International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 10 Issue 5 ǁ 2022 ǁ PP. 68-85

Formulation and Optimization of Gastroretentive Mucoadhesive Microspheres of Irbesartan using Chitosan

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ABSTRACT: *The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and then maintain the desired drug concentration. A well designed controlled drug delivery system can overcome some of problems of conventional therapy and enhance therapeutic efficacy of the given drug. There are various approaches in delivering therapeutic substance to the target site in sustained and controlled release fashion. One such approach is using microspheres as carriers for drug. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers which are biodegradable in nature ideally having particle size less than 200µm.Various synthetic and natural materials are used for the preparation of microspheres. Microspheres are having wide range of applications because of controlled and sustained release. It is a very important carrier for safe and effective in vivo drug delivery.*

Keywords: *Microspheres, Irbesartan, Chitosan, microencapsulation, mucoadhesive microsphere*

Date of Submission: 15-05-2022 Date of acceptance: 29-05-2022

I. INTRODUCTION

Microspheres are solid, approximately spherical particles ranging 1-1000µm in size. They are made up of polymeric substances, in which the drug is dispersed throughout the microsphere matrix. The substances used in the formulation are biodegradable, synthetic polymers, and natural products. The natural polymers of choice are albumin and gelatin and the synthetic ones are polylactic acid and polyglycolic acid. The polymers used to manufacture microspheres are chosen according to their solubility, stability profile, safety, and economic suitability. [1, 2]

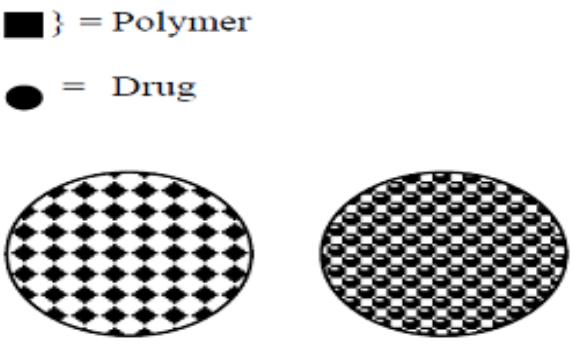

Figure 1: Microsphere

Microspheres are multiparticulate drug delivery systems that are prepared to obtain prolonged or controlled drug delivery to improve bioavailability, stability also target the drug to a specific site at a predetermined rate. Microspheres are characteristically free-flowing powders having a particle size ranging from 1-1000µm consisting of proteins or synthetic polymers. [3]

Classification of microspheres:
(a) Microcapsules
(b) Micromatrices
Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersing throughout the microsphere's matrix. They are made up of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products. [4]

Types of microspheres
1. Bioadhesive microspheres
2. Mucoadhesive microspheres
3. Magnetic microspheres
4. Floating microspheres
5. Radioactive microspheres
6. Polymeric microspheres

a. Biodegradable polymeric microspheres
b. Synthetic polymeric microspheres

Mucoadhesive microspheres
Mucoadhesive microspheres are of great pharmaceutical interest due to their adhesive nature to the mucous membrane of the nasal cavity, eye, and urinary tract. These systems are well suited for both systemic as well as localized. Mucoadhesive microspheres either consist of an entire mucoadhesive polymer or have an outer coating. Better absorption with improved bioavailability of various drugs due to high contact of dosage with mucous membrane and specific drug targeting to the particular site are the main advantages that make them an effective drug delivery carrier for a variety of drugs. [5]
The materials used in the preparation of Microspheres are as follows:
Polymers are generally used in the preparation of microspheres. They are classified into two types:
1. Natural polymers- Chitosan, Gelatin, Starch
2. Synthetic polymers- Lactides, Polymethyl Methacrylate (PMMA) [7]

Advantages of microsphere delivery system: [8]
- ❖ Better processability, improves solubility, dispersibility, flowability etc.
- ❖ Shelf-life enhancement by preventing degradative reactions.
- ❖ Safe handling of toxic materials.
- ❖ Masking of odor or taste.
- ❖ Enzyme and microorganism immobilization.
- ❖ Controlled and targeted drug delivery.
- ❖ Proper handling.
- ❖ Improved bioavailability & stability.

II. METHODOLOGY:

Preformulation Studies
Preformulation studies can be defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients.
Characterization of drug molecule is very important and basic step of preformulation studies. [9]
Drug characterization
The drug was characterized by various official tests of identification. It includes determination of Melting point, solubility, absorption maxima (λ max), UV spectroscopy, and partition coefficient.
Melting point determination
Melting point was determined by capillary fusion method. A small amount of drug was filled in capillary and it was placed in melting point apparatus. Then, the temperature at which drug crystals started melting and turned into liquid was noted down. [10]
Partition coefficient
A shake-flask technique was used to determine log P values. The partition coefficient was calculated by using the formula:

$$P.C. = C_o/C_w$$

Where, P.C. = Partition coefficient;
C_o = Concentration of drug in n-octanol phase;

C_w = Concentration of drug in distilled water

Determination of Ultraviolet absorption maxima (λ_{max})

The molecules present in the drug solution absorb light of particular wavelength when exposed to light in UV region of spectrum.

The absorption of light depends upon the type of electronic transition associated with the absorption. The 0.01% w/v solution of the drug in distilled water was scanned between 200-400 nm and absorption maximum was determined spectrophotometrically (Shimadzu-1700 Japan). [11]

Preparation of Calibration Curves

i. Preparation of PBS 7.4- Dissolve 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8 g of sodium chloride in sufficient amount of water to produce 1000 mL (Indian Pharmacopoeia, 2007). Adjust the pH, if necessary.

ii. Preparation of standard stock solution of drug in PBS 7.4

A standard stock solution of drug was prepared by dissolving accurately weighed 10 mg of in PBS 7.4 using magnetic stirrer and the volume was made up to 100 ml, to obtain a stock solution of 100 µg/ ml.

iii. Calibration curve in distilled water- The calibration curves of drug was prepared in phosphate buffer and distilled water. The method estimated the drug concentration in the range of 2-20 µg/mL in both media and it followed the Beer's Lambert law in the same concentration range. 10 mg accurately weighed drug was dissolved in 100 mL with the different media resulting in a stock solution of 100 µg/mL. From the stock solution, aliquots of 0.2, 0.4, ……, 1.8, 2.0 mL were withdrawn in a series of 10 mL volumetric flasks and diluted to 10 mL with media. This gave a concentration range of 2, 4….. 18, 20 µg/mL. The absorbance of each solution was measured in U.V spectrophotometer at λ_{max} 246 nm. [12]

Solubility studies

For determination of qualitative or crude solubility, a known amount of the drug (10 mg) was suspended in the various solvents (dil. sulphuric acid, dil. hydrochloric acid, sodium bicarbonate, methanol, ethanol, propanol-2-ol, acetonitrile) and shaken for 30 min in water bath shaker. The solubility was observed by visual inspection. For quantitative solubility study, a defined quantity (10 mg) of drug was taken in each thoroughly cleaned test tube. 10 mL of different solvents (dil. sulphuric acid, dil. hydrochloric acid, sodium bicarbonate, methanol, ethanol, propanol-2-ol, acetonitrile) were added and test tubes were tightly closed. After shaking for 24 h the mixture was filtered. The drug in the supernatant solution was determined spectrophotometrically at λ_{max} 246 nm. [13]

Drug polymer interaction studies

Desired quantity of drug with specified excipients (chitosan) in the ratio 1:1, 1:2 and 1:0.5 w/w drug-polymer were taken and mixed thoroughly and filled in dried vials. The vials were sealed and kept at 45°C for two weeks. The vials were examined daily at regular interval for discoloration, clump formation and liquefaction. The infrared absorption spectra of physical mixture of polymer and drug were run for drug excipients compatibility studies between 400 cm^{-1} 4000 cm^{-1} by using Perkin Elmer FTIR spectrophotometer (RXIFT-IR system, USA). [14]

Formulation of chitosan- Irbesartan gastro-retentive mucoadhesive microspheres:

Preparation of Chitosan solution

For the formulation of mucoadhesive microspheres, chitosan solution of different concentration was prepared with the help of glacial acetic acid (1% w/v) and distilled water. The following mixture of Chitosan was kept at room temperature (20°C) for 24 hours with continuous stirring. The mixture was stirred magnetically until the polymer was completely dissolved and a viscous solution is formed. The solution was filtered through glass-wool to remove undissolved particles of chitosan. [15]

Preparation of drug solution

Irbesartan (IRB) is insoluble in aqueous solvents. To prepare the drug mixture about 300mg of Irbesartan drug was mixed with few ml of water and triturated vigorously until damp mass/ mixture was obtained. It was stirred until a homogenous mixture is formed. [16]

Preparation of continuous phase

The continuous phase for the preparation of microspheres is prepared by adding light liquid paraffin and heavy liquid paraffin in the ratio of 1:1 and Span 80 in 0.5 % w/v concentration as surfactant.

Drug loading and preparation of microspheres [16, 17]

Aqueous drug solution is gradually added to the chitosan solution with continuous stirring. The mixture is then stirred for further 24 hours at room temperature (20°C). This solution is further added to continuous phase under constant stirring (1800 rpm) using three blade propeller stirrer to form a w/o emulsion. This procedure is followed by addition of 0.25 ml of gluteraldehyde (25% *v/v*) dropwise at 15, 30, 45 and 60 min, respectively. The stirring is continued for 3 to 4 hrs. The microspheres so obtained are separated by centrifugation and

washed with petroleum ether to remove liquid paraffin. The microspheres are suspended in 5% *w/v* sodium bisulfite solution and stirred for 15 min to remove residual gluteraldehyde. Final washing is done with distilled water. The microspheres are dried and stored in a vacuum desiccator. The polymer to drug ratio and stirring speed are varied in batches and is depicted in Table 1

S. No.		1	2	3	4	5	6	7	8	9
Batch		**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
Variables coded	**X1**	-1	-1	-1	0	0	0	1	1	1
	X2	-1	0	1	-1	0	1	-1	0	1

Variable level	Low (-1)	Medium (0)	High (1)
Polymer-drug ratio (X1)	1:1	3:1	6:1
Stirring speed (X2) rpm	500	1000	1500

Table 1 Irbesartan Microspheres Batches using 3^2 Full Factorial Design Layout

Optimization of Microspheres Formulation Using 3^2 Full Factorial Designs

A statistical model incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^{\ 2} + b_{22}X_2^{\ 2}$$

Where, Y is the dependent variable, b_0 is the arithmetic mean response of the nine runs, and bi is the estimated coefficient for the factor Xi. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors were simultaneously changed. The polynomial terms ($X_1^{\ 2}$ and $X_2^{\ 2}$) were included to investigate non-linearity. On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables i.e. polymer-to-drug ratio (X_1) and the stirring speed (X_2) on dependent variables % mucoadhesion, the time required for 80 % drug dissolution (t80), drug entrapment efficiency, particle size and swelling index. [18]

Characterization of Chitosan Irbesartan mucoadhesive microspheres:

Determination of Particle size

The particle size of the microspheres was determined by using optical microscopy method. Approximately 300 microspheres were counted for particle size using a calibrated optical microscope.

Determination of Scanning Electron Microscope

A scanning electron microscope was used to characterize the surface topography of the microspheres. The microscope was equipped with electron optical system consisting of 0.5–30 kV capacity electron gun and an electron detector. The microspheres were placed on a metallic support with a thin adhesive tape and were coated with gold under vacuum. The surface was scanned and photographs were taken at 30 kV accelerating voltage for the drug loaded microspheres. [19, 20]

Angle of repose: It is a maximum angle possible between the surface of pile and the horizontal plane. The lesser the angle of repose, more is the free flowing granules and vice-versa.

$$\tan\theta = h/r$$

$$\theta = \tan^{-1} h/r$$

Where, h = height of pile, r = radius of the pile base

Angle of repose	Flow Property
< 25	Excellent
25-30	Good
30-40	Moderate to passable
> 40	Poor

Table 2 Comparison between angle of repose and flowability

Bulk density: It is the ratio of weight of powder to the volume it occupied and expressed in gm/cm^3. The bulk density depends on the particle size distribution, shape of particles and tendency of particles for adhesion.

The bulk density and bulkiness were determined by following method: A sample of about 50gm of granules was carefully transferred into 100ml graduated cylinder and the bulk volume (Vb) and weight of the powder (M) was determined. The bulk density was calculated using equation,

$$\rho b = \frac{M}{Vb}$$

Where, ρb = Bulk density, M= Mass of granules in grams, Vb= Bulk volume of granules in cm^3

Tapped Density: After measuring the bulk volume the same measuring cylinder was set into tap density apparatus. The tap density apparatus was set to 100 taps drop from a height of 1 inch at 2 seconds interval. The minimum volume (Vt) occupied in the cylinder and the weight (M) of the blend was measured.

$$\rho t = \frac{M}{Vt}$$

Where M = Weight of powder, V t = Volume after tapping

Compressibility index: It is one of the most important parameter to characterize the nature of powders and granules. The simplest way of measurement of free flow of powder is compressibility. The indication of the ease with which a material can be induced to flow is given by compressibility index.

I = [(Vb - Vt) / Vb] x 100 Where, Vb = Bulk volume, Vt = Tapped volume.

Hausner ratio: Hausner ratio is an indirect index of ease of powder flow. Hausner's ratio is an important character to determine the flow property of powder and granules. [21, 22]

Hausner ratio = ρt / ρd Where, ρt = Tapped density, ρd = Bulk density

Carr's Index (%)	Hausner's Ratio	Flowability
<10	1-1.11	Excellent
11-15	1.12-1.18	Good
16-20	1.19-1.25	Fair
21-25	1.26-1.34	Passable
26-31	1.35-1.45	Poor
32-37	1.46-1.59	Very Poor
>38	>1.60	Very very poor

Table 3 Comparison between compressibility index, Hausner's ratio and flowability

Determination of Swelling Index [23]

The swelling ability of the microspheres in physiological medium was determined by allowing the microspheres to swell to their equilibrium in phosphate buffer pH 6.8. Accurately weighed quantity of microspheres (50 mg) was immersed in a little excess of phosphate buffer (pH 6.8) and kept for 10 hr. At every 1 hr interval, the microspheres were removed, blotted with a piece of paper towel to absorb excess buffer on surface and then reweighed. The difference in weight initially and after swelling was found out up to 10 hr.

The following formula was used for calculation of percentage of swelling:

$$S_{sw} = \left(\frac{Ws - Wo}{Wo}\right) \times 100$$

Where, S_{sw} = Percentage swelling of microspheres;

W_o = Initial weight of microspheres; and

W_s = Weight of microspheres after swelling.

Determination of mucoadhesion

A simulated mucosa with agar-agar and mucine is employed. Briefly, 17 g of agar–agar is mixed with 700 mL of water in a beaker. The mixture is stirred and heated to 95°C for 15 min. After cooling at 70°C, 2.5 g of mucin is added and the final mixture is homogenized. Finally, before reaching room temperature, 30 mL of the mixture is added to beakers with a 6 cm diameter and left to solidify at room temperature. Once the simulated mucosa is produced, 100 mg of formulation is deposited on it and left to rest for 2 min. Then, the formulation is covered with 60 mL of simulated gastric fluid (2g sodium chloride, 3.2g purified pepsin, and 7mL hydrochloric acid in 1 L of purified water, final pH = 1.2) and an orbital agitation at 150 rpm at 37°C is applied. Mucoadhesion differences are obtained based on the time required to detach each formulation from the simulated gastric mucosa (indirect measurement of mucoadhesion as resistance to agitation, measured by the naked eye). [24]

Drug Entrapment Efficiency

100mg of accurately weighed microspheres are crushed in a glass mortar-pestle and the powdered microspheres are suspended in 10 mL phosphate buffer (pH 7.8). After 24 h the solution is filtered and the filtrate is analysed for the drug content. The drug entrapment efficiency is calculated using the following formula:

$$\frac{Practical\ drug\ content}{Theoretical\ drug\ content} \times 100.$$

***In Vitro* Drug Release Studies**

The *in vitro* dissolution studies are performed by USP-30 type I dissolution apparatus at 50 rpm. The dissolution medium consisted of 0.1N hydrochloric acid for first 2 h and the phosphate buffer with pH 6.8 for the next 3 to 12 h (900 mL) and the medium is maintained at 37°C ± 0.5°C. This simulated the gastrointestinal pH. An aliquot (5 ml) is withdrawn at specific time intervals and replaced with the same volume of fresh medium at same temperature. The withdrawal sample is filtered through 0.45 μm filter paper. Next, its drug content is determined by UV-visible spectrophotometer at 246 nm. The samplings are performed in triplicate manner (n =

3). Mean percent cumulative drug release is plotted against time of release. The dissolution profile of all the formulations is subjected to kinetic modelling such as zero-order, first order, Higuchi and Korsmeyer–Peppas models to know the drug release mechanisms. [25]

Determination of Release Kinetics [26]

The mathematical models are used to evaluate the kinetics and mechanism of drug release. The model that gives high correlation coefficient (r) value is considered as the best fit of the release data.

Different mathematical models used are:

1. Zero order release model
2. First order release model
3. Higuchi release model
4. Korsmeyer- Peppas release model

Zero order release model [27]

It describes the systems where the drug release rate is independent of its concentration of its dissolved substances. The equation for zero order release is:

$$\mathbf{Q_t = Q_0 + K_0 t,}$$

Where, Q_t = Initial amount of drug,

Q_0 = Cumulative amount of drug release at time t,

K_0 = Zero order release constant

First order release model

It describes that release is concentration dependent. This model has been also used to describe absorption and elimination of drug. The first order equation is:

$$\mathbf{LogQ_t = Log\ Q_0 + Kt/2.303}$$

Where, Q_t = Initial amount of drug,

Q_0 = Cumulative amount of drug release at time t,

K = Zero order release constant,

t = Time in hours

Higuchi release equation [28]

The Higuchi equation suggests that the drug release by diffusion mechanism. Higuchi's model as cumulative percent drug dissolved vs. square root of time. The Higuchi equation is:

$$\mathbf{Q = K_H\ t^{1/2}}$$

Where, Q = Cumulative amount of drug release at time t,

K_H = Higuchi constant, t = time in hours

Korsmeyer- Peppas model

Korsmeyer- Peppas developed a simple, semi-empirical model, relating exponentially the drug release to the elapsed time (t). The equation for Korsmeyer- Peppas model is:

$$\mathbf{F = (M_t / M) = K_m t^n\ 'or'\ M_t / M = at^n}$$

Where, F = Fraction of drug release at time t,

M_t = amount of drug release at time t,

M = Total amount of drug in dosage form,

K_m = Kinetic constant,

n = Diffusion exponent for the drug release that is dependent on slope of dosage form, t = Time in hours

If diffusion is the main drug release mechanism, a graphical representation of the log cumulative percentage drug release vs. log time should originate as a straight line. Under some experimental situations, the release mechanism deviates from the Fickian diffusion following an anomalous diffusion or non-fickian diffusion, which refers to the combination of both diffusion and erosion controlled rate release. [29]

Release exponent (n)	Drug transport mechanism	Rate as a function of time
n = 0.45	Fickian diffusion	$t^{-0.5}$
0.45 < n < 0.89	Non- fickian diffusion	t^{n-1}
n = 0.89	Case II transport	Zero order release
n > 0.89	Super case II transport	t^{n-1}

Table 4 Diffusion exponents and solute release mechanism from formulations

Stability studies

Stability study of the formulation is conducted at different temperature conditions according to ICH guidelines at $25^oC \pm 2^oC$ / 60 % ± 5% RH for real and $40^o C \pm 2^o C$ / 75 % ± 5% RH for accelerated stability studies as per ICH guidelines for a period of 3 months. Samples are withdrawn at 1 month time intervals and evaluated for physical appearance, drug entrapment and drug release. [30]

III. RESULTS AND DISCUSSION

PREFORMULATION STUDY :

Determination of melting point

Melting point of Irbesartan was determined by capillary fusion method. Results were shown in table 5.

Method	Melting Point of Irbesartan				
	Experimental value (°C)				Literature Value
Capillary fusion method	A_1	A_2	A_3	Average	180-181°C
	179	180	180	180	

Table 5 Comparative values of melting points used to identify drug

Partition Coefficient

The partition coefficient of Irbesartan in n-octanol: PBS 7.4 was found to be 10.1 ± 0.06. This indicated that Irbesartan is hydrophobic in nature.

Determination of absorption maxima

The solution of Irbesartan (0.01% w/v) in phosphate buffer was scanned between 200–400 nm and an absorption maxima was determined spectrophotometrically. It exhibited absorption maxima at 246 nm.

Preparations of calibration curves

A simple, reliable and reproducible method for estimation of Irbesartan was required to estimate the drug content in dissolution media and in various other experimental protocols. In order to estimate drug in experimental protocols, standard curves were prepared in phosphate buffer saline (PBS) 7.4. The method estimated the drug concentration in PBS 7.4 followed the Beer's Lambert law in the concentration ranges (0.5-3.0 µg/ml) at 246 nm (Table 6). ;

The estimation procedure was found to be fairly reproducible and fairly sensitive. The method is convenient, inexpensive, reproducible and sensitive.

Solubility study

The solubility of irbesartan in different mediums such as dilute sulphuric acid, dilute hydrochloric acid, sodium bicarbonate, methanol, ethanol, propanol-2-ol, acetonitrile was determined. The amount of the drug dissolved was analyzed spectrophotometrically using UV Visible spectrophotometer and the solubility (mg) was tabulated in table 7 and represented in figure 3.

S. No.	Conc.(µg/ml)	Absorbance at λ_{max} 246 nm
		PBS 7.4
1	0	0
2	2	0.2017
3	4	0.364
4	6	0.5401
5	8	0.7010
6	10	0.8053
7	12	0.8801

Table 6 Calibration data of Irbesartan

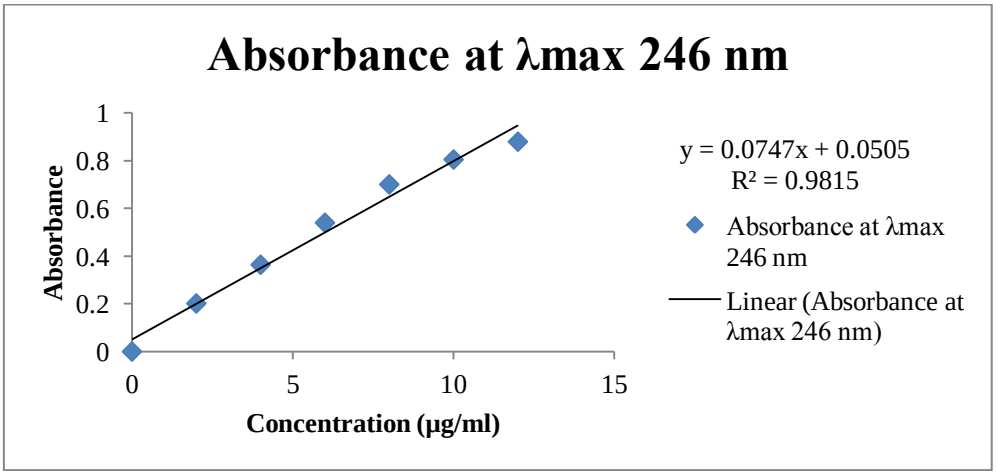

Figure 2 Calibration curve of Irbesartan in PBS 7.4

Solvent	Solubility studies	Solubility (mg/ml)
Methanol	Freely Soluble	1.083 ± 0.15
Dil. H2SO4	Slightly soluble	0.85 ± 0.23
Dil. HCl	Slightly soluble	0.77 ± 0.65
NaHCO3	Freely Soluble	1.025 ± 0.12
Ethanol	Freely Soluble	1.095 ± 0.78
Propanol-2-ol	Sparingly Soluble	0.117 ± 0.21
Acetonitrile	Sparingly Soluble	0.108 ± 0.04
Water	Practically Insoluble	0.08 ± 0.08

Table 7 Solubility of Irbesartan in various solvents (mean ± S.D., n=3)

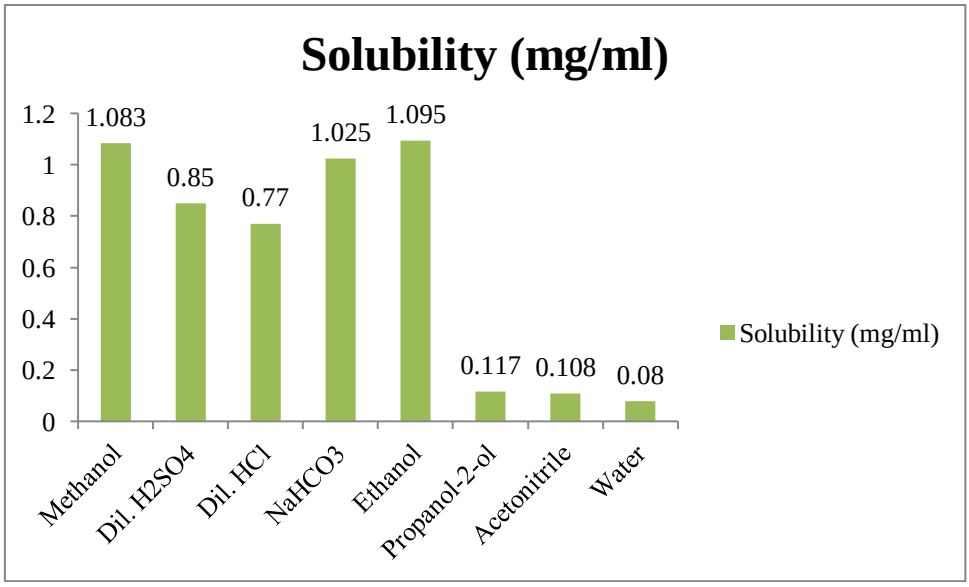

Figure 3 Solubility studies of Irbesartan

Drug excipient interaction study

The FTIR spectra of Irbesartan and mixture of drug with excipient (Irbesartan and chitosan) were shown in Figures 4-5. FTIR-spectra of Irbesartan showed the characteristic peaks of different functional groups as shown in Table 8.

S. No.	Functional Group	Peaks Obtained (cm^{-1})
1	N-H	3742.57
2	C-H	3047.65, 3121.96
3	C=N	1731.16
4	C=O	1648.51
5	N-H	1613.68
6	C-H	1437.09, 1433.55

Table 8 FTIR spectral assignment of Irbesartan

The FTIR spectra of physical mixture of drug and excipients showed no significant changes in the characteristic peaks of the drug which shows that there were no interaction between drug and excipients. The infrared spectra of Irbesartan presented characteristic peaks depicted in table 8.

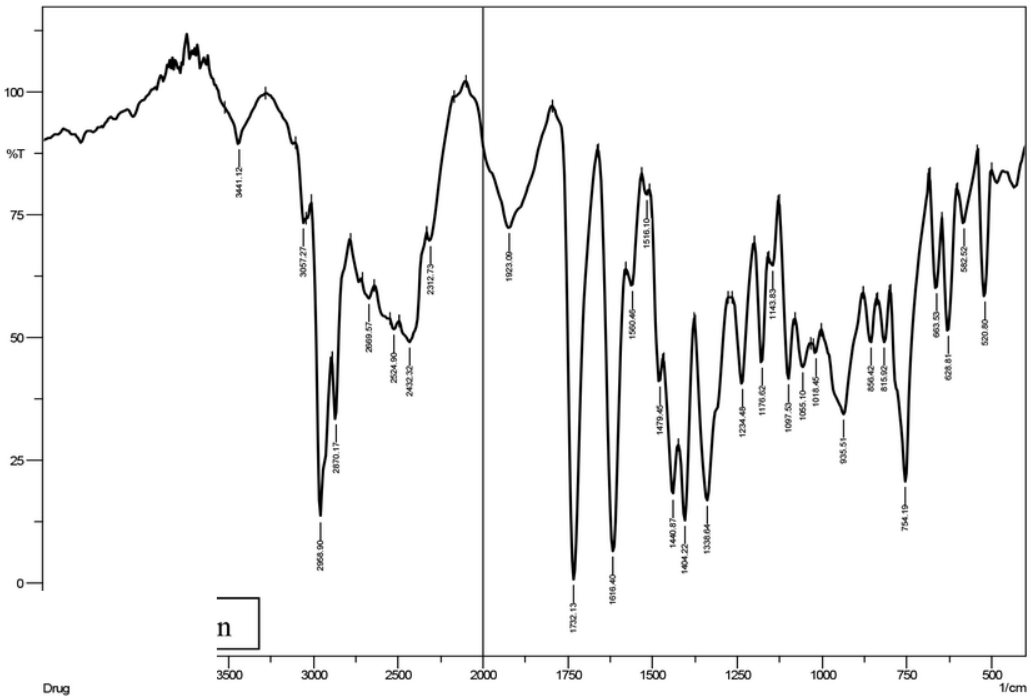

Figure 4 FTIR Spectra of Irbesartan

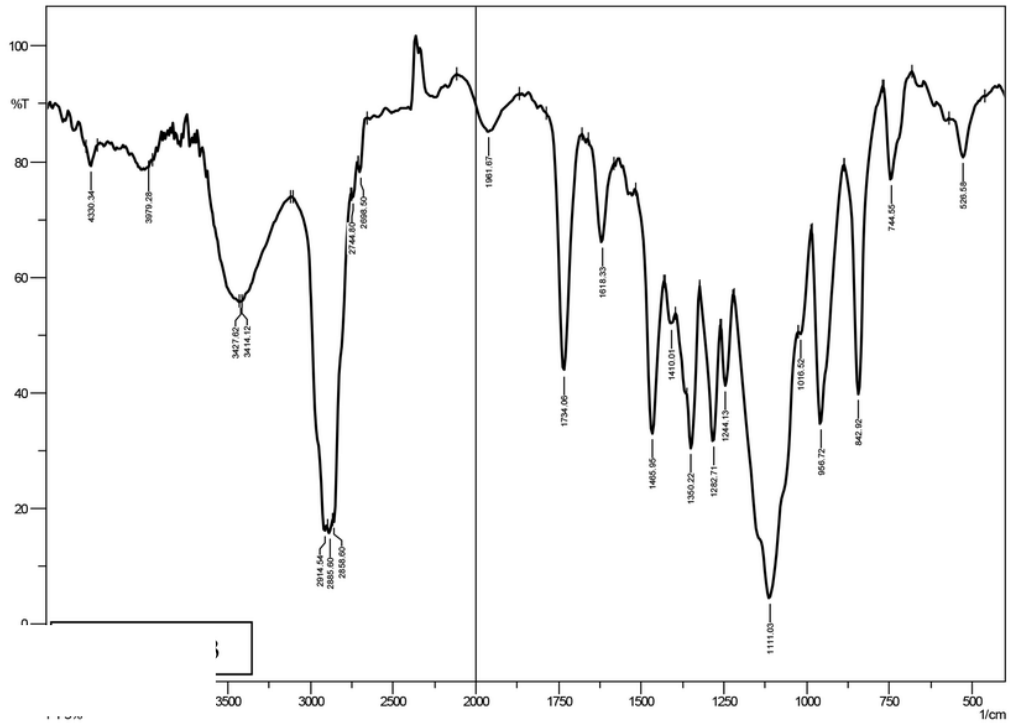

Figure 5 FTIR Spectra of Irbesartan with chitosan

Formulation of Gastro-Retentive Mucoadhesive Microspheres of Irbesartan

The mucoadhesive microspheres of irbesartan using chitosan were prepared. Chitosan was selected as a polymer for the preparation of mucoadhesive microspheres owing to its biodegradable and mucoadhesive properties. Different concentrations of glacial acetic acid from 1% w/v to 6% w/v were used for preparing the polymer solution, but no significant effect of concentration of acetic acid was observed on percentage mucoadhesion or drug entrapment efficiency, therefore 1% w/v of acetic acid was used. This finding could be owing to good solubility of chitosan in acetic acid.

One of the important factors related to microspheres as reported by Lee et al., is the viscosity of the polymer solution. Polymer concentrations of 0.5%, 1%, and 2% w/v were selected for preliminary trials. Flake formation was observed when chitosan concentration was used at a level of 0.5% w/v, whereas maximum sphericity was observed at the 1% w/v level. The chitosan solution was found to be too viscous to pass through the syringe when used at the 2% w/v level. Therefore, 1% w/v of chitosan in 1% v/v acetic acid was found to be the optimum concentration for the polymer solution.

A 1:1 mixture of heavy and light liquid paraffin was found to be suitable as the dispersion medium. Preliminary trial batches were prepared to study the effect of the volume of cross-linking agent (glutaraldehyde), time for cross-linking, and stirring speed on the percentage mucoadhesion, drug entrapment efficiency, and characteristics of the microspheres. The volume of glutaraldehyde was varied from 5 to 50 mL. Discrete spherical microspheres were obtained using 30, 40, and 50 mL of glutaraldehyde. Batches prepared using 5 and 10 mL of glutaraldehyde yielded irregular microspheres. The higher amount of glutaraldehyde appears to favor the cross-linking reaction, and hence spherical free-flowing microspheres were obtained. Microspheres of batches prepared using 30 mL of glutaraldehyde showed good percentage mucoadhesion, but drug entrapment efficiency was below 60%. Batches prepared using 40 mL of glutaraldehyde also showed good mucoadhesion as well as 70% drug entrapment efficiency. In the microspheres of batches prepared using 50 mL of glutaraldehyde the drug entrapment efficiency was above 72%, but mucoadhesion decreased. The decrease in mucoadhesion could possibly be attributed to the greater amount of cross-linking agent giving a more rigid cross-linked polymer whose adhesion is decreased. Thus, we can conclude that 40 mL of glutaraldehyde was the optimum amount. Increase in the cross-linking time (1 to 3 h) in all preliminary trial batches inversely affected the percentage mucoadhesion. The cross-linking polymer probably becomes more rigid and thus muco-adhesiveness decreases. The cross linking time did not have a significant effect on the percentage drug entrapment efficiency.

On the basis of the preliminary trials a 3^2 full factorial design was employed to study the effect of independent variables (i.e. polymer-to-drug ratio [X1] and the stirring speed [X2]) on dependent variables percentage mucoadhesion, % drug release, drug entrapment efficiency, particle size and swelling index. The results depicted in Table 4.5 clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 9 batches (B1 to B9).

Characterization of Gastro-Retentive Mucoadhesive Microspheres of Irbesartan:

Determination of Particle size

The particle size of the microspheres was determined by using optical microscopy method. Approximately 300 microspheres were counted for particle size using a calibrated optical microscope. The results of the particle size are depicted in table 9 and figure 6. The mucoadhesive microspheres of all the batches of the factorial design were spherical and free flowing. They ranged from a particle size of 57.2 to 80.2 µm and showed good correlation co-efficient (0.9698). The results indicated that the effect of X1 (polymer-to-drug ratio) is more significant than X2 (stirring speed). Thus, as the stirring speed increases, the particle size decreases which directly affects the percentage mucoadhesion.

Determination of Scanning Electron Microscope

Surface morphology of microspheres of formulation was investigated with the Scanning electron microscope. SEM and photomicrograph is shown in figure 7. The particle surface was found to be slightly wrinkled, irregularly shaped and discrete. The smoothness of the surface may increase with the increasing concentration of polymer. Very less particulate matter of the drug were seen on the surface of the microparticles indicating uniform distribution of the drug in the polymer network.

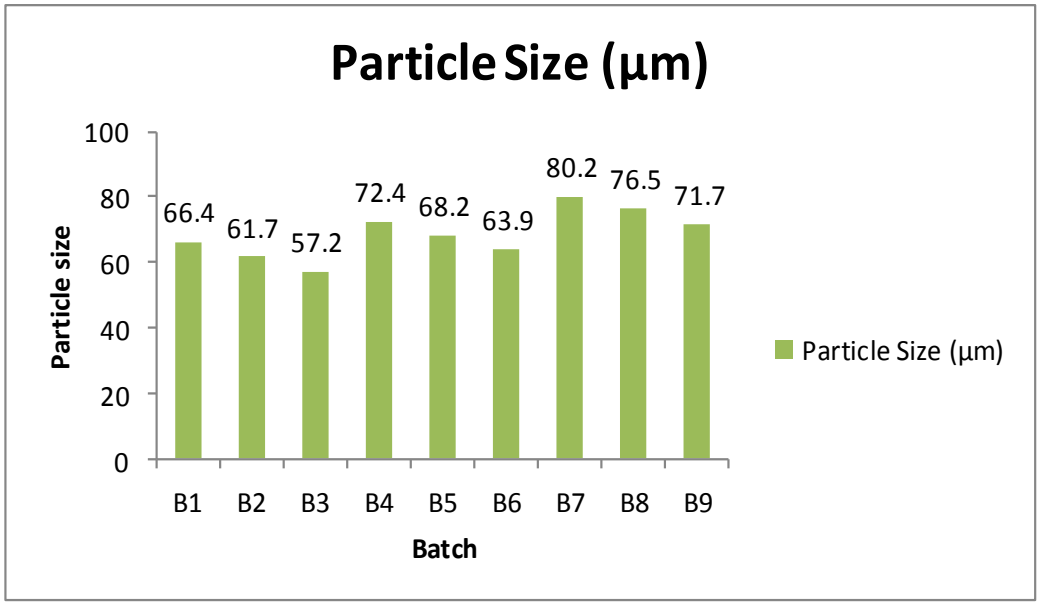

Figure 6 Particle size of Mucoadhesive Microspheres of Irbesartan

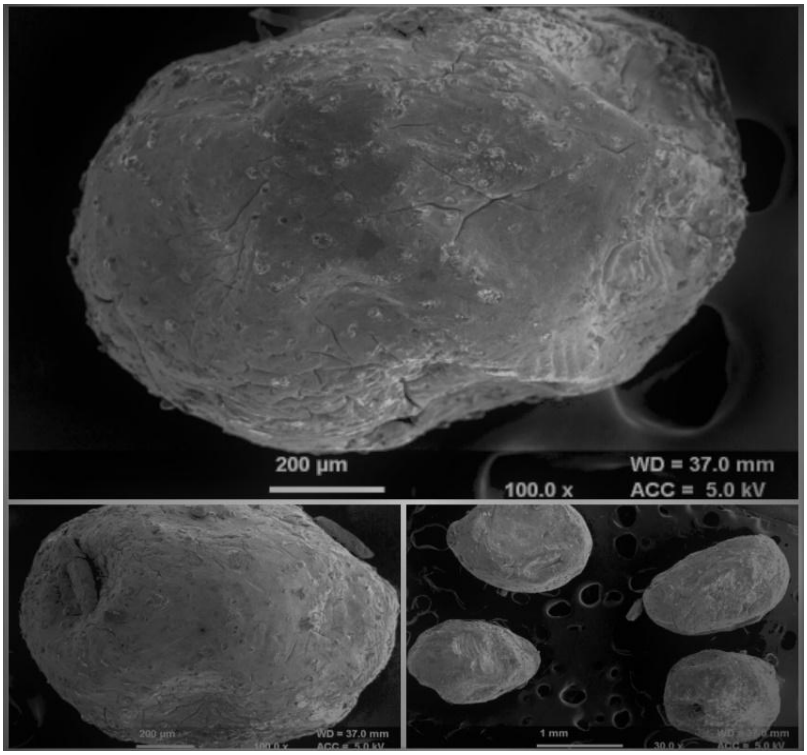

Figure 7 Scanning electron photomicrographs of Mucoadhesive Microspheres of Irbesartan

Batch	Angle of Repose	Bulk Density	Tapped Density	Carr's Index	Hausner's Ratio
B1	25.4 ± 0.12	0.564 ± 0.025	0.532 ± 0.018	9.91 ± 0.33	1.11 ± 0.014
B2	25.7 ± 0.22	0.565 ± 0.036	0.540 ± 0.022	10.80 ± 0.42	1.12 ± 0.012
B3	26.5 ± 0.58	0.554 ± 0.021	0.575 ± 0.014	12.12 ± 0.44	1.13 ± 0.015
B4	27.8 ± 0.41	0.564 ± 0.089	0.564 ± 0.017	12.01 ± 0.38	1.14 ± 0.012
B5	26.4 ± 0.02	0.574 ± 0.018	0.568 ± 0.089	12.41 ± 0.44	1.12 ± 0.011
B6	27.2 ± 0.07	0.561 ± 0.80	0.531 ± 0.182	9.081 ± 0.80	1.13 ± 0.12
B7	28.7 ± 0.17	0.572 ± 0.08	0.522 ± 0.251	10.12 ± 0.012	1.14 ± 0.64
B8	28.5 ± 0.23	0.592 ± 0.123	0.531 ± 0.93	11.32 ± 0.02	1.25 ± 0.097
B9	25.4 ± 0.68	0.581 ± 0.180	0.562 ± 0.11	12.18 ± 0.018	1.14 ± 0.109

Table 9 Characterization of Mucoadhesive Microspheres of Irbesartan

Angle of Repose

The Chitosan Irbesartan microspheres were evaluated for angle of repose to determine the flow properties. The angle of repose was found in the range of 25.4 to 28.7 which indicated that granules have good flow properties. The results were tabulated in Table 10 and figure 8.

S. No	Batch	Height of Pile (h) in cm	Diameter of pile (r) in cm	Angle of repose (θ)
1	B1	6	26	24.7
2	B2	7	29.2	25.4
3	B3	6	23	25.7
4	B4	6	24	26.5
5	B5	6	23	27.8
6	B6	7	28	26.4
7	B7	7	27	27.2
8	B8	6	22.1	28.7
9	B9	7	26	28.5

Table 10 Angle of repose of Chitosan Irbesartan microspheres

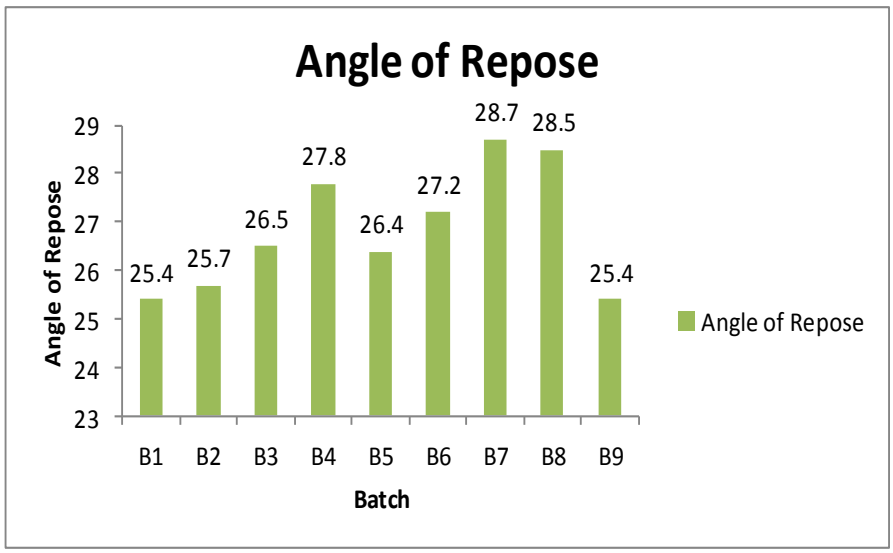

Figure 8 Angle of Repose for Chitosan Irbesartan microspheres

Bulk Density

The Chitosan Irbesartan microspheres were evaluated for bulk density to determine the flow properties. The bulk density was found in the range of 0.554 to 0.592 gm/cm^3 which indicated that granules have good flow properties. The results were tabulated in Table 11 and figure 9.

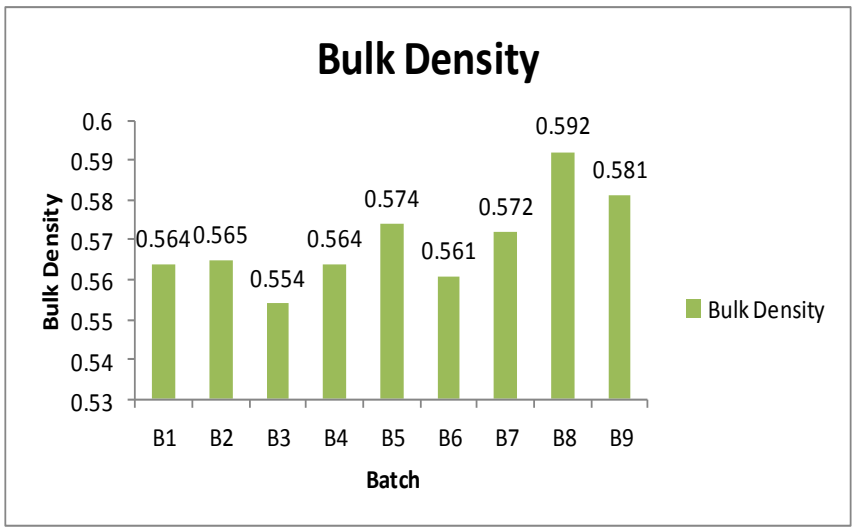

Figure 9 Bulk Density for Chitosan Irbesartan microspheres

Tapped Density

The Chitosan Irbesartan microspheres were evaluated for tapped density to determine the flow properties. The tapped density was found in the range of 0.522 to 0.575 gm/cm^3 which indicated that granules have good flow properties. The results were tabulated in Table 11 and figure 10.

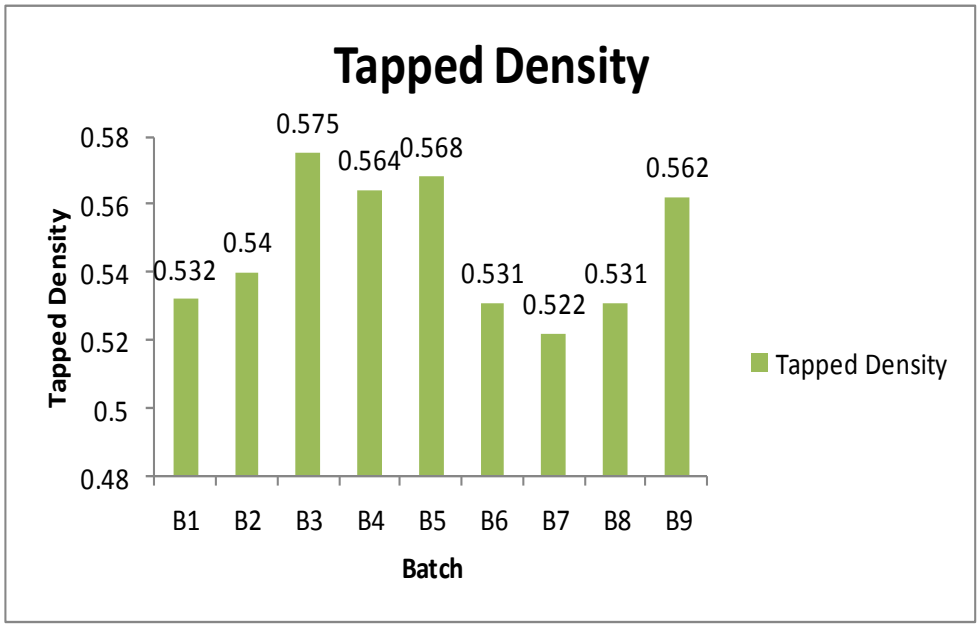

Figure 10 Tapped Density for Chitosan Irbesartan microspheres

Compressibility Index (Carr's Index)

Compressibility index was found to be in the range of 9.081 to 12.41 for all batches of formulation of Chitosan Irbesartan microspheres. This indicates granules were having good flow property. The results were tabulated in Table 11 and figure 11.

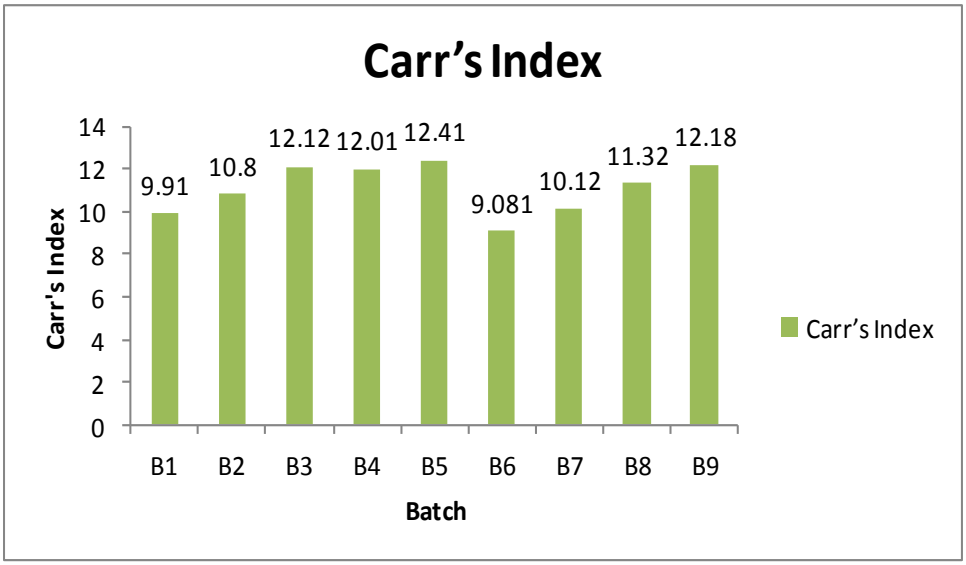

Figure 11 Compressibility Index for Chitosan Irbesartan microspheres

Hausner's Ratio

Hausner's ratio was found in between 1.11 to 1.25 for all batches of formulation of Chitosan Irbesartan microspheres. This indicates granules were having good flow property. The results were tabulated in Table 11 and figure 12.

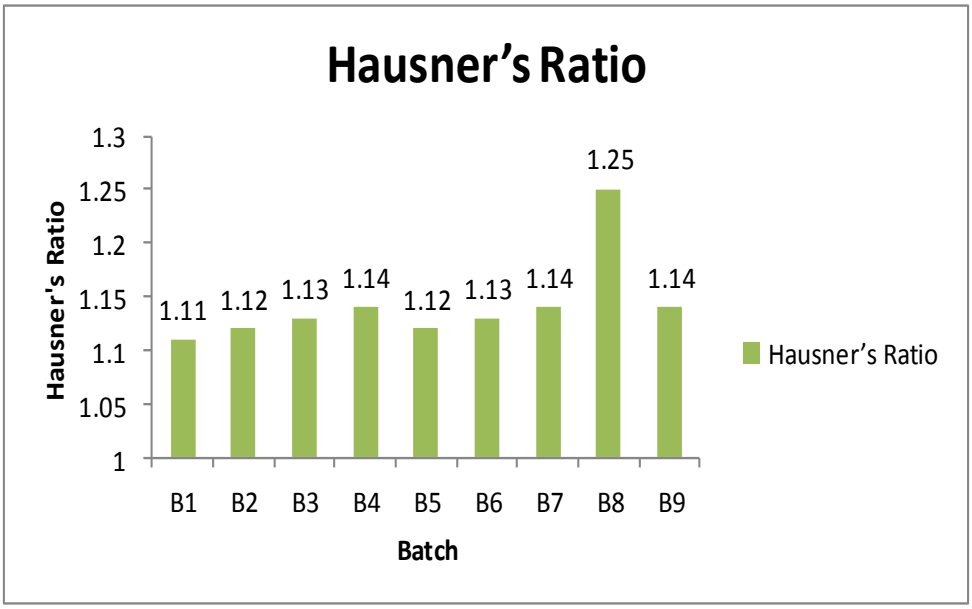

Figure 12 Hausner's Ratio for Chitosan Irbesartan microspheres

Determination of Swelling Index

The amount of polymer directly affected the solvent transfer rate and thus as the polymer concentration increased the swelling index also increased. The % swelling index varied from 60.4 to 78.2 and showed good correlation coefficient (0.9907). Thus, we can conclude that the amount of polymer and stirring speed directly affects the percentage mucoadhesion and swelling index. The % swelling index of each batch is shown in table 10 and depicted in figure 13

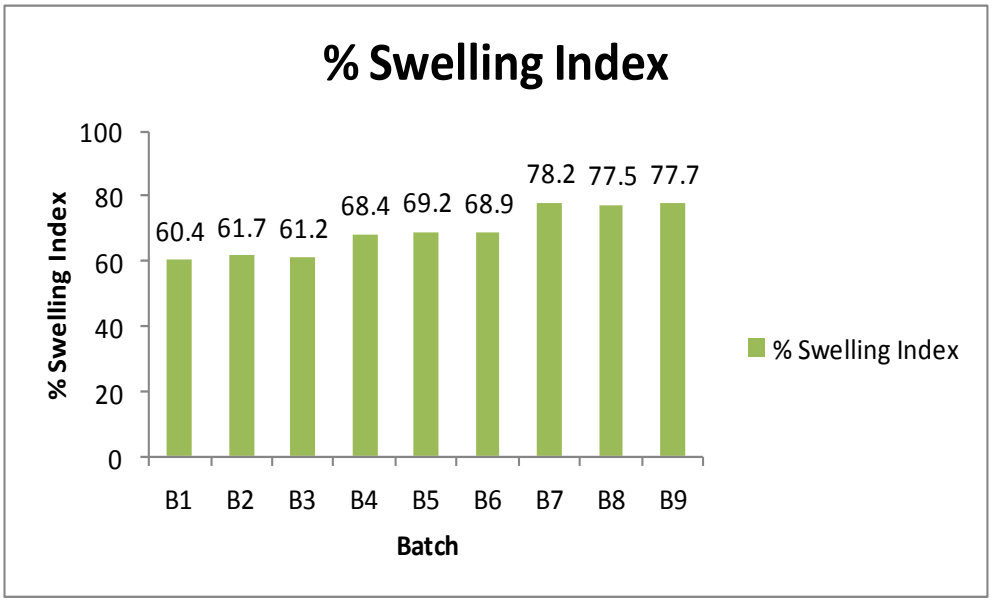

Figure 13 % Swelling index for Chitosan Irbesartan microspheres

Determination of mucoadhesion

The *in vitro* wash-off test for % mucoadhesion after 1 h varied from 57 to 78.2 and showed good correlation coefficient (0.9967). Results of equation indicate that the effect of X1 (polymer-to-drug ratio) is more significant than X2 (stirring speed). Moreover, stirring speed had a negative effect on the percentage mucoadhesion (i.e. as the stirring speed increased, the percentage mucoadhesion decreased). This finding may be attributed to the change in particle size that affects mucoadhesion. As the polymer-to-drug ratio increases, the % mucoadhesion also increases because m\ore amount of polymer results in higher amount of free –NH2 groups, which are responsible for binding with sialic acid groups in mucus membrane and thus results in

increase in mucoadhesive properties of microspheres. . The % mucoadhesion of each batch is shown in table 4.6 and depicted in figure 14.

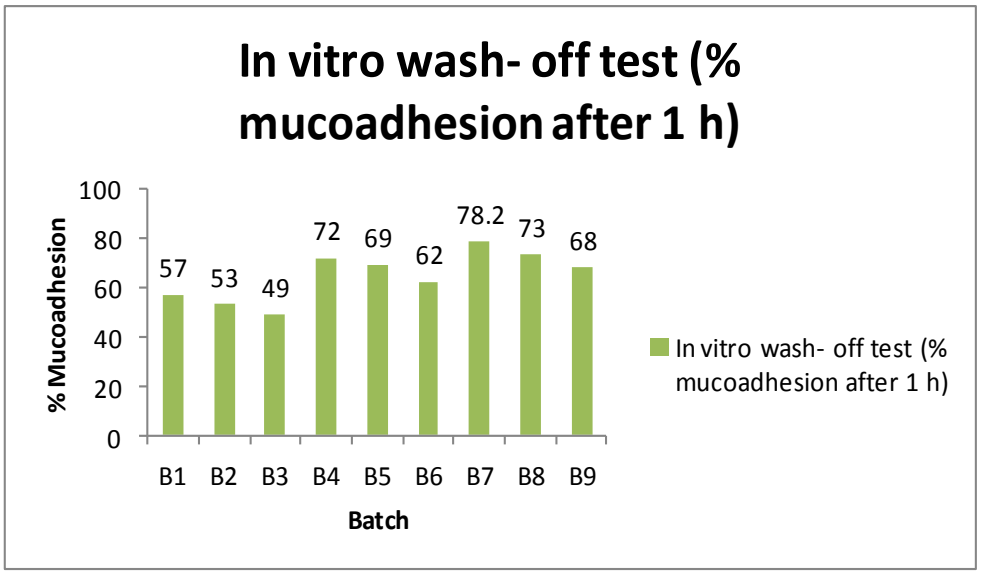

Figure 14 % Mucoadhesion for Chitosan Irbesartan microspheres

Drug Entrapment Efficiency

The drug entrapment efficiency varied from 38% to 72% and showed good correlation co-efficient (0.9998). Results of equation indicate that the effect of X1 (polymer-to-drug ratio) is more significant than X2 (stirring speed). Moreover, stirring speed had a negative effect on the per drug entrapment efficiency (i.e. as the stirring speed increased, the particle size decreased, and thus drug entrapment efficiency decreased). The results for drug release of each batch is shown in table 4.6 and depicted in figure 15.

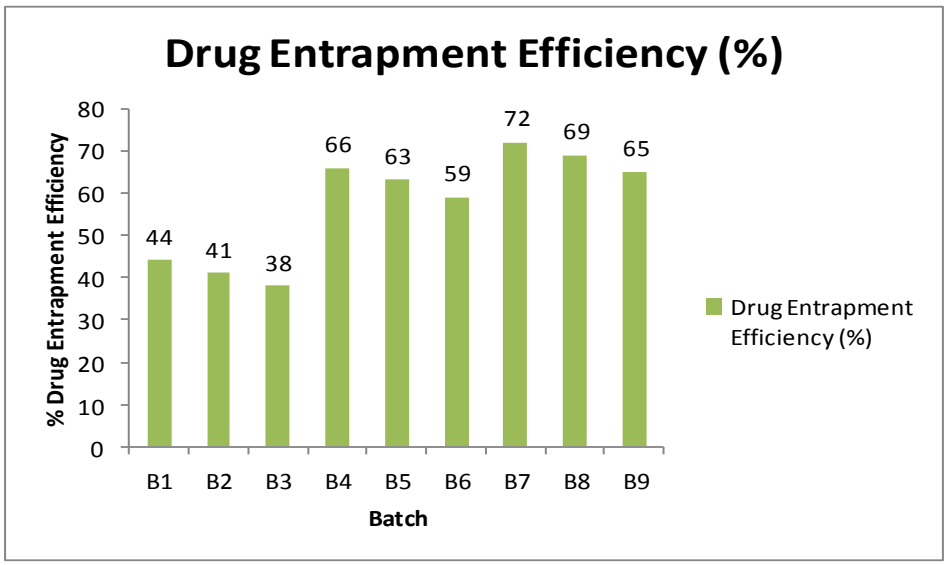

Figure 15 % Drug Entrapment Efficiency for Chitosan Irbesartan microspheres

Characterization of Mucoadhesive Microspheres of Irbesartan					
S. No.	**Batch**	**Particle Size (µm)**	**% Swelling Index**	***In vitro* wash- off test (% mucoadhesion after 1 h)**	**Drug Entrapment Efficiency (%)**
1.	B1	66.4	60.4	57	44
2.	B2	61.7	61.7	53	41
3.	B3	57.2	61.2	49	38
4.	B4	72.4	68.4	72	66
5.	B5	68.2	69.2	69	63
6.	B6	63.9	68.9	62	59

7.	B7	80.2	78.2	78.2	72
8.	B8	76.5	77.5	73	69
9.	B9	71.7	77.7	68	65

Table 11 Characterization of Mucoadhesive Microspheres of Irbesartan

***In Vitro* Drug Release Studies**

Results depicted in Table 4.8 indicate that the % drug released *in vitro* is highly dependent on the polymer-to-drug ratio and stirring speed. The stirring speed has a negative effect on drug release because as the particle size increases the drug releases decreases. Higher levels of polymer-to-drug ratio favour the cross-linking reaction and thus higher drug release is obtained. Batch B7 exhibited a high drug release of and seems to be a promising candidate for achieving drug release upto 10 h. The drug release profile of batch B7 is shown in Figure 16. The figure reveals that drug release rate was slowed after 4 h.

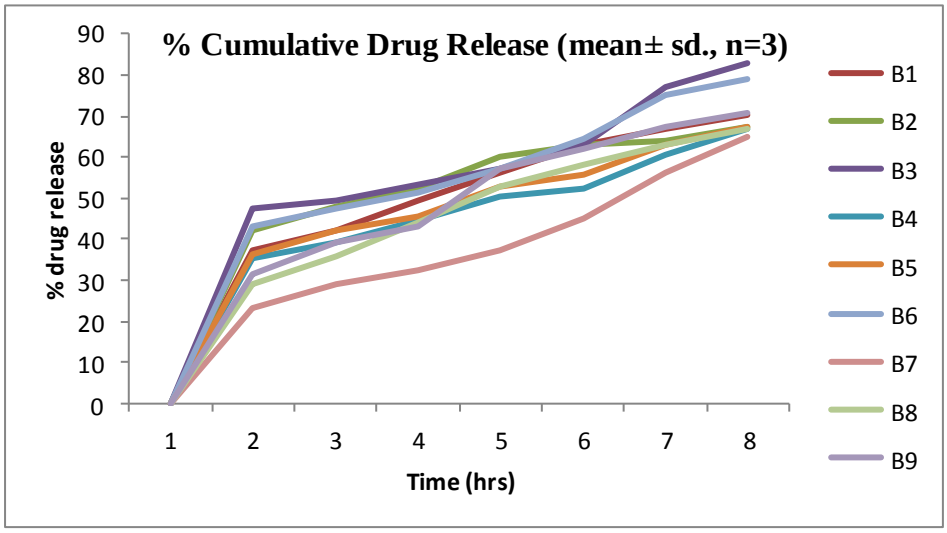

Figure 16 % cumulative drug release of different batch of Chitosan Irbesartan microspheres

Determination of Release Kinetics

The mathematical models were used to evaluate the kinetics and mechanism of drug release. The model that gave high correlation coefficient (r) value was considered as the best fit of the release data (Martin, 1994). Data of *in vitro* release were fitted to different Equation and kinetic models to explain the release kinetics of Irbesartan from the mucoadhesive microspheres. The data were processed for regression analysis using MS-Excel statistical functions. To know the order of reaction from these formulations, the data were treated according to first-order (log cumulative percent drug remaining vs. time), Higuchi's (cumulative percent drug released vs. square root of time), and Korsmeyer Pappas's (log cumulative percent drug released vs. log time) Equations along with zero order (cumulative amount of drug released vs. time) Equation.

Time (hrs)	**% Cumulative Drug Release (mean± sd., n=3)**								
	B1	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
0	0	0	0	0	0	0	0	0	0
1	37.15±0.32	42.23±0.25	47.33±0.23	35.20±0.12	36.23±0.25	43.33±0.23	23.15±0.32	29.23±0.25	31.33±0.23
2	42.24±0.69	47.95±0.32	49.32±0.52	39.14±0.91	41.95±0.32	47.32±0.52	29.24±0.69	35.95±0.32	39.32±0.52
4	49.19±0.32	52.29±0.68	53.12±0.15	44.59±0.28	45.29±0.68	51.12±0.15	32.19±0.32	44.29±0.68	43.12±0.15
6	56.35±0.54	59.87±0.69	57.35±0.39	50.35±0.43	52.87±0.69	57.35±0.39	37.35±0.54	52.87±0.69	57.15±0.79
8	62.95±0.25	62.91±0.36	63.21±0.52	52.52±0.05	55.91±0.36	64.21±0.52	44.95±0.25	57.91±0.36	62.01±0.27
10	67.02±0.65	64.17±0.25	77.28±0.02	60.49±0.08	63.17±0.25	75.28±0.02	56.02±0.65	63.17±0.25	67.57±0.14
12	70.12±0.82	67.22±0.02	83.05±0.12	66.75±0.78	67.22±0.02	79.05±0.12	65.12±0.82	67.02±0.32	70.75±0.57

Table 12 % cumulative drug release of different batch of Mucoadhesive microspheres of Irbesartan

The release kinetics showed all formulations followed first order kinetics and the and the value ranged from 0.8782 – 0.9710 and therefore the formulations considered as following slow first order kinetics. The highest regression value was obtained with RCP3 which shows coacervation phase separation method seems to be yielding best results. Peppas n value ranged from 0.5786 – 0.6212. These values indicate all formulations followed non-fickian diffusion mechanism of release.

Formulation	Zero order model	First order model	Higuchi model	Korsmeyer-Peppas model	
	r^2	r^2	r^2	r^2	Slope (n)
B1	0.8825	0.8782	0.8811	0.9487	0.5786
B2	0.8355	0.7390	0.9181	0.9526	0.5887
B3	0.8112	0.9074	0.9163	0.9539	0.5902
B4	0.8238	0.9023	0.9183	0.9631	0.5996
B5	0.8184	0.9405	0.9156	0.9710	0.6019
B6	0.8393	0.9218	0.9153	0.9789	0.6036
B7	0.9582	0.9710	0.9150	0.9972	0.6212
B8	0.9088	0.9134	0.9120	0.9858	0.6074
B9	0.9323	0.9540	0.9102	0.9946	0.6102

Table 13 *in vitro* drug release profile

Stability studies

The prepared tablets were subjected to stability studies at 25±2°C/60±5% RH for real and 40 ±2°C/75±5% RH for accelerated studies as per ICH guidelines for a period of 3 months. Samples were withdrawn at 1 month time intervals and evaluated for physical appearance, drug entrapment and drug release. The results for stability studies were tabulated in table 4.10 (a & b).

From stability studies, it was found that tablets remained stable even after exposing to high temperature/moisture conditions at first and third month of time interval. Both the real and accelerated stability studies showed stability of tablets at varied temperature and moisture conditions. No major change was observed in physical appearance, drug entrapment and drug release.

PARAMETERS	Storage Conditions: 25±2°C/60±5% RH			
	Initial	1 month	2 month	3 month
Color	White to off white color	White to off white color	White to off white color	White to off white color
Drug Entrapment	72 %	71.89%	71.88%	70.97%
In vitro release after 12 hrs (%)	65.12 %	66.85 %	67.25 %	67.78 %

Table 14 (a) Results for stability studies

PARAMETERS	Storage Conditions: 40 ±2°C/75±5% RH			
	Initial	1 month	2 month	3 month
Color	White to off white color	White to off white color	White to off white color	White to off white color
Drug Entrapment	72 %	70.89%	70.68%	70.97%
In vitro release after 12 hrs (%)	65.12 %	67.85 %	67.97 %	68.27 %

Table 14 (b) Results for stability studies

IV. CONCLUSION

The Mucoadhesive Microspheres of Irbesartan were prepared by using Chitosan. The particle size of microspheres was determined by optical microscopy. The % drug released *in vitro* is highly dependent on the polymer-to-drug ratio and stirring speed. The *In Vitro* dissolution studies showed that Irbesartan Mucoadhesive Microspheres formulation B7 exhibited a high drug release of and seems to be a promising candidate for achieving drug release upto 10 h than other formulations. Hence, prepared Mucoadhesive Microspheres may be an effective strategy for the development of easy, reproducible and cost effective method for safe and effective Mucoadhesive drug delivery. In conclusion, microspheres are a promising approach for the formulation of drug compounds with poor aqueous solubility. The objective of our investigation was to formulate microspheres to enhance bioavailability and solubility. With further development of this technology, microspheres will continue to enable novel applications in drug delivery and solve problems associated with the delivery of poorly soluble drug.

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