Journal of PharmaSciTech
ISSN: 2231 3788 (Print)
2321 4376 (Online)
Research Article

Preparation and Characterization of Sodium Alginate and Gellan Gum Hydrogel Beads for Bio-Sensing Application

Jessica Kharwanniang[1], Biplab Debnath[2], Nilimanka Das[1*]

[1]*Regional Institute of Pharmaceutical Science & Technology, Abhoynagar, Agartala- 799 005, Tripura, India*
[2]*Department of Pharmaceutical Sciences & Technology, Birla Institute of Technology, Mesra, Ranchi-835215, India*
[*]***Correspondence:*** E-mail: aandeehere@yahoo.co.in (Tel.: +91-8974215254)

Abstract

During pregnancy baby remains protected in mother's womb in a fluid called Amniotic fluid. But many a time unfortunately the amniotic sac ruptures leading to secretion of this fluid and exposing the baby under adverse conditions & even death. As a preventive measure to protect the mother and baby, it is important to identify the liquid secreted from vagina at the earliest. It has seen that the pH of normal vaginal secretion is 3.8-4.5, but the amniotic fluid secreted is having a pH varies from 7.2 to 7.5. Hence our work is focused to develop gellan gum hydrogel to detect such type of fluid. Bromothymol Blue, a chromomeric dye that exhibit color change at different pH environment is entrapped in gellan gum hydrogel matrix using calcium chloride (0.5M) as a cross-linking agent. Preliminary results show that 1% gellan gum containing BTB (0.1%) can produce hydrogel that may serve the purpose. The hydrogel beads efficiently changes color when exposed to a pH range of 1 to 8. The hydrogel beads produce yellow color at pH 4 and blue color at pH 8 within very short time (within seconds). The beads re-usability is documented by switching over to different pH values instantly. Thus conclusion may be made that gellan gum hydrogel is smart enough to act as a bio-sensor.

Keywords: Amniotic fluid, sodium alginate, gellan gum, bromothymol blue, hydrogel, biosensor.

Introduction

During pregnancy baby remains protected in mother's womb in a fluid called Amniotic fluid. But many a time unfortunately the amniotic sac ruptures leading to secretion of this fluid and exposing the baby under adverse conditions & even death. As a preventive measure to protect the mother and baby, it is important to identify the liquid secreted from vagina at the earliest. The pH of normal vaginal secretion is 3.8-4.5, but the amniotic fluid secreted is having a pH varies from 7.2 to 7.5.

Hence all our efforts were oriented towards developing a hydrogel that can sense the fluid and turn up with a color change. To materialize the quest, our work was focused to develop Bromothymol Blue (BTB) entrapped pH sensitive Gellan gum (GG) and Sodium alginate (ALG) hydrogel that may exhibit color change in-situ. Thanks to the tailorable properties of natural polymers that helped the formulation scientist to transform them suitably for particular application. Hydrogel is one such formation that has gained huge attention recently. It is a three-dimensional cross-linked polymer architecture that are smart enough to respond to the environmental stimuli like pH, temp, ionic strength, electric field, presence of enzyme etc. and exhibit excellent biocompatibility [1]. Hence such biomaterials are used widely in different field of pharmaceutical and biomedical engineering [2]. Hydrogels are capable of delivering genetically engineered pharmaceuticals like protein and peptides and improve the therapeutic efficacy and safety in comparison to conventional methods [3].

Materials and Methods

Materials

Sodium alginate was purchased from Loba Chemie, India. Gellan gum was procured from Sigma, USA. Bromothymol Blue and Calcium chloride dihydrate are procured from Merck, India. Double distilled water is prepared in our laboratory and used for the study. All other chemicals and reagents used in the study are of analytical grade.

Preparation of BTB loaded ALG smart hydrogel beads

ALG dispersion (0.5-2% w/v) is prepared in double distilled water taking in a beaker. Then BTB (0.1-1% w/v) is added slowly in the ALG dispersion with continuous stirring to dissolve it completely for 30 minutes using a magnetic stirrer. Then this dispersion is added drop wise in 20 ml of 0.5M Calcium Chloride Dihydrate solution with constant stirring at 100 rpm using magnetic stirrer (UPtin 2MLH). Immediately BTB loaded ALG hydrogel beads were produced and the curing time was 15 min. The beads are then washed by distilled water to remove the excess amount of calcium chloride present on the surface of the beads. The calibration curve for BTB is given in Figure 1.

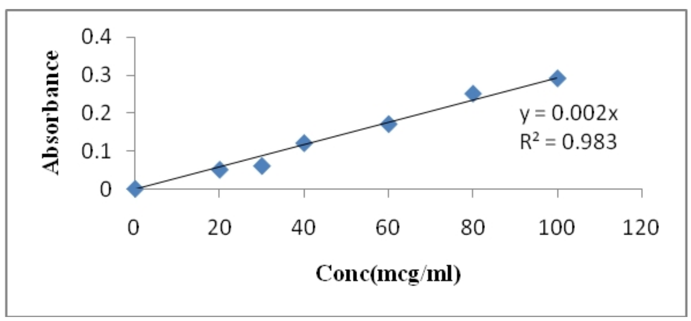

Figure 1: Calibration curve of bromothymol blue in distilled water at 520 nm

Preparation of BTB loaded GG smart hydrogel beads

GG dispersion (0.5–2.0% w/v) is prepared in double distilled water taking in a beaker. Then BTB (0.1 – 1.0% w/v) is added slowly in the GG dispersion with continuous stirring to dissolve it completely for 30 minutes using a magnetic stirrer. Then this dispersion is added drop wise in 20 ml of 0.5M Calcium chloride dihydrate solution with constant stirring at 100 rpm using magnetic stirrer (UPtin 2MLH). Immediately BTB loaded GG hydrogel beads were produced and the curing time was 15 min. The beads are then washed by distilled water to remove the excess amount of calcium chloride present on the surface of the beads. The formulation variables are enlisted in Table 1 and Table 2.

Table 1. Different compositions of bromothymol blue-loaded alginate hydrogel formulations

Sl. No.	Sodium Alginate (% w/v)	Calcium chloride dihydrate Solution (M)	BTB (% w/v)
1	0.5	0.5	1
2	0.5	0.5	0.5
3	0.5	0.5	0.1
4	1	0.5	1
5	1	0.5	0.5
6	1	0.5	0.1
7	1.5	0.5	1
8	1.5	0.5	0.5
9	1.5	0.5	0.1
10	1.75	0.5	1
11	1.75	0.5	0.5
12	1.75	0.5	0.1
13	2	0.5	1
14	2	0.5	0.5
15	2	0.5	0.1

Table 2. Different compositions of bromothymol blue-loaded gellan gum hydrogel formulations

Sl. No.	GG (% w/v)	Calcium chloride dihydrate Solution (M)	BTB (% w/v)
1	0.5	0.5	1
2	0.5	0.5	0.5
3	0.5	0.5	0.1
4	1	0.5	1
5	1	0.5	0.5
6	1	0.5	0.1
7	1.5	0.5	1
8	1.5	0.5	0.5
9	1.5	0.5	0.1
10	1.75	0.5	1
11	1.75	0.5	0.5
12	1.75	0.5	0.1
13	2	0.5	1
14	2	0.5	0.5
15	2	0.5	0.1

Swelling study

Hydrogels are cross-linked three dimensional architecture that posses a unique property of imbibing huge quantum of water without dissolving in it. Thus the swelling property is very much integral to confirm the formation. In this study hydrogel beads were exposed in pH 1.2 HCl-KCl buffer of pH 1.2 for first 2 h followed by next 4 h exposure in phosphate buffer of pH 7.2. Swelling study is carried out by taking 30 mg of dried beads. The swelling ratios are calculated using the following formula

$Q_s = (W_s - W_d)/W_d)$

Where, Q_s is the swelling ratio; W_s is the weight of the swollen sample; and W_d is the weight of the dried sample.

Results and Discussion

In the present work we have attempted to fabricate polysaccharide based smart hydrogels for bio-sensing application. Polysaccharide based materials have unique properties due to the presence of carboxylic, amine or hydroxyl and other functional groups in their backbone which render these polyelectrolytes unique for application in most contemporary fields of biomedical vicinity. They could be fabricated or tailored to meet the goal in predetermined fashion.

Hence, we have chosen two such polymers of polysaccharide origin and these are sodium alginate and gellan gum. Sodium alginate is well known for its unique applications in biomedical engineering. Therefore an attempt has been made to prepare calcium chloride cross-linked sodium alginate entrapped bromothymol blue hydrogel beads for bio-sensing application (Figure 2). BTB is a weakly acidic dye (pKa 7.2) which changes color with environmental pH fluctuations (Figure 3).

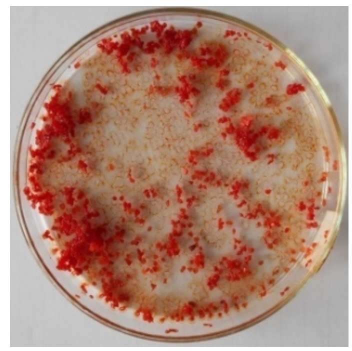

Figure 2. Bromothymol blue entrapped gellan gum hydrogel beads

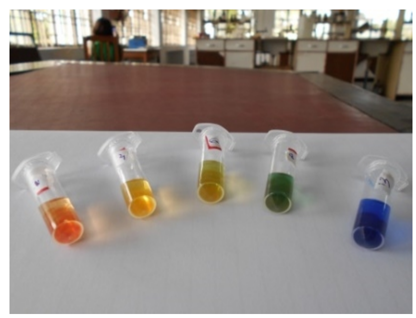

Figure 3: Hydrogels smartly changing color at different pH values (pH 1.0, pH 4.0, pH 6.0, pH 8.0 and pH 10.0, respectively).

Hydrogel formation of similar kind was also attempted with gellan gum. For the preparation of BTB loaded hydrogel beads, sodium alginate was used in the concentration range of 0.5-2.0% w/v. Different formulations were developed using BTB concentration of 0.1-1.0% w/v. The nascent hydrogel beads made of 1.5% w/v sodium alginate and 1.0% BTB could not be transformed into spherical shape and after drying adhere strongly to the surface of petridish. This may be attributed to the lower gel strength as the polymer concentration was inadequate. However, very little amount whatever recovered exhibit different colors when exposed in different pH. Another bead formation made of 1.75% w/v sodium alginate and 1% BTB transformed into spherical shape and retained the morphology even after drying. After fixing the optimum sodium alginate concentration at 1.75% w/v, BTB concentrations were manipulated between 1.0 and 0.1% w/v to study the hydrogel formation that could serve the purpose. The optimum BTB concentration that could serve the purpose was found to be 0.1% w/v. Then in another set of experiment, formation of smart hydrogel beads were attempted using gellan gum and found to exhibit the similar characteristic with much lower gellan gum concentration i.e. 1.0% w/v. Hence our further study was concentrated on gellan gum hydrogel beads.

It has been observed that gellan gum beads shows color change faster in alkaline pH (within seconds) and comparatively slower in acidic pH. This is because gellan gum is made of gallic acid which contains carboxylic acid (COOH) functional group. This COOH group remains unionized in acidic pH results in less porous cross-linked architecture. That is why the color exhibited by BTB is slower. On the contrary to that the functional group of gellan gum beads ionizes in alkaline pH and becomes more porous translating into rapid color change. This is very much evident from the swelling study (Table 3).

Table 3. The swelling data of hydrogel beads

Sl. No.	Time (hour)	Swelling Ratio	Swelling Ratio (%)
HCl-KCl Buffer pH 1.2			
1	00	0	0
2	0.5	0.1	10
3	1	0.107	10.7
4	1.5	0.117	11.7
5	2	0.143	14.3
Phosphate Buffer pH 7.2			
6	2.5	1.96	19.6
7	3	2.23	22.3
8	4	3.18	31.8
9	5	3.57	35.7
10	6	3.83	38.3

Gellan gum hydrogel beads exhibit reusability. Figure 4 shows that the red colored beads (pH 1.0) immediately transform into blue when few drops of alkaline solution of pH 10 are added onto it.

Figure 4: Reuse ability of gellan gum smart hydrogel

To study the stimuli responsive behavior, gellan gum hydrogels were characterized by swelling property. The hydrogel beads were allowed to swell in HCl-KCl buffer of pH 1.2 for 2 h with predetermined time interval. Maximum swelling ratio (%) in HCl-KCl buffer and phosphate buffer was 14% and 38% respectively (Table 3). Hydrogel is three-dimensional cross-linked architecture that swell in aqueous environment. The swelling pattern is a kind of confirmation of hydrogel formation. The swelling ratio may describe smaller sized pores in acidic pH while comparatively bigger sized pores in alkaline pH.

Conclusion

The pH of normal vaginal secretion is 3.8-4.5, but the amniotic fluid is having a pH of 7.2 -7.5. The BTB entrapped GG hydrogel smartly sense pH fluctuation and responds accordingly by changing color. So, this smart hydrogel may be used as a bio-sensor to detect the presence of amniotic fluid.

Acknowledgement

The author's are gratefully acknowledging the support extended by Regional Institute of Pharmaceutical Science & Technology (RIPSAT), Agartala, Tripura, India to carry this piece of work.

Declaration of Interest

The authors do not have any conflict of interest.

References

[1] Prashant PK, Vivek BR, Deepashree ND, Pranav PP. Hydrogels as a drug delivery system and applications: a review. Int J Pharm Pharm Sci 2012; 4: 1-7.

[2] Das N, Bera T, Mukherjee A. Biomaterial hydrogels for different biomedical applications. Int J Pharm Bio Sci 2012; 3: 586-595.

[3] Das N. Preparation methods and properties of hydrogel: a review. IJPPS 2013; 5: 112-117.