Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

APPLICATION OF 2^5 FACTORIAL DESIGN IN OPTIMIZATION OF SUPERPOROUS HYDROGEL SYNTHESIS CONTAINING NATURAL MUCILAGE

PALLAVI KHANEKAR, MUNIRA MOMIN*, SUPRIYA MHATRE

Oriental College of Pharmacy, Sector-2, Sanpada, Navi Mumbai, Maharasthra 400705, INDIA
Email: munira_momin@yahoo.com

Received: 11 Apr 2014 Revised and Accepted: 16 May 2014

ABSTRACT

Objective: An experimental design, based on 2^5 factorial designs was used to study the influence of formulation parameters on a swelling and gelling behaviour of synthesized superporous hydrogel. Although a vast variety of formulations parameters could affect the hydrogel networks, attempt was made to synthesize a natural super-disintegrant, i.e., fenugreek mucilage, based super-porous hydrogel.

Methods: A 2^5 factorial design was adopted to study the effect of various parameters for synthesis of superporous hydrogel using fenugreek mucilage as a foam stabilizer. The gelation features during the synthesis process, including inhibition period, exothermic period were observed. For this study, five formulation variables i.e, type of monomer, amount of cross-linker, redox initiator, redox activator and level of foam strengtheners were chosen and their effects were examined within the frame of a 2^5 factorial design.

Results: The responses examined were inhibition period, exothermic period and gelling feature along with the physical appearance during gelation and after drying. These responses found to be dependent on the concentration of cross-linker and redox activator ($P< 0.05$). The resultant hydrogel was subjected to SEM study and it clearly showed that fenugreek mucilage at higher level could stabilise the foam during gelation process which led to better and enhanced porous network formation.

Conclusion: The synthesized superporous hydrogels can be used in for those formulations where fast swelling and superabsorbent properties are critical.

Keywords: Superporous hydrogel, Fenugreek mucilage, Foam strengtheners, Factorial design.

INTRODUCTION

A superporous hydrogel (SPH) is a 3-dimensional network of hydrophilic polymers. It can accommodate a large amount of fluid quickly due to presence of interconnected microscopic pores [1]. As compared to conventional hydrogels like, acrylamides and cellulosic derivatives, highly cross-linked SPHs possess numerous times more surface area and shorter diffusion distance. These features allow dried SPHs to take up and hold large amount of fluid and remain swollen on contact with fluid. The hydrogels and SPH being biocompatible and biodegradable has role in biomaterial science, surgical materials, drug delivery system etc. Drugs which are absorbed from duodenum or lower part of stomach has very narrow absorption window. For these types of drugs, gastroretentive drug delivery system proves advantageous for increasing the gastric retention time [2, 3].

Superporous hydrogels are gaining popularity as a gastro-retentive drug delivery system. When drug loaded superporous hydrogel are administered, the super swollen hydrogel retains inside the stomach either by floating or remain near pylorus sphincter due to porous and larger volume. Higher mechanical strength, swelling capacity and stability in acidic condition of stomach pH 1.2 are the most desired characteristics of any SPH [4, 5, 9]. Swollen hydrogel should be strong enough to withstand with pressure, abrasion and shear forces generated in stomach by gastric fluid [6]. The swollen hydrogel is capable of bearing pressure more than 50-70 cm water pressure. A few aspects of superporous hydrogel including synthesis, pore structure, gastric retention properties, characterization, swelling dependence on compression stress, pH, have been studied. But, little attention has been paid to the events that occur during cross-linking and gel formation [7,10].

The properties of hydrogel are dependent on the cross-linking events during the gel formation, particularly when the reaction is carried out in the presence of oxygen. In conventional hydrogel synthesis, reactions continue during the final drying process and influenced the degree of cross-linking and gel consistency even after its gel formation. Thus, the swelling properties of the final dried polymer are not affected only by the inhibition period of polymer gel formation events, but also by the drying process. While, during the synthesis of superporous hydrogels, to maintain the porosity and further stop the cross-linking process, gels are immediately dehydrated in n-hexane once the cross-linked gel is formed [10].

The aim of the present experimental design is to study the effects of various processing variables at various stages of SPH formations, i.e, the effect of monomer, cross linker, type of acids as co-monomers & foaming aids, redox initiator and Redox activator for polymerization process. Also, attempt has been made to use natural polymer, flaxseed mucilage as a foam strengthener and its effect on swelling behavior of resultant acrylamide based superporous hydrogel. A statistical analysis was carried out using 2^5 factorial design to study the most effective levels of each selected parameters for the synthesis of SPH.

MATERIALS AND METHODS

Materials

Acrylamide (AAm), Acrylic acid (AA), Citric Acid (CA), Span 80, (S. D. Fine Chem. Limited) Mumbai, India, N,N'-methylenebisacrylamide (BIS) (Loba Chemie laboratory reagents fine chemicals, India), Ammonium peroxydisulfate (APS,), (Thomas baker Chemicals, India), N,N,N',N'-tetramethylethylenediamine (TMEDA,) (Himedia Laboratories, India), Trigonella foenum-graecum Linn. Obtained from local market at Mumbai, India & authenticated by a botanist at Poona, India. Isolation of mucilage was done by the method developed at our laboratory [11]. Double Distilled water was used throughout experiment. All solutions were freshly made at room temperature before use.

Experimental Method

The superporous hydrogel was prepared as per the method described by Hitesh et.al. Briefly, all the ingredients except sodium bicarbonate were used as a solution in double distilled water [10]. To make the superporous hydrogel, a monomer, cross-linker, water, foam stabilizer, polymerization initiator and foaming agent etc were added sequentially to a test tube. All these ingredients were mixed in

an order and quantity mentioned by Hitesh et.al. Briefly, 300 µL of 50 % w/v aqueous AM solution (base monomer) was placed into a test tube; to this solution, 200 µL, 50% v/v of AA or CA solution (co-monomers), 70 µL of Bis (1, 3%) w/v (cross-linker), 30 µL span 80 (10%) v/v (Foam stabilizer), 25 µL of APS (20,40%) w/v (Redox-initiator), 25µL of TEMED (20,40%) v/v (Redox Activator), 400 µL Fenugreek mucilage (FNM) aqueous solution (4,6%) w/v (Foam strengthener) were added one by one. In this procedure, polymerization was allowed to continue for approximately 10 minutes. After adding each ingredient to the test tube, the reaction mixture was shaken vigorously and temperature was noted. Finally, sodium bicarbonate (200 mg) was added very quickly to the solution and mixed with glass rod. Synthesized SPHs were removed with blunt forceps, allowed to dry in oven at 60^0C + 0.5 ^{0}C for 48 hrs, and cut into small pieces (1 cm^2) of required size. The SPHG was submerged in hexane overnight. This treatment dehydrated the SPH rapidly. The dehydrated SPH was removed with the help of blunt forceps and put it into oven at 60^0C for 48 hrs for drying completely.

Evaluation parameters during synthesis of sph

The following parameters were studied and observations were made during the process of polymerization.

Effect on inhibition period

The inhibition period is observed during cross-linking process of monomers. This period is normally affected by the type and amount of redox initiator, co-monomer, redox activator and cross-linker. The inhibition period (in minutes) of gelation during the process of polymerization was noted down after addition of different concentration of redox initiator, co-monomer addition, cross linker and redox activator.

Effect on Exothermic period

The change in temperature is noted during the process of cross-linking of polymerization. The change in temperature during the gel formation after addition of different concentration of redox initiator, co-monomer addition, cross linker and redox activator was noted down.

Evaluation of dried SPH

Swelling ratio and gelling property

The synthesis of SPH was carried out using two different acids, citric acid and acrylic acid. Several batches were prepared using citric acid/acrylic acid with other SPH ingredients. After complete polymerization process of SPH, to stop the further reaction, the resultant mass was completely dehydrated using n-haxane as mentioned in methodology.

The dried mass was cut into small pieces and swelling index was measured by the method described Hitesh et.al. The results are depicted in the table 1 and 2 and Figure 1.

Bulk density & Tapped density

The compressibility parameters like, bulk and tapped density as important factors when the material is to be used for tablet or capsule formulations. The resultant dried powdered SPH was subjected to bulk and tapped density measurement. The results are depicted in Table 2

Load–Deformation Measurement

A simple instrument fabricated in our lab was used to measure load-deformation of dried SPH. The individual hydrogel having size 2 cm were placed on stationary upper jack. Weights were applied to the lower movable jack. The corresponding weights required to deformation & break the hydrogel ribbon was measured. The results are presented in Table 2.

Statistical analysis study of selected parameters

The 2^5 factorial design was adopted to study the effect of selected independent variables like, concentration of monomer, cross linker, type of acids as co-monomers & foaming aids, foam strengthener, redox initiator and redox activator on the swelling and gelling of the resultant SPH. Statistical analysis software (State-Ease Design Expert™, version8) was used to study the effect of each selected variables on the swelling and gelling of the synthesized SPH.

RESULTS AND DISCUSSION

A total of 32 batches as per the 2^5 factorial design were prepared with different concentration of monomer, cross linker, type of acids as co-monomers & foaming aids, foam strengthener redox initiator and Redox activator.

In qualitative terms, increased concentrations of the foam stabilizer, redox couple and cross-linker (in a lower range of concentration) resulted in reduced inhibition and exothermic periods and also an increased temperature during gelation was observed.

Fig. 1: Swelling of superporous hydrogels.

Effect of the Redox Initiator Concentration

In the present study, ammonium persulphate at 20%w/v and 40%w/v was used as a redox initiator during the synthesis of SPH. It was observed that high concentrations of initiator, although the oxygen can still take part in the reaction, there was a sufficient amount of initiator radicals for normal polymerization to occur.

Therefore, the inhibition period was shortened at high initiator concentration. Likewise, polymerization took place faster at high initiator concentrations; and hence the gelation or exothermic reaction occurred faster.

Effect of the Stabilizer Concentration

Span 80 was used as foam stabilizer of the foam which was generated by the carbon dioxide originating by addition of the sodium bicarbonate. Span 80 does not contribute chemical structure of the polymer but, it very active as a surface active agent to create highly porous polymer structure.

Effect of type of co-monomer

Acrylic acid and citric acid were explored to see the effect of type of acid as a co-monomer during the synthesis of SPH. An aqueous AAm-

based hydrogel formulation has a pH around 4, at this pH, polymerization occurs rapidly even at high water dilution. Acid addition reduces the AAm solution to pH 2; polymerization is retarded at this pH even at low water dilution. Therefore, the inhibition and exothermic periods are extended upon acid addition. The heat generated during this period can cause more monomer to polymer conversion for the systems at low water dilution or at high monomer concentrations. On the other hand, the heat generated raised the water temperature for systems with high water dilution. Therefore, more heat can be lost during the exothermic period via convection and conduction rather than to attain higher reaction rates. During this process of exothermic reactions of polymerization, effect of type of acid was also observed. Batches prepared with citric acid and acrylic acid showed different physical properties like, swelling and gelling. The results are depicted in table 1 and 2.

Effect of the Cross-linker Concentration

Theoretically, the cross-linker can shorten the inhibition and exothermic periods simply by raising the reaction viscosity created by the cross-links between the growing chains. Although the observation at low concentration is as expected, this dual behavior of the bisacrylamide (BIS) cross-linker at its low and high concentrations can presumably be accounted for in terms of its interaction with another variable like water.

Increased BIS concentration showed negative effect on swelling capacity, increases in density and decrease in porosity. Hence, increase in the cross-linkers concentration is beneficial for mechanical stability of SPH. Also, decreasing porosity leads to decrease in drug release by diffusion.

Table 1: Variables and parameters 2^5 factorial experimental design

S. No.	Types of acids 50% v/v	BIS	SPAN 80	APS	TEMED	Fenugreek Mucilage	Swellability Status	Physical appearance
1.	AA	1%	10%	20%	20%	4%	Swellable	Tough
2.	AA	3%	10%	20%	20%	4%	Highly swellable	Very tough
3.	CA	1%	10%	20%	20%	4%	Good swellable	Soft
4.	CA	3%	10%	20%	20%	4%	Swellable	Soft
5.	AA	1%	10%	40%	20%	4%	Swellable	Soft
6.	AA	3%	10%	40%	20%	4%	Swellable	Tough
7.	AA	3%	10%	40%	40%	4%	Swellable	Soft
8.	CA	1%	10%	40%	20%	4%	Swellable	Soft
9.	CA	3%	10%	40%	20%	4%	Swellable	Very tough
10.	CA	1%	10%	40%	40%	4%	Not Swellable	Oily
11.	CA	3%	10%	40%	40%	4%	Swellable	Tough
12.	AA	3%	10%	40%	40%	4%	Swellable	Tough
13.	AA	1%	10%	20%	40%	4%	Slightly swellable	Grease like
14.	AA	3%	10%	20%	40%	4%	Highly swellable	Tough
15.	CA	1%	10%	20%	40%	4%	Slightly swellable	Viscous liquid
16.	CA	3%	10%	20%	40%	4%	Slightly swellable	Soft
17.	AA	1%	10%	20%	20%	6%	Swellable	Soft
18.	AA	3%	10%	20%	20%	6%	Highly Swellable	Tough
19	CA	1%	10%	20%	20%	6%	Swellable	Soft
20.	CA	3%	10%	20%	20%	6%	Good swellable	Very tough
21.	AA	1%	10%	40%	20%	6%	Swellable	Tough
22.	AA	3%	10%	40%	20%	6%	Swellable	Soft
23.	CA	3%	10%	40%	40%	6%	Semi soluble	Grease like
24.	AA	1%	10%	40%	40%	6%	Swellable	Soft
25.	CA	3%	10%	40%	20%	6%	Swellable	Very tough
26.	CA	1%	10%	40%	40%	6%	Semi soluble	Grease like
27.	CA	3%	10%	40%	40%	6%	Swellable	Soft
28.	AA	3%	10%	40%	40%	6%	Highly swellable	Tough
29.	AA	1%	10%	20%	40%	6%	Slightly Swellable	Liquid
30.	AA	3%	10%	20%	40%	6%	Slightly swellable	Tough
31.	CA	1%	10%	20%	40%	6%	Swellable	Soft
32.	CA	3%	10%	20%	40%	6%	Swellable	Tough

Effect of foam strengthener

The effect of FNM at different concentration was studied on the final product. The higher concentration of FNM resulted in SPH with improved gelling characteristics and retention of pores formed due to foam former.

Effect on the Inhibition Period

To start the exothermic reaction, maximum retardation or inhibition was observed as the concentration of acid was increased. On the other hand, increased TMEDA, APS and Span 80 concentrations resulted in promoting the gelation process. Fenugreek Mucilage and BIS (in range of higher concentration) showed nearly negligible positive effects.

Effect on the Exothermic Period

Increased co-monomer addition (AAc) similarly resulted in increased duration of inhibition and exothermic events. On the other hand, increased TMEDA, Span 80 and APS concentration led to a fast gelation process. Again, increased Fenugreek Mucilage and BIS concentration (at its lower concentration range) showed comparatively negligible negative effect, respectively.

Swelling ratio and gelling property

All the prepared batches were subjected to gelling intensity test. Not all the batches showed good gelling properties. Batches with 3% AA and CA showed gelling at the end of the polymerization. Also, batches containing Span 80 at 10% v/v, TEMED and APS at 20% v/v with FNM 6%w/v showed better gelling property. Hence, batches 1, 2,11,12,14, 18, 21, 28, 30 and 32 were selected for further studies. (Table 2)

Swelling index was carried out in double distilled water and pH 1.2 for all above mentioned selected batches. No difference was observed on swelling ratio and time required for SPH to swell for the media used. Hence, it can be revealed that swelling property of resultant SPH is pH independent. All the batches showed excellent swelling property and swell in less than a minute (32-48 Sec).

Bulk and Tapped Density

The selected batches were subjected to density measurement as per the official method.

The results given in table 2 reveal that all the selected batches of SPH show excellent compressibility (1.12-1.66 gm/ml).

Table 2: Physical properties of selected batches of SPH (n=3)

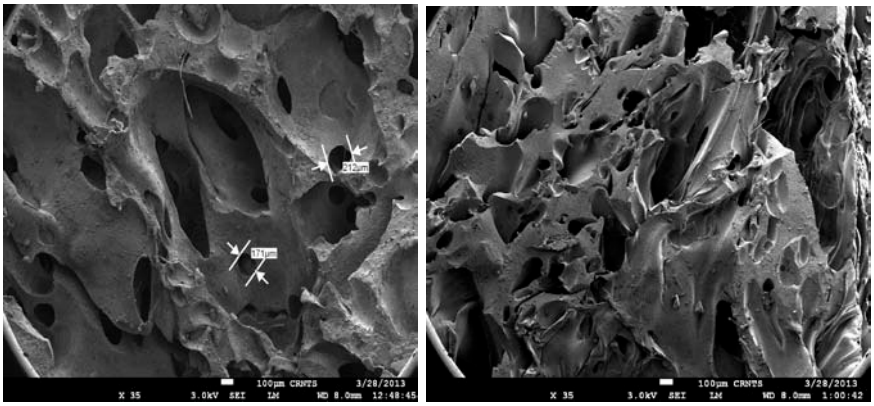
Batch No.	Load deformation used (mg)	Density (gm/ml)*		Swelling Time (Sec)*	
		Bulk	Tapped	H_2O	pH1.2
1	40	1.25±0.03	1.14±0.02	48±1	46±2
2	30	1.66±0.012	1.5±0.014	35±1	39±3
11	30	1.66±0.015	1.5±0.011	48±2	43±1
12	40	1.12±0.021	1.12±0.01	45±4	43±1
14	40	1.42±0.011	1.4±0.021	45±1	42±2
18	15	1.11±0.017	1.28±0.017	32±2	30±4
21	40	1.42±0.01	1.4±0.011	42±3	46±2
28	30	1.11±0.02	1.125±0.031	43±2	42±2
30	40	1.42±0.022	1.16±0.026	38±4	40±2
32	15	1.25±0.011	1.14±0.01	42±2	44±1

*n=3

Scanning Electron Microscopy

The synthesized SPH with fenugreek mucilage (FN-SPH) were subjected to Scanning Electron Microscopy. The figure 2 shows the comparison of SEM pictures of FN-SPH and SPH synthesized without fenugreek mucilage. The picture (a) reveals that the size of the pores is big while in figure (b) indicates that addition of fenugreek mucilage gives stability to the pore structure.

In FN-SPH denser multiple porous structures were observed.



(a) (b)

Fig. 2: SEM photographs of SPH (a) and FN-SPH (b)

Fig. 3: 3-D Plot for effect of parameters on gelling nature of SPH

Load deformation study

The load-deformation behavior of all the selected batches showed non-linear results.

All the batches showed elastic nature of the material. From the composition of all the selected batches it can be revealed that the elasticity of the resultant SPGH is largely based on cross linker concentration (3%w/v) and type of acid (AA) added. The increase in concentration of cross linker increased the elasticity and toughness of the SPH. Also, addition of FNM at 6%w/v affected the toughness of the SPH.

Statistical analysis: factorial design

The 2^5 factorial design and its statistical analysis revealed that amount of cross-linker (P=0.002) & redox activator (0.0155) has significant effect on polymerization during synthesis of SPH using Fenugreek mucilage as a foam strengthener. Counter plot and 3D graph (fig 3 and 4) also shows significant effect of the selected independent variables. On gelling & swelling properties of resultant SPH. The ANOVA based polynomial equation is as follow,

For AA: $$Y\ Gelling = 1.81 - 0.094X1 + 0.593X2 - 3.125X3 - 0.0343X4 + 0.094\ X5\ (P = 0.0002, r2 = 0.994)$$

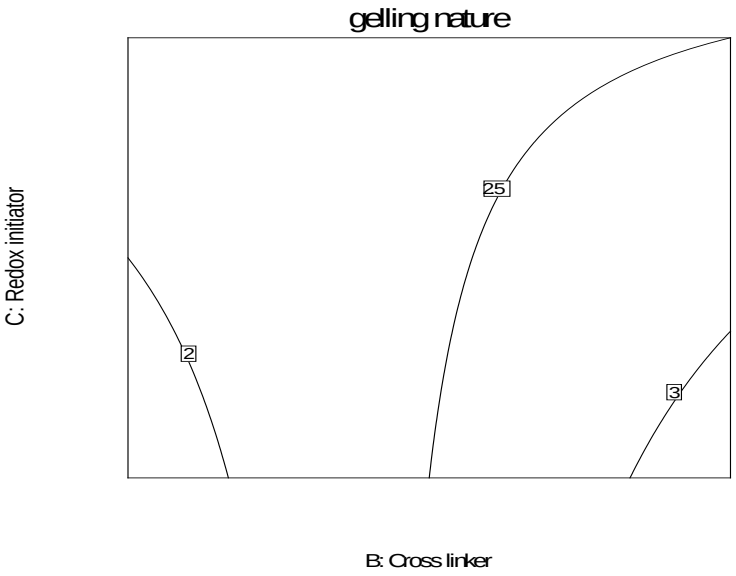


Fig. 4: Contour plot for effect of parameters on gelling nature of SPH.

CONCLUSION

A novel composite SPH was synthesized using natural mucilage, i.e. Fenugreek mucilage. The SEM was carried out to study the effect of FNM on pore formation of synthesized SPH. The synthesis parameters were optimized & statistical analysis was carried out using ANOVA for 2^5 factorial design. Among the selected variables, effect of cross-linker (Methylene bisacrylamide) & redox activator (Tetramethyl ethylenediamine) showed significant effect on swelling and gelling properties of the synthesized FN-SPH. In this complex synthesis with more than 5 variables, a 2^5 factorial design proved to be a promising tool to obtain the optimized condition for polymerization based synthesis. The resultant SPH was found to be PH-independent in their swelling & gelling capacity. The present investigation explores the application of natural mucilage, the one with good swelling properties; in synthesis of superporous hydrogel based drug delivery system.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Chen J, Park H, Park K, Superporous hydrogels:Fast responsive hydrogel systems. Proc ACS Div Polymer Material Science Eng.1998;79:236-37.
2. Chen J, Blevins WE, Park H, Park K. Gastric retention properties of superporous hydrogel composites. Journal of Control Release. 2000;64 (1–4):39–51.
3. Agyilirah GA, Green M, DuCret R, Banker GS. Evaluation of the gastric retention properties of a cross-linked polymer coated tablet versus those of a non-disintegrating tablet. Int. J. Pharm. 1991;75:241–47.
4. Dorkoosh FA, Brussee J, Verhoef JC, Borchard G, Rafiee-Tehrani M, Junginger HE. Preparation and NMR characterization of superporous hydrogels (SPH) and SPH composites, Polymer 2000;41:8213-20.
5. Nagpal M, Singh S, Mishra D, Superporous hydrogels as gastroretentive devices. Acta Pharmaceutica Scientica, 2011;53:7-24.
6. Park K. Superporous hydrogels for pharmaceutical and other applications. Drug. Deliver. Technol. 2002;38:40-44.
7. Palapparambil SG, Debajyoti R, Prafulla KS, Characteristics of xanthan gum-based biodegradable superporous hydrogel, International Journal of Biological Macromolecules 2009;45:364–71.
8. Pourjavadi A, Ayyari M, Amini-Fazl MS, Taguchi optimized synthesis of collagen-g-poly(acrylic acid)/kaolin composite superabsorbent hydrogel, European Polymer Journal. 2008;44:1209–16.
9. Harika D, Sunitha R, Srivalli KP, et.al. Superporous Hydrogels-Versatile Drug Release Retardants, International Journal of Advances International Pharmaceutical Sciences, 2011;2(4):329-39.
10. Hitesh C, Chhagan NP. Chitosan superporous hydrogel composite-based floating drug delivery system:newer formulation approach. J Pharm Bioall. 2010;2(2):124-31.
11. Pooja A, Munira M, Mayur I, et.al. Comparative evaluation of fenugreek and flaxseed as pharmaceutical expedients, International journal of pharma world research.2012;3:119-2