Superporous hybrid hydrogels based on polyacrylamide and chitosan: Characterization and *in vitro* drug release

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**Abstract**

**Objective:** Current research was aimed at the development of the drug delivery systems based on the superporous hydrogels (SPH) with the desired swelling and the mechanical properties. **Materials and Methods:** Superporous hydrogel composites (SPHCs) and superporous hybrid hydrogels (SPHHs) based on the chitosan and the polyacrylamide were synthesized using the gas blowing technique. The prepared hydrogels were evaluated for swelling studies, mechanical strength and scanning electron microscopy. The selected hydrogels were loaded with the drug (verapamil hydrochloride) by aqueous loading method. Drug integrity with in polymeric network was evaluated via fourier transform infrared spectroscopy (FTIR), X-ray diffraction (X-RD), differential scanning calorimetry (DSC), proton nuclear magnetic resonance (¹HNMR) studies. *In vitro* drug release studies were carried out using the united state pharmacopeial (USP) dissolution apparatus (type II). **Results and Discussion:** The mechanical strength was observed to be higher in SPH hybrids in comparison to that in SPHCs while no significant difference was observed in swelling behavior. *In situ* crosslinking of chitosan with glutaraldehyde (GA) may be responsible for high mechanical strength. The equilibrium swelling time was slight higher in SPHH than in SPHCs. The integrity of pores was maintained in ethanol treated hydrogels as observed in scanning electron micrographs. Whereas, freeze dried SPH samples showed non-uniform pores. No drug polymer interaction was observed as indicated by DSC, FTIR, X-RD and NMR studies. However, the crosslinking of chitosan with GA was clearly indicated by these studies. The *in vitro* drug release studies from SPH hybrids indicated initial fast release (65%) with in first 2 h and then sustained release at the end of 24 h (95%). The addition of hydroxypropyl methyl cellulose with drug; however, leads to a significant decrease in drug release (56% at the end of 24 h). **Conclusion:** Superporous hybrid hydrogels can be promising devices for the sustained delivery of drug candidates to the gastrointestinal region.

**Key words:** Equilibrium swelling, *in situ* crosslinking, integrity, mechanical strength, porous network

**INTRODUCTION**

Several difficulties are faced in designing controlled release systems for better absorption and enhanced bioavailability. One of such difficulties is the inability to confine the dosage form in the desired area of the gastrointestinal (GI) tract. Drug bio-disponibility is a crucial facet in therapeutic effectiveness.

Over the past three decades, the pursuit and exploration of devices designed to be retained in the upper part of the GI tract has advanced consistently in terms of technology and diversity, encompassing a variety of systems and devices such as floating systems, raft systems, expanding systems, swelling systems, bioadhesive systems, and low-density systems.¹²³

One of the essential factors is the residence time of the drug at the absorption site. Many drugs categorized as once-a-day delivery have been demonstrated to have suboptimal absorption due to dependence on the transit time of the dosage form, and making traditional extended release development challenging. Therefore, a system designed for longer gastric retention will extend the time within, which the drug absorption can occur in the small intestine. Superporous hydrogels (SPH) with average pore size >100 μm, swell to equilibrium size within a minute, due to rapid water uptake by capillary wetting through numerous interconnected open pores.¹ Conventional SPH have the major disadvantage of their weak mechanical strength.¹⁴⁵ Further, improvement in mechanical properties is deemed necessary in case of SPH, which can be achieved by synthesis of superporous
hydrogel composites (SPHCs)\textsuperscript{[6-9]} or interpenetrating/hybrid hydrogel network.\textsuperscript{[10,11]}

The use of various polymers has been reported e.g. chitosan, chitin, algimates and gelatin are among widely used the natural polymers while polyacrylamide, poly (acrylic acid), poly (vinylpyrrolidone) are among synthetic one used to generate polymeric networks and enhance mechanical strength.\textsuperscript{[12-14]}

Chitosan is a copolymer of N-acetyl D-glucosamine and D-glucosamine. It is a semi rigid polysaccharide and is biodegradable, biocompatible, and of relatively low toxicity.\textsuperscript{[15]}

Verapamil hydrochloride (HCl) is a calcium channel blocker; biopharmaceutics classification scheme (BCS) class II drug. The drug shows poor bioavailability (22 ± 8%) due to its extensive first pass effect. It is more soluble at acidic pH (1-3). With a short elimination half-life (4.0 ± 1.5 h), the drug can be a suitable candidate for controlled release formulation.\textsuperscript{[16,17]}

Current research work includes synthesis and characterization of SPH composites and superporous hybrid hydrogels (SPHH) using polyacrylamide as primary polymer; chitosan as secondary polymer. The prepared SPHCs and SPHH were evaluated for swelling and mechanical strength studies and further characterized by scanning electron microscopy (SEM). Drug loading was carried out in selected SPHH, which were further characterized by fourier transform infrared spectroscopy (FTIR), DSC, X-ray diffraction (X-RD), proton nuclear magnetic resonance (\textsuperscript{1}HNMR), in vitro drug release studies.

\textbf{MATERIALS AND METHODS}

\textbf{Materials}

Verapamil HCl was kindly gifted by Abott Health-care Pvt. Ltd. Baddi, India; Chitosan (80% deacetylated) was kindly gifted by Central Institute of Fisheries Technology, Kochi; acrylic acid and acrylamide was purchased from Central drug house (CDH) Pvt Ltd, India; Pluronic F 127 (PF 127) was purchased from Sigma Life Sciences; N, N'-methylene bis acrylamide (BIS), ammonium per sulfate (APS), N, N, N', N'- tetraethylmethylenediamine (TEMED), Sodium bicarbonate were purchased from Loba Chemie, Mumbai, India. All the chemicals used were of suitable analytical grade and used as received.

\textbf{Methods}

\textbf{Synthesis of SPH by gas blowing technique}

Gas blowing technique\textsuperscript{[18]} was used to synthesize SPHC and SPHH. All the ingredients including monomers (acrylamide [50%]), crosslinker (BIS), foam stabilizer (10% PF 127), acrylic acid-25 µl, secondary polymer (chitosan), glutaraldehyde (GA) (10 µl), and reaction initiator pair (20% APS-20% TEMED) were taken in a test tube (20 mm outer diameter and 150 mm length) and test tube was shaken thoroughly after addition of each ingredient. pH of monomer solution was kept between 5 and 6 using acrylic acid before addition of sodium bicarbonate (90 mg).

Immediate stirring is required to uniformly distribute generating gas bubbles. The volume of the final solution in the test tube usually increased to 2-10 times the original solution volume. Simultaneous mixing is required at this step, which leads to foaming and gelation. The resulting SPH were dried under two different conditions: (i) ethanol dehydrated for 2 h and then oven dried at 55°C overnight; (ii) freeze dried for 24 h. The dried hydrogels were kept in desiccator until further characterization.

SPHC were synthesized by simple addition of chitosan in the reaction mixture.

SPHH were synthesized by chitosan and GA in the reaction mixture where \textit{in situ} crosslinking of chitosan occurs within the reaction mixture.

The composition of different SPHC and SPHH is shown in Table 1. The composition using the chitosan solution and GA as cross linker was based on supporting literature.\textsuperscript{[19]} However, some trial batches using varying amounts of APS-TEMED (30, 40, and 50 µl) and sodium bicarbonate was varied from 90 mg to 120 mg. The composition in which uniform foaming and gelation appears (resulting uniform porous hydrogels) was selected.

\section*{EVALUATION OF SPHC AND SPHH (WITHOUT DRUG)}

\textbf{Swelling studies}

\textbf{Equilibrium swelling ratio}

Completely dried SPH was weighed and kept in excess of swelling medium (distilled water at 37°C) until the equilibrium swelling was achieved and the hydrogel sample was again weighed. The results were obtained in triplicate.

The swelling ratio was calculated as:

$$Q = \frac{(M_s - M_d)}{M_d}$$

Where Q is the swelling ratio, $M_s$ is the mass of the hydrogel in swollen state; $M_d$ is the mass of the hydrogel in the dried state.

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|}
\hline
\textbf{Name of ingredient} & \textbf{SPHC} & \textbf{SPHH} \\
\hline
Acrylamide (50%) & 500 µl & 500 µl \\
N, N'-methylenebisacrylamide & 200 µl & 200 µl \\
Pluronic F 127 (10%) & 100 µl & 100 µl \\
Distilled water & - & - \\
Acrylic acid (pH-5-6) & 25 µl & 25 µl \\
Chitosan & 500 µl & 500 µl \\
Glutaraldehyde (50%) & - & 10 µl \\
Ammonium per sulfate (20%) & 40 µl & 40 µl \\
N, N', N'-tetraethylmethylenediamine (20%) & 40 µl & 40 µl \\
NaHCO\textsubscript{3} & 90 mg & 90 mg \\
\hline
\end{tabular}
\caption{Composition of superporous hydrogel composites and superporous hydrogel hybrids}
\end{table}

SPHC: Superporous hydrogel composites, SPHH: Superporous hybrid hydrogels, TEMED: Tetraethylmethylenediamine.
Equilibrium swelling time
The swelling time was determined in triplicate and the hydrogel samples were kept in excess of swelling medium (distilled water at 37°C) and the time required for equilibrium swelling was noted.

Density
Solvent displacement method was used for the determination of density. The pre-weighed hydrogel sample was immersed in hexane in a graduated cylinder. Initial volume of hexane was noted and the increase in volume was also noted. The results were obtained in triplicate. Density was calculated as:

Density = Mass of superporous hydrogel / Volume of solvent displaced

Mechanical properties
Compression force (N) was determined using TA.XT Plus Texture Analyzer (Stable Micro Systems, UK) using a cylindrical aluminum probe (P75) having a pretest speed of 2.00 mm/sec, test speed of 1 mm/sec and posttest speed of 2 mm/sec up to a distance of 3 mm. The swollen hydrogel sample was placed on a disk shaped platform. Compression force was estimated as the peak value in the force-time plot.

SEM
The dried hydrogels were cut in transverse section and mounted on a double sided tape on aluminum stubs and were sputter coated with gold using the fine coat ion sputter and then micrographs were recorded using the scanning electron microscope (JEOL, JSM-6100, Japan) to study the porous nature of hydrogels.

DRUG LOADING
Verapamil HCl (120 mg) was loaded into selected hydrogels SPHH using the method of soaking or equilibration. The amount of water required for complete swelling was determined and thereafter drug was dissolved in the pre-determined amount of water. The SPH sample was kept in the drug solution and left until all the solution was sucked up. Finally, the completely swollen hydrogel was freeze dried. The swollen hydrogels were kept in petri plates, covered with Aluminum foil, making holes in foil layer. The petri plates were kept in lyophilizer chamber for 24-48 h.

Two formulations of SPHH were prepared-SPHH1: Without hydroxypropyl methyl cellulose (HPMC) K4M only pure drug was loaded; SPHH2: drug was first mixed with HPMC K4M using the drug to HPMC K4M ratio of 2:1 then loaded by soaking method.

EVALUATION OF DRUG LOADED SPH
Drug loading capacity
Drug loaded SPHH samples were triturated and an equivalent weight of 10 mg of the drug was dissolved in 100 ml of simulated gastric fluid (SGF) (pH 1.2) and the mixture was filtered and the analyzed spectrophotometrically at 278 nm.

Drug loading capacity: a/b × 100.

Where a is calculated amount of drug; b is theoretical amount of drug loaded.

FTIR
The IR spectra of drug, chitosan, acrylamide, HPMC and drug loaded SPHH were recorded using KBr pellet method over the scanning range of 400-4000 cm⁻¹ using the FTIR spectrophotometer (Perkin Elmer Spectrum 400). The FTIR spectrum was recorded to check the interaction of the hydrogel with the drug.

X-RD analysis
The X-RD studies were carried out to monitor the changes in crystallinity characteristics of the drug when the drug was loaded into hydrogel polymeric network. The freeze dried drug loaded hydrogel was powdered in a mortar and then the X-RD patterns were measured using the X-ray diffractometer (X’pert PRO, PAN analytical, Netherland) using the Ni filtered, CuKα radiation with a voltage of 45 kV and 40 mA current.

'HNMR studies
'HNMR studies were carried out using the cryo-magnet spectrometer 400 MHz Fourier Transformed FT-NMR spectrometer (Bruker) using D₂O and chemical shifts were recorded in ppm downfield from internal reference tetramethylsilane.

In vitro drug release
The in vitro drug release of verapamil HCl from various batches of SPHH was carried out using USP dissolution apparatus (type II) at 37 ± 0.5°C at a paddle speed of 50 rpm in 900 ml of SGF (pH 1.2) for 24 h. At specified intervals, 10 ml of the dissolution medium was withdrawn and an equivalent volume of fresh dissolution medium was replaced. The samples were analyzed at 278 nm using the UV-VIS (ultra violet – visible) spectrophotometer (Shimadzu, Japan).

RESULTS AND DISCUSSION
Equilibrium swelling ratio
Equilibrium swelling ratio of SPHC was found to be higher than that of SPHH. The effect of drying conditions was also observed on swelling behavior of hydrogels. Ethanol dehydrated SPHC showed equilibrium swelling ratio of (116.2 ± 11.95) whereas freeze dried SPHC showed equilibrium swelling ratio of (102 ± 3.89). Similarly, ethanol dehydrated SPHH showed equilibrium swelling ratio (110.18 ± 0.14) and freeze dried SPHH showed equilibrium swelling ratio of (93.43 ± 0.76). A slight decrease in swelling ratio in freeze dried samples may to due distortion in the pores or disrupted capillary channels of hydrogel network.
**Equilibrium swelling time**

Equilibrium swelling time was observed to be less than 5 min in CSPH and SPHC. Ethanol dehydrated SPHC showed equilibrium swelling time of (2.68 ± 0.55) whereas, freeze dried SPHC showed equilibrium swelling time of (4.78 ± 0.35). Similarly, ethanol dehydrated SPHH showed equilibrium swelling time (4.887 ± 0.14) and freeze dried SPHH showed equilibrium swelling time of (5.45 ± 0.76). The uniformity in capillary channels in ethanol dehydrated samples lead to swell them in the least time, which is not the case with freeze dried samples which take comparatively more time. *In situ* crosslinking (SPHH) also affect the swelling ratio (which is comparatively less than SPHC) as well as swelling time (comparatively more than SPHC).

**Density**

The density of all the SPH batches was observed to be less than 1 i.e. they can act as floating devices. Ethanol dehydrated SPHC showed density value of (0.68 ± 0.05) whereas, freeze dried SPHC showed density value of (0.41 ± 0.03). Similarly, ethanol dehydrated SPHH showed density value of (0.51 ± 0.04) and freeze dried SPHH showed density value of (0.42 ± 0.06). Freeze dried samples were found to be least dense.

**Mechanical strength/compressibility**

The SPH should have desired mechanical strength to withstand the pressure of gastric fluids. Higher compression strength was observed in SPHH than in SPHC. SPHC were observed to be fragile under compression of more than 3N while SPHH were observed elastic and did not break under a compression force of 9-10 N [Figure 1a and b]. Same results were also observed in freeze dried SPHH samples. This may be due to *in situ* crosslinking of chitosan with GA in polymeric network of SPHH.

**SEM**

The integrated pore structure with interconnected channels was observed in SEM images of ethanol dehydrated-oven dried batches [Figure 2a]. Non-uniformity in pores with distorted capillary network was observed freeze dried batches [Figure 2b].

**Drug loading capacity**

SPHH were selected for further drug loading and characterization studies due to desired mechanical and swelling characteristics. The drug was uniformly loaded into selected SPHH and drug loading was observed in the range of 97-98% for both the formulations (SPHH1 and SPHH 2).

**FTIR**

The spectra of pure verapamil exhibits peaks at 2575 and 2541 cm⁻¹ (aldehydic C-H stretching), 2236 (C-N stretching vibrations), 1592, 1518 and 1462 (C = C in the aromatic ring), 1259 (C-O stretching in aromatic and aliphatic ring), 1027 (C-N aliphatic stretching), 813 and 768 (Meta substituted benzene). The FTIR spectra of HPMC showed the peak at 3421.04 cm⁻¹ due to OH vibrational stretching; symmetric stretching mode of methyl and hydroxyl propyl groups were found at 2928.96 cm⁻¹ in which all the CH bonds extend and contract in phase; the peak at 1621 cm⁻¹ indicated the presence of stretching vibration six membered cyclic rings. While the asymmetric bending vibrations of the methoxy group normally appeared in the region 1500-1450 cm⁻¹; the symmetric vibrations were mostly displayed in the range 1400-1350 cm⁻¹. The FTIR spectra of acrylamide showed the presence of the band at 3354.19 cm⁻¹ that can be assigned to symmetrical and asymmetrical stretching of N–H group. The characteristic C = O stretching vibration bands of amide and acid groups have been observed at 1673.6 cm⁻¹ and 1720 cm⁻¹ respectively, and the peak for –CH = CH₂ group at 1620 cm⁻¹ was observed. The FTIR spectra of chitosan showed peak at 2919 cm⁻¹ indicated alkane C-H stretching vibration.

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**Figure 1:** Force versus time plots of superporous hydrogel composite and superporous hybrid hydrogels
while the other peaks at 1379 cm$^{-1}$ and 1420 cm$^{-1}$ indicated alkane C-H bendings. The broad stretching peak at 3370 cm$^{-1}$ can be attributed to either the hydrogen bonded O-H or N-H (primary amines) bonds or both. The peak at 1595 cm$^{-1}$ is due to the bending vibration of the N-H bonds of the primary amine group. The peaks at 1152 cm$^{-1}$ corresponds to the anti-symmetric stretching of the C-O-C bridge while the peak at 1078 cm$^{-1}$ is due to the skeletal vibrations involving the C-O stretching and are generally regarded as the fingerprint peak for the saccharide structure of chitosan. The FTIR measurements of chitosan and cross-linked chitosan incorporated in SPHH polymeric network confirm a complete reaction of the amino groups. The peaks at 1572 cm$^{-1}$ indicated the formation of imine bonds due to cross-linking reaction of free amino groups of chitosan with the aldehydeic groups of GA. The cross-linked chitosan with GA also showed reduced absorption for the O-H and N-H stretching vibration peaks. The FTIR spectra of SPHH showed the presence of the characteristic peaks of the drug showing that there was no drug polymer interaction [Figure 3].

**X-RD**

X-RD of verapamil HCl showed numerous characteristic peaks
at 2θ of 10.59°, 14.45°, 17.07°, 18.1°, 18.84°, 20.29°, 21.32°, 23.06°, 23.75°, and 26.29°. X-RD of HPMC showed the amorphous behavior of HPMC due to the presence of broad peaks with the peak of maximum intensity at 5.82°. The X-RD of chitosan showed two small peaks at 8.5° and 11.2° and a broad peak over 18°-25° indicating amorphous nature [Figure 4]. Although, the crosslinking of chitosan with GA in SPHH polymeric network was clearly observed by the presence of more peaks at 29° and 42°, which leads to consequent increase in crystallinity of chitosan. Figure 4 shows the overlay diagram of X-RD of drug, polymers and drug loaded SPHH. The incorporated drug remain intact in polymeric network of SPHH, which is clearly indicated by the presence of characteristic peaks of drug in the diffusion pattern of drug loaded SPHH. However, minor shifts in characteristic peaks of drug and reduced diffraction intensity of verapamil HCl suggests a reduction in quality of the crystals (e.g. change in crystal size) due to the presence of higher amount of polymers.

**1H NMR studies**

The presence of various characteristic peaks of pure drug verapamil HCl in proton NMR spectra [Figure 5] of drug loaded SPHH indicated drug integrity with in polymer network.

**In vitro drug release**

The drug release studies were carried out to (i) evaluate the sustain release characteristics of SPHH as drug delivery system for verapamil HCl; (ii) evaluate the effect of addition of HPMC on release characteristics. Initially, fast drug release in 2 h (65%) was observed in SPHH, which was then sustained at the end of 24 h (95%). This may be due to the drug present on the surface of hydrogel and later on the drug from within the polymeric network release slowly by diffusion. The addition of HPMC K4M leads to a significant decrease in drug release at the end of 24 h (nearly half i.e., 56%). The drug particles mixed with HPMC K4M may act as matrix systems from which drug particles diffuse and further drug release from acrylamide network occur again by diffusion. In vitro drug release was compared with the marketed product (Calaptin 120 SR), which shows constant sustained effect with 80% drug release at the end of 24 h [Figure 6].

**CONCLUSION**

SPH have been evolved to address the need for pharmaceutical applications such as gastroretentive drug delivery. SPHs with enhanced mechanical properties (SPHC, SPHH) were prepared by introducing another polymer network and increased the feasibility of using them as oral sustained release devices particularly for gastric retention. The interconnected pore structures are not destroyed by penetrating polymer networks thereby maintaining high and fast swelling characteristics with improved mechanical strength.

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