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Research Article

FORMULATION, DEVELOPMENT AND IN VITRO EVALUATION OF CANDESARTAN CILEXETIL MUCOADHESIVE MICROBEADS

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ABSTRACT

Mucoadhesive microbeads of candesartan cilexetil was designed in order to obtain a unique drug delivery system which would remain in the small intestine and prolong the residence time at the absorption site by intimate contact with the mucus layer thereby increase bioavailability, reduce the frequency of dose administration and also to prolong the drug release. The mucoadhesive microbeads were prepared by ionotrophic gelation method using sodium alginate, xanthan gum, pectin in combination with 1% chitosan using different crosslinking agents including calcium chloride (ca^{2+}), zinc chloride (zn^{2+}), barium chloride (ba^{2+}) and lead nitrate (pb^{2+}) ions in different ratios. The microbeads were evaluated for percentage yield, bead size, entrapment efficiency, swelling index, entrapment efficiency, mucoadhesion study by *in vitro* wash off test and scanning electron microscopy (SEM) analysis were investigated. Infrared spectroscopy studies (IR) confirmed the absence of any drug interaction with polymers. DSC analysis revealed that the drug was uniformly distributed in the beads. The mean particle size increases with increasing the polymer concentration. SEM photomicrograph showed microbeads with rough surface and spherical shape. The entrapment efficiency of all formulations was in the range between 70% and 90%. The swelling index decreased with increase in sodium alginate and divalent ions concentration. The results of *in vitro* mucoadhesive showed that the microbeads were remained adhered to mucus membrane for longer period of time. *In vitro* drug release studies were performed in simulated gastric fluid [SGF, pH 1.2] for 2 hours and phosphate buffer [pH 6.5] for 10 hours at 37±2^{0}C. *In vitro* drug release followed zero order kinetics, Korse Meyer & Peppas model. The diffusional exponent, n, (0.43 and 0.85) specified anomalous transport or non fickian type and controlled by diffusion through swollen matrix. The above observations suggested that the candesartan cilexetil can be developed as mucoadhesive drug delivery system with sodium alginate using calcium chloride as cross linking agent and 1% chitosan as mucoadhesive polymer.

Keywords: Candesartan cilexetil, Sodium alginate, Microbeads, Ionotrophic gelation method, Mucoadhesion.

INTRODUCTION

Oral route has been commonly adopted and most convenient route of drug delivery. Oral route of administration has been received more attention in pharmaceutical field because of the more flexibility in the designing of the dosage form than drug delivery design for other routes. By considering the conventional dosage form of the drug and drug profile data, such as dose, absorption and elimination rate constant, metabolic properties, drug properties and the quantity of the drug needed, one can determine the desired release rate of the drug from the controlled release dosage form. To achieve and maintain the drug concentration in the body within the therapeutics range required for medication, it is necessary to take this type of drug delivery system several times a day this yield undesirable seesaw drug level in body. A number of advancement has been made recently in the development of new technique for drug delivery, the technique capable of regulating the rate of drug delivery system.[1]

Multiparticulate system made up of natural biodegradable polymers have been paid considerable attention for several years in controlling and sustaining of release rate of drugs. Recently, dosage forms that can precisely control the release rates and targets drugs to a specific body site have made enormous impact in the formulation and development of novel drug delivery systems.[2] Oral multiunit dosage forms such as microcapsules and microspheres have received much attention as modified/controlled drug delivery systems. Microbeads are small, solid and free flowing particulate carriers containing dispersed drug particles either in solution or crystalline form that allow a sustained release or multiple release profiles of treatment with various active agents without major side effects.[3] Additionally, the beads maintain functionality under physiological conditions, can incorporate drug to deliver locally at high concentration ensuring that therapeutic levels are reached at the target site while reducing the side effects by keeping systemic concentration low. It will therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by coupling the bioadhesive characteristics to microbeads and develop bioadhesive microbeads. Bioadhesive microcapsules have advantages such as efficient absorption and enhanced bioavaibality of drugs owing to high surface to volume ratio, a much more intimate contact to mucus layer and specific targeting of drugs to the absorption site.[4]

Alginate (polysaccharide) is obtained from marine brown algae, alginate can be considered as block polymers which mainly consist of mannuronic acid (M), guluronic acid (G) and mannuronic-guluronic (MG) blocks. The gelation of alginate is caused by forming an egg-box junction to associate divalent metal ions with the GC block of alginate polymer chain. The medicinal use of sodium alginate as a matrix material to achieve controlled release drug delivery is due to its hydrogel forming properties. Pectin serves as a thickening and gelling agent. Pectin is also used for their perfect biocompatibility. In this case xanthan gum was used to regulate the drug release pattern. Chitosan was selected as a polymer in preparation of mucoadhesive microcapsules because of their good mucoadhesive and biodegradable properties. Candesartan cilexetil, (±)1-{[(cyclohexyloxy)carbonyl]oxy}ethyl 2-ethoxy -1- {[2'-(1*H*-tetrazol-5-yl)- biphenyl -4-yl]methyl}-1*H*-benzimidazole-7-carboxylate, a nonpeptide, is a selective AT1 subtype angiotensin II receptor antagonist indicated for the treatment of hypertension alone or in combination with other antihypertensive agents. Candesartan cilexetil [CC], is a prodrug that is hydrolyzed to candesartan during absorption from the gastrointestinal tract. CC has log P value of 6.1 and the aqueous solubility of CC is less than $5x10^{-5}$ g/L which may be the reason for very low bioavailability i.e. about 15%. Half life of candesartan cilexetil 5.1-10 hours.[5] The purpose of present investigation is to develop mucoadhesive beads with different crosslinking agents like calcium chloride, zinc chloride, lead nitrate, barium chloride and chitosan as mucoadhesive polymer with drug Candesartan cilexetil by ionotrophic gelation method.

MATERIALS AND METHODS

Candesartan cilexetil was obtained as a gift sample from Ranbaxy laboratories, Gurgaon. Sodium alginate was purchased from Loba Chemie, Mumbai. Zinc chloride, calcium chloride and xanthan gum

were purchased from Nice chemical private limited, Kochi. Chitosan was purchased from Hi media laboratories Pvt limited, Mumbai.

Formulation of mucoadhesive alginate beads

The mucoadhesive alginate beads were prepared by ionotrophic gelation method as per the composition shown in Table 1. An aqeous solution of various concentrations of sodium alginate, xanthan gum and pectin is prepared with vigorous stirring to form a clear solution. Pectin and xanthan gum is used in low concentration whereas high concentration will result in much higher viscosity of solution which was difficult to process for preparing the microbeads. To this solution the drug candesartan cilexetil is added slowly and stirred continuously until a uniform dispersion is obtained. The dispersion is kept undisturbed for 30 minutes. The resultant bubble free, homogeneous dispersion is extruded into polyvalent ion solutions (200 ml) containing 1% chitosan using a hypodermic syringe with 21 gauge needle and stirred at 100rpm in magnetic stirrer. The gel beads are cured in gelation medium for 15 mins and then collected by decantation technique and the product thus separated is washed with acetone for two times and dried at room temperature for 24 hours[6].

Table 1: Preparation of Mucoadhesive Alginate Beads

Batch code	Drug (%w/v)	Sodium alginate (%w/v)	Pectin (%w/v)	Xanthan gum (%w/v)	Cross linking agent(%w/v)				Chitosan (%w/v)
					Zinc chloride	Barium chloride	Calcium chloride	Lead nitrate	
F1	2	1.40	0.40	0.20	2	-	-	-	1
F2	2	2.80	0.80	0.40	4	-	-	-	1
F3	2	4.20	1.20	0.60	6	-	-	-	1
F4	2	5.60	1.60	0.80	8	-	-	-	1
F5	2	7.00	2.00	1.00	10	-	-	-	1
F6	2	1.40	0.40	0.20	-	2	-	-	1
F7	2	2.80	0.80	0.40	-	4	-	-	1
F8	2	4.20	1.20	0.60	-	6	-	-	1
F9	2	5.60	1.60	0.80	-	8	-	-	1
F10	2	7.00	2.00	1.00	-	10	-	-	1
F11	2	1.40	0.40	0.20	-	-	2	-	1
F12	2	2.80	0.80	0.40	-	-	4	-	1
F13	2	4.20	1.20	0.60	-	-	6	-	1
F14	2	5.60	1.60	0.80	-	-	8	-	1
F15	2	7.00	2.00	1.00	-	-	10	-	1
F16	2	1.40	0.40	0.20	-	-	-	2	1
F17	2	2.80	0.80	0.40	-	-	-	4	1
F18	2	4.20	1.20	0.60	-	-	-	6	1
F19	2	5.60	1.60	0.80	-	-	-	8	1
F20	2	7.00	2.00	1.00	-	-	-	10	1

Preformulation studies

FTIR studies

FT-IR spectra (Spectrum RX-1 Perkin-Elmer, German) for the drug and various physical mixtures are obtained in a FT-IR spectroscopy in the transmission mode with the wave number region 4000-500cm^{-1}. KBr pellets are prepared by gently mixing 1mg sample powder with 100mg KBr.[7]

DSC studies

To detect any interaction between drug and polymer the DSC thermograms of pure drug, polymers and pnysical mixture of drug and polymers were taken using DSC200 TA Instruments, USA. The samples are heated in sealed aluminium pan at a rate of 10°C/min over the temperature range of 5-400°C under a nitrogen flow of 20 lb/cm^2. [8]

Evaluation of mucoadhesive alginate beads

Percentage yield

The percentage yield of microcapsules of various batches are calculated using the weight of final product after drying with respect to the initial total weight of the drug and polymer used for preparation of microbeads and percent yields is calculated as per the formula mentioned below.[9]

$$\text{Percentage yield} = \left(\frac{\text{Amount of dried microbeads}}{\text{Amount of drug+ Amount of polymer}} \right) \text{X } 100$$

Bead size measurement

Bead size is measured by using Leica image analyzer [France]. Fifty number of completely dried alginate beads were used to measure their size[10].

Drug entrapment efficiency

Drug loaded microbeads (100 mg) are crushed in glass mortar and pestle and suspended in 100 ml of phosphate buffer (pH 6.5) solution and kept for 24hr. It is stirred for 5 minute and filtered by whatmann filter paper. Drug content in the filtrate is determined by spectrophotometrically[11].

Entrapment efficiency is calculated using the reported formula[12].

$$\text{Entrapment efficiency} = \left(\frac{\text{Actual \% drug content}}{\text{Theoretical \% drug content}} \right) \text{X } 100$$

$$\text{Actual drug content mg (\%)} = \left(\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \right) \text{X } 100$$

$$\text{Theoretical drug content mg (\%)} = \left(\frac{\text{Weight of drug in microbeads}}{\text{Microbeads sample weight}}\right) X\ 100$$

Swelling studies

Swelling studies of alginate beads are carried out in two media. The beads are immersed in the simulated gastric fluid (SGF, pH 1.2) for first 2 hours and simulated intestinal fluid (SIF pH 6.5) for next 10 hours. Accurately weighed amounts of beads (2.5g) are immersed in 25 ml of SGF and at fixed time intervals the beads are separated from the medium using a stainless steel grid. Immediately, they are wiped gently with tissue paper and weighed. After two hours the beads were transferred to 25ml of SIF and at fixed time intervals the beads are separated from the medium using a stainless steel grid and wiped gently with tissue paper and weighed on electronic balance[13]. The swelling index of the beads with respect to time is calculated according to the formula:

$$\text{Swelling index} = \left(\frac{Ws - Wi}{Wi}\right) X\ 100$$

Where, *Wi* is the initial weight of beads

Ws is the weight of swollen beads

***In vitro* dissolution studies**

An accurately weighed amount of drug loaded alginate beads equivalent to 16 mg are taken for *in vitro* dissolution studies. The beads are filled into hard gelatin capsules and it is coated with Eudragit L 100 by dipping and drying method. The study is carried out in the USP Type II apparatus using 900 ml of buffer solution. The rotating speed of paddle is maintained at 50 rpm at 37±1°C. First two hour study is carried out in pH 1.2 and next ten hour study is carried out in phosphate buffer pH6.5. Samples are withdrawn every 15 mins for first one hour and then for every 30 mins. 10 ml of sample is withdrawn from buffer medium, diluted with fresh medium and make upto 100ml. At the same time 10 ml of fresh medium was added to the dissolution medium to maintain the sink condition. 10 ml fresh medium and analyzed for candesartan cilexetil content by the absorbance of the sample was determined by UV-Visible spectrophotometer at 257nm.[14]

Release kinetics

In order to understand the mechanism and kinetics of drug release, the drug release data of the in-vitro dissolution study are analyzed with various kinetic equations like zero-order, higuchi and peppas equation. Coefficient of correlation (r) values are calculated for the linear curves obtained by regression analysis of the above plots.[15]

***In vitro* wash off test**

The mucoadhesive property of microbeads was evaluated by *in vitro* adhesion testing method known as the was-off method. The time taken for detachment of beads from sheep intestinal mucosa is measured in 0.1N hydrochloric acid (pH 1.2) and 0.05M phosphate buffer (ph 6.5). This is evaluated by an *in vitro* adhesion testing method, known as wash off method. A piece of sheep intestinal mucosa (2×2 cm) is mounted onto glass slide (3×1 inch) with cyanoacrylate glue. The beads (50 nos) are counted and spread over the wet rinsed tissue specimen and immediately thereafter the support is hung on the arm of a USP tablet disintegrating test machine. By operating the disintegration machine the tissue specimen is given a slow regular up and down movement. The slides move up and down in the test fluid at 37 ± 0.5^0 C. The number of beads adhering to the tissue is counted at 2-hour intervals up to 12 hours [16].

Scanning electron microscopic studies

The morphology and surface structure of beads are observed using SEM photographs taken with SEM analyser. The beads are made conductive by sputtering thin coat of platinum under vacuum and then the images are recorded with at magnification of 25X.

RESULTS AND DISCUSSION

The objective of the present work is to develop different mucoadhesive formulations of candesartan cilexetil. Alginate beads of candesaratan cilexetil were formulated by ionotrophic gelation method. Twenty formulations of alginate beads were prepared using different concentrations of sodium alginate and different cross linking agents as shown in Table 1. The results observed are mentioned in the following sections.

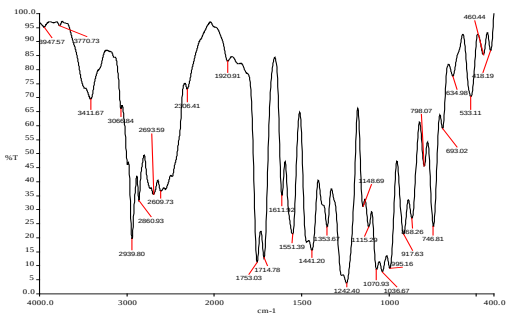
Physical characterization

In all formulations the alginate beads were more or less spherical in shape and the exterior surfaces were rough. The spherical shape of the beads in wet state was usually lost after drying especially for beads prepared with low concentration of sodium alginate and cross linking agent. With the increase of sodium alginate concentration the shape of the beads retained considerably.

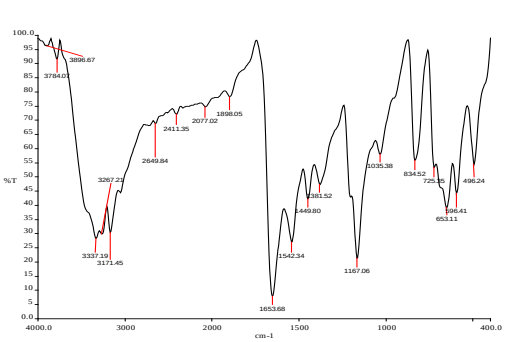
Preformulation studies

FTIR studies

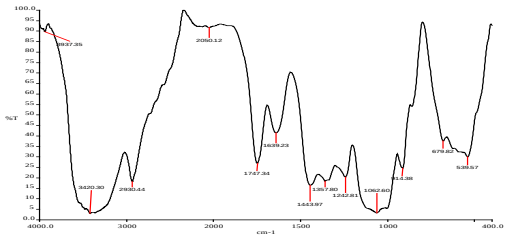
To check the compatibility of drug with various polymers, IR spectra of drugs, polymers and combination of the drug and polymers were taken. FTIR spectra of candesartan cilexetil, sodium alginate, xanthan gum, pectin and chitosan were recorded in KBr pellets and are presented in Figure 1.The IR spectral analysis of candesartan cilexetil pure drug alone showed that principal peaks were observed at wave numbers 3411.67cm^{-1}, 2939.80cm^{-1}, 1753.03cm^{-1}, 1551.14cm^{-1} and 798.02cm^{-1}. Further in the physical mixture of sodium alginate, xanthan gum, pectin, chitosan and candesartan cilexetil, the major peaks of were observed at 3412.95cm^{-1}, 2940.58cm^{-1}, 1714.69cm^{-1}, 1548.86cm^{-1} and 746.67cm^{-1} suggesting that there is no interaction between the polymers and drug used in the present study.



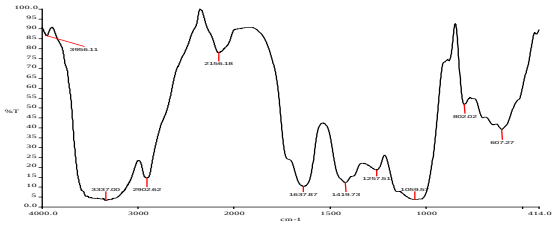
Pure drug



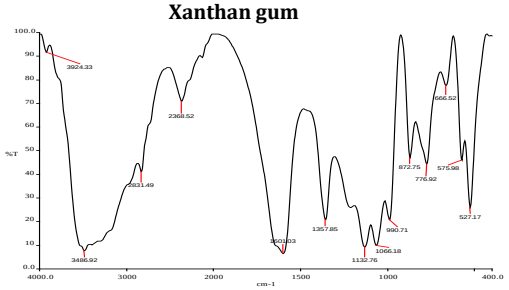
Sodium alginate



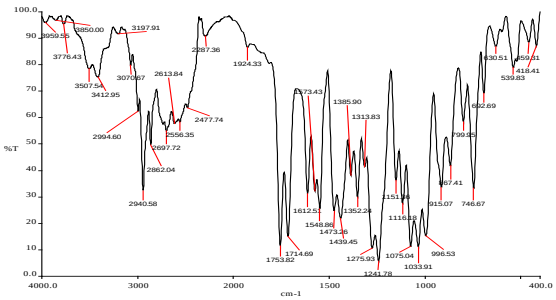
Xanthan gum



Pectin

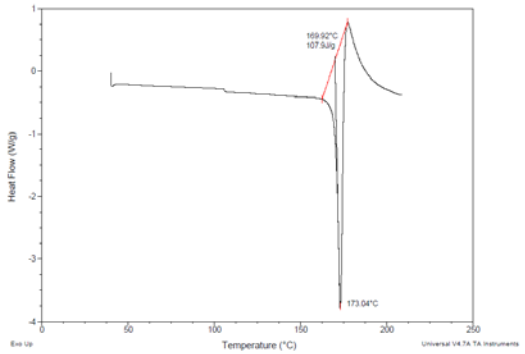


Chitosan

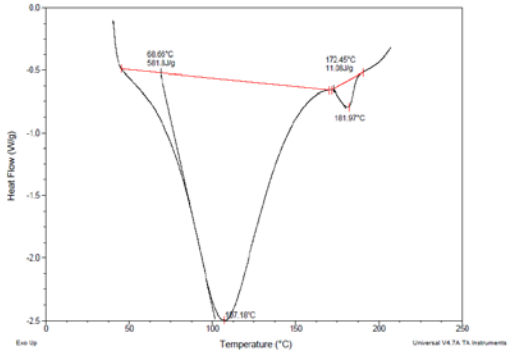


Drug + sodium alginate +xanthan gum + pectin + chitosan

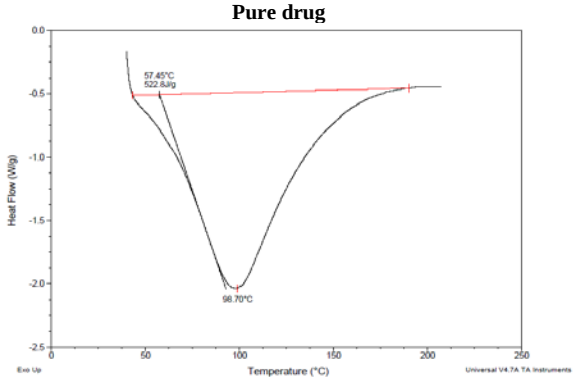
Fig. 1: FTIR Spectra



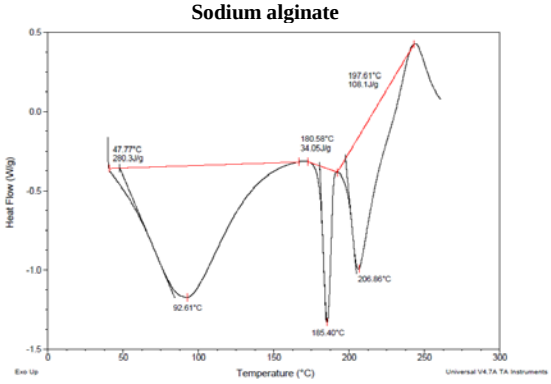
Pure drug



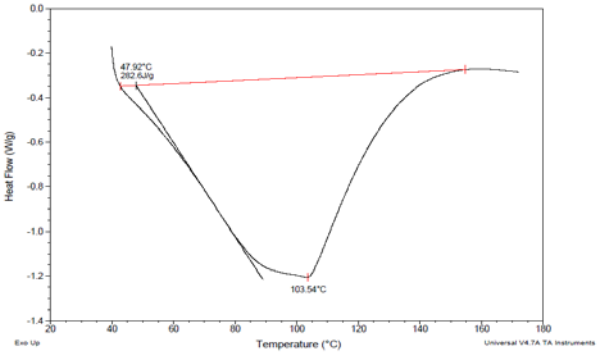
Sodium alginate



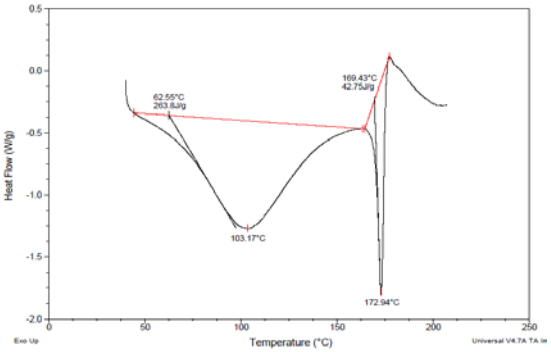
Pectin



Xanthan gum



Chitosan



Drug + sod.alginate+ xanthan gum+Pectin+chitosan

Fig. 2: DSC thermogram

DSC studies

The DSC thermogram of pure drug and the different polymers were shown in the Figure 2. A sharp exothermic peak at about 173°c was observed for pure candesartan cilexetil. It was observed that the large exothermic peak of pure drug was a bit smaller and shifted to 172.94°c in physical mixture revealing its unchanged nature. This indicates that the drug has not undergone any chemical interaction with the polymer backbone.

Evaluation of mucoadhesive alginate beads

Percentage yield

As shown in Table 2 the percentage yield of alginate beads prepared by ionotrophic gelation method were found to be between 76% and 94%. It was found that percentage yield of alginate beads prepared in calcium chloride was greater than barium chloride, zinc chloride and lead nitrate. A significant decrease in the production yield was observed with increase of alginate concentration. The probable reason behind this may be due to high viscosity of the solution which decreases its syringe ability resulting in blocking of needle and wastage of the drug polymer solution which ultimately decreased the production yield[17].

The order of percentage yield was

Zinc chloride>calcium chloride>barium chloride>lead nitrate

Table 2: Determination of percentage yield

Formulation code	Percentage yield(%w/v)
F1	89.00
F2	76.00
F3	82.28
F4	84.48
F5	86.23
F6	91.75
F7	94.13
F8	93.83
F9	92.69
F10	87.07
F11	90.40
F12	90.26
F13	91.12
F14	90.24
F15	88.02
F16	81.20
F17	80.90
F18	87.18
F19	84.97
F20	80.41

Bead size

By image analyzer it was found that the particle size was very well within a narrow size range between 1.073mm and 1.938mm. The mean particle size was different among the formulations as shown in Table 3. The effect of concentration of polymer on the size of beads formed were studied and it was found that there was increase in the average diameter of particles as there was an increase in the concentration of polymer.

The most important property of alginates is their ability to form gels by reaction with divalent cations such as ca^{2+}. [18] The gelation and cross linking of the polymers are mainly occurred by exchange of sodium ions from the guluronic acids with the divalent cations.

Effect of sodium alginate on bead size

The result indicated that as the amount of sodium alginate was increased, the particle size is also proportionately increased. This could be attributed to an increase in relative viscosity at higher concentration of sodium alginate results in the formation of larger particles.

Effect of crosslinking agent on bead size

Calcium chloride produced larger and spherical beads than other divalent ions. This may be attributed to greater availability of calcium binding sites in the polymeric chains and consequently the greater degree of cross linking as the quantity of sodium alginate increased, resulting in the formation of nonporous microbeads.[19]

The order of bead size was

Calcium chloride > barium chloride> zinc chloride>Lead nitrate

Table 3: Determination of particle size

Formulation code	Mean particle size in mm
F1	1.073
F2	1.115
F3	1.298
F4	1.524
F5	1.636
F6	1.091
F7	1.406
F8	1.483
F9	1.514
F10	1.794
F11	1.116
F12	1.528
F13	1.715
F14	1.803
F15	1.938
F16	1.390
F17	1.469
F18	1.452
F19	1.605
F20	1.763

Drug entrapment efficiency

The drug entrapment efficiency of all formulations was in the range between 70% and 90%. This is probably due to more firmness in the alginate-chitosan complex during gelation caused by increased ionic interactions between the carboxylate groups in the alginate and the protonated amine groups in the chitosan. The results of drug entrapment efficiencies were shown in Figure 3.

Effect of sodium alginate on entrapment efficiency

Drug entrapment efficiency of alginate beads increases with increase in concentration of polymers. The higher viscosity of the polymer solution at the highest polymer proportion would be expected to decrease the diffusion of the drug into the external phase which would result higher entrapment efficiency. This may be attributed to the greater availability of active binding sites in polymeric chains and consequently the greater degree of cross linking as the quantity of alginate increased.[20]

Effect of cross linking agents on entrapment efficiency

The entrapment efficiency was improved in ca^{2+} than other divalent ions, it may be due to high affinity towards alginate which produces non porous beads than that of Ba^{2+}, zn^{2+} and Pb^{2+} ions as cross linking agent.[21]

The order of entrapment efficiency was

Calcium chloride>zinc chloride >barium chloride>lead nitrate

Swelling studies

Swelling of the dry beads is mainly attributed to the hydration of the hydrophilic groups of alginate and chitosan. In this case free water penetrates inside the beads in order to fill the inert pores among polymer chains, contributing to a greater swelling degree.

At pH 1.2, the swelling degree of the beads was limited due to the reduced chemical potential of the network resulted from protonation of carboxylic acid groups. The initial increase of swelling degree is mostly driven by counter ions which neutralize – NH_3^+ groups of polymer chain in the network.

All the formulations exhibited significant swelling rates when exposed to the slightly alkaline medium. This swelling mechanism in this case is related with the polyvalent ions and Na^+ exchange.

All beads began to swells presumably due to an increase in the electrostatic repulsive forces at a pH above the pka of the uronic acid groups on the alginate.

When the swelling degree of the formulations was compared, the lowest swelling ratio was obtained in pH 1.2. In pH 6.5, the alginate beads swelled and they were not broken in these pH values after 8 hours. These results suggest that alginate beads do not disintegrate in the stomach and thus resulted in release of candesartan cilexetil in intestinal fluids.

The swelling degree of calcium- alginate beads was lower than that of zinc-alginate, barium-alginate, and lead-alginate beads. The increase in concentration of ca^{2+} ions in the gelation medium increases the availability of ca^{2+} ions which increase the number of interactions with coo^{-} groups present in alginate. This resulted in increased crosslinking density which hindered inward diffusion of swelling medium.[22]

Fig 4 shows the swelling behavior of beads, as a function of pH.

The order of swelling index was

Lead nitrate>barium chloride>zinc chloride>calcium chloride

***In-vitro* dissolution studies**

The results of *in-vitro* drug release studies from the mucoadhesive alginate beads are shown in Figures 5A, 5B, 5C and 5D respectively.

The beads did not show any drug release at pH 1.2 and it released the drug at pH 6.5. Above pH 6.0 Eudragit L 100 coating started to dissolve and exposed the alginate beads for drug release. So it protected the release of drug from the acidic medium to minimize the side effects.

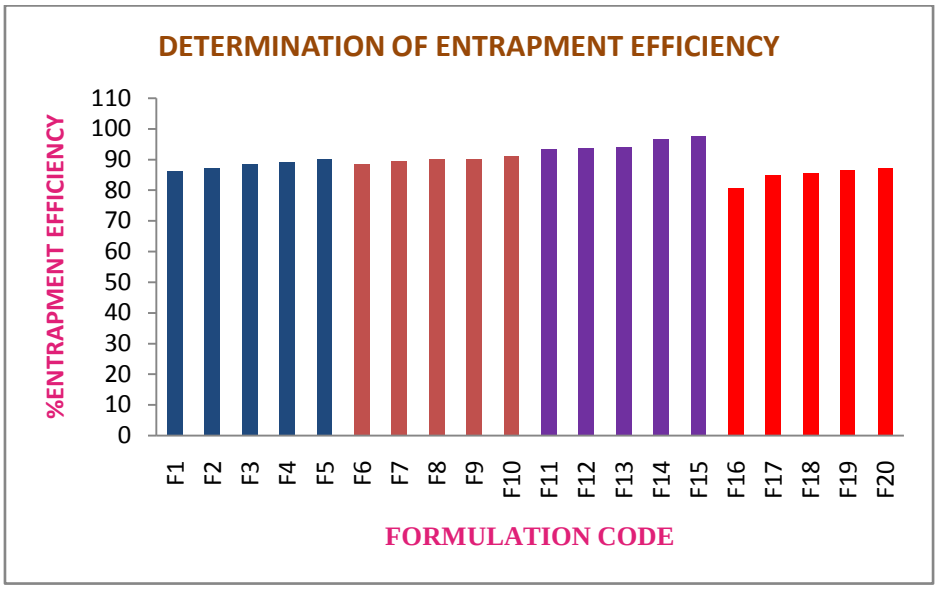


Fig. 3: Determination of entrapment efficiency

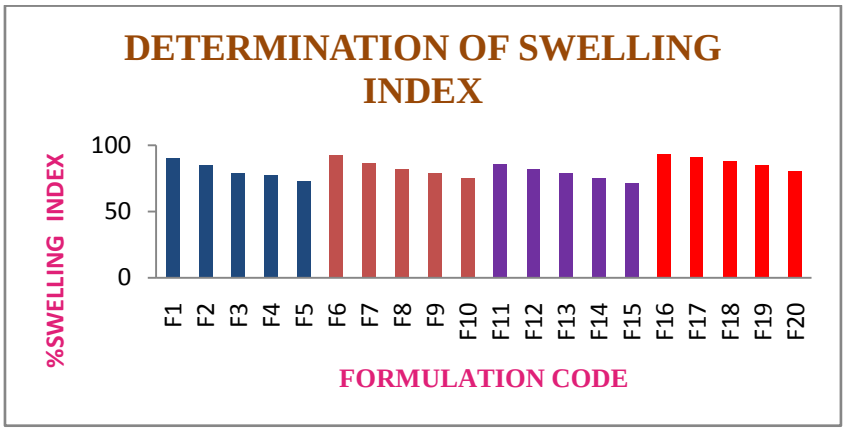


Fig. 4: Determination of swelling index

Effect of cross linking agents on drug release

The formulations F1, F2, F3, F4 and F5 were prepared with 2%, 4%, 6%, 8%, 10% barium chloride as cross linking agent showed the cumulative percentage of drug release 78.12%, 76.07%, 72.72%, 71.17%, 70.01% respectively at the end of 12 hours. Among the formulations F1, F2, F3, F4 and F5, the formulation F5 showed more controlled release, this may be due to the increase in concentration of barium chloride and polymers.

The formulations F6, F7, F8, F9 and F10 were prepared with 2%, 4%, 6%, 8%, 10% zinc chloride as cross linking agent showed the cumulative percentage of drug release 80.01%, 77.03%, 75.59%, 74.91%, 73.40% respectively at the end of 12 hours. Among the formulations F6, F7, F8, F9 and F10, the formulation F10 showed more controlled release, this may be due to the increase in concentration of zinc chloride and polymers.

The formulations F11, F12, F13, F14 and F15 were prepared with 2%, 4%, 6%, 8%, 10% calcium chloride as cross linking agent showed the cumulative percentage of drug release 75.12%, 73.71%, 71.72%, 69.62%, 67.75% respectively at the end of 12 hours. Among the formulations F11, F12, F13, F14 and F15 the formulation F15 showed more controlled release, this may be due to the increase in concentration of calcium chloride and polymers.

The formulations F16, F17, F18, F19 and F20 were prepared with 2%, 4%, 6%, 8%, 10% lead nitrate as cross linking agent showed the cumulative percentage of drug release 81.08%, 79.76%, 78.42%, 76.76%, 75.80% respectively at the end of 12 hours. Among the formulations F16, F17, F18, F19 and F20, the formulation F20 showed more controlled release, this may be due to the increase in concentration of lead nitrate and polymers.

The formulations F5, F10, F15 and F20 containing 10% barium chloride, 10% zinc chloride, 10% calcium chloride and 10% lead nitrate respectively showed more sustained release with the increase in percentage of cross linking agents. This indicates that the release rate is retarded due to increase in percentage of cross linking agents because of strong bonds between sodium alginate and divalent ions, Hence drug release is retarded in the following order.

Lead nitrate > zinc chloride > barium chloride> calcium chloride

Although alginate is able to form complex with divalent ions like zn^{2+}, ba^{2+}, ca^{2+} and pb^{2+}, the association is stronger with calcium than other ions. This is because calcium is more densely cross linked with alginate This may be due to the increased interaction between Na^{+} and Ca^{2+}, forming closer network which decreased the diffusion of drug outwards the alginate beads.

The batch containing sodium alginate-chitosan and calcium chloride showed sustained release of drug upto 12hr when compared with other cross linking agents. This may be due to stronger affinity of ca^{2+} ions towards alginate than other cross linking agents' results in resistance of the polyelectrolyte complex against drug diffusion and slower release of drug from alginate beads.[23]

Increase in the concentration of calcium ions with sodium alginate permitting a higher extent of cross linking that led to stronger bead formation, providing greater resistance to drug diffusion from the alginate beads.[24]

Effect of sodium alginate on drug release

As increasing the percentage of sodium alginate in formulation could led to a greater viscosity of the solution, and hence large drops needs to be dripped out of the needle. As a result, larger beads were formed and diffusivity decreased. As the polymer to the drug ratio was increased the extent of drug release decreases. The decrease in the rate and extent of the drug release is due to the higher density of polymer matrix that results in increased diffusion pathlength through which the drug molecule have to traverse.[25]

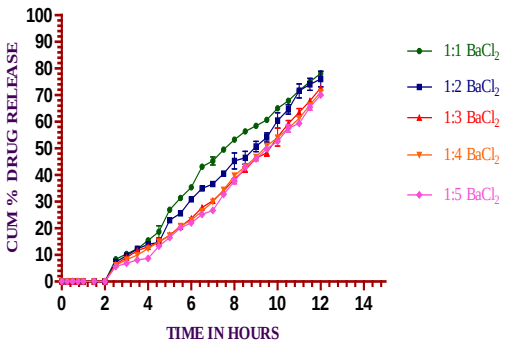


Fig. 5a: *In vitro* release profile of F1 to F5

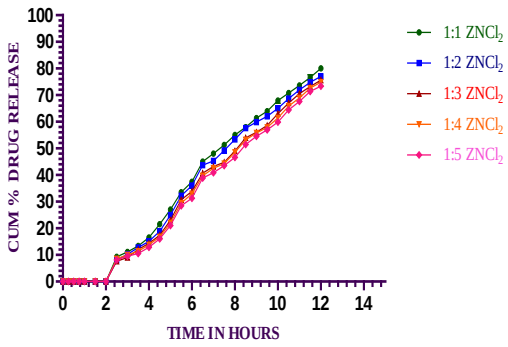


Fig. 5b: *In Vitro* release of F6 to F10

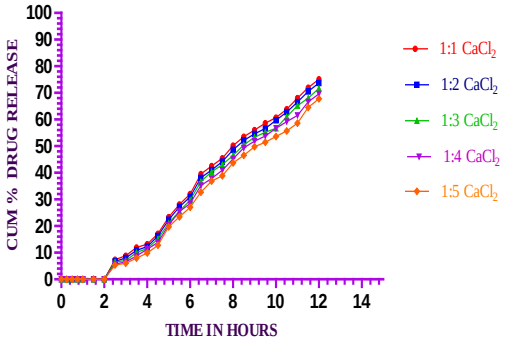


Fig. 5c: *In vitro* release profile of F11 to F15

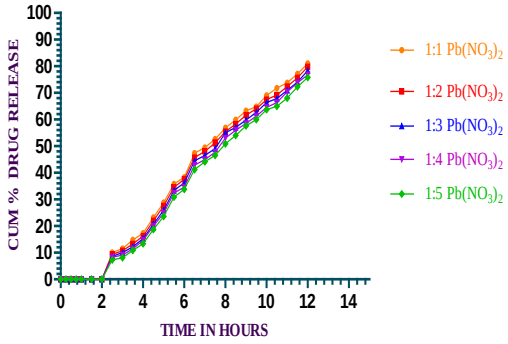


Fig. 5d: *In vitro* release profile of F16 to F20

Release kinetics

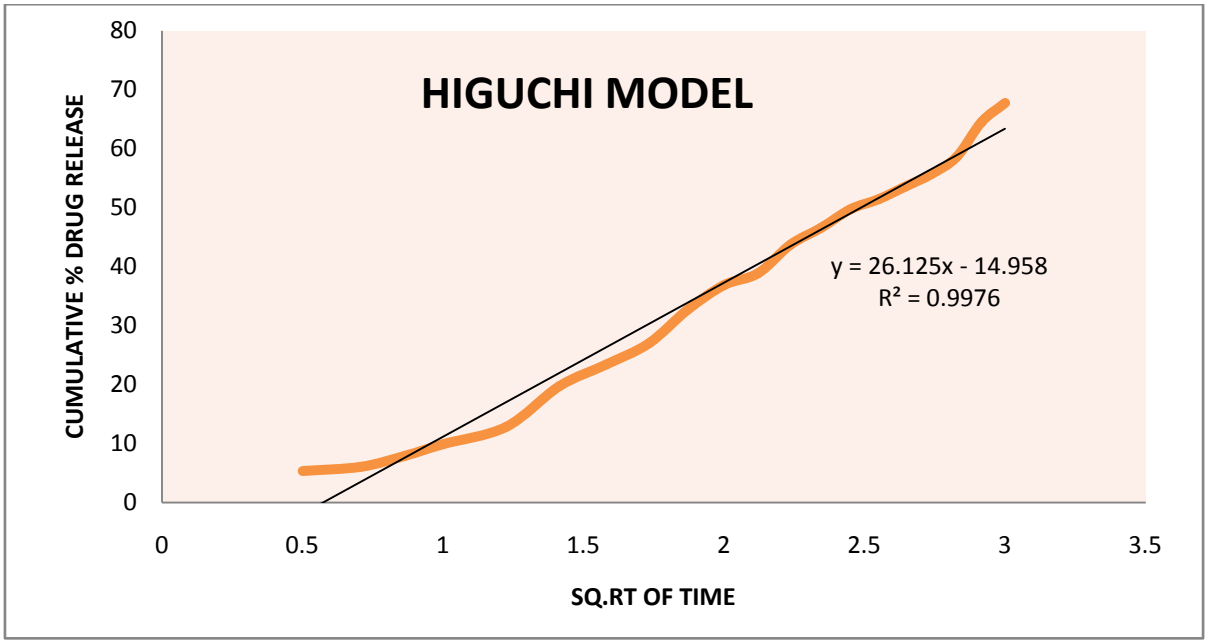
Further the drug releases were subjected for mathematical treatment to check whether the release is following first order kinetics or zero order kinetics. The correlation co efficient values are shown in Table 9.

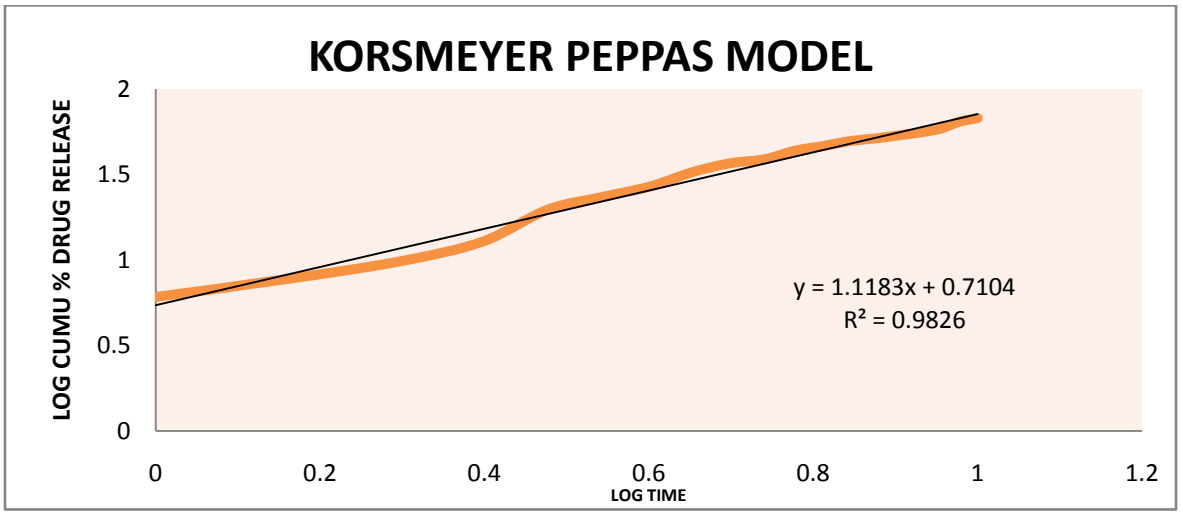
The r values of zero order plots were between 0.981 to 0.986 and first order plot between 0.925 and 0.982. The r^2 values indicate all these formulations followed zero order kinetics.

The values of co efficient of correlation were found to be best fitted to Korse meyer peppas and Higuchi model. The R^2 values are closer to one in zero order kinetics, so it follows zero order kinetics.

The diffusional exponent, n, specifies the mechanism of release. For alginate beads, values of 'n' between 0.43 and 0.85 are an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport or non-fickian diffusion). Values above 0.85 indicate case II transport which relate to polymer

relaxation during gel swelling[26]. The release kinetics of best formulation is shown in Fig- 9.





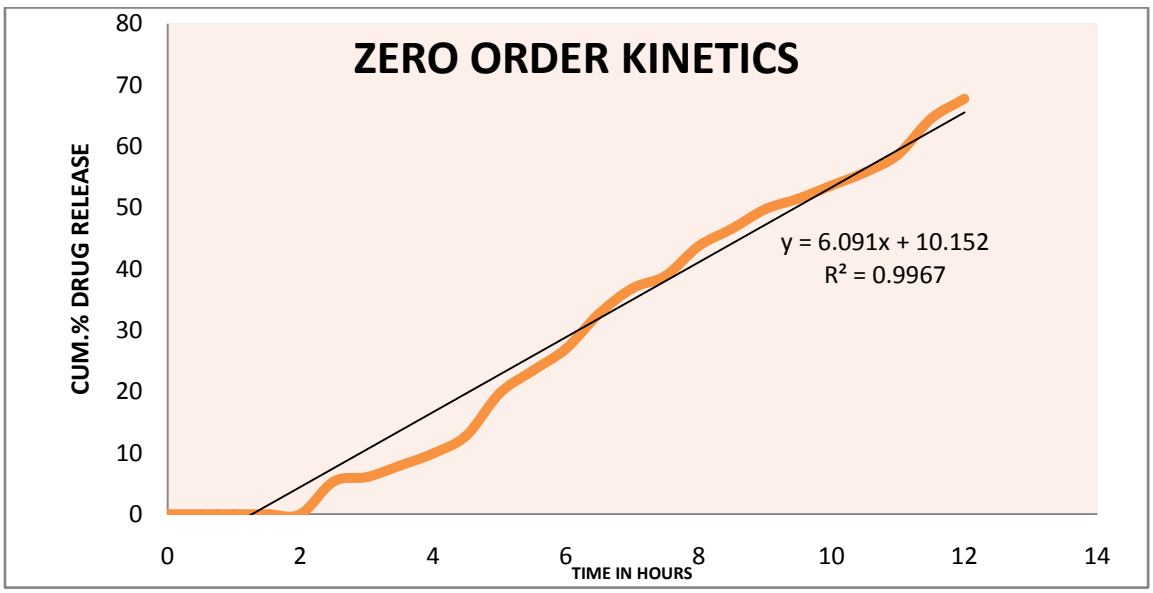


Fig. 6: Release kinetics of selected formulation

Table 7: Release kinetics of all formulation

Formulation code	Zero order		First order		Higuchi model		Korsmeyer peppas model		Hixson crowell	
	R^2	K_0	R^2	K_1	R^2	k_H	R^2	N	R^2	k_{HC}
F1	0.987	6.511	0.98	-0.065	0.989	29.49	0.986	0.934	0.969	-0.192
F2	0.987	6.954	0.974	-0.022	0.981	28.18	0.98	0.85	0.964	-0.002
F3	0.988	6.107	0.925	-0.052	0.978	25.89	0.974	0.786	0.963	-0.161
F4	0.981	6.664	0.958	-0.053	0.974	25.89	0.97	0.96	0.964	-0.151
F5	0.982	5.974	0.943	-0.05	0.986	24.48	0.98	0.915	0.963	-0.15
F6	0.984	7.443	0.964	-0.068	0.991	30	0.982	0.974	0.968	-0.19
F7	0.987	7.245	0.981	-0.065	0.99	29.58	0.983	0.931	0.967	-0.191
F8	0.982	6.994	0.98	-0.061	0.986	28.89	0.98	0.931	0.966	-0.184
F9	0.982	6.888	0.978	-0.06	0.982	28.41	0.981	0.637	0.966	-0.18
F10	0.981	6.746	0.978	-0.058	0.98	28.04	0.979	0.603	0.965	-0.175
F11	0.987	6.875	0.981	-0.059	0.986	28.48	0.985	0.875	0.966	-0.17
F12	0.989	6.736	0.982	-0.057	0.988	28.17	0.985	0.837	0.961	-0.174
F13	0.988	6.545	0.974	-0.002	0.989	26.92	0.986	0.85	0.964	-0.002
F14	0.991	6.637	0.975	-0.052	0.99	27.04	0.984	0.76	0.966	-0.161
F15	0.996	6.099	0.982	-0.048	0.997	26.12	0.982	0.71	0.959	-0.153
F16	0.983	7.54	0.975	-0.07	0.983	29.96	0.981	1.02	0.966	-0.201
F17	0.982	7.392	0.968	-0.067	0.982	29.68	0.98	0.98	0.976	-0.195
F18	0.981	7.259	0.98	-0.065	0.981	29.46	0.976	0.943	0.979	-0.191
F19	0.982	7.133	0.979	-0.063	0.98	29.18	0.979	0.915	0.967	-0.187
F20	0.986	6.999	0.976	-0.063	0.987	28.98	0.982	0.879	0.976	-0.182

***In vitro* wash off test**

The results of in vitro wash off test for selected formulations were shown in Table 8. Mucoadhesive property was studied on the selected formulations (F5, F10, F15 and F20) of alginate beads by *in vitro* wash off test method. The percentage of alginate beads attached to the goat intestinal mucosa after 12 hours is shown in Table 10. Among the selected formulations the alginate beads prepared with drug: polymer 1:5 using calcium chloride as cross linking showed good mucoadhesive property. The following stages may have occurred during mucoadhesion. Initially, an intimate contact i.e., (wetting) between the mucus gel and the swelling of mucoadhesive polymer, which makes the polymer strands to relax, this is followed by the penetration of the mucoadhesive polymer into the mucus gel network and finally the formation of secondary chemical bond between the mucus and the mucoadhesive polymer. It was found that the pH of the medium is important for the hydration, solubility and mucoadhesion of the polymer.[27]

Table 8: In vitro wash off test

S. No.	Formulation code	%Mucoadhesion after 12 hours
1	F5	56%
2	F10	64%
3	F15	72%
4	F20	52%

Scanning electron microscopy analysis

A SEM photograph of formulation (F-15), a single bead taken at 25X magnification, was shown in Figure 10. As seen from the Figure 8, the drug loaded alginate bead was almost of spherical in shape and have rough surface.

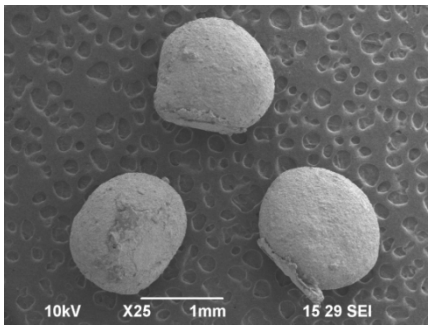


Fig. 8: SEM photograph of selected formulation

CONCLUSION

It was found that sodium alginate along with pectin and xanthan gum substantially controlling the release of candesartan cilexetil from the microbeads. Results of the *in vitro* drug release indicated that the controlled drug release upto 12 hours. These studies demonstrated that candesartan cilexetil can be encapsulated into microbeads having sodium alginate, pectin and xanthan gum backbone by ionic gelation technique having good yield, particle size, entrapment efficiency and *in vitro* drug release profile of micro beads. The microbeads also showed considerable swelling behavior in phosphate buffer pH 6.5. The prepared microbeads showed controlled drug delivery behavior as can be inferred from the release kinetics data, as it follows zero order as well as Higuchi model which confirm its diffusion controlled release behavior although the Korsemeyer-Peppas plot shows anomalous transport suggests that the release is controlled by diffusion as well as by other factors like swelling of the microbeads.

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