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Original Research Paper

COMPARATIVE EVALUATION OF ALGINATE BEADS PREPARED BY IONOTROPIC GELATION TECHNIQUE

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ABSTRACT

The present study involves preparation of alginate beads by microencapsulation and evaluation of various parameters like average size, swelling, mechanical stability and degradation of the beads. In the present work microcapsules were prepared by using Ionotropic gelation technique where gelation of anionic polysaccharide sodium alginate, the primary polymer of natural origin, was achieved with oppositely charged calcium ions, acting as counter ion, to form instantaneous micro particles**.** Sodium alginate was chosen as the material for preparation of the carrier matrix because it is a natural, biodegradable, biocompatible, non toxic orally and hydrophilic polymer suitable for the entrapment of water soluble drugs. In this study different sizes of needles (26 G, 24 G, 23 G, 22 G, 21 G, 20 G, and 18 G) and two different concentrations of sodium alginate (0.5 M and 1 M) were used for the preparation of empty alginate beads. From this, the needle having maximum accommodation (18 G) and lower concentration of sodium alginate (0.5 M) has been selected. The criterion behind this selection is degradation and swelling of the beads. The obtained results were encouraging. More the concentration of sodium alginate more is the stability of the bead. The degradation and swelling are less. This result can be used further for the development of *in vivo* procedures for determining the immunoenhancer activity of monocytes as well as vincristine sulphate.

Keywords: Microencapsulation, Ionotropic gelation technique, Alginate beads, Swelling, Mechanical stability, Degradation.

INTRODUCTION

The aim of cell therapy is to replace, repair, or enhance the function of damaged tissues and organs. Several factors complicate the development of cellular therapies. The primary importance is protection of the implanted cells from the host's immune system.[1] The limitations of the current treatment protocols are long – term failure and the high (partially unrealistic) costs. Immuno isolated transplantation (i.e., encapsulated – cell therapy) is one of the most promising approaches to overcome the limitations of the current treatment protocols. To avoid life – time immunosuppressant therapy while excluding an immune response in the host, the transplants must be enclosed in immunoprotective capsules or devices. Studies with macro capsules (e.g. hollow fibres, diffusion chambers) made up of different materials have shown a number of drawbacks that stand in the way of their clinical use. Apart from surgery and retrieval problems, non- specific fibrotic overgrowth, necrosis of the encapsulated cells due to unfavorable (disk and tube) geometries, and thus diffusion limitations, breakage and the problems resulted in the early failure of the grafts. In contrast, microcapsules that are produced from hydro – gels offer potential solutions to these problems. Over the past two decades, a number of microcapsules made up of different hydro gels (e.g., alginate, agar, agarose, gellan, gum, chitosan, synthetic polymers) have been developed and tested. This research has shown the feasibility of alginate-based microcapsules for transplantation of laboratory cell lines as well as of allo – and xenogeneic tissue.[2]

In the present work microcapsules were prepared by using ionotropic gelation technique where gelation of anionic polysaccharide sodium alginate, the primary polymer of natural origin, was achieved with oppositely charged calcium ions, acting as counter ion, to form instantaneous micro particles**.** Various parameters like swelling, mechanical stability of the beads and their degradation will be studied.[3]

MICROENCAPSULATION[4,5,6]

Microencapsulation is described as a process of enclosing micron-sized particles of solids or droplets of liquids or gases in an inert shell, which in turn isolates and protects them from the external environment. This capsule must be a physical barrier that prevents the entrance of lymphocytes and other cells of the immune system into the capsule, however, it must be permeable to low molecular weight substances such as nutrients, electrolytes, oxygen and glucose. Microencapsulation is shown in Figure 1and Ionotropic gelation technique is shown in Figure 2.

Source of Alginate

Alginates are produced by brown seaweeds (*Phaeophyceae*, mainly *Laminaria*). Commercial varieties of alginate are extracted from seaweed, including the giant Kelp *Macrocystis pyrifera*, *Ascophyllum nodosum*, and various types of *Laminaria* like *Laminaria hyperborean*.

Structure of Alginate

Alginate polymers are a family of linear unbranched polysaccharides which contain varying amounts 1, 4 – linked β – D – Mannuronic acid (M – residue) and 1, and 4-linked α – L – guluronic acid residues (G – residue). These monomers are connected in blocks of M homopolymers (M-M-M), G homopolymers (G-G-G) and MG heteropolymers, which can be alternated (M-G-M-G) or not. Structural units of alginate are seen in Figure 3.

Molecular Structure

Alginates are not random copolymers but, according to the source *algae*, consist of blocks of similar and strictly alternating residues (that is, MMMMMM, GGGGGG and GMGMGMGM), each of which have different conformational preferences and behavior. As examples, the **M**/**G** ratio of alginate from *Macrocystis pyrifera* is about 1.6 whereas that from *Laminaria hyperborea* is about 0.45. Alginates may be prepared with a wide range of average molecular weights (50 - 100000 residues) to suit the application. Molecular structure of alginate is seen in Figure 4.

Reasons for Selecting Sodium Alginate

Sodium alginate was chosen as the material for preparation of the carrier matrix because it is a natural, biodegradable, biocompatible, non toxic orally and hydrophilic polymer suitable for the entrapment of water soluble drugs. Another advantageous property is their inability to reswell in acidic environment whereas they easily reswell in alkaline environment. So acid-sensitive drugs incorporated into the beads would be protected from gastric juice. Therefore, alginate is used as entrapment matrix for cells and enzymes.[7,8]

MATERIALS AND METHODS

Chemicals

Sodium alginate (SD Fine Chemical LTD),Calcium chloride (SD Fine Chemical LTD) ,Sodium chloride (Himedia), Disodium Hydrogen Phosphate (Himedia), Potassium Hydrogen Phosphate (Himedia), Double distilled water, Milli Q water (Millipore),0.05M Phosphate Buffer Saline (pH-5.5) (Himedia).

Equipments Used

pH meter (Eutech), Magnetic Stirrer (Remi equipments Pvt. LTD), Syringe and glass apparatus (Borosil), Needles (18 G, 20 G, 21 G, 22 G, 23 G, 24 G, 26 G),Weighing Balance (Denver), Centrifuge (Remi instrument Pvt. LTD), Laminar air flow unit (Scientech), Incubator (Scientech), Micropipettes (Tarson), Test Tubes (Borosil) and Petri dishes (Riviera).

Preparation of Empty Alginate Beads

Method of Preparation

The beads were prepared by *ionotropic gelation technique*. The sodium alginate (0.5 M) dispersion was extruded drop wise into 100 ml of calcium chloride solution using 5 ml hypodermic syringe with 18 gauge needle and stirred at 100 rpm for 15 minutes. The beads were then separated by filtration. The separated alginate beads were washed with distilled water and dried in a filter paper. The beads of different sizes were prepared using different size needles (18 G, 20 G, 21 G, 22 G, 23 G, 24 G and 26 G) and their diameters were compared (It needs special mention that as the size of needle increases, the diameter of the needle decreases).The alginate beads prepared with different sizes of needles were compared. In the same way beads are prepared using another concentration of sodium alginate (1M) using different size needles.

Measurement Size of Empty Alginate Beads

The bead was spread over a flat surface using a spatula. The diameter was then measured using a calibrated scale.

Swelling Test of Empty Alginate Beads

A Petri plate was taken and filled with Phosphate Buffer (pH 7.4). Its weight was measured using a digital balance. About 10 beads were taken and their weight was measured using a digital balance. The beads were then placed in Petri dish containing phosphate buffer. Swelling rate was determined by measuring the weight

periodically for 100 minutes. Beads with different sizes were also studied in the same manner and their swelling was compared.

Degradation Study of Empty Alginate Beads

A Petri plate was taken and filled with Phosphate buffer (pH 7.4) and its weight was measured using a digital balance. About 10 beads were taken and their weight was measured using a digital balance. The beads were then placed in Petri dish containing Phosphate buffer (pH 7.4). The beads were then periodically weighed for 14 days. Beads of different sizes were also studied in the same manner and their degradation was compared.[9]

Mechanical Stability Test of Empty Alginate Beads

About 300 beads were taken in a Petri dish. They were subjected to shearing force at 133 rpm using a magnetic needle and magnetic stirrer for 1 hour. The number of beads that were damaged was counted after 1 hour.[10] Percentage stability of beads was calculated by using the following formula:

$$\% \text{ stability} = 100 - \% \text{ Damaged}$$

$$\% \text{ Damaged} = \frac{\text{Number of beads damaged}}{\text{Total number of beads taken}}$$

Beads with different sizes were also studied in the same manner and their mechanical stabilities were compared.

RESULTS AND DISCUSSION

Preparation of Empty Alginate Beads

Empty alginate beads were prepared using different sizes of needles namely 18 G, 20 G, 21 G, 22 G, 23 G, 24 G and 26 G. Two concentrations of sodium alginate (0.5 M and 1.0 M) were used for preparation of beads along with 2.5 M Calcium chloride.

Measurement of Size of Empty Alginate Beads

Sizes of 100 beads that were prepared using different sizes of needles were measured. Averages were calculated using the following formula:

$$\text{Average Diameter of one bead} = \frac{\text{Total diameter of all beads}}{\text{Total number of beads taken}}$$

The sizes of beads are tabulated in Tables 1 to 4.Comparision of average sizes of beads prepared by using different needles is done graphically and shown in Figures 5 and 6.

From Figure 5, for 0.5 M sodium alginate the linearity of the line with 18 G size needle was not in expected lines. This might be attributed to the fact that though there is an increase in droplet size, other factors like gelling capacity, concentration of bath solution would have limited increase in bead size. From the Figure 6, for 1 M sodium alginate the linearity of the line with 18 G size needle was not in expected lines. This might be attributed to the fact that though there is an increase in droplet size, other factors like gelling

capacity, concentration of bath solution would have limited increase in bead size.

Swelling Test for Empty Alginate Beads

Swelling test was performed by using phosphate buffer of pH 7.4 for 100 minutes. The results are as shown in Tables 5 and 6. Comparison of Swelling of beads prepared by using different sizes needles is done graphically and shown in Figures 7 and 8.

From Figures 7 and 8, for both the concentrations swelling of beads is increased with time. Size 18 G needles have highest swelling and 26 size needles have the least. This indicates that the beads absorb the PBS (pH 7.4) and increase in weight. The rate of swelling with second concentration was found to be lower when compared to the swelling rate obtained with that of first concentration.

Degradation Test of Empty Alginate Beads

Degradation test was performed by taking 10 beads in phosphate buffer (pH 7.4) and its weight was noticed for 14 days. The results are as shown in Tables 7 and 8. Comparison of Degradation of beads prepared by using different sizes needles is done graphically and shown in Figures 9 and 10.

From the Figures 9 and 10, the least increase in weight was with 26 G needle and highest was with 18 G. When we observe the beads prepared with 18 G needle to 26 G needles there is good increase in weight. This shows that upon degradation of beads, particulate matter is broken down and surface area is also increased. Due to increase in surface area, there is increase in swelling of bead in PBS. This was observed by the particulate matter seen in media. By the end of 14 days, there is complete degradation of bead. But the degradation with second concentration was found to be lower than that of first concentration.

Mechanical Stability Test of Empty Alginate Beads

About 300 beads were subjected to shearing force by using a magnetic agitator for 60 minutes and the number of damaged beads was counted. The percentage of damaged beads was calculated using the formula as given earlier. The results are as shown in Tables 9 and 10. Comparison of Mechanical stability of beads prepared by using different sizes needles is done graphically and shown in Figures 11 and 12.

From the Figures 11 and 12, Mechanical stability is an indication of strength of the bead. The test was done to analyze the stress induced damage to the beads. From the above results it is evident that the stability of beads is high, i.e., 87 -89 %. This indicates that beads are quite strong and can withstand stress. The highest stability was with 18 G and 20 G needles. The mechanical stability was found be to be almost similar for both the concentrations.

CONCLUSION

In this study different sizes of needles (26 G, 24 G, 23 G, 22 G, 21 G, 20 G, and 18 G) and two different concentrations of sodium alginate (0.5 M and 1 M) were used for the preparation of empty alginate beads. From these, the needle having maximum accommodation (18G) and lower concentration of sodium alginate (0.5 M) has been selected. The criterion behind this selection is degradation and swelling of the beads. The obtained results were encouraging. More the concentration of sodium alginate more is the stability of the bead. The degradation and swelling are less. This result can be used further for the development of *in vivo* procedures for determining the immune enhancer activity of Monocytes as well as Vincristine sulphate.

Table 1: Size of the beads prepared by using 18 G needles: (0.5 M Sodium Alginate and 0.25 M Calcium chloride)

S.No.	**Size of the beads (mm)**									
1-10	3.9	4.0	3.9	4.1	3.9	4.0	3.9	3.9	3.9	4.0
11-20	4.2	3.9	3.9	4.1	3.9	4.0	3.9	4.2	4.0	3.9
21-30	4.0	3.9	4.0	3.9	4.1	4.0	4.2	4.2	3.9	4.0
31-40	4.2	3.9	4.1	4.0	3.9	4.2	4.0	3.9	4.2	3.9
41-50	3.9	4.0	3.9	4.2	3.9	3.9	3.9	4.1	3.9	4.0
51-60	3.9	3.9	4.0	4.1	3.9	3.9	4.1	3.9	4.0	3.9
61-70	3.9	4.2	4.0	4.1	4.0	3.9	4.2	4.0	4.1	3.9
71-80	4.0	4.0	4.0	3.9	4.2	3.9	4.0	3.9	4.0	3.9
81-90	3.9	4.1	4.0	3.9	4.0	4.2	4.0	3.9	4.2	3.9
91-100	4.0	3.9	4.1	3.9	3.9	4.0	4.0	3.9	4.2	3.9

Table 2: Average bead size of beads obtained with different size of needles (First concentration)

Needle size (Gauge)	26	24	23	22	21	20	18
Average bead size (mm) n=100	2.22	2.95	3.04	3.35	3.62	3.97	3.99

Table 3: Size of the beads prepared by using 18 G needles: (1.0 M Sodium alginate and 0.25 M Calcium chloride)

S.No.	Size of the beads (mm)									
1-10	3.8	3.9	3.8	4.0	3.8	3.9	3.8	3.8	3.8	3.9
11-20	4.1	3.8	3.8	4.0	3.8	3.9	3.8	4.1	3.9	3.8
21-30	3.9	3.8	3.9	3.8	4.0	3.9	4.1	4.1	3.8	3.9
31-40	4.1	3.8	4.0	3.9	3.8	4.1	3.9	3.8	3.9	3.8
41-50	3.8	3.9	3.8	4.1	3.8	3.8	3.8	4.0	3.8	3.9
51-60	3.8	3.8	4.1	3.9	3.8	4.0	3.8	3.8	3.9	3.8
61-70	3.8	4.1	3.9	4.0	3.9	3.8	4.1	3.9	4.0	3.8
71-80	3.9	3.9	3.9	3.8	4.1	3.8	3.9	3.8	3.9	3.8
81-90	3.8	4.0	3.9	3.8	3.8	4.1	3.9	3.8	4.1	3.8
91-100	3.9	3.8	4.0	3.8	3.9	3.9	3.8	4.1	3.8	3.9

Table 4: Average bead size of beads obtained with different size of needles (second concentration)

Needle size (Gauge)	26	24	23	22	21	20	18
Average bead size(mm) n=100	2.12	2.84	2.94	3.24	3.52	3.85	3.87

Table 5: Swelling test for empty Alginate beads (First concentration)

Needle size	Weight of the bead (gm) AT							
	0 Min	10 Min	25 Min	40 Min	55 Min	70 Min	85 Min	100 Min
18	4.26	15.56	31.11	46.67	64.44	80.00	115.56	142.22
20	4.20	12.96	27.78	40.74	55.56	68.52	94.44	114.81
21	3.98	12.90	27.42	38.71	53.23	70.97	82.21	98.39
22	3.88	11.43	22.82	34.29	48.59	60.00	72.86	84.29
23	3.97	11.01	21.56	33.82	46.32	58.66	69.32	79.41
24	3.83	9.54	18.02	30.96	43.87	53.43	65.78	75.32
26	3.75	7.11	16.36	28.87	41.79	50.26	61.66	72.11

Table 6: Swelling test for empty Alginate beads (Second concentration)

Needle size	Weight of the bead (gm) AT							
	0 Min	10 Min	25 Min	40 Min	55 Min	70 Min	85 Min	100 Min
18	4.12	14.65	30.09	45.3	63.69	79.00	112.65	138.2
20	4.03	12.85	26.58	39.82	54.36	77.25	92.43	110.81
21	3.78	11.90	25.72	37.61	51.93	68.96	80.98	95.93
22	3.68	11.23	23.68	33.19	47.66	65.32	69.88	80.66
23	3.54	10.86	20.96	32.46	45.32	56.36	67.43	77.14
24	3.38	9.32	18.54	29.98	43.78	52.13	64.98	72.67
26	3.25	7.01	15.48	27.42	40.97	49.32	60.76	70.79

Table 7: Degradation test for empty Alginate beads (First concentration)

Day	Weight (gm) according to needle size (Gauze)						
	18	20	21	22	23	24	26
1	4.10	3.99	3.89	3.83	3.80	3.72	3.70
2	4.36	3.97	3.96	3.85	4.29	4.06	3.81
3	4.50	3.98	4.03	3.87	4.49	4.21	3.89
4	4.66	4.11	4.16	3.91	4.51	4.38	4.10
5	4.88	4.54	4.35	4.05	4.59	4.51	4.15
6	5.09	4.96	4.56	4.19	4.64	4.62	4.22
7	5.23	5.11	4.80	4.40	4.71	4.65	4.29
8	5.36	5.55	5.10	4.82	4.98	4.78	4.32
9	5.54	5.90	5.33	5.03	5.05	4.79	4.39
10	5.79	6.14	5.42	5.25	5.22	4.82	4.44
11	6.28	6.32	5.94	5.66	5.36	5.05	4.49
12	6.54	6.53	6.02	5.98	5.43	5.33	4.54
13	6.92	6.65	6.36	6.05	5.96	5.43	4.62
14	7.30	6.99	6.79	6.54	6.32	5.98	4.76
Increase in weight(gm)	7.30-4.10 =3.20	6.99-3.99 =3.00	6.79-3.89 =2.9	6.54-3.83 =2.71	6.32-3.80 =2.52	5.98-3.72 =2.2	4.76-3.70 = 1.06
% increase in weight	78.14%	75.18%	74.55%	70.75%	66%	59%	28.6%

Table 8: Degradation test for empty Alginate beads (Second concentration**)**

Day	Weight (gm) according to needle size (Gauze)						
	18	20	21	22	23	24	26
1	3.96	3.89	3.83	3.75	3.61	3.56	3.50
2	3.70	3.95	3.97	3.95	3.75	3.61	3.62
3	3.85	4.05	4.08	4.02	3.86	3.71	3.75
4	3.98	4.19	4.23	4.15	3.91	3.95	3.89
5	4.26	4.39	4.37	4.27	4.03	4.11	3.97
6	4.45	4.65	4.56	4.54	4.18	4.20	4.05
7	4.59	4.80	4.75	4.70	4.32	4.30	4.16
8	4.78	5.19	4.98	4.97	4.49	4.44	4.25
9	4.99	5.29	5.16	5.05	4.66	4.59	4.39
10	5.32	5.36	5.42	5.16	4.92	4.68	4,51
11	5.64	5.66	5.66	5.32	5.01	4.86	4.67
12	5.98	5.89	5.72	5.49	5.25	4.97	4.79
13	6.36	5.99	5.88	5.59	5.32	5.09	4.88
14	6.67	6.03	5.97	5.66	5.40	5.26	4.95
Increase in weight (gm)	6.67-3.96 =2.71	6.03-3.89 =2.14	5.97-3.83 =2.14	5.66-3.75 =1.91	5.40-3.61 =1.79	5.26-3.56 =1.70	4.95-3.50 =1.45
% Increase in weight	68.43%	55.01 %	55.87%	50.93%	49.58%	47.75%	41.42%

Table 9: Mechanical stability of empty Alginate beads (First concentration)

Size of the needle (Gauge)	Number of damaged beads	% of damaged beads	% Stability
18	32	10.66 %	89.33%
20	32	10.66 %	89.33%
21	33	11%	89%
22	33	11%	89%
23	37	12.33%	87.67%
24	37	12.33%	87.67%
26	36	12	88

Table 10: Mechanical stability of empty alginate beads (Second concentration)

Size of the needle (Gauge)	Number of damaged beads	% of damaged beads	% Stability
18	32	10.66 %	89.33%
20	32	10.66 %	89.33%
21	33	11%	89%
22	33	11%	89%
23	36	12%	88%
24	36	12%	88%
26	37	12.33%	87.67%

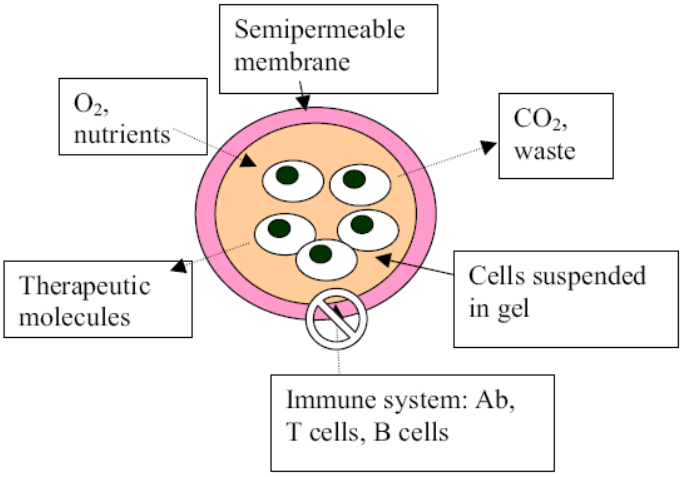

Figure 1**:** Micro Encapsulation

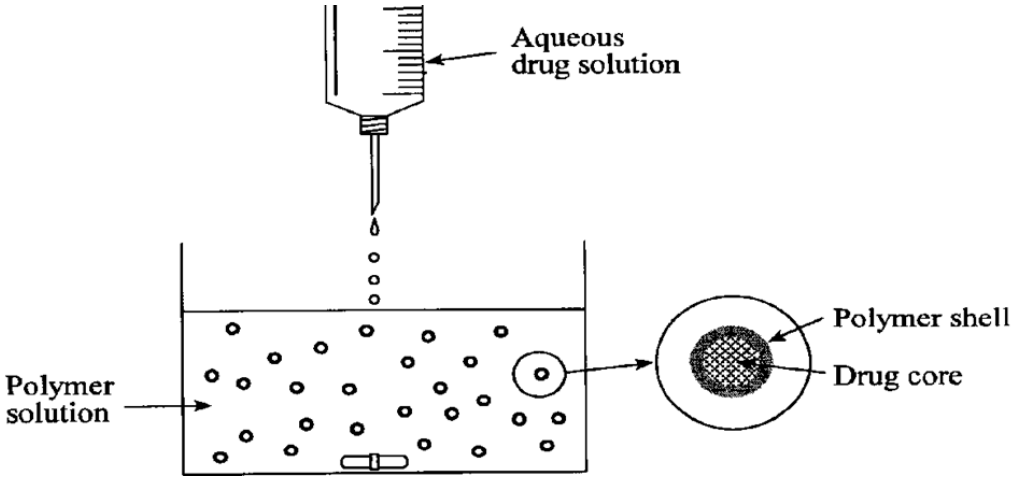

Figure 2: Ionotropic gelation technique

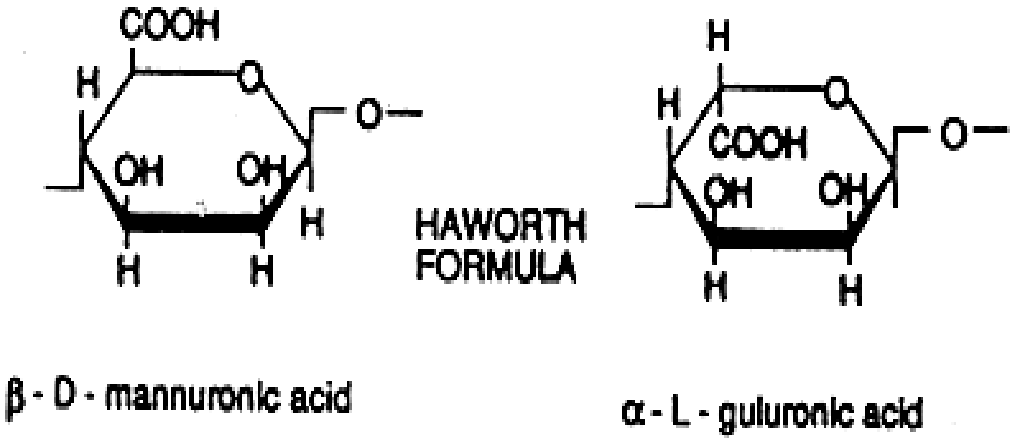

Figure 3: Structural units of alginate

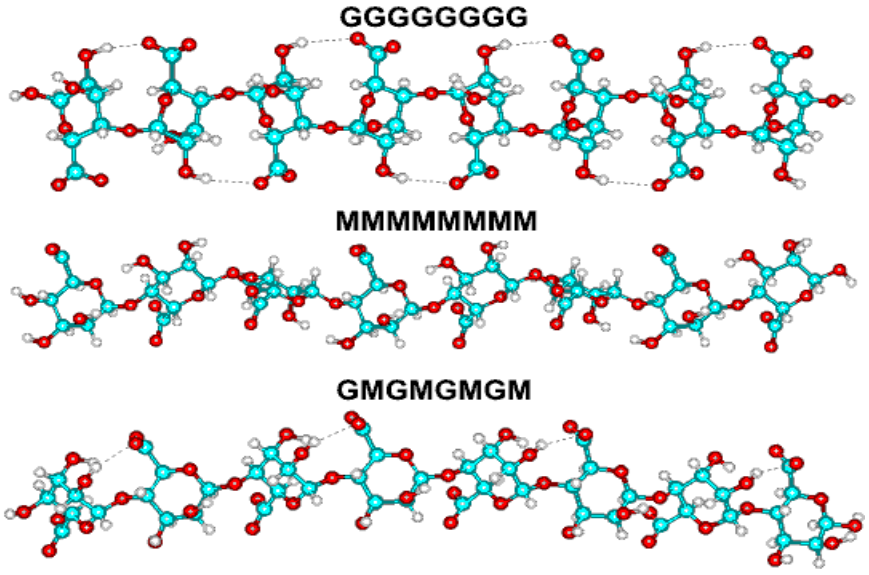

Figure 4: Molecular structure of alginate

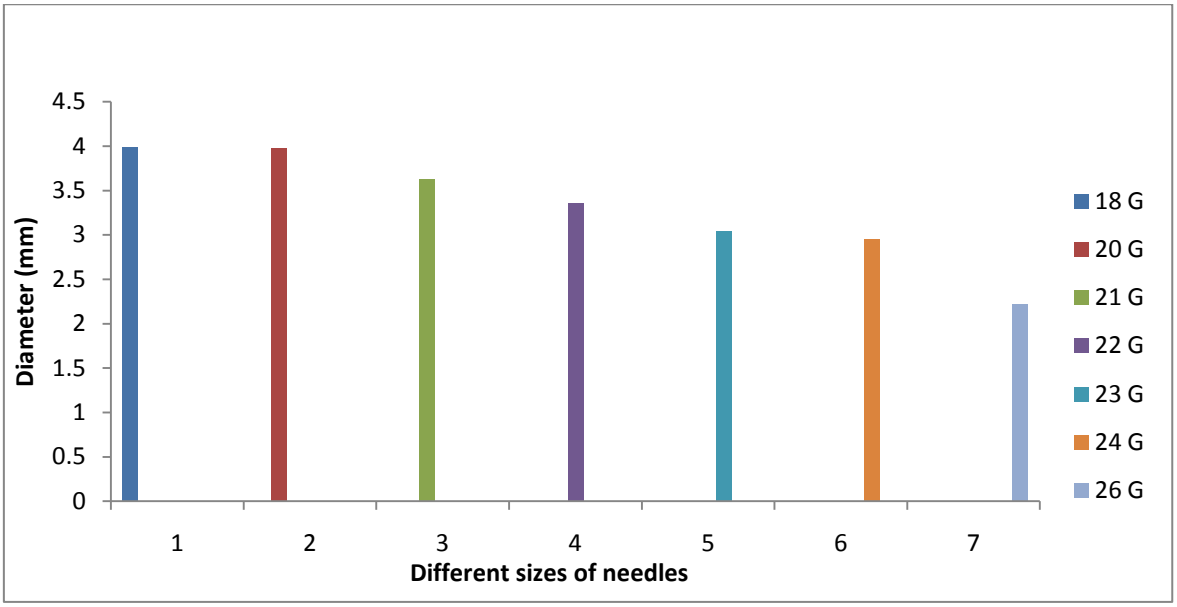

Figure 5: Average bead size (First concentration)

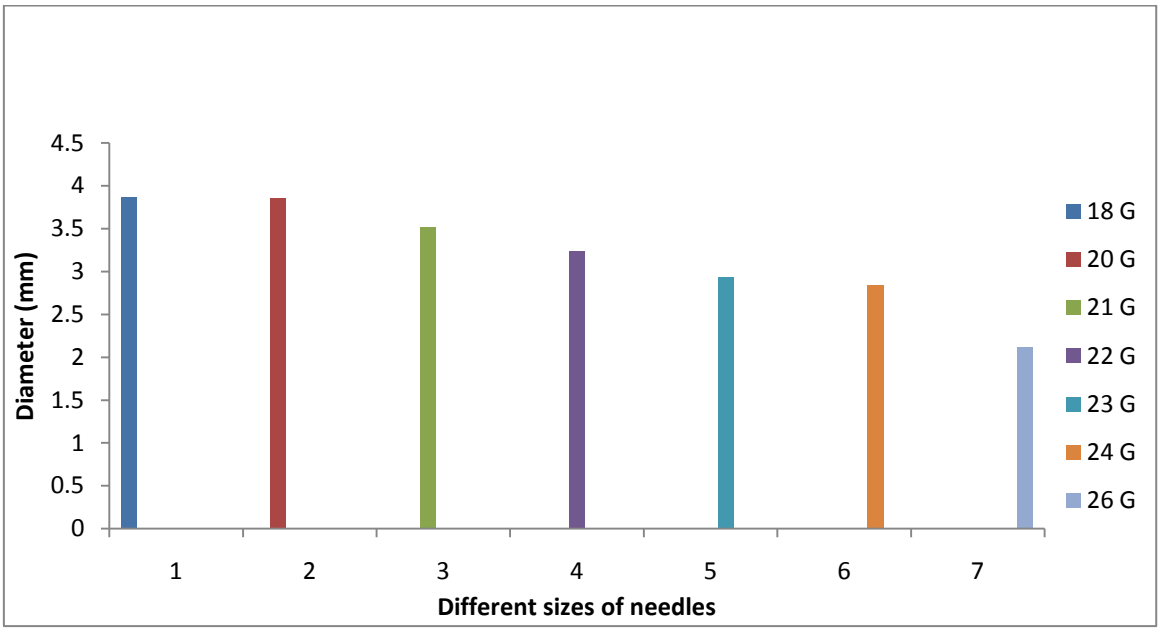

Figure 6: Average bead sizes (Second concentration)

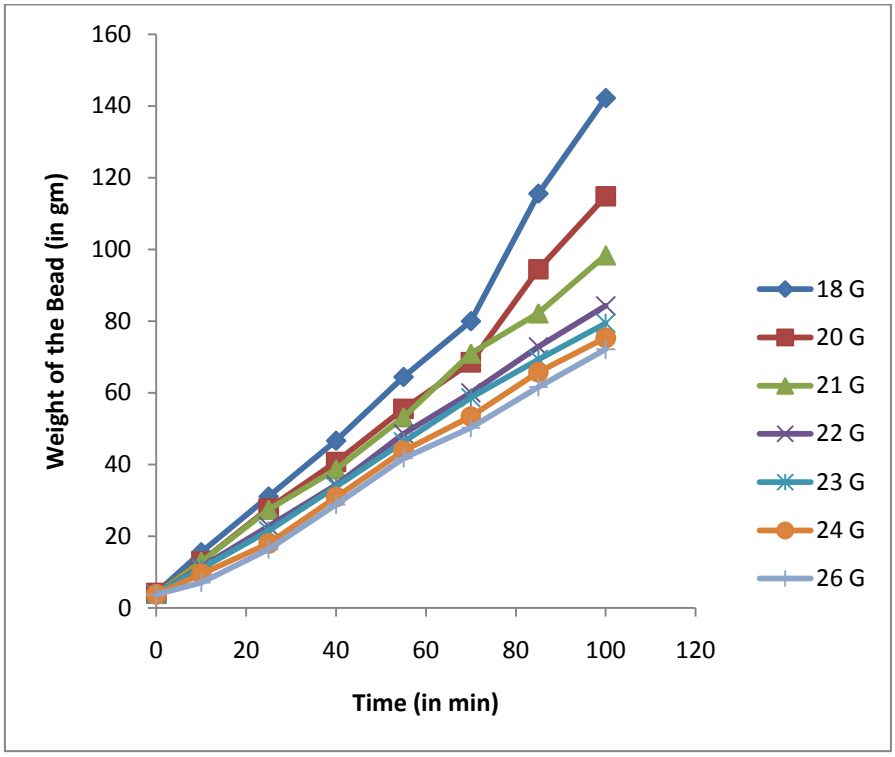

Figure 7: Swelling test (First concentration)

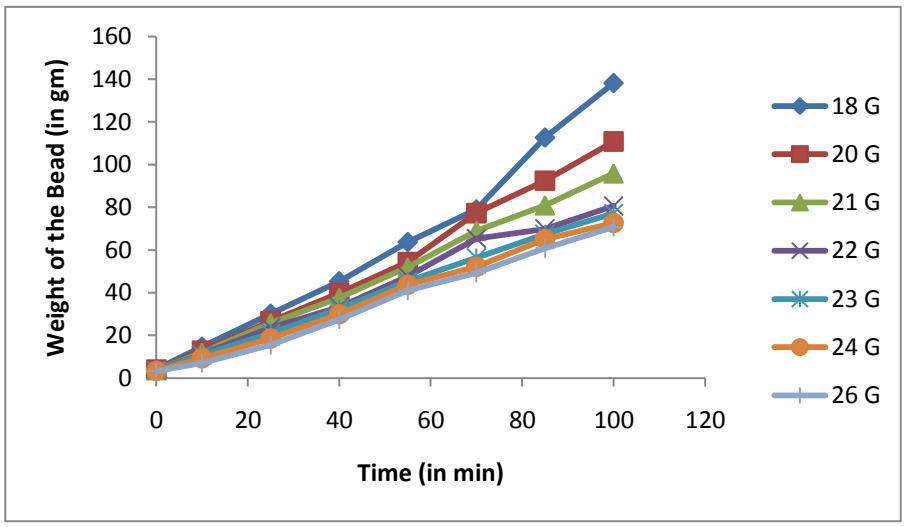

Figure 8: Swelling test (Second concentration)

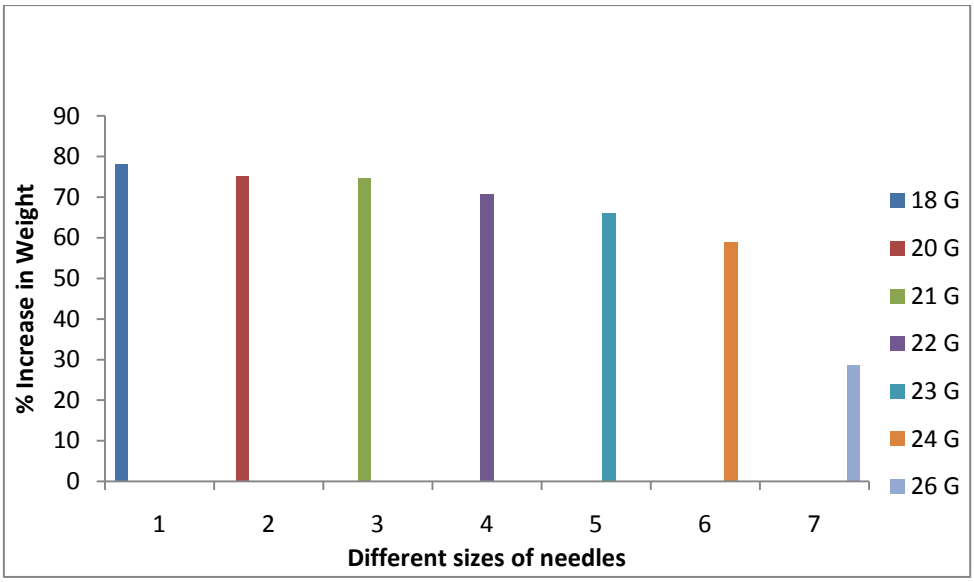

Figure 9: Degradation test (First concentration)

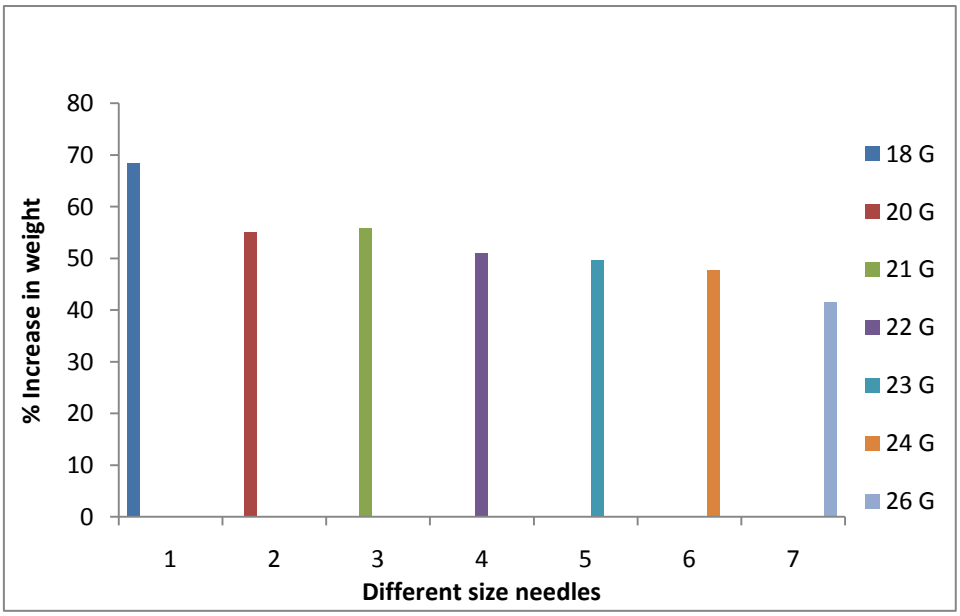

Figure 10: Degradation test (Second concentration)

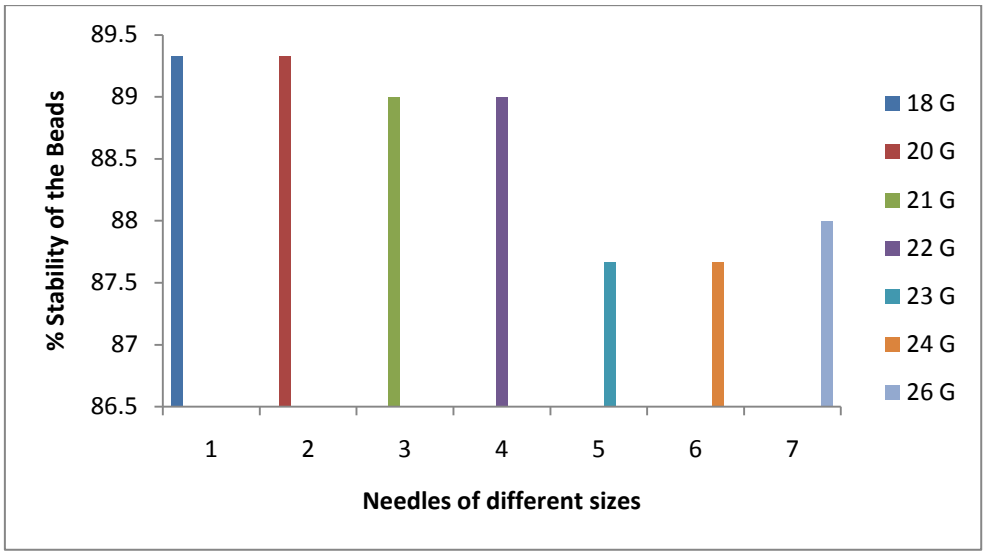

Figure 11: Mechanical stability test (First concentration)

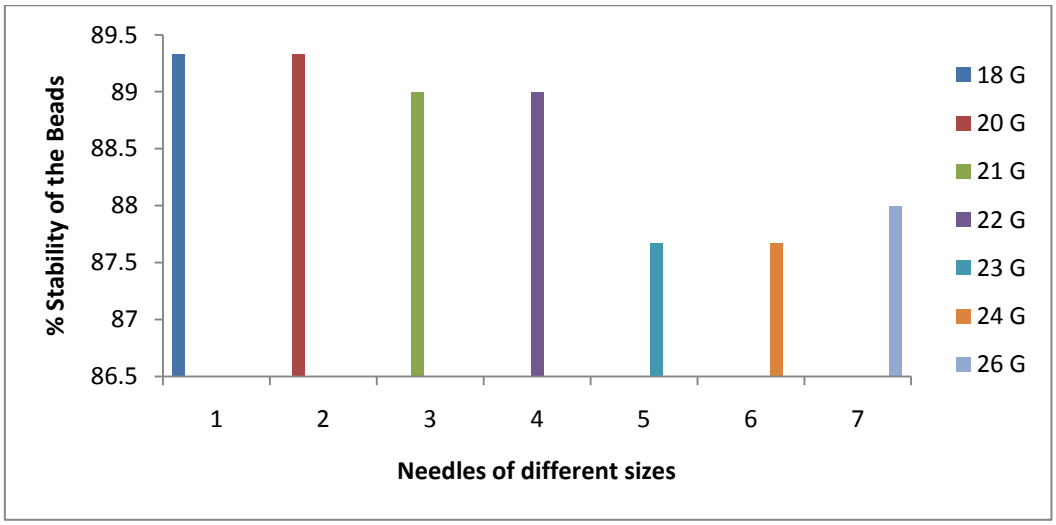

Figure 12: Mechanical stability test (Second concentration)

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