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Effect of Process Variables on the Size Distribution of Aluminium Chloride Cross-linked Sodium Alginate Microspheres

Jitendra Kawadkar [1*], Meenakshi K. Chauhan [1], and Maheshwari Maharana [2]

[1] *Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), University of Delhi, New Delhi, 110017, India.*

[2] *Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh, 201303, India.*

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ABSTRACT

In the present study, spherical microspheres of diclofenac sodium using sodium alginate as the hydrophilic carrier and aluminium chloride as a cross-linker were prepared. The shape, surface and size characteristics were determined by binocular microscopy. The microspheres were found to be discreet and spherical in shape. Results of rheological study of polymer solutions showed the increase in viscosity with the increase in polymer concentration and increase in concentration of cross-linker (from 20.77 cP to 1283 cP and from 580.42 cP to 4800.82 cP, respectively). Micromeritic properties of microspheres were studied. It was observed that mean particle size of the microspheres increased from 81.76 ± 5.21 µm to 185.7 ± 2.47 µm and 57.46 ± 4.91 µm to 223.5 ± 5.71 µm with an increase in the concentration of polymer and drug:polymer ratio respectively. The increase in cross-linker concentration and stirring rate gave decrease in mean particle size of the microspheres from 165.7 ± 3.17 µm to 125.9 ± 4.78 µm and 290.5 ± 6.32 µm to 52.16 ± 7.39 µm, respectively. The desired size of microspheres can be obtained by using these process variables. Further these findings help the industry to scale up the commercial production.

Keywords: Sodium alginate, microspheres, aluminium chloride, size distribution

INTRODUCTION

Alginates have a long history of use in numerous biomedical applications, including drug delivery systems, as they are biodegradable, biocompatible and mucoadhesive polymers [1]. Alginate polymers are also hemocompatible, have not been found to accumulate in any major organs and show evidence of *in vivo* degradation [2]. Sodium alginate is a sodium salt of alginic acid, a naturally occurring polysaccharide obtained from marine brown algae. It contains two uronic acids, a-L-guluronic and ß-D-mannuronic acids, and is composed of homopolymeric blocks and blocks with an alternating sequence [3, 4].

Sodium alginate is used in a variety of oral and topical pharmaceutical formulations and it has been specifically used for the aqueous microencapsulation of drugs, in contrast to more conventional solvent based systems [2, 5]. Recently it has been employed as a matrix for entrapment of drugs [6, 7, 8] macromolecules [9], and biological cells [10].

The interaction between polyvalent cations like Ca^{2+} or Al^{3+} and negatively charged polymer like sodium alginate has been used in the past to prepare microspheres [11]. The cations as cross-linkers used for gelation can be divided into two major categories: Low molecular weight counterions (e.g. $CaCl_2$, $BaCl_2$, $MgCl_2$, $CuCl_2$, $ZnCl_2$, $CoCl_2$, pyrophosphate, tripolyphosphate, tetrapolyphosphate, octapolyphosphate, hexametaphosphate, $AlCl_3$ and [Fe(CN)6]-4 /[Fe(CN)6]-3) and high molecular weight ions (*e.g.* octyl sulphate, lauryl sulphate, hexadecyl sulphate, cetylstearyl sulphate) [12]. The addition of cations was able to extend the drug release. This phenomenon is related to an in situ gel formation between the cations and the anionic polymer. The occurrence of in situ gel formation depends on the concentration of the cations [13]. The mechanism of gelation of sodium alginate with calcium was studied by using rheological measurements [14] and it was reported that alginate solution containing a calcium concentration just below the gel point formed swollen aggregates of alginate molecules in the continuous phase. The gelation progress seemed to pass through a pregel state corresponding to clusters of alginate molecules before a continuous and infinite gel was formed. The trivalent Al^{3+} is accepted to form a three-dimensional network by a trivalent bonding structure with the sodium alginate [15]. This three-dimensional bonding results in extended cross-linking through the whole bead. This is because the cross-linking occurs in two different planes at the same time resulting in compacting the alginate molecules (Figure 1).

Microsphere technology is an established technique that has been used to deliver several different types of drugs including antigens, steroids, peptides, proteins, and antibiotics by injection or oral administration. Biodegradable polymer microparticles, either microspheres or microcapsules, are often employed as supports to deliver bioactive compounds. Alginate gel microspheres have been conventionally prepared using extrusion by dropping an alginate solution through a needle into an aqueous cross-linker solution, but this approach has several inconveniences, namely, the limitation in reducing microsphere diameter, the teardrop shape of the microparticle produced, and the difficulty in industrial scale-up. The use of an emulsification/external gelation method by adding an alginate solution and cross-linker solution separately in an emulsion phase could permit the reduction of microsphere diameter. When the cross-linker was used in this method the increased concentration of cross-linker reduced the intermolecular space. That would be the reason of decrease in size on increase in concentration of cross-linker [16]. A lot of work has been done using $CaCl_2$ therefore $AlCl_3$ was selected as a cross-linker because aluminum ions have an extra positive charge compared to calcium ions thus each molecule of aluminum is able to bind to one more alginate molecule. Because of this, $AlCl_3$was capable of forming a gel more quickly than $CaCl_2$ [17].

In this study, the effect of process variables e.g. polymer concentration, D:P ratio, concentration of cross-linker, and stirring rate, on the size distribution of sodium alginate microspheres was investigated. $AlCl_3$ was used as a cross-linker and the gelation effect of sodium alginate with aluminium counter ions was also studied by rheological measurement.

MATERIALS AND METHODS

Materials

Sodium alginate was obtained from Central Drug House, New Delhi, India. Diclofenac sodium was received as gift sample from H-Jules Corporation, Nagpur, India. Light liquid paraffin, heavy liquid paraffin, span-80, methanol, isopropyl alcohol, aluminium chloride, petroleum ether were purchased from Rankem, RFCL, New Delhi, India. All these chemicals were used without any previous analysis.

***Corresponding author.**

Jitendra Kawadkar
Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR),
University of Delhi,
New Delhi, 110017, India.
Tel.: +91 9971160504.
Telefax: +91-011-29554649.
E-mail:j_kawadkar@rediffmail.com

METHODS

Preparation of alginate microspheres

Emulsification method was used for the preparation of sodium alginate microspheres. Appropriate amounts of sodium alginate were dissolved in milli-Q water (Millipore, Milford, MA, USA) and then different amounts of diclofenac sodium drug were added in the polymer solution. Air bubbles entrapped in solutions are removed by applying bath sonication (Ultrasonic bath sonicator, Metrex Scientific Instruments, New Delhi, India). The solution (2 ml) was then added drop-wise with the help of syringe (23 gauge needle) into the oil phase (30 ml) containing light liquid paraffin (20ml) and heavy liquid paraffin (10ml) premixed with 2% w/v Span-80. Stirring was continued at the time of addition of aqueous phase into the oil phase by an overhead mechanical stirrer (Icon, Deepak Enterprises, New Delhi, India) to form a stable water/oil emulsion system at room temperature (25 ± 2°C). 5ml of aluminium chloride solution in a mixture of methanol and isopropyl alcohol (2:3) was added dropwise to the emulsion and stirred to assure efficient cross-linking. Stirring was continued for up to 3 hours to allow the formation of solid microspheres. The solution was allowed to stand for 24 hours. Then solution was centrifuged at 1500 rpm for 3 mins. The oil was decanted and the collected microspheres were washed twice with petroleum ether (5 ml) and thrice with isopropyl alcohol (5 ml) and finally dried at 40°C temperature in a hot air oven (Icon, Deepak Enterprises, New Delhi, India) for 24 hours, and then stored in dessicator for further studies Different process variable are given in Table 1.

Measurement of Polymer Solution Viscosity

The rheometer (Rheometer-RheolabQC, Anton Paar, Austria), used for the study of viscosity of polymer solutions, was cup and bob type. Polymer solutions of different concentrations (5% w/v, 10% w/v and 20% w/v) were prepared in milli-Q (Millipore, Milford, MA, USA) water and 3 ml of each solution was used for the viscosity study one by one. Viscosities of different concentrations of sodium alginate are reported in table 2 the rheogram is shown in Figure 2. Effect of absence and presence of cross-linker ($AlCl_3$) on viscosity of sodium alginate solution was also studied using rheometer (Table 3 and Figure 3).

Measurement of Size Distribution of Microspheres

The particle size distribution of different formulations were determined by laser differection particle size analyzer (Beckman Coulter Counter, LS 200, US). Suspension of 50 mg microspheres was prepared in 5 ml of water containing 1% w/v Tween-80. Bath sonication (Ultrasonic bath sonicator, Metrex Scientific Instruments, New Delhi, India) was applied to decrease the chances of aggregation of particles in suspension. Then suspension was added into the sample cell with the help of micropipette (Lobalife, Mumbai, India). Stirring was done at 50% rpm with obscuration at 10%. Detector flux more than 10^4. Same procedure was carried out for 3 runs.

Micromeritic study

The microspheres were characterized for their micromeritic properties, such as bulk density, tapped density, compressibility index and flow properties. The tapping method was used to determine the tapped density and percent compressibility index [18] as follows:

Bulk density, Tapped density, Consolidation index, Angle of repose

$$\text{Bulk density} = \frac{\text{mass of microparticles}}{\text{volume of microparticles}}$$

$$\text{Tapped density} = \frac{\text{mass of microparticles}}{\text{volume of microparticles after tapping}}$$

$$\%\ \text{Compressibility index} = (1 - V / V_0) \times 100$$

Here V and V0 are the volumes of the sample after and before the standard tappings, respectively.

Angle of repose q of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method [19].

Photomicrograph Observation of Microspheres

The morphology of alginate microspheres were observed by a binocular microscope (Motic, BA400, China).The alginate microspheres were dispersed in distilled water, and then the one drop of dispersion was dropped on the glass slide and covered it with glass coverslip. Finally, the sample was observed at 10X magnification by microscope and images were taken.

RESULT AND DISCUSSION

Diclofenac sodium is an ideal candidate for incorporation in a controlled release device to diminish its adverse effects after oral administration. Microspheres were prepared successfully by typical emulsification method using sodium alginate as a polymer and $AlCl_3$ as a cross-linking agent. The effect of various process variables was analyzed on the size distribution of alginate microspheres.

Table 1: Preparation of microspheres at different process variable.

Formulation no.	Polymer concentration (% w/v)	Cross-linker concentration (M)	D:P ratio	Stirring rate (rpm)
1.	**5**	2	1:2	1200
2.	**10**	**2**	1:2	1200
3.	**20**	2	1:2	1200
4.	10	**0.5**	1:2	1200
5.	10	2	**0:1**	1200
6.	10	2	**1:2**	1200
7.	10	2	**2:1**	1200
8.	10	2	1:2	**600**
9.	10	2	1:2	**1200**
10.	10	2	1:2	**1800**

Bold numbers are showing the factors which are compared in respect of particle size distribution

Table 2: Viscosity of different concentrations of sodium alginate.

Different concentrations of sodium alginate (% w/v)	Shear Rate [1/s]	Shear Stress [Pa]	Viscosity [cP]
5	105	2.18	20.77
10	105	13.99	133.20
20	105	134.71	1283

Table 3: Viscosity of sodium alginate solution with and without $AlCl_3$.

Different volume of cross-linker (µl)	Shear Rate [1/s]	Shear Stress [Pa]	Viscosity [cP]
0	105	69.45	580.42
20	105	158.27	1900.47
40	105	159.64	1980.69
60	105	162.37	2170.45
120	105	168.79	2218.58
480	105	224.57	4800.82

Table 4: Particle Size, bulk density, tapped density, consolidation index, and angle of repose of sodium alginate microspheres.

Process variables	Micromeritic properties				
	Average Particle Size (µm)	Bulk Density (gm/ml)	Tapped Density (gm/ml)	Consolidation index (%)	Angle of repose(q)
Polymer concentration					
5%w/v	81.76 ± 5.21	0.253 ± 0.11	0.292 ± 0.08	15.4 ± 1.89	19.48 ± 2.16
10%w/v	138.6 ± 4.41	0.365 ± 0.17	0.423 ± 0.12	13.7 ± 2.51	22.45 ± 2.21
20%w/v	185.7 ± 2.47	0.780 ± 0.21	0.877 ± 0.34	11.0 ± 1.78	23.27 ± 1.52
Cross-linker concentration					
0.5M	165.7 ± 3.17	0.318 ± 0.16	0.378 ± 0.11	15.8 ± 2.17	28.72 ± 2.71
2M	125.9 ± 4.78	0.314 ± 0.12	0.404 ± 0.16	22.2 ± 2.42	21.28 ± 2.18
Drug:polymer ratio					
0:1	57.46 ± 4.91	0.281 ± 0.09	0.318 ± 0.16	11.6 ± 1.29	15.64 ± 1.69
1:2	91.76 ± 5.82	0.341 ± 0.16	0.401 ± 0.11	14.9 ± 2.14	17.48 ± 2.87
2:1	223.5 ± 5.71	0.485 ± 0.18	0.583 ± 0.19	16.8 ± 2.16	31.78 ± 2.46
Stirring rate					
600rpm	290.5 ± 6.32	0.602 ± 0.18	0.765 ± 0.13	21.3 ± 2.89	34.89 ± 3.12
1200rpm	138.6 ± 5.81	0.328 ± 0.14	0.422 ± 0.17	22.2 ± 2.12	22.45 ± 2.21
1800rpm	52.16 ± 7.39	0.295 ± 0.12	0.329 ± 0.06	10.3 ± 1.85	15.28 ± 3.18

Effect of different polymer concentrations on viscosity

With increase in the polymer concentration (5% w/v, 10% w/v and 20 %w/v), the density of the solution increases. The increased density of polymer solution increases the resistance to flow. It has been found that as the polymer concentration increases, the viscosity also increases. In our results, increasing concentration of sodium alginate concentration from 5% w/v to 20% w/v showed viscosity from 20.77 cP to 1283 cP (Table 2).

Effect of different concentrations of cross-linker on viscosity

In this study $AlCl_3$ was used as a cross-linker. Aluminium ion (Al^{3+}) which is a trivalent cation combines with a–L-guluronic acid of alginate in a planar three-dimensional manner, producing the “egg-box” structure. It reduces the intermo-

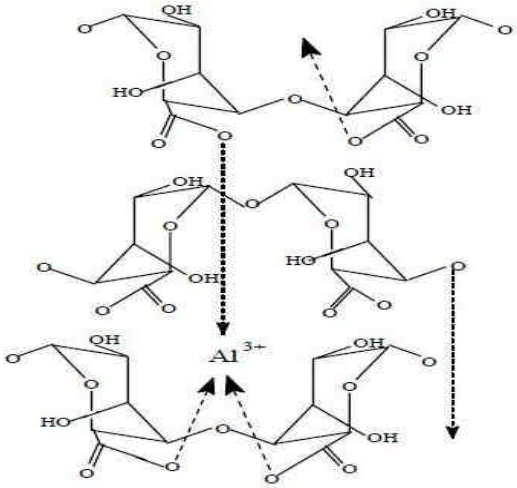

Figure 1. .Expected three-dimensional interaction between Al^{3+} and alginate molecules.

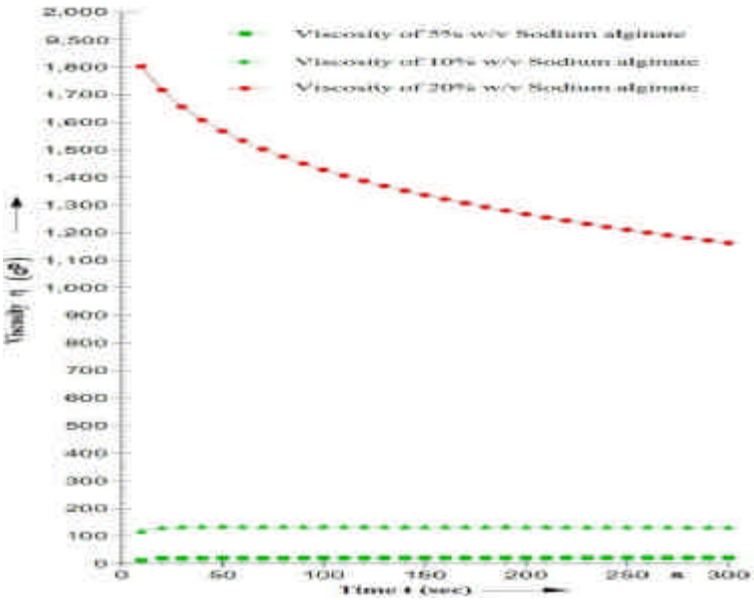

Figure 2. *Rheogram of different concentrations of sodium alginate.*

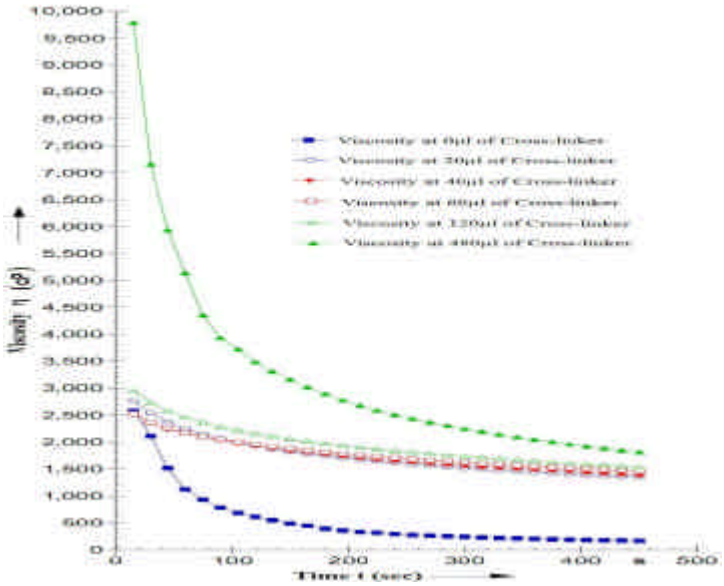

Figure 3. Rheogram of sodium alginate solution with and without $AlCl_3$.

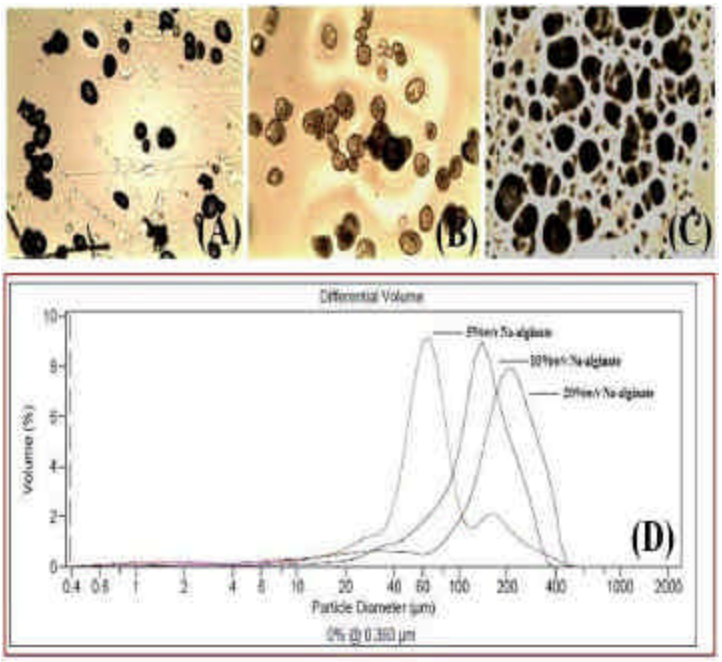

Figure 4. *Photomicrographs of microspheres prepared by using different polymer concentration: (A) 5% w/v, (B) 10% w/v, (C) 20% w/v. (D) Particle size distribution of microspheres prepared by using different polymer concentration.*

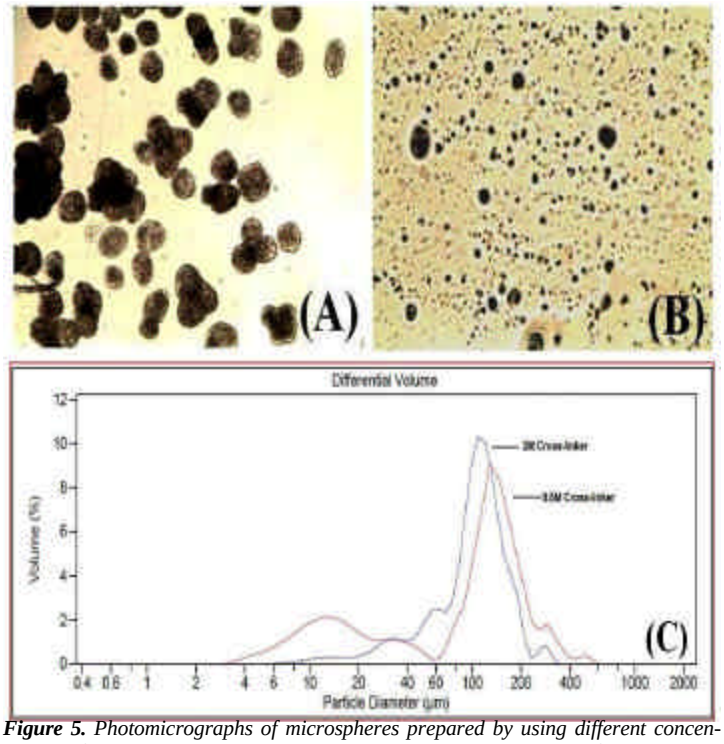

Figure 5. *Photomicrographs of microspheres prepared by using different concentration of $AlCl_3$: (A) 0.5 M, (B) 2 M. (C) Particle size distribution of microspheres prepared by using different concentration of $AlCl_3$.*

lecular space. So the polymer becomes more dense leading to the formation of a better cross-linked gel. As the concentration of cross-linker increases, the density of the polymer solution also increases, thus the viscosity rises (Table 3).

Effect of polymer concentration on particle size

The microspheres obtained by varying the concentration of polymer solution, were slightly irregular with lower polymer concentration which changed to regular spherical shape with higher polymer concentration. The average particle size of

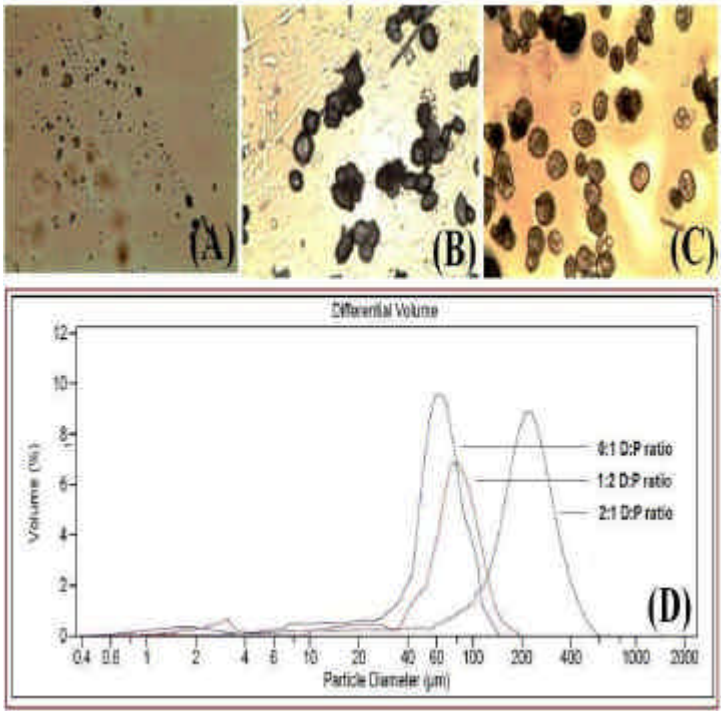

Figure 6. Photomicrographs of microspheres prepared by using different D:P ratio: (A) 0:1, (B) 1:2, (C) 2:1. (D) Particle size distribution of microspheres prepared by using different D:P ratio.

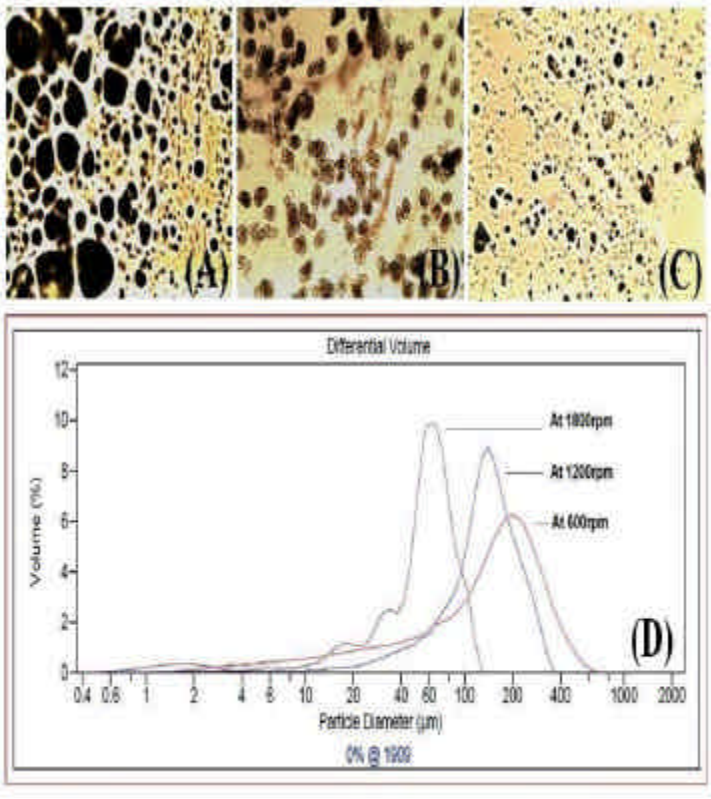

Figure 7. *Photomicrographs of microspheres prepared at different stirring rate: (A) 600 rpm, (B) 1200 rpm, (C) 1800 rpm. (D) Particle size distribution of microspheres prepared at different stirring rate.*

the microspheres obtained increased from 81.76 ± 5.21 µm to 185.7 ± 2.47 µm with increasing sodium alginate concentration. This observation may be attributed to an increase in the viscosity of the dispersed phase, making the coalescence of emulsified dispersed droplets easier.

At the time of addition of aqueous phase into the oil phase, the polymer solution of higher concentration made larger drops as compared to the solutions of lower polymer concentration and this result in bigger microspheres (Table 4 and Figure 4).

Effect of cross-linker concentration on particle size

In the presence of trivalent cations such as aluminium, alginate forms a gel due to the stacking of guluronic acid blocks with the formation of "egg-box" aluminium-linked junctions. It was seen that with increase in concentration of cross-linker solution particle size decreases (Figure 6), it may be due to the formation of tight cross-linked network. It was in agreement with the results observed by rheology study of the viscosity of alginate solution in presence of different concentrations of $AlCl_3$. Here it was found that the cross-linker 0.5 M and 2 M gave average particle size of 165.7 ± 3.17 µm and 125.9 ± 4.78 µm, respectively.

Effect of D:P ratio on particle size

Microspheres were prepared using different drug-polymer ratios (0:1, 1:2, 2:1). The drug-polymer ratio was varied by changing the amounts of polymer and the drug. The size of placebo microspheres (0:1 D:P ratio) was smaller than the other two type of formulation batches because the solution of polymer without drug was less viscous than the other two. The 1:2 D:P solution was more viscous than 0:1 D:P ratio but less viscous than 2:1 D:P ratio. Therefore the particle size of this batch (1:2 D:P ratio) was higher than the batch of 0:1 D:P ratio and smaller than the batch of 2:1 D:P ratio. The particle size of microspheres prepared from 2:1 D:P ratio was higher. It might be due to the more viscous solution of the 2:1 D:P ratio and also the excess amount of drug embedded on the surface of microspheres. In this work the average particle size of sodium alginate microspheres for drug-polymer ratios 0:1, 1:2, and 2:1 was reported in Table 4 and showed in Figure 6.

Effect of stirring rate on particle size

Stirring rate influenced particle size distribution of microspheres. By increasing the stirring speed from 600 to 1800 rpm, the particle size of microspheres decreased (Figure 7). The average particle size of microspheres prepared using various stirring rate (*i.e.,* 600, 1200, and 1800 rpm) were showed in Table 4. The higher stirring rate of 1800 rpm causes high turbulence, frothing and adhesion to the container wall that resulted in irregularly shaped microspheres. The desired spherical and not aggregated microspheres were obtained at stirring rate of 1200rpm. Any increase in average particle size at lower stirring rate as 600 rpm can be attributed to increased tendency of globules to coalescence and aggregates. This was expected because high stirring rates provide the shearing force needed to separate the oil phase into smaller droplets. It was shown that microspheres prepared in this study at stirring rates of 600 rpm and 1200 rpm were spherical with smooth surfaces. However, increasing the stirring rate to 1800 rpm caused microspheres to become slightly irregular.

Micromeritic study

Alongwith other parameters, the micromeritic properties of the sodium alginate microspheres were also investigated. The values of bulk density, tapped density, consolidation index, and % yield of all the batches are reported in Table 4. It was found that the bulk density, and tapped density of microspheres prepared by increasing polymer concentration, increases because the size of microspheres increases and therefore size of void rises. The consolidation index values were all less than 18, suggesting good flowability of microspheres.

With the process variable cross-linker concentration, the microspheres having 0.5 M cross-linker had low value of bulk density and tapped density and the microspheres having 2 M cross-linker had high value of bulk density and tapped density. The consolidation index was found 15.8% which is less than 18 indicating good flowability and 22.2% which is greater than 18 showing passable flowability, respectively.

The process variable D:P ratio 0:1, 1:2, and 2:1 gave the microspheres of bulk density and tapped density in increasing order. The consolidation index was found 11.6%, 14.9% and 16.8%, respectively. The consolidation index values were all less than 18, indicating good flowability of microspheres. Also the process variable stirring rate resulted in microspheres with bulk density and tapped density in decreasing order. Consolidation index for them was found to be 21.3%, 22.2% and 10.3%, respectively. The microspheres obtained from stirring rate 600 rpm and 1200 rpm have consolidation index 21.3%, and 22.2% which are more than 18,

suggesting good flow property of microspheres, and the microspheres obtained from stirring rate 1800 rpm have 10.3% consolidation index which is less than 18, showing excellent flowability of microspheres.

CONCLUSIONS

In this study alginate microspheres were prepared by emulsification method. The significant effect of process variables such as polymer concentration, cross-linker concentration, D:P ratio, and stirring rate have been seen on the size of microspheres. The size of microspheres increases with the increase in polymer concentration and D:P ratio. While it decreases with the increase in cross-linker concentration and stirring rate. The desired size of microspheres can be obtained by using the process variables studied. Viscosity of polymer solution increases with the increase in polymer concentration and decreases with the increase in cross-linker concentration.

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