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PREPARATION AND EVALUATION OF INSULIN MICROPARTICLES BASED GEL FROM NATURAL SOURCES FOR NASAL DELIVERY

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ABSTRACT: Ionotropic gelation, a characteristic property, has become one of the easy, simple and mild ways of encapsulating variety of drugs, enzymes, and proteins, prepared by various Polysaccharides. The externally crosslinked beads with calcium chloride and zinc chloride were suitable for water soluble and low molecular weight drugs. Insulin, encapsulated in a calcium pectinate gel / Sodium alginate bead by dispersing the insulin and core cross linking agent in a solution of pectin, beads are prepared by spraying the dispersion in calcium chloride by Inotropic gelation. The purpose of present research work was to study effect of different concentration of sodium alginate and pectin solution on the drug entrapment, drug release and stability of Insulin. Insulin is a high molecular weight drug, widely used to treat diabetic patients. The beads were prepared with factorial design and evaluated for, entrapment efficiency, stability and bioadhesivity. The formulated gel was evaluated for administration in the nasal cavity for treatment of diabetics.

INTRODUCTION: The polysaccharides are widely used in oral drug delivery systems because of the simplicity in obtaining desired drug delivery system and drug release profile, to regulate the release of the drug by control of cross-linking, insolubility of crosslinked beads in gastric environment and broad regulatory acceptance. These include sodium alginate, pectin, chitosan, xantan, guar gum, starch, dextran, gellan [1-4]. Among the various applications, polysaccharides are used for oral controlled release matrices, floating or bioadhesive sustained release beads or tablets, enteric effect, colon targeting of drug and for pulsatile drug release [5-9].

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The extent and rate of crosslinking depends on the valency, molecular size, concentration of crosslinking agent, Hardening Agent and speed and curing time during processing [10- 11]. Solubility, molecular size and ionic nature of drug determine the entrapment and drug and drug release. The water soluble and low molecular weight drugs have poor entrapment as compared to insoluble and large molecular Weight drugs [12]. To obtain the higher entrapment efficiency in high molecular weight proteins like insulin with the help of internal cross-linking agents.

The drug entrapment and drug release may be governed by extent of surface and core crosslinking of bead, which is function of penetration of cation into the bead, its molecular size and valency (35 grade). The beads structured with closely packed polymer arrangement and egg-box or three-dimensional bonding may have different drug holding and releasing abilities. The inclusion of core cross linking may be effective for structuring

the core of bead and thus enhancing the entrapment efficiency of drug. The purpose of present research work was to study the effect of core crosslinking and surface crosslinking of pectin on the entrapment efficiency, swelling and drug release of Insulin from cross linked pectinate beads. Calcium carbonate and calcium chloride solution were used as core and surface crosslinking agents respectively. Insulin is a high molecular weight drug, widely used in diabetic patients. Insulin is a peptide hormone produced by beta cells in the pancreas. It regulates the metabolism of carbohydrates and fats by promoting the absorption of glucose from the blood to skeletal muscles and fat tissue and by causing fat to be stored rather than used for energy. The beads were evaluated for micromeritic properties, entrapment efficiency surface topography, and differential scanning calorimetry, swelling study and dissolution study.

MATERIALS: Pectin (LM-104 AS) was the generous gift from CPKelco Pvt. Ltd. (Mumbai. India) Insulin was purchased from the marketed product. Calcium chloride purchased from Sisco Research Lab. Pvt. Ltd. (Mumbai, India). All other chemicals were of analytical reagent grade.

METHODS:

Fabrication of Insulin Loaded Microparticles:

Development Study#1:

Sodium Alginate/Pectin Solution Preparation: The sodium alginate/pectin does not forma a clear solution immediately, Hence the required % of alginate/pectin solution was prepared by weighing the powder and adding the required quantity of water in a beaker and kept in a 2-8°C temperature for 12 hours to swell and form a clear solution. The different % of pectin/sodium alginate solution is prepared by using the different amount of pectin/sodium alginate with same amount of water.

Mixing Uniformity: The mixing uniformity time is to identify the constant time for the entire microparticle size preparation to obtain the uniform dispersion of drug (insulin) in the pectin/alginate solution. To identify the mixing time in the higher concentration of alginate solution (4%) was selected as a worst-case concentration to optimize the mixing time. The solution was checked for the uniformity of drug by sampling in different areas by using micro pipette and different time point. Based on the outcome of the result the time point was fixed the mixing to obtain the mixing throughout the process. The mixing time is selected as 5 min, 10 min and 15 min. Once the mixing time is fixed, for all the preparation the same mixing time will be followed. The study was given in the **Table 1.**

TABLE 1: INSULIN DISPERSION TIME IN ALGINATE/PECTIN SOLUTION OPTIMIZATION

Mixing time optimization study (Stirrer RPM:100)	
Sampling time	**Sampling locations**
5 min	Top, middle and bottom
10 min	Top, middle and bottom
15 min	Top, middle and bottom

Microparticle Preparation: The microparticles were prepared by ionotropic gelation method (Acarturk and Takka 1999) 3000 IU equivalent insulin solution was added to 100 mL of pectin/sodium alginate solution (3% w/v) and stirred for 4minutes using the magnetic bead with 500 RPM/min to form a uniform dispersion of pectin/alginate and insulin.

The solution is sprayed through the spray gun using peristaltic pump with 10 RPM and atomization air pressure 3 kg/cm^2, in the vessel containing calcium chloride solution with magnetic bead stirring. The process is illustrated in **Fig. 1.**

FIG. 1: ILLUSTRATION OF MICROPARTICLE PREPARATION

The main function of the spray gun in the microparticle preparation is to divide the pectin solution stream (as delivered by the pumping device) into very fine droplets. This division is known as atomization and is achieved by forcing a fine stream of liquid through a fine nozzle into the pressurized zone (achieved by compressed air).

The droplet size depends on the nozzle size and available air pressure. These fine droplets, after leaving the nozzle, a micron size or Nano size depends on the air pressure ravels in certain distance in the atmosphere and forms a fine globule and reaches calcium chloride solution. The inotropic gelation takes place in the calcium chloride solution and becomes solid nature.

The curing time was optimized for the curing in calcium chloride as 10 minutes for all trials. The cured microparticles were filtered and dried. Total 16 initial trials were done to finalize the polymer, solution concentration, internal cross-linking agent, peristaltic pump RPM and air pressure for the optimization process. The trial was shown in **Table 2.**

TABLE 2: INITIAL TRIAL FOR THE MICROPARTICLE PREPARATION

Batch- sodium alginte and pectin solution	Specification		
	Peristaltic pump rpm	Air pressure	Internal crosslinking agent ($CACO_3$)
A1 -0.5%	10	3 kg/cm^2	-
A2-1.0%	10	3 kg/cm^2	-
A3-1.5%	10	3 kg/cm^2	-
A4-2.0%	10	3 kg/cm^2	-
A5-2.5%	10	3 kg/cm^2	-
A6-3.0%	10	3 kg/cm^2	-
A7-3.5%	10	3 kg/cm^2	-
A8-4.0%	10	3 kg/cm^2	-
A9 -0.5%	10	3 kg/cm^2	10 mg/ml
A10-1.0%	10	3 kg/cm^2	10 mg/ml
A11-1.5%	10	3 kg/cm^2	10 mg/ml
A12-2.0	10	3 kg/cm^2	10 mg/ml
A13-2.5%	10	3 kg/cm^2	10 mg/ml
A-14-3.0%	10	3 kg/cm^2	10 mg/ml
A-15-3.5%	10	3 kg/cm^2	10 mg/ml
A-16-4.0%	10	3 kg/cm^2	10 mg/ml

The solution concentration, air pressure and the Flow rate (peristaltic pump RPM) was optimized through design of experiments. The micro particles were prepared using pectin/sodium alginate with and without internal cross-linking agent with sodium bicarbonate to check the entrapment efficiency.

Selection of Process Parameter: Based on the initial trial, the process parameters like peristaltic pump RPM, air pressure and the concentration of the internal cross-linking agent were finalized.

Effect of Drying Methods: Since, insulin is a thermolabile molecule, the effect of drying may impact on the quality of the product. Hence three different drying methods were selected to check the drug product stability during drying.

The selected drying methods were:

1. Oven drying
2. Vacuum oven drying
3. Freeze drying

The washed beads were divided into three portions and dried in three different methods like oven drying (OD), freeze drying (FD) and vacuum oven drying (VOD) are carried out for 24 hours. A control batch using pure pectin/sodium alginate microparticles was also prepared using the above procedure with 1% pectin/sodium alginate solution. The obtained microparticles were dried in three methods. Those are oven drying (Metalab, Mumbai), Vacuum oven drying (Metalab, Mumbai) freeze drying (LyoproHeto 3000).

Drying Optimization Study: Drying study to keep the loss on drying to keep the minimum water content to promote the stability of insulin. The placebo gel was prepared with the same concentration and prepared the micro particles with the gram level and the same parameters were kept in the hot air oven, vacuum oven drier and freeze drier. The samples were removed in the different frequency of time and the water loss was checked by loss on drying. Total moisture content was kept Not more than 2% W/W. Based on the drying study the different time was fixed to obtain a same water content in different drying methods. The drying methods were validated by same placebo preparation work was carried out three times and

the moisture content check were conducted, and the drying time was validated. Based on the drying time validation the same time was used to dry the particles in the drug-loaded microparticles.

Oven Dried Particles: The one portion of microparticles were kept in the cleaned butter paper layered petri dish and loaded in the hot air oven (Metalab, Mumbai) at 40°C for 24 hours gives oven dried (OD) beads. The oven dried (OD) beads were collected and kept in the double lined poly bag with the silica gel 1 gram pouch was kept in between poly bag to avoid the moisture interruption in the stability of the insulin.

Vacuum Oven Dried Particles: The second portion of the micro particles of sodium alginate was loaded in the butter paper lined petri-dish and loaded in the vacuum oven with the pressure (Metalab, Mumbai) in 100mmHg pressure at 40°C for 24 hours. The vacuum oven dried (VOD) beads were collected and kept in the double lined poly bag with the silica gel 1 gram pouch was kept in between poly bag to avoid the moisture interruption in the stability of the insulin.

Freeze-dried Particles: The third portion of microparticles were loaded in the 10 mL glass vials and kept in the deep freezer and loaded at -40°C for 3 hours to obtain the frozen beads and the same was kept immediately in the freeze drier (LyproHeto 3000) for 24 hrs under Vacuum (Vaccubrand) 0.06 hecta pascal at room temperature. The Freeze dried (FD) beads were collected and kept in the double lined poly bag with the silica gel 1 gram pouch was kept in between poly bag to avoid the moisture interruption in the stability of the insulin.

Stability Study for Selection of Drying Process: To evaluate the suitability of the drying method, the stability was conducted in the 25°C/60 % RH for 1 month, 3 months to get the final drying process. The stability details were given in **Table 3.**

TABLE 3: STABILITY STUDY FOR SELECTION OF DRYING PROCESS

Stability Condition 25ºC/60%RH	Time Points		
	Initial	1 Month	3 Month
Description	√	√	√
Assay	√	√	√

Selection of Process for the Final Evaluation: The microparticles were dried in the three different drying methods and loaded for stability in the accelerated condition. The microparticles assay was checked to identify any degradation. Based on the assay results the microparticles drying process shall be selected.

Development Study#2: The goal of the development study #2 was to evaluate the risk assessment of the selected excipients and process variables with response of critical parameters in formulation. The significant factors were considered as optimizing the risk with level of change in quantity of solution concentration, atomization air pressure and peristaltic pump RPM. This study will give an understanding if there is any interaction between these variables in the entrapment efficiency and particle size of the formulation. This DOE study helps to establish the robustness of the proposed formula. A full factorial design of experiments (2^3DOE) with one center point was used to study the impact of these three process variables on the response. The combination of solution concentration ranged from 2 %, 3 % and 4 % on evaluating its critical attributes on final proposed formula and its interaction on selected responses of the formulation. These levels are followed with acceptance range limit of atomization air pressure and peristaltic pump RPM. The atomization air pressure selected for formulation studies due to, the air pressure has an impact on the particle size of the proposed formulation. The acceptable range of atomization air pressure with respect to getting particle size range from 80-120µm are ranges from 2.0 kg/cm^2, 3.0 kg/cm^2 and 4.0 kg/cm^2 were selected to evaluate its impact on selected response variables and interaction of this parameter with variable factor in the formulation study. The peristaltic pump RPM and the concentration of pectin/ sodium alginate was optimized with three different RPM of as 5RPM, 10RPM and 15RPM used in this formulation to evaluate the impact of particle size of product as response variables.

The process optimization DOE variables were fed in minitab 18 with one centre point to identify the curvature effect. A total of 9 experiments with different combinations were conducted and the results were evaluated for the particle size and entrapment efficiency. The process variables were illustrated in **Table 4.** The results were uploaded and analysed statistically by using minitab 18. The statistical tools used for the analysis was pare to chart, half normal plot and contour plot with center point to check the curvature effect and without center point for the entrapment efficiency and particle size.

TABLE 4: 2^3 FULL FACTORIAL DOE: STUDY OF EXCIPIENTS AND PROCESS VARIABLES

Factors: Formulation Variables			Levels		
			-1	0	+1
A	Solution Concentration (%)		2	3	4
B	Peristaltic Pump RPM		5	10	15
C	Atomization Air Pressure (kg/cm^2)		2	3	4
Response		**G**	**Acceptable Ranges**		
1	Particle size	Maximize	80 – 120 µm		
2	Entrapment efficiency	Maximize	80- 100 %		

Yield and Drug Content: Microparticles samples were weighed, and process yield were calculated. Microparticles (10 mg) were weighed accurately and extracted using 20 mL of 6.8 pH phosphate buffer in shaking for one hours in rotary shaker (Steelmet Industries, Pune, India). After filtration through membrane filter of pore size 0.45 µm and sufficient dilutions samples were analyzed spectrometrically at 220 nm (Jasco v- 500, Tokyo, Japan). Drug content was calculated from the standard curve of insulin in 6.8 pH phosphate buffer. The entrapment efficiency is calculated using the following formula.

EE= (actual drug content in beads / theoretical drug content in beads) x 100

Particle size Analysis by Malvern: Particle size and particle size distribution of micro particles were determined using particle analyzer (Mastersizer 2000 ver.2.00, Malvern instruments Ltd., Malvern, UK). Using water as a medium. Based on the result of entrapment efficiency and particle size the final formulation will be selected and mixed with biogel. The insulin bio gel was evaluated for suitability of nasal delivery of insulin.

Formulation of Insulin Loaded bio Gel:

Dispersion of Insulin Microparticles in Gel and Content Uniformity: The gel consists of required quantities of gum, Methylparaben sodium (0.05%) and Propylparaben sodium (0.02%) and water. The Methylparaben sodium and Propylparaben sodium were dissolved in the purified water. The required gum (3.0%) was added after dissolving the methylparaben sodium and Propylparaben sodium in the solution and allowed to swell. The dispersion was mixed using overhead stirrer to get the uniform, clear, smooth gel. Now the insulin micro particles were added in the gel and mixed with the overhead stirrer with rpm of 100. The insulin microparticles were dispersed in the gel equivalent to 100 IU/g.

Evaluation of Content Uniformity: To obtain uniform therapeutic efficiency, the content uniformity of the active pharmaceutical ingredient in gel formulation is an important and deciding factor. To confirm the uniformity, microparticles were mixed in the gel, sampled from different locations and analyzed for uniformity of insulin. The content uniformity was checked for variable mixing time 10, 15 and 20 minutes. For performing content uniformity, the gel equivalent to the 10 IU/mg was taken from 3 different locations from the container, active pharmaceutical ingredient was extracted and analyzed by using UV spectrometer at 220 nm. The specification for the content uniformity was 80-120%. The results were illustrated in the **Table 5.**

TABLE 5: TIME OPTIMIZATION STUDY FOR INSULIN MICROPARTICLE CONTENT UNIFORMITY

Mixing time optimization study (Stirrer RPM:100)		
Sampling time	**Sampling locations**	**Results**
5 min	Top, middle and bottom	85.6 ± 12 %

10 min	Top, middle and bottom	95.6 ± 9 %
15 min	Top, middle and bottom	90.1 ±12 %

Evaluation of Insulin Loaded Bio-gel: The evaluation of homogeneity, appearance, spreadability, pH, freeze thaw study, and viscosity was carried out as per earlier procedure in the bio gel preparation. The IR spectroscopy, DSC-TGA, XRD and SEM studies were carried out to study the nature of microparticles and gel formulation.

***In-vitro* Release:** The *in-vitro* release of insulin bio gel was carried out in pH 5.5 phosphate buffer to have bio-relevant nature in the nasal cavity. The accurately weighed nasal gel equivalent 50 IU in placed in the dissolution apparatus with 500 ml of media with 37±2°C. The 2 ml samples were withdrawn, and the fresh medium was replaced for each withdrawal. The sampling time points were 15, 30, 60, 120, 180 & 240 minutes. The withdrawn samples were analyzed spectrophotometrically at 220nm.

Stability Monitoring Insulin Bio Gels: To conduct stability study, the final insulin gel formulation was packed in the glass vial with rubber stopper followed by aluminum seal to conduct the stability study. The packed samples were loaded in different conditions like 2-8°C, and 25°C/45% RH to evaluate the stability. The stability schedule is given in **Table 6.** The loaded samples were analyzed for the description, assay, pH and dissolution periodically.

TABLE 6: STABILITY MONITORING CONDITIONS FOR INSULIN BIO GELS

Conditions	1 month	2 months	3 months	6 months	12 months
25°C/45%RH	√	√	√	√	√
2-8°C	√	√	√	√	√

RESULTS AND DISCUSSION:

Evaluation of Biogel Properties:

Homogeneity and Appearance: The appearance of the almond gel was the white translucent gel and looked homogeneous when particles were fine. Hence, particle size of the almond gum is significant in this process. Hence the almond gum powder passed through #100 mesh was used to get uniform gel throughout the process. The appearance of garlic bio gel is fine, smooth gel with stickiness. The swelling nature of garlic polymer is comparatively less and forms homogeneous gel. The gel prepared with banana skin polymer was homogeneous, white colored and slippery in nature. The tomato skin biogel looked homogeneous, off-white colored with slight characteristic odor.

Spreadability: The spreadability was carried out for all four-gel formulation and results were presented in **Table 7**. Based on results, the spreadability of almond biogel and garlic biogel has comparable spreadability. The Banana biogel and Tomato biogel have less spreadability than other two biogel formulations.

TABLE 7: SPREADABILITY STUDY OF BIOPOLYMERS

S. no.	Biopolymer	Spreadability
1	Almond biogel	17.8 ±2.8
2	Garlic biogel	20.8± 1.8
3	Banana skin biogel	32.8± 2.7
4	Tomato skin biogel	47.5± 3.8

pH Measurement of the Gels: The pH of the gel was carried out using 2% gels. The experiment was conducted triplicate to get the mean value. The results were presented in **Table 8**. Based on results, the pH of the almond gum has the equivalent pH nature of nasal cavity and insulin stability pH range. Hence, stability of insulin may be promoted by the product nature of gel itself. The garlic, tomato skin biopolymer has pH in the acidic side and banana polymer has in the basic side. The 1% gel and 2% gel with microparticles also show the same pH range. The gel pH is not impacted or altered by addition of insulin microparticles.

TABLE 8: pH MEASUREMENT OF BIO GELS

S. no.	Gel sample (2%)	pH
1	Almond polymer Gel	5.6± 0.3
2	Garlic polymer Gel	4.1± 0.4
3	Banana skin polymer Gel	6.7± 0.3
4	Tomato skin polymer Gel	4.5± 0.5

Freeze thaw Study: The product exposed to extreme conditions during transportation. Hence, freeze thaw study was carried out to check the physical appearance, assay and change in pH. The study conducted for 7 cycles, after completion of 7 cycles the appearance, pH and assay were checked, and the results were presented below **Table 9**. Based on the study results the almond bio gel gives goods stability to insulin followed by banana skin bio gel. The other two formulations show degradation in assay. This may be due to the changes in the pH of the product.

TABLE 9: FREEZE THAW STUDY OF BIOGELS

Almond bio gel	Appearance	PH	Assay
Initial	White clear gel	5.7	96%
After 7 Cycle	White clear gel	5.9	92%
	Garlic Bio gel		
Initial	Light yellow gel	3.6	97%
After 7 Cycle	Dark yellow gel	2.7	65%
	Banana skin Bio gel		
Initial	White gel	6.7	96%
After 7 Cycle	Off white gel	5.6	87%
	Tomato skin Bio gel		
Initial	White to off white gel	4.2	93%
After 7 Cycle	off white gel	6.7	78%

Viscosity: The viscosity of the gel was carried out in the 1%, 2%, 3% and 4% W/W gel. The torque value observed in the gel was converted and given as viscosity. The viscosity results were presented in **Table 10.** The almond biogel possesses the highest viscosity than all other natural polymers.

TABLE 10: VISCOSITY OF BIO GEL

Concentration	1%	2%	3%	4%
Almond Bio gel (CPS)				
Viscosity	180± 10.2	250±22.5	320±18.8	480±32.2
Garlic Bio gel (CPS)				
Viscosity	80± 11.8	100± 14.8	120±12.9	160±20.3
Banana skin Bio gel (CPS)				
Viscosity	89± 8.6	104± 11.2	147± 14.8	182± 22.5
Tomato skin Bio gel (CPS)				
Viscosity	76± 10.8	82± 14.2	92± 18.5	122±22.8

Measurement of Mucoadhesion Strength: The gum/extract swells in the presence of water and forms a gel structure. Due to the nasal application, application of gel in the nasal cavity also to be considered. Hence the gel was considered for the pourable nature also. Hence, mucoadhesive strength is comparatively less when compared to the dry gum/extract. The mucoadhesive strength of gum/extract is presented in the **Table 11**. The almond gel possesses the highest mucoadhesive strength than the other natural polymers.

TABLE 11: MUCOADHESIVE STRENGTH OF BIOGELS

S. no.	Formulation	Mucoadhesive strength (mN)[a]
1	Almond biogel	28 ± 5.2
2	Banana skin biogel	24± 4.2
3	Tomato skin biogel	18±3.8
4	Garlic biogel	15± 4.5

Toxicological Study: The toxicological study was not conducted as the published literature concluded the extracts has no toxic effects and safe to animals and human beings. The literatures were given below. The acute toxicity study was carried out and concludes and safe up to 200 mg/kg [13]. Acute toxicity study for the garlic extract was carried out by the researchers in the Wister rats and concluded as there is no toxic activity [14]. The toxicity study for the banana skin extract was carried out in the Swiss albino mice and reported safe [15]. Toxicology study of the tomato pomace with skin was studies

and reported as safe for the consumption [16]. Based on the literature support the four polymers were considered as safe and further toxicology study was not conducted.

Development Study#1: Based on the Pre-Formulation study and the literature review, the initial development study started to prepare the insulin loaded microparticles. To prepare the micro particles the solution concentration 0.5 - 4% of the solution were used and the mixing uniformity of insulin the solution was checked. The samples from the top middle and bottom of the beakers were withdrawn and analyzed. The results were shown below in **Table 12** based on the results of the 10 minutes selected for the mixing time of API in the polymer solution.

TABLE 12: CONTENT UNIFORMITY OF INSULIN AT DIFFERENT TIME POINT AND LOCATION

Time/ RPM:100	Top	Middle	Bottom
5 min	92±4.5%	94± 5.2%	105± 3.8%
10 min	97±%2.8	98± 4.5%	99± 2.6%
15 min	96±%4.8	95±5.8%	92± 4.2%

Microparticle Preparation: To prepare Microparticles, new innovative and reproducible with less manual intervention method was selected. The automated process reduces the manual intervention and improves the quality of the product. The micro/nano particles were prepared by pumping the solution in the peristaltic pump. The pumped liquid is surrounded by a stream of air, friction between liquid and air stream causes atomization. Based on the flow of liquid and air pressure the particle size is determined. The atomization of liquid and the effect of viscosity on the particle size is described in Fig**. 2**. to prepare microparticles, low viscous solutions of 0.5, 1.0, 1.5, 2, 2.5, 3.0 & 4.0% concentration of solution were prepared. The prepared solutions were stored in 2°C-8°C at the same temperature the insulin is mixed and sprayed in the calcium chloride solution.

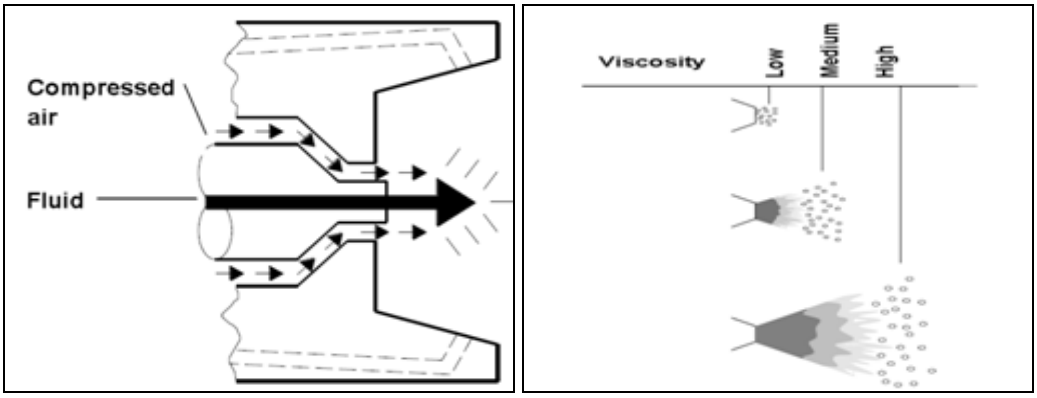

FIG. 2: SOLUTION FLOW DIAGRAM FROM THE SPRAY GUN

Selection of Polymer Concentration: The initial trials with different concentration of sodium alginate/pectin solution (0.5%, 1%, 1.5%, 2%, 2.5%, 3% & 4%) and atomization air pressure (3 kg/cm^2) and different peristaltic pump RPM were taken th and finalized. The stabilty data of the initial and upto three months were compard for the sodium alginate and pectin based microparticles, the sodium alginate deads were shown promising stability than the pectin beads. Hence the sodium alginate beads were finalized for further study. The mixing uniformity of the insulin in the solution was checked in the three places for every time point and found 10 minutes mixing with 100RPM is good in getting uniform mixing. The results of the content uniformity in 10 minutes were found 95±4.8%. The observations were presented in **Table 13.**

TABLE 13: STABILITY RESULTS OF INSULIN ALGINATE MICROPARTICLE

25ºC/60%RH	Initial	1 Month	3 Month
Description	off white microparticles	off white microparticles	off white microparticles
Assay	96.2%	94.8%	93.9%

The initial trials with 0.5%, 1.0% and 1.5% pectin and sodium alginate micropartiles were not in the significant round shaped particles. The concentration above 2.0% the satissfactory shape of the particles were formed. Hence, it was planned to select the pectin/alginate solution above 2.0%. The internal crosslinking agents were used to promote the entrapment efficiency and checked for the initial stability study.

TABLE 14: STABILITY RESULTS OF INSULIN PECTIN MICROPARTICLE

25ºC/60%RH	Initial	1 Month	3 Month
Description	off white microparticles	off white microparticles	off white microparticles
Assay	97.2%	92.8%	88.6%

Selection of air Pressure and Pump RPM: Based on the initial trial results, the 10 RPM of peristaltic pump and 3.0 kg/cm^{2}produced the desirable particle size for use in the nasal drug delivery. Hence the atomization pressure selected as 3.0 kg/cm^2 and peristaltic pump RPM is selected as 10 RPM.

Selection of Drying Method: The evaluation of the three drying methods was done and selected based on output in the particle shape, size, temperature, stability and time for drying. The oven drying method takes around 24 hours at 40° C to provide the dried particle. The particles were retained on the #100 sieve. Hence the particle size is above 150 microns. Freeze drying does not require the temperature; however, the resulting particles were retained on the #100 mesh and highly porous in nature. The vaccum oven dried particles were around in shape, 4 hours with 40°C is required for drying. The particles obtained passed through the #100 mesh. Based on the evaluation of the three methods vacuum drying is selected as ideal methotd for drying the insulin microparticles.

Stability Study of Micro Particles: The stability study of the micro particles with batch number A-6 and A-14 with vaccum oven drying were placed in the 25°C/40% RH. The stability results were interpreted to select the polymer for the further process. The results were presented below in **Table 16** for alginate based microparticle and **Table 15** for pectin based microparticles. Based on the stability results, the sodium alginate microparticles showed good stability than the pectin beads. Hence, the sodium alginate was taken for further microparticle preparation.

TABLE 15: PROTOTYPE FORMULA

Ingredients	Concentration
Insulin	30IU/mg
Sodium Alginate/pectin concentration	3%
Peristaltic pump RPM	10

Prototype Formula: Based on the initial results and trials, the prototype formula was derived. The formula is represented in the **Table 15.**

Statistical optimization methods were used to optimize the parameters which are involved in the micro particle preparation. The particle size of the solution depends on its solution viscosity, solution flow rate and atomization air pressure. Based on the initial trial and the prototype formula was finalized and the design space was identified with DOE trials in the development study#2.

TABLE 16: INITIAL EXPERIMENTAL RESULTS-PARTICLE SIZE

Batch- sodium alginte and pectin solution	Specification		Particle size	
	Peristaltic pump rpm	Air pressure	Sodium alginate	Pectin
A1 -0.5%	10	3 kg/cm^2	Not done	Not done
A2-1.0%	10	3 kg/cm^2		Not done
A3-1.5%	10	3 kg/cm^2		38 µm
A4-2.0%	10	3 kg/cm^2	45 µm	49 µm
A5-2.5%	10	3 kg/cm^2	52 µm	58 µm
A6-3.0%	10	3 kg/cm^2	65 µm	63 µm
A7-3.5%	10	3 kg/cm^2	72 µm	78 µm
A8-3.5%	10	3 kg/cm^2	85 µm	88 µm
A12- 2.0% with 10 mg $CaCO_3$	10	3 kg/cm^2	52 µm	58 µm
A13-2.5% with 10 mg $CaCO_3$	10	3 kg/cm^2	66 µm	69 µm
A14-3.0% with 10 mg $CaCO_3$	10	3 kg/cm^2	72 µm	78 µm
A-15-3.5% with 10 mg $CaCO_3$	10	3 kg/cm^2	85 µm	92 µm
A-16-4.0% with 10 mg $CaCO_3$	10	3 kg/cm^2	90 µm	98 µm

Development Study#2: The goal of the formulation development study was to evaluate the risk assessment of the selected excipients and process variables with response of critical parameters in formulation. The significant factors were considered as optimizing the risk with level of change in quantity of solution concentration, atomization air pressure and peristaltic pump RPM and to understand if there was any interaction of these variables with response to particle size and entrapment efficiency in the formulations. This study also wants to establish range of the process variables of the proposed dosage form. To study the robustness of the formulation, three formulation factors on the response variables were selected. The 2^3 full factorial design of experiments (DOE) with one center point was selected for this study. The different combination of experimental outcomes in the Minitab 18 is represented in **Table 16.** The selection solution concentration ranged from 2 %, 3 % and 4 % on evaluating its critical attributes on final proposed formula and its interaction on selected responses of the formulation. These levels are followed with acceptance change limit of atomization air pressure and peristaltic pump RPM.

The atomization air pressure selected for formulation studies were based on air pressure has an impact on the particle size in the proposed formulation. The acceptable range of atomization air pressure with respect to getting particle size range from 80-120µm are ranges from 2.0 kg/cm^2, 3.0 kg/cm^{2}and 4.0 kg/cm^2 were selected to evaluate its impact on selected response variables and interaction of this parameter with variable factor in the formulation study. The peristaltic pump RPM was optimized with three different RPM of as 5 RPM, 10 RPM and 15 RPM used in this formulation to evaluate the impact of particle size of product as response variables. The outcome of particle size analysis and entrapment efficiency was presented in the **Table 17.**

TABLE 17: EXPERIMENTAL RESULTS: 2^3FULL FACTORIAL DOE TO PROCESS VARIABLES AND EXCIPIENTS

Batch No.	Factors: Formulation Variables			Responses	
S. no.	A: Solution concentration (%)	B: Peristaltic Pump RPM	C: Atomization Air Pressure (kg/cm^2)	Y1: Particle size	Y2: Entrapment efficiency
	%	%	(mm)	(µm)	(%)
1	2	15	2	96.74	90
2	2	5	4	21.7	50
3	4	5	2	108.6	85
4	2	15	4	43.32	55
5	3	10	3	104.46	88
6	4	15	4	112.32	95
7	2	5	2	105.72	90
8	4	5	4	28.03	45
9	4	15	2	110.53	92

CONCLUSION:

Formulation Development Study Conclusions: The formulation composition was finalized based on Formulation Development Studies #1 and #2.

The prototype formula arrived based on the initial experimental studies by analyzing the process and product parameters in the first study.

In the second study, DOE trials were conducted to identify the critical parameters which may affect the quality of the product like particle size and entrapment efficiency. The atomization air pressure is a critical parameter for the particle size and critical parameter for the entrapment efficiency. The prototype Formula was presented in **Table 18.**

TABLE 18: PROTOTYPE FORMULA FOR INSULIN MICROPARTICLE BASED BIOGEL

Ingredients	Concentration
Insulin	30IU/mg
Sodium Alginate/pectin concentration	3%
Peristaltic pump RPM	10
% calcium chloride solution	2%
Atomization air pressure	3 kg/cm^2

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