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Preparation and Characterization of Chitosan-based Superporous Hydrogel Composite

Chavda HV, Patel CN, Karen HD

Shri Sarvajanik Pharmacy College, Hemchandracharya North Gujarat University, Nr. Arvind Baug, Mehsana, Gujarat-384 001, India

Address for correspondence: *Mr. Hitesh Chavda; E-mail: hvchavda@sspcmsn.org*

ABSTRACT

The synthesis of superporous hydrogel composites (SPHCs) with various concentrations of chitosan as a composite material was carried out by solution polymerization. The characterization studies were performed by measurement of apparent density, porosity, swelling studies, mechanical strength studies, and scanning electron microscopy (SEM). In double distilled water, SPHCs showed tremendous increase in their equilibrium swelling capacity. But, when the same SPHCs were placed into simulated gastric fluid for swelling, they showed very less equilibrium swelling capacity. The chitosan altered swelling characteristics in both the swelling media. SPHCs showed improved mechanical strength as the chitosan concentration increased. SEM images clearly indicated the formation of interconnected pore, capillary channels, and the cross-linked chitosan molecules were observed around the peripheries of pores.

Key words: Chitosan, penetration pressure, superporous hydrogel composite, swelling studies

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INTRODUCTION

Hydrogels are cross-linked hydrophilic polymers, with a network structure, which are able to imbibe large amounts of water, and are water insoluble.[1–3] For the pharmaceutical applications they are unique carriers for controlled drug delivery, release control can be governed by both swelling and biodegrading properties.[4–6] The swelling properties of hydrogels are mainly related to the elasticity of the network, the presence of hydrophilic functional groups in the polymer chains, the extent of cross-linking, and porosity of the polymer. The physical characteristics of hydrogels including their swelling ratio also depend on the balance between attractive and repulsive ionic interactions and solvent-mediated effects.[7,8] Owing to their high water affinity and biocompatibility, hydrogels based on poly (acrylic acid) and its derivatives,[9,10] chitosan,[11] alginate[12] and collagen,[13] have attracted the attention. However, these nonporous hydrogels swell slowly and exhibit low loading capacities,[14,15] which restrict their use in effective drug delivery. A new generation of hydrogels, which swells and absorbs water very rapidly, has been developed. Examples of this new generation are superporous hydrogels (SPHs), which swell to equilibrium size in a short period of time.[16–20] The first approach for SPH synthesis involves copolymerization/crosslinking of co-monomers using multifunctional co-monomer, which acts as crosslinking agent. Chemical initiator initiates the polymerization reaction. Gas blowing techniques are used to synthesize SPHs. The commonly used foaming agents are inorganic carbonates such as sodium carbonate and sodium bicarbonates, which have been safely applied in drug delivery systems. The second method involves crosslinking of linear polymers by irradiation or by

chemical compounds.[21] Several important properties of SPHs, such as fast swelling, large swelling ratio, and surface slipperiness, make them an excellent candidate material to develop gastric retention devices.[22] Due to poor mechanical strength of conventional SPHs (CSPHs), they are difficult to handle without breaking.[23]

SPH composites (SPHCs), as the second generation of SPHs, possess improved mechanical properties with composite agents such as Ac-Di-Sol serving as the local point of physical entanglement of the formed polymer chains.[24,25] SPHC containing Carbopol also exhibits enhanced *in vitro* muco-adhesive force over CSPHs.[26]

The objective for the current investigation was to synthesis SPHCs containing chitosan as a composite material to improve the characteristics of CSPHs. Acrylic acid and acrylamide were chosen as the base monomers for their high water affinity and fast copolymerization velocity,[27] while chitosan was selected as the second polymer component for its biocompatibility,[28] and water solubility.[29]

MATERIALS AND METHODS

Materials

Chitosan was obtained as a gift sample from Mahtani Chitosan Pvt. Ltd., Gujarat, India. Acrylamide was obtained from Burgoyne Burbidges and Co. Pvt. Ltd., Mumbai, India. Acrylic acid, N,N'-methylenebisacrylamide, Span 80, ammonium persulphate, and N,N,N',N'-tetramethylethylenediamine were purchased from SD Fine Chem Ltd, Mumbai, India. Double distilled water (DDW) was prepared in laboratory. Simulated gastric fluid (SGF) with pH of about 1.2 was prepared in laboratory by dissolving 2 g of sodium chloride, 3.2 g pepsin, and 6.8 ml of hydrochloric acid in DDW to 1 L. All other chemicals used were of analytical grade and used as obtained.

SPHC synthesis

All ingredients except sodium bicarbonate were used as solution in DDW. The pH of the monomer solutions was adjusted to 5.5 with 5 M sodium hydroxide solution. When the pH monomer solution was lower than 5, carbon dioxide formation took place before the polymerization commences and no pores were formed inside the synthesized SPHCs.

For the synthesis of SPHC of poly (acrylamide-co-acrylic acid), the following substances were added subsequently into a test tube at 25°C: acrylamide 50%; acrylic acid 50%; methylenebisacrylamide 2.5%; span 80 10%; ammonium persulphate 20%; tetramethylethylenediamine 20%; chitosan aqueous solution 6%, and 200 mg of sodium bicarbonate as shown in Table 1. In this procedure, polymerization was allowed to continue for approximately 10 mins. After adding each substance to the test tube, the reaction mixture was vigorously shaken. Finally, sodium bicarbonate was added very quickly to the solution and mixed with a spatula. If sodium bicarbonate was not added quickly enough, the polymerization had already been started by ammonium persulphate as initiator, under this condition, some clamps were formed additionally and homogenous SPHC polymer was not obtained.

After synthesis of SPHCs, they were removed with the forceps, allowed to dry in oven at 60°C for 48 hrs, and cut into pieces of required size. SPHC was submerged in hexane. This treatment dehydrates the SPHCs quickly as well as provides drying. Thereafter, the SPHCs were removed with forceps and put in an oven at 60°C for 48 hrs in order to ensure that the SPHCs have been dried completely. These SPHCs were stored in airtight container until further use.

Scanning electron microscopy analysis

Dried SPHCs were cut to expose their inner structure and used for SEM studies. The morphology and porous structures of the SPHCs were examined using ESEM EDAX XL-30 Scanning Electron Microscope (Philips, Netherlands), with an operating voltage of 30 kV.

Density and porosity measurements

For density measurement, the solvent displacement method was used. Dried SPHCs were used for density measurements, which actually show the apparent densities of the SPHCs. Pieces of SPHCs were taken and weighed in order to determine the mass of each piece. A piece of

Table 1: Formulation of SPHCs

Ingredients	Batch				
	CH1	CH2	CH3	CH4	CH5
AM (50% w/v)	300 µl	300 µl	300 µl	300 µl	300 µl
AA (50% v/v)	200 µl	200 µl	200 µl	200 µl	200 µl
BIS (2.5% w/v)	70 µl	70 µl	70 µl	70 µl	70 µl
Span 80 (10% v/v)	30 µl	30 µl	30 µl	30 µl	30 µl
APS (20% w/v)	25 µl	25 µl	25 µl	25 µl	25 µl
TEMED (20% v/v)	25 µl	25 µl	25 µl	25 µl	25 µl
Chitosan solution (6% w/v)	-	200 µl	400 µl	600 µl	800 µl
Sodium bicarbonate	200 mg	200 mg	200 mg	200 mg	200 mg

AM: Acrylamide; AA: Acrylic acid; BIS: N,N'-methylenebisacrylamide, APS: Ammonium persulphate, TEMED: N,N,N',N'-tetramethylethylenediamine

the polymer was immersed in a predetermined volume of hexane in a graduated cylinder, and the increase in the hexane volume was measured as the volume of the polymer. The density was calculated from eqn. 1:

$$Density = M_{SPHC}/V_{SPHC}, \quad (1)$$

where, V_{SPHC} is the volume of solvent displaced by SPHC and M_{SPHC} is the mass of the SPHC.

For porosity measurement, dried hydrogels were immersed in hexane over night and weighed after excess hexane on the surface was blotted. The porosity was calculated from eqn. 2:

$$Porosity = V_P/V_T, \quad (2)$$

where, V_P $(= V_T - V_{SPHC})$ is the pore volume of SPHC and V_T is the total volume of the SPHC. Total volume of SPHC can be measured from its dimensions, as it is cylindrical in shape.

Swelling studies

The equilibrium swelling ratio can be calculated from eqn 3:

$$Q = (M_s - M_d)/M_d, \quad (3)$$

where, Q is the equilibrium swelling ratio, M_s is the mass in the swollen state, and M_d is the mass in the dried state. At the beginning of each experiment, the dried gel was measured gravimetrically to obtain M_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 M_s.[16]

Mechanical strength studies

The penetration pressure (PP) of the SPHCs was measured using a bench comparator as described by Chen *et al.* with modifications.[24] The fully swollen hydrogel was put longitudinally under the lower touch and weights were successively applied to the upper touch until the polymer completely fractured. The compressive force could be read from the gauge, and the penetration pressure[25] could be calculated from eqn. 4:

$$PP = F_u/S, \quad (4)$$

where, F_u is the ultimate compressive force at complete breakage of the polymer and S is the area of the lower touch.

RESULTS AND DISCUSSION

SPHC synthesis

In the synthesis procedure of SPHC, acrylic acid and acrylamide are the monomers. Methylenebisacrylamide is used as a cross-linker, and span 80 is used as a foam stabilizer, which is formed by carbon dioxide originating from sodium bicarbonate. To obtain homogeneous SPHCs with as many pores as possible, polymerization should take place when the foam was stabilized. Here span 80 was used instead of Pluronic F127 as reported by Dorkoosh *et al.* [30] Span 80 does not contribute to the chemical structure of the polymer, but is very important as a surface-active agent to create the highly porous polymer structure. Ammonium persulphate is used as a polymerization initiator and tetramethylethylenediamine as a catalyst. One of the important factors that influence the synthesis of the SPHCs was the pH of the acrylic acid monomer solution. At the pH 5.0, SPHCs with well-distributed pores were produced because of the stability and the proper formation rate of the foam.

Synthesis of homogeneous SPHC with large numbers of pores was also dependent on amount of chitosan present. Only when amount of chitosan was less than 47.06% of the CSPH, homogeneous hydrogels with large numbers of interconnected pores could be obtained. When the amount is increased above 47.06%, hydrogel was nonhomogeneous in that only a few pores were maintained. As a thickening agent, chitosan enhanced the viscosity of the stock solution, which efficiently prevented bubbles from escaping from the solution and the residual gas bubbles were able to form inter-connected channels. In addition, chitosan was favorable for foam generation and stabilization when used together with span 80. Good solubility of the chitosan solution at pH values lower than 6,[29] it could be readily blended with the stock solution and well distributed in the SPHs, yielding a homogeneous SPHC.

Scanning electron microscopy analysis

Figure 1 shows the SEM pictures of SPHC and CSPH. Both CSPH and SPHC possessed large numbers of pores, indicating that formation of hydrogel would not destroy the superporous structure. White fibers on the peripheries of the inner pores were observed in SPHC while not in CSPH, which was primarily determined to be the chitosan molecules.

The fully swollen CSPH was transparent in water and lots of bubbles could be seen within the hydrogel. By comparison, white fibers could be observed in the swollen SPHC, which

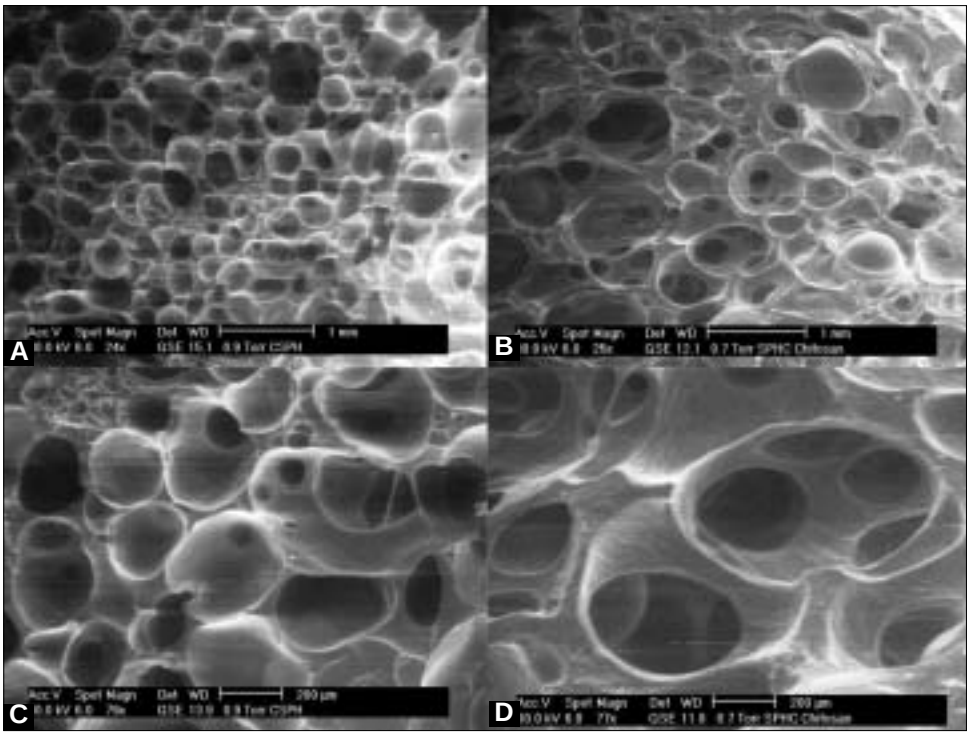

Figure 1: (A) SEM image of CSPH under magnification 1 mm; (B) SEM image of SPHC under magnification 1 mm; (C) SEM image of CSPH under magnification 200 µm; (D) SEM image of SPHC under magnification 200 µm

appeared as netlike distribution. Such difference indicated that the white fibers could be the chitosan molecules and they were well distributed in the polymer to form a three-dimensional network, which primarily confirmed formation of the SPHC.

Density and porosity measurements

Apparent densities and porosities of SPHCs are shown in Table 2. Apparent density increases, while the porosity of SPHC decreases with increase in chitosan concentration. Increasing concentration of chitosan prevented the bubbles from escaping from the solution mixture as well as it decreases the pore size of SPHC due the accumulation of the chitosan at the periphery of the pore. At higher concentration excessive water was introduced into the system, leading to collapse of some of the bubbles and a corresponding significantly lower porosity than CSPH.

Table 2: Apparent density and porosity of SPHCs

Batch	Apparent density (g/cc)	% Porosity
CH1	0.50 ± 0.03	84.08 ± 3.02
CH2	0.40 ± 0.03	83.62 ± 3.05
CH3	0.58 ± 0.04	82.84 ± 3.51
CH4	0.66 ± 0.03	78.13 ± 4.03
CH5	0.82 ± 0.02	69.27 ± 2.54

Data are expressed as the mean of three experiments

Swelling studies

Swelling parameters of SPHCs in DDW and in SGF are shown in Tables 3 and 4, respectively. Figure 2 shows swelling ratios of SPHCs in DDW and SGF. An increase in the chitosan concentration led to a slower swelling and decreased equilibrium swelling ratio of the SPHCs. Through entanglement with the cross-linked chitosan

Table 3: Swelling parameters of SPHCs in DDW

Batch	Size of swollen SPHC (mm^2)	Swelling time (min)	Swelling ratio
CH1	3392 ± 76	4	287.37 ± 16.01
CH2	2568 ± 90	7	163.24 ± 11.11
CH3	1852 ± 94	9	115.26 ± 8.29
CH4	1469 ± 95	12	91.86 ± 8.72
CH5	1055 ± 52	16	65.84 ± 3.05

Data are expressed as the mean of three experiments. Initial size of all the SPHCs is 100 mm^2.

Table 4: Swelling parameters of SPHCs in SGF

Batch	Size of swollen SPHC (mm^2)	Swelling time (min)	Swelling ratio
CH1	605 ± 28	5	11.27 ± 0.63
CH2	458 ± 20	9	7.49 ± 0.25
CH3	340 ± 16	11	4.44 ± 0.18
CH4	244 ± 5	16	3.55 ± 0.10
CH5	176 ± 7	19	2.63 ± 0.18

Data are expressed as the mean of three experiments. Initial size of all the SPHCs is 100 mm^2.

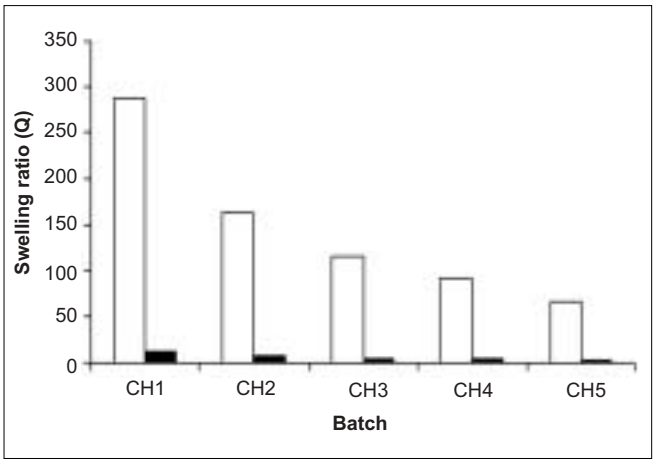

Figure 2: Swelling ratios of SPHCs in DDW (□) and SGF (■)

network, flexibility of the polymeric chains was greatly restricted. Hydrogen bonds between chitosan and P (acrylamide-co-acrylic acid) reduced the ability of the polymer to form hydrogen bonds with water molecules, thus limiting its water absorption. Therefore, a dense chitosan network would further restrict the swelling of the polymer. However, compared to nonporous hydrogels, SPHCs still possessed significantly faster swelling rate and larger equilibrium swelling ratios owing to their porous structures.

Mechanical strength studies

A SPHC should be able to withstand the pressure expected in the stomach during repeated gastric contractions, especially the housekeeper waves. Formulation variables, such as the amount of crosslinker, type of monomer, amount of blowing agent as well as process variables all affect the mechanical properties of the SPHCs. SPHCs when swollen in DDW showed mechanical strength that can withstand the pressure during gastric contraction, but when compared to that of in SGF it was less (data not shown). SPHCs showed too good penetration pressure more than 500 cm water, when swollen in SGF. The maximum pressure during the gastric contraction was reported to range from 50–70 cm water.[31] Increase in the amount of chitosan helped bring about a denser chitosan network and a smaller equilibrium swelling ratio, thus enhancing the elasticity of the polymer.

CONCLUSIONS

SPHC based on chitosan improve the mechanical stability, while the mechanical stability of CSPH is too poor. The porosity of SPHC decreases with increase in chitosan concentration, however, Batch CH2 and CH3 did not show much change in porosity, indicating at these concentrations of chitosan the porous structure of SPHC was not altered. The equilibrium swelling ratio and size of SPHCs decreased with the increase in chitosan content in DDW and SGF. It also reflects, the equilibrium swelling ratio of the SPHC was pH dependent, as in SGF with pH 1.2 equilibrium swelling ratio was less compared to DDW with pH 7.0 for all the concentration of chitosan. Mechanical properties of the SPHCs were significantly improved and could be altered by varying the chitosan content in SGF. In DDW, mechanical properties of the SPHCs were not improved significantly.

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