 μl; (▲) 600 μl. Data were depicted as the mean ± SD of three experiments.

very high cross-linking degree of the Carbopol® 974P, which resulted in filling more room of the pores inside the gel. This swelling behavior may make it a candidate in double phase time-controlled release system proposed by Dorkoosh [9].

3.3. Density studies

Since the SPH and SPHCcs possess lots of pores, the density of them should be lower compared with the conventional hydrogels. As shown in Table 1, with the increase of the amount of the aqueous Carbopol® solution, the density of the synthesized superporous hydrogels decreased. It is likely that SPHCcs contained some of pores connected each other, thus leading to larger occupied volume. The density of the SPH composites containing Carbopol® 974P was slightly larger than that of the SPH composites containing Carbopol® 934P. This may be due to the very high cross-linking degree of the Carbopol® 974P, which reduced the amount of the occupied volume. This result is consistent with the swelling ratio studies.

3.4. In vitro bioadhesion studies

Table 2 showed both the SPH and SPHCcs adhered rapidly to the intestinal mucosa due to fast swelling and their substances with functional groups to form hydrogen bridges (COOH) to interact with the mucus layer. An increase in Carbopol® content gave rise to an increase in adhesion in every polymer tested. However, the adhesive force reduced when the amount added of 1.3% aqueous Carbopol® solution increased to 600 μl. This may be due to the following fact: the component and the swelling rate of the polymer are two primary factors affecting the bioadhesive force. As a rule, the larger the amount of the adhesive ingredient and the faster the swelling rate of the polymer, the stronger the adhesive force. When the Carbopol® content is less, the polymer could swell quickly and the amount of adhesive component play a dominant role. The swelling rate is primarily responsible for the adhesive capacity when the Carbopol® content is more. The decreasing of swelling rate results in the

Table 1
Density of SPH and SPHCcs

1.3% (W/V) aqueous Carbopol® 934P solution		1.3% (W/V) aqueous Carbopol® 974P solution	
Amount added (μl)	Density[a] (g/cm^3)	Amount added (μl)	Density[a] (g/cm^3)
0[b]	0.78 ± 0.08	0 [b]	0.78 ± 0.08
200	0.67 ± 0.04	200	0.73 ± 0.06
400	0.69 ± 0.01	400	0.72 ± 0.05
600	0.65 ± 0.03	600	0.68 ± 0.01

[a] Data were expressed as the mean ± SD of three experiments.
[b] The hydrogel was SPH when the amount of aqueous Carbopol® solution was zero.

Table 2
Bioadhesive force of SPH and SPHCcs

1.3% (W/V) aqueous Carbopol® 934P solution		1.3% (W/V) aqueous Carbopol® 974P solution	
Amount added (μl)	Bioadhesive force[a] (N/m^2)	Amount added (μl)	Bioadhesive force[a] (N/m^2)
0[b]	3332.4 ± 842.3	0 [b]	3332.4 ± 842.3
200	5588.8 ± 1840.3	200	5916.0 ± 1377.4
400	7669.6 ± 977.9	400	8601.2 ± 1388.9
600	2712.8 ± 772.1	600	3754.9 ± 561.7

[a] Data were expressed as the mean ± SD of six experiments.
[b] The hydrogel was SPH when the amount of aqueous Carbopol® solution was zero.

reduction of bioadhesive force. The quicker swell of SPHCcs is beneficial to their bioadhesive capacity.

The adhesive force of the SPH composites containing Carbopol® 974P was slightly higher than that of the SPH composites containing Carbopol® 934P. This was in accordance with the fact that the adhesive capacity of Carbopol® 974P is superior to that of Carbopol® 934P.

4. Conclusions

New SPH composites as candidates for TMD were prepared from free radical copolymerization using APS and TEMED as an initiator system at various amounts of aqueous Carbopol® solution. The hydrogels possessed the structure approved by scanning electron microscopy that the inner surface had higher porosity and the outer surface was non-porous, which resulted in the enhancement of mechanical strength. SPHCcs have fast swelling rate and high swelling ratio because of the high porosity and interconnection among some of the pores within these polymers. The density, swelling ratio and in vitro bioadhesive force are influenced by the category of aqueous Carbopol® solution. The swelling ratio and the density decreased when increasing the content of aqueous Carbopol® solution. SPHCcs can quickly adhere to the intestinal mucosa owing to the carboxylic groups in the structure and fast swelling, thus suggesting their potential use for mucosal drug delivery, especially for effective peroral delivery of peptide and protein drugs.

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