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An investigation into the effects of pH and material concentration on the morphology of Chitosan-Alginate microspheres prepared using an Ionic gelation techniques

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Abstract

The aim of this study was to develop microspheres as a drug delivery system using natural polymeric materials including Alginate and Chitosan using an Ionotropic Gelation method of synthesis. In this study, several parameters which affect the functionality of the microspheres were examined. They include; the concentrations of Chitosan, Sodium Alginate, Calcium Chloride, and the pH value of the gelation medium. The effect of these parameters on the product were determined by observing the morphology of microspheres using light microscopy and Scanning Electron Microscopy (SEM). Overall, the Chitosan-Alginate microspheres were successfully prepared with using a Chitosan concentration at 0.25% w/v, Sodium Alginate and Calcium Chloride concentration of between 4% to 6% w/v.

Keywords: *Chitosan, Alginate, Microsphere, Ionic gelation method.*

1. Introduction

Most of the conventional drugs available have been associated with numerous issues such as: degradation by body biological factor, hepatic first pass effect, side effects, compliance issues etc. One of the novel approaches offered by the pharmaceutical industry is to use microspheres as drug carriers. Microspheres are spherical shaped particles with a size of less than 1000 μm that can be synthesized from either natural or synthetic polymers (Kadam & Suvarna, 2015). Microspheres have been widely used as a drug delivery system for ophthalmic, gene, nasal, buccal, gastrointestinal, vaginal, and anti-cancer drug. Some of the advantage offered by microspheres are:

i. Protected from various kinds of enzymes and metabolism.
ii. Surpass the hepatic first pass metabolism.
iii. Reduce unwanted side effects.
iv. Smaller dosage.
v. Improve drug bioavailability.
vi. Prolongs therapeutic effect of the drug.
vii. Improve patient compliance.
viii. Can be designed as a specific target site drug.

As presented in Figure 1, Chitosan is a structural polysaccharide mainly found in shrimp and other crustacean shells and has been widely used in the formulation of microspheres. It has some unique features such as mucosadhesion, non-toxic, biodegradable and displays certain anti-microbial properties (Arora & Budhiraja, 2012). Chitosan is a weak base (pKa value ~ 6.5) which dissolves and produces a high degree protonated amine when exposed to aqueous acid (Rora, Upta, Arang, & Udhiraja, 2011). As a result of this, it is a cationic charged polymer, which interacts with counter ions such as Alginate, resulting in a gel-like structure.

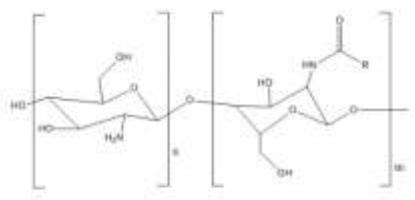

Figure 1: Chitosan

As shown in Figure 2, Alginate is made up of both homopolymeric sequences and heteropolymeric sequences of α-L-guluronic acid (G) and β-D-mannuronic acid (M) (Sosnik, 2014). Alginate can be found naturally in marine brown algae such as *Ascophyllum nodosum, Macrocystispyrifera, Laminara hyperborean* (Takka & Gürel, 2010). The stability and drug release of Alginate based microspheres can be enhance by introducing a cross linking agent such as Calcium ions to the formulation. When Alginate is mixed with Calcium ion through ionic interaction, it results in the 'egg-box model' as shown in Figure 3. This phenomenon offers significant application to drug delivery systems. The model can be used to encapsulate drugs from various kinds of unwanted environments.

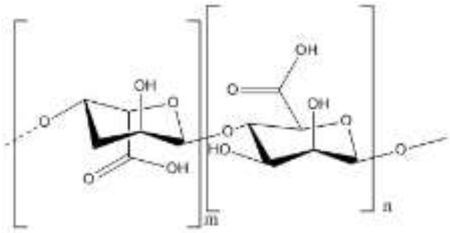

Figure 2: Alginate

There are several limitations when using Sodium Alginate alone in the formulation, such as low entrapment efficiency which cause burst release of the drug, high porosity, and the microspheres produced are weak. However, when Sodium Alginate interacts with Ca^{2+} ions, it forms ionic bonds and produce a polymeric film (Benavides, Cortés, Parada, & Franco, 2016). By adding Chitosan into the formulation, it enhances the strength of the weak film, providing positive charges for mucoadhesion purpose, avoiding the burst release effect and enhancing drug entrapment in the polymeric matrix system (Yadav, Khan, Bonde, & Mishra, 2017).

Figure 3: Schematic diagram of the 'egg-box model' structure(Axelos & Thibault, 1991).

The Ionic Gelation method is one of the methods used to produce microspheres. This method uses mild conditions with no high shear force required. It uses the concept of crosslinking polyelectrolytes in the presence of counter ions (Usmiati, Richana, Mangunwidjaja, Noor, & Prangdimurti, 2014). The mechanism of this method occurs via polyelectrolyte interaction between negatively charged Alginate carboxyl group and positively charged Chitosan amine group (Ribeiro, Silva, Ferreira, & Veiga, 2005). The interaction results in the gelation of the polymer and it then swells upon interaction with the simulated body fluid and releases the drug particles (Patil, Chavanke, & Wagh, 2012).

In this work, Chitosan-Alginate microspheres were prepared using the Ionic Gelation method with Calcium ions as the cross linking agent. The study was carried out to determine the effect of the concentration of Chitosan, Sodium Alginate, Calcium Chloride, and the pH of the gelation medium on the formation of microspheres.

2. Materials and Methods

2.1 Materials and Instrumentation

Chitosan medium molecular weight (75-85% deacetylation; viscosity 200-800cps; product no. 448877; CAS no.9012-76-4), Sodium Alginate (viscosity 5.0-40.0 cps; product no. W201502, CAS no. 9005-38-3), Dioctyl Sodium sulfosuccinate (DOSS; assay ≥97%; MW 444.56; EC no. 209-406-4), Glacial Acetic Acid (Product no. A6283, MW 60.05; CAS no. 64-19-7; Reagent ≥99%), Sodium hydroxide (ACS reagent ≥ 97%; Product no. 221465; CAS no. 1310-73-2), Calcium Chloride (MW 111.0; CAS no. 10043-52-4; assay ≥93%) were all purchased from Sigma Aldrich (Wicklow, Ireland).

2.2 Synthesis of Chitosan-Alginate microspheres

Sodium Alginate powder with various concentrations (2%-6% w/v) were weighed using an analytical balance. The Sodium Alginate powder was then dissolved in distilled water (25mL) with vigorous stirring. Dioctyl Sodium sulfosuccinate (DOSS) (0.075% w/v) was added by stirring it into the mixture. The solution was then left to stir for 3 hours to achieve a homogenous state.

Chitosan with a concentration of 0%, 0.25%, 0.75% and 1% (w/v) was dissolved in glacial acetic acid (2% v/v) in a 50 mL beaker. Distilled water (48mL) was added and the mixture was homogenized using a magnetic stirrer. When the solution becomes clear, Calcium Chloride with concentrations varying from 2%-6% w/v was added. The preparation was then stirred for another 30 minutes. When the solution became transparent, the solution pH was adjusted to 5-6 using 1M Sodium Hydroxide (NaOH). The pH was measured using a calibrated pH meter.

The prepared Sodium Alginate solution were withdrawn using a 10 mL syringe and the rest of the mixture was left to stir. The Chitosan-Calcium Chloride solution was setup on a hot plate stirrer and the temperature was set to 37°C and the stirring speed at 700 rpm. The syringe containing Sodium Alginate was setup to be 2.5 cm away from the gelation medium using a retort stand. A needle size of 25G was used when the disperse solution was sprayed into the Chitosan-Calcium Chloride solution.

The microspheres were left for gelation for a period of 90 minutes. After that, the microspheres produced were rinsed with water and allowed to dry in an oven at of 37 ± 0.5 °C for 12 hours. Figure 4 provides the illustration of the apparatus setup for this experiment.

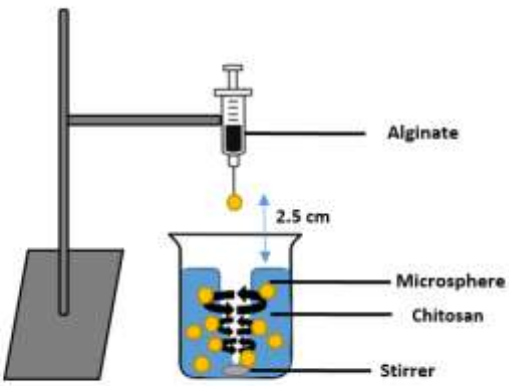

Figure 4: Apparatus setup for microsphere preparation

2.3 Formulation

Several experiments were carried out to examine the formulation parameters that can affect the formation of microspheres namely; the concentration of Chitosan (Table 1), the concentration of Sodium Alginate (Table 2), the concentration of Calcium Chloride (Table 3), and the pH of the gelation medium (Table 4).

Table 1: Formulation of different concentrations of Chitosan in order to optimize microspheres formation

No.	Sodium Alginate (% w/v)	Chitosan (% w/v)	Calcium Chloride (%w/v)
01	6	0	6
02	6	0.25	6
03	6	0.75	6
04	6	1	6

Table 2: Formulation of different concentrations of Sodium Alginate in order to optimize microsphere formation

No.	Sodium Alginate (% w/v)	Chitosan (% w/v)	Calcium Chloride (% w/v)	pH
05	2	0.25	1	6
06	4	0.25	1	6
07	6	0.25	1	6
08	8	0.25	1	6

Table 3: Formulation with different concentrations of Calcium Chloride in order to optimize microspheres formation

No.	Sodium Alginate (% w/v)	Chitosan (% w/v)	Calcium Chloride (%w/v)
09	4	0.25	1
10	4	0.25	2
11	4	0.25	4
12	4	0.25	6
13	6	0.25	1
14	6	0.25	2
15	6	0.25	4
16	6	0.25	6

Table 4: Formulation of different pH values in order to optimize microspheres formation

No.	Sodium Alginate (% w/v)	Chitosan (% w/v)	Calcium Chloride (%w/v)	pH
17	4	0.25	2	2.5
18	4	0.25	2	4
19	4	0.25	2	5
20	4	0.25	2	6

2.4 Surface morphology

The surface morphology of the samples was examined using a Mira SEM (TESCAN, Oxford Instruments UK) setting the magnification bar at 200 μm and the surface elemental composition were examined using the EDX system. Prior to introduction to the microscope, the samples were placed on an aluminum stub and gold coated using a Baltec SCD 005 gold coated instrument for 110 seconds at 0.1 mBar vacuum in order to provide electrical conductivity on the surface of the sample. Two different formulations with the same concentration of Sodium Alginate and Chitosan, 4% w/v, and 6% w/v respectively with different pH (5 and 6) as tabulated in Table 5 were investigated for this purpose. The element presence on the surface of the microspheres were presented in weight percentage.

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Table 5: Formulation of samples for surface morphology

No.	Sodium Alginate (% w/v)	Chitosan (% w/v)	Calcium Chloride (%w/v)	pH
21	4	0.25	6	5
22	4	0.25	6	6

2.5 Swelling study

50mg of the Chitosan-Alginate microspheres was placed in a separate petri dish containing 50 ml of buffer pH 1.2 in an incubator maintained at 37 ± 0.5°C. At pre-determined time intervals, the percent swelling index was calculated by reweighing the microspheres with the swelling index (%) calculated using the formula shown in Equation 1 (Arora & Budhiraja, 2012). Six formulations were carried out to determine the swelling properties of the blank microspheres. The six formulas (Table 6) chosen had the best surface morphology.

$$Swelling\ index = \frac{(Wt - Wo)}{(Wo)}\ x\ 100\%$$

W_t: Weight at pre-determined time point; W_o: Weight at initial

(Equation 1)

Table 6: Formulation concentrations of the microspheres with the best morphology used to study

No.	Sodium Alginate (% w/v)	Chitosan (% w/v)	Calcium Chloride (%w/v)
11	4	0.25	4
12	4	0.25	6
15	6	0.25	4
16	6	0.25	6
23	2	0.25	4
24	3	0.25	6

2.6 Fourier Transform Infrared Spectroscopy

The functional groups of the Chitosan-Alginate microspheres were studied using a Perkin Elmer Spectrum One FTIR Spectrometer. The sample was crushed into tiny particles using a mortar and pestle and placed on the ATR crystal. Data was collected in the spectral range of 4000-650 cm^{-1} at 21°C, utilising a 4 scan per sample cycle and fixed universal compression force of 80 N. Subsequent analysis was carried out using Spectrum software (Perkin Elmer). IR analysis was used to determine the composition of the microspheres. This was compared with the composition of the individual components used to make up the microspheres.

3. Results and Discussion

3.1 Results

3.1.1 The effect of Chitosan

The influence of the Chitosan concentrations (Table 1) was investigated using light microscopy as shown in Figure 5. From these images, it can be observed that when Chitosan is absent (0%) and only Calcium Chloride is present, the Sodium Alginate sprayed produced a spherical shaped microsphere. The presence of 0.25% w/v Chitosan produces a spherical shape microsphere with a transparent appearance. However, when the concentration was increased to 0.75% w/v and 1% w/v respectively, there was no evidence any spherical morphology. Also, the higher concentration of Chitosan (1%) produced a structure with an opaque appearance.

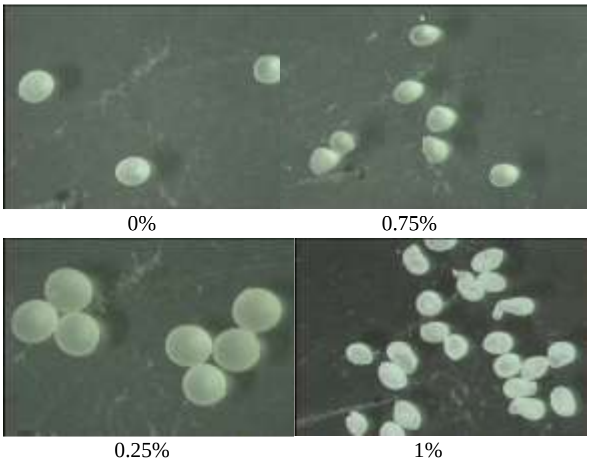


Figure 5: Light microscopy imaging of microsphere with different concentration of Chitosan.

3.1.2 The effect of Sodium Alginate

The influence of Sodium Alginate on microsphere morphology was determined by adjusting the concentration of Sodium Alginate to 2%, 4%, 6% and 8 % (w/v) respectively (Table 2). Light microscopy and SEM images of Sodium Alginate (Figure 6) demonstrated various forms of microsphere morphology.

The results indicated that when 2% w/v of Sodium Alginate was used, a large number of pores were present on the surface of the microsphere which indicating that gelation process has not entirely occurred. However, it was observed that there was a slight improvement when the concentration was increased to 4% w/v (Figure 6).

When microspheres with 4% w/v of Sodium Alginate were observed via SEM, it showed that the microspheres produced a spherical-like shape with a rough surface structure. Whereby, the rough surface structure indicating formation of salt crystallization taking place on the surface of the bead.

The SEM image showed a high-resolution view of the morphology of microspheres and demonstrated a consistent spherical shape. It was observed that the microspheres with 6% w/v of Sodium Alginate provided the optimal morphology in comparison to the other formulations.

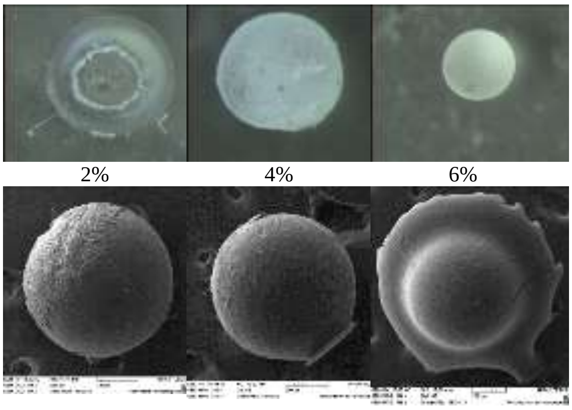


Figure 6: Light microscopy and SEM image of microsphere with different concentration of Sodium Alginate.

However, microspheres were not formed when the concentration of Sodium Alginate was increased to 8% w/v. At this concentration, egg-like structures were observed. Also, when the concentration was increased up to 8% w/v, the viscosity of the samples increased and as a result, it was difficult to inject the solution out of the syringe.

3.1.3 The effect of Calcium Chloride

The purpose of the addition of Calcium Chloride is to encourage the egg box shaped polymerisation. The concentration of Calcium Chloride was varied from 1% to 6%**.** Based on the investigation, it was demonstrated that the Calcium Chloride concentration affected the gelling structure of the microsphere morphology.

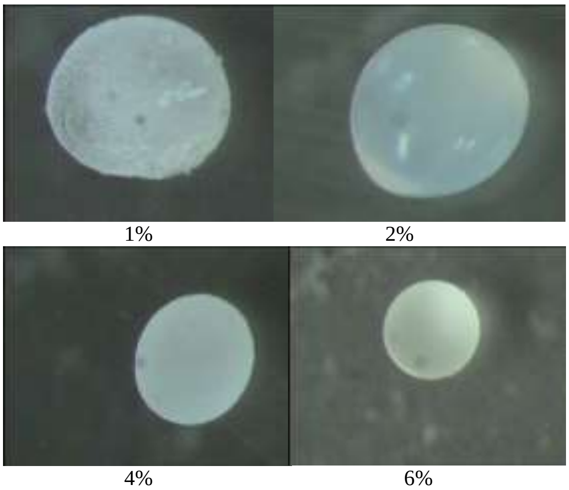
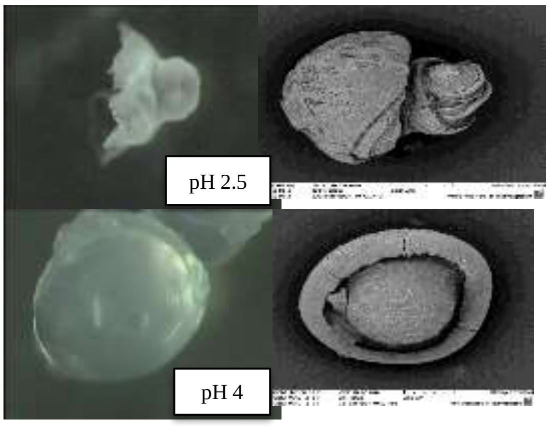


Figure 7: Image of microsphere with different concentration of Calcium Chloride taken using light microscopy.

Microspheres with a Calcium concentration of 1% (Sample 9, from Table 3) produced a spherical shape with a rough surface. When concentrations were increased from 2% to 4% and 6% w/v, the surfaces of microspheres were notably smoother and at all three concentration the microspheres were transparent spherical shapes. The smoothest spherical shape was evident when a 2% Calcium Chloride solution was used (Sample 10, Table 3). However above 6% the microsphere shrunk and the spherical shape were not formed.

3.1.4 The effect of pH of the Gelation medium

As presented in Figure 8, the influence of pH greatly influenced the shape of the material. Without the addition of NaOH, the pH of the solution was 2.5 which results in the formation of a mushroom shaped microsphere.



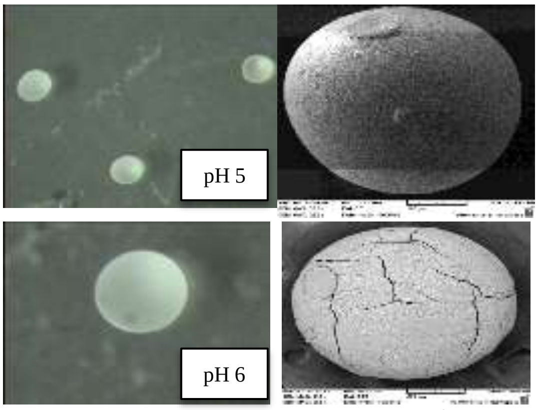


Figure 8: Light microscopy and SEM image of Chitosan-Alginate microspheres.

When the pH value was increased to pH 4, a ring structure encased the microsphere which shows how the formation of the microsphere evolve to create spherical geometries. This was confirmed when the pH was increased to 5. However, pH 6 and above resulted in the breakdown of the microsphere as indicated by the appearance of cracks on the surface.

3.1.5 Surface morphology

Expanding on the findings from section 3.1.4, the formulations as outlined in Table 5 were evaluated with the only difference being the change in pH [5 and 6]. The SEM images of a microsphere loaded with 6% $CaCl_2$, 4% Sodium Alginate and 0.25% Chitosan (Sample 21, Table 5) prepared at pH 5 are presented in Figure 9.

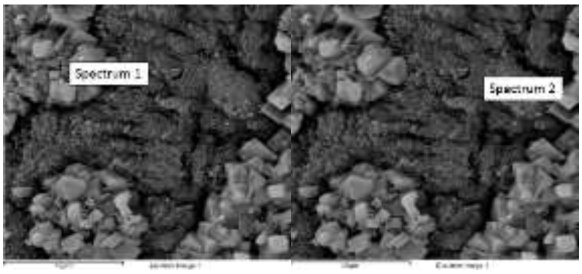


Figure 9: SEM images of the surface of microspheres sample 21 highlighting two EDX spectrum points.

From the images, a crystalline structure on the outer layer of the microspheres was evident and EDX was utilised to determine the components of the crystalline structure as presented in both Table 7 and 8.

Table 7: EDX result of Spectrum 1

Element	Weight (%)
C	19.80
O	8.80
Na	25.69
Cl	38.65
Ca	6.76

From Table 7, the white crystalline structure (spectrum 1, Figure 9) contains a high Chloride (38.65%) and Sodium (25.69%) concentration. This is a result of a reaction which occurred between Sodium Alginate and Calcium Chloride which produced NaCl as a by-product.

Table 8: EDX result of Spectrum 2

Element	Weight (%)
C	26.90
O	32.78
Na	3.48
Cl	14.59
Ca	22.25

The information provided in Table 8 represents the data from the dark area on the microsphere surface as shown in Spectrum 2 (Sample 21, Figure 9). From this spectrum point, the presence of Oxygen (32.78%), Carbon (26.90%) and Calcium (22.25%) were also evident. Hence, one can conclude that the presence of these elements beneath the crystalline structure is Calcium Alginate formed from the reaction, this is verified by the increase in calcium concentration.
The SEM images of a microsphere loaded with 6 %, $CaCl_2$, 4 %, Sodium Alginate and 0.25% Chitosan prepared at pH 6 is presented in Figure 10.

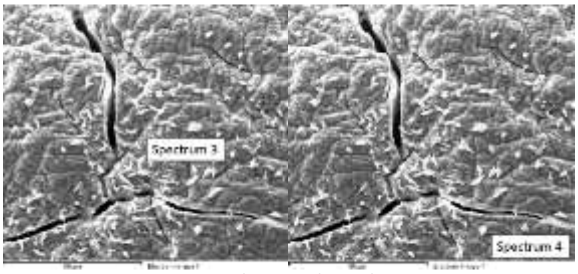


Figure 10: SEM images of the surface of microspheres sample 22 highlighting two EDX spectrum points.

On the surface of the microsphere, the image shows the presence of material precipitation as well as fracture lines. The surface characteristic of the microsphere was further investigated using an energy dispersive X-ray to determine the component of the crystalline structures.

Table 9: EDX result of Spectrum 3

Element	Weight (%)
C	27.30
O	44.42
Na	0.69
Cl	3.53
Ca	24.07

From Table 9, the white precipitation structure in Spectrum 3 of Figure 10 contains high amounts of Oxygen (44.42%), Calcium (24.07%) and Carbon

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(27.03%). This may be due to the residue from the Chitosan that is not completely dissolved in the preparation.

Table 10 shows that spectrum 4 contains the presence of Oxygen (32.74%), Carbon (30.01%) and Calcium (21.33%). Hence, through the presence of these elements Calcium Alginate has formed from the reaction.

Table 10: EDX result of Spectrum 4

Element	Weight (%)
C	30.01
O	32.74
Na	3.03
Cl	12.89
Ca	21.33

3.1.6 Swelling study of Chitosan-Alginate microspheres

The swelling capacity of the microspheres was investigated by immersing the samples in a buffer pH 1.2 for the durations of 0.5, 1, 1.5, 2 and 4 hours respectively at a temperature of 37 °C ± 0.5. The formulations chosen were previously presented in Table 6 and the associated graphs showing the swelling profiles for the blank microspheres are presented in Figure 11.

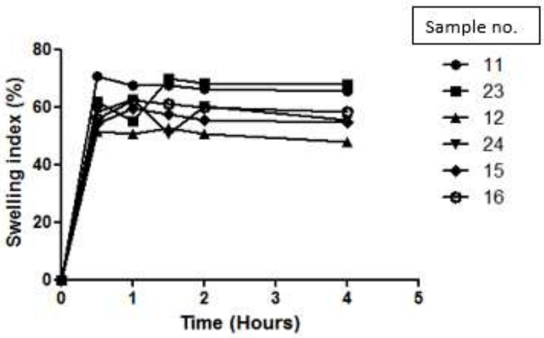


Figure 11: The swelling index graph of the different formulations of Chitosan-Alginate microspheres.

From the information derived from Figure 11, the tabulated swelling index values are presented in Table 11. From this information, all formulations show a rapid increase in the swelling index to more than 50% in the first 30 minutes of the experiment.

The penetration of the buffer medium which caused swelling of the samples was observed to be highest during the first 30 minutes. Formulation 11 produced the highest swelling index (70.78%) in the first 30 minutes of the study while formulation number 12 had the lowest swelling index (51.32%).

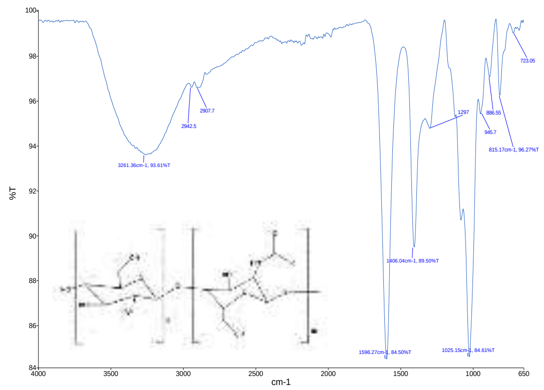
Table 11: Swelling index of formulated Amoxicillin loaded microsphere (n=3)

Time (hours)	Formulation number and swelling index					
	11	12	15	16	23	24
0.5	70.78	51.32	54.19	55.84	61.84	58.17
1	67.53	50.66	59.35	62.34	55.26	62.75
1.5	67.53	52.63	57.42	61.04	69.74	50.66
2	66.23	50.66	55.48	59.74	68.42	60.13
4	65.58	48.03	54.84	58.44	67.76	55.56

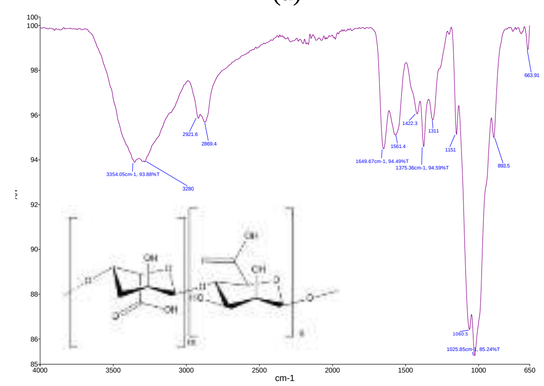
Each formulation produced the same profile of swelling, except for formulation 23 which contained 2% w/v of Sodium Alginate and 4% w/v of $CaCl_2$. Formulations 24 and 16 have almost similar character in swelling profile of the Chitosan-Alginate microspheres.

After 4 hours of exposure in the media, the microspheres remained intact. These experiments were carried out in triplicate.

3.1.7 FTIR analysis



(a)



(b)

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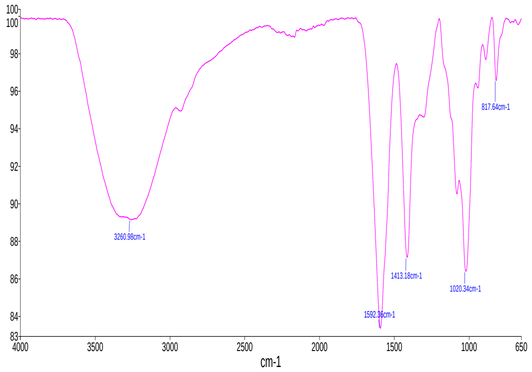

(c)

Figure 12: Infrared spectra of (a) Sodium Alginate, (b) Chitosan and (c) Chitosan-Alginate Microspheres, respectively.

The Sodium Alginate spectrum (Figure 12a) produced a band at 3261cm^{-1} indicating the presence of the stretching vibrations of O-H bonds. Bands at 2907cm^{-1} corresponds with the presence of aliphatic C-H group and bands at 1596 and 1406cm^{-1} correspond with the asymmetric and symmetric stretching of carboxylate ions respectively. The bands at 1025cm^{-1} and 945 cm^{-1} can explain the presence of Alginate structure which consists of the C-O stretching vibrations (Daemi & Barikani, 2012).

The FTIR spectrum of Chitosan is shown in Figure 12b where the bands at 3354cm^{-1} indicates the presence of hydroxyl (O-H) and (N-H) amine group stretching. Bands at 2869cm^{-1} reveals the presence of aliphatic C-H stretch on the material and bands showing at 1649cm^{-1} is due to the stretching of C=O in the primary amide group. The absorption peak at 893 cm^{-1} is the ring stretching of the characteristic bond for the β-1-4 glycosidic linkage (Wanule, Balkhande, Ratnakar, Kulkarni, & Bhowate, 2014). It was noted that the band of the O-H bonds stretching vibration on the Chitosan-Alginate microspheres (Figure 12c) at 3260cm^{-1} indicating the Alginate. The band at 1020cm^{-1} indicate the ether functional group from Chitosan and Alginate.

3.2 Discussions

3.2.1 The preparation of Chitosan-Alginate microspheres

In this study, Chitosan Alginate microspheres were successfully produced using two natural polymers namely, Chitosan and Alginate. These two materials undergo electrostatic interaction in the presence of Calcium ions which help to cross-link the polyelectrolyte complexes. The polyelectrolyte complex formed by the reaction occurs between the amino groups of Chitosan and the carboxyl group of Alginate. Preparation was carried out using an ionic gelation method where the Alginate solution was sprayed into the Chitosan containing Calcium Chloride solution. Figure 13 illustrates the interaction between Chitosan-Alginate complexes.

There are several factors from the experiment setup that can affect the microsphere formation. These includes needle size, stirring speed, and the distance between the tip of the needle and the surface of gelation medium.

A study by Arya et al., discussed that a smaller diameter needle size will result in sputtering and microspheres will not be produced (Arya, Chakraborty, Dube, & Katti, 2008). Flowing of the disperse phase out of the small internal diameter of the needle did not allow the microsphere to achieve the spherical shape before it was sprayed out from the needle and thus resulted in sputtering. In this study, needle size of 18G, 22G and 25G have been used to achieve the optimal spherical sizes.

Stirring is required to ensure the homogeneity of the mixture and to provide a turbulent force throughout the emulsion. A high speed stirring rate would provide a less uniform distribution energy and thus would lead to a wider size of the microsphere (Silva, Ribeiro, & Vit, 2006). In our study, it was observed that the microspheres aggregated when the disperse phase droplet fell into the gelation medium. However, when adding the stirring in the gelation medium, the droplets undergoes gelation and produce a spherical gel beads.

It was noted that a 700 rpm stirring speed produce the best and most spherical beads compared to a speed of 500 rpm. The reason for high turn out of beads produced from 700 rpm is due to the higher availability of Amino groups to react with carboxyl groups of Alginate. When the stirring speed is 500 rpm, it was observed that the beads sediment in the bottom part of the beaker and this decreased the volume of the gelation medium.

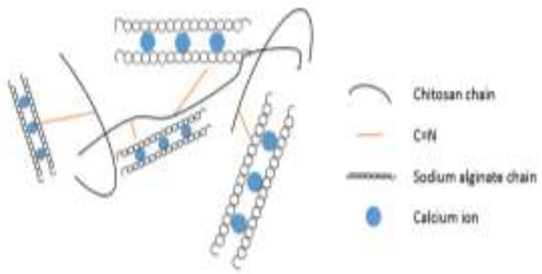

Figure 13: Illustration diagram of the interaction between Chitosan chain and crosslink of Calcium ion and Alginate (Tang, Sun, Fan, & Zhang, 2012).

The distance between the tip of the needle and the surface of the gelation medium is an important factor as the droplets have to undergo the evaporation process which in turn helps it to mould and become a spherical shaped microsphere. In this study, it was observed that there was no spherical beads formed when the distance of the needle was close to the surface of the gelation medium. Later, the experiment setup (as shown in Figure 4) was changed by increase the distance between the tip of needle and surface of gelation medium to 2.5 cm after which spherical beads were produced.

After spraying the dispersed phase into the gelation medium, the microsphere formed were set to undergo the gelation process for 90 minutes. The volume of the distilled water can have an affect on the morphology of the beads. Small volumes of water will not remove the precipitation completely from the surface of the beads as shown in the Figure 9. High concentration of NaCl on the surface of the microspheres observed.

3.2.2 The effect of Chitosan

The effects of concentration of Chitosan was investigated by observing the morphology of the beads produced. As previously shown in Figure 5, increased concentration of Chitosan will damage the structure of the microsphere produced. A solution of 0.25% w/v of Chitosan concentration produced the best spherically structure microspheres.

Theoretically, the electrostatic interaction between the amine group on Chitosan and the carboxyl group of Alginate leads to the formation of a polyelectrolyte complex. This should lead to the potential entrapment of the drug in the polymeric-rich matrix chain. High concentrations of Chitosan will increase the degree of the matrix and thus increase the entrapment efficiency of the drug as theoretically shown in Figure 14. As the Chitosan concentration increases, the matrix formed becomes more compact leaving less spaces to encapsulate the drug resulting in a lower drug release rate.

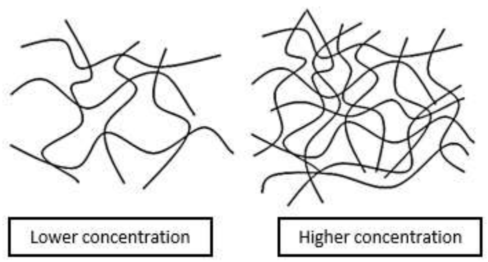

Figure 14: Chitosan concentration vs polymeric matrix network formation.

The role of Chitosan concentration on microspheres encapsulation of drug was not studied for this paper. However, it is expected that as the concentration of Chitosan increases the rate of release of the drug will drop off due to a more compact matrix being formed.

3.2.3 The effect of Sodium Alginate

The effect of Sodium Alginate concentration on microsphere production was investigated by adjusting its concentration from 2%, 4%, 6% and 8% and observing the effect on the morphology of the microspheres produced. It was observed that a spontaneous gelation occurred when a drop of Alginate solution was dropped into the gelation medium containing Ca^{2+} ion and Chitosan.

As demonstrated by the microscopy image in Figure 6, it reveals that the concentration of Alginate does play a role in microsphere formation. When the concentration is below 4%, holes or voids can be observed on the surface of the microsphere. The presence of pores could indicate that the microsphere was not being produced when the concentration of sodium Alginate is insufficient. Similarly, when an 8% solution of Alginate was used, the microsphere formed an egg yolk structure and no spherical shape was seen. This is due to the increase of the viscosity of the solution prepared.

It was noted that when the concentration of Alginate is high, a higher force was required to produce a droplet at the tip of the needle. Another possible explanation for this situation is due to the difference in density between the disperse phase and gelation medium. Based on the observation, the droplet is struggling to overcome the surface of the gelation medium that leads to the formation of the egg yolk structure micro particles.

3.2.4 The effect of Calcium Chloride

Calcium, a divalent cation is frequently incorporated into microsphere formulation to act as an ionic crosslinking agent. When reacting with a negative charge Alginate, Ca^{2+} ions will cross-link and form an egg-box model like structure as shown in Figure 3. The study of the effect of Calcium ions on the microsphere formation examined using utilising light microscopy. Viewing the microscopy image in Figure 7, it can be observed that the presence of Calcium provides gel properties in the microsphere formation.

Microspheres produced using 1% of Calcium were revealed to have a rough morphological structure and no gel-like structure was observed on the microsphere The presence of Ca^{2+} in Alginate-Chitosan microsphere formulation improves the characteristics such as increasing the gelling properties, permeability reduction and enhancing the ability of Chitosan and Alginate as drug carriers (Mennini, Furlanetto, Cirri, & Mura, 2012). However, when the concentration was increased up to 6%, a better spherical structure and smooth surface was produced.

The presence of Calcium ions is believed to be aiding the gelation process of the Chitosan-Alginate reaction. The presence of Ca^{2+} ions as a crosslinking agent with Alginate solution providing aggregation (Baimark & Srisuwan, 2014).

3.2.5 Effect of pH

The stability of the microspheres at different pH levels was investigated by observing the morphology of the microspheres produced; where the pH of the gelation medium was adjusted by adding 0.1 M of NaOH. The pH of pure gelation medium which contains Chitosan and Calcium Chloride was measured at pH 2.5. In order for the polyelectrolyte complex to form, the pH values have to be in between the dissociation constant (pKa) of Chitosan (6.5) and Alginate (3.65) monomer (Kulig, Korzycka, Andrzej, & Marycz, 2016). This explains the reason behind microspheres produced at pH levels of 2.5 and 4 as demonstrated in Figure 8 which indicates that the microspheres produced were shrunk, partially formed and no spherical shape was observed.

According to Takka and researchers (2010), Chitosan and Alginate both show an opposite effect when exposed to different pH levels. Alginate will precipitate in a low pH environment and will dissolve at a higher pH level. However, Chitosan is insoluble at higher pH values and dissolves at a lower pH value (Takka & Gürel, 2010).

3.2.6 Swelling study of Chitosan-Alginate microspheres

The amino groups of Chitosan interact with a fraction of the hydrophilic groups that adhere to the surface of Calcium Alginate beads via a polyelectrolyte complex. When exposed to low pH media, the amino group will produce a repulsive force which leads to a swelling increment and allows water to penetrate into the beads. The hydration of the hydrophilic group causes increased weight of the Calcium Alginate beads (Pasparakis & Bouropoulos, 2006).

In this study, the process occurred after 30 minutes when the microspheres were placed in the phosphate buffer of pH 1.2. Later, the swelling index of all six formulation decrease and achieve steady state till the end of a 4 hour period. This was due to the equilibrium achieved between the osmotic pressure and repulsive force.

4. Conclusions

Chitosan-Alginate microspheres were successfully prepared using an Ionic gelation method. The present study demonstrated various parameters that effect the production of microspheres which include the concentration of Chitosan, Sodium Alginate and Calcium Chloride. It was also noted that needle size, stirring and the distance of the tip of needle and the gelation medium does affect the formation of the microspheres. Chitosan was required to produce the polymeric network structure inside the microspheres. When 0.25% w/v of Chitosan was used, it produces a well-rounded shape microsphere and when the concentration was increased to more than 0.25% w/v, an irregular shaped microsphere was produced. The porosity of the microspheres was not observed when Sodium Alginate concentration was in between 4% and 6% w/v. When the concentration of Sodium Alginate was increased to 8%, it increased the viscosity of the disperse solution and made it difficult to produce the droplet at the tip of the needle. Calcium ion as the cross linking agent demonstrated that it also produces the gelling properties on the surface of the microspheres. The gel layer was observed when the concentration was more than 2% w/v. The study has provided an overview of the optimum concentration of each parameters to produce a microspheres. However, a more in-depth study is required to study the effect of each parameter when the drug is accommodated in the microspheres.

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