INTERNATIONAL JOURNAL OF PHARMACY AND ANALYTICAL RESEARCH

ISSN:2320-2831

IJPAR |Vol.8 | Issue 4 | Oct - Dec - 2019
Journal Home page: www.ijpar.com

Research article *Open Access*

Formulation and evaluation of irbesartan microspheres

Y. Deepika*, Dr. A. Pavani and Dr. M. Bhagavan Raju

Department of Pharmaceutics, Sri Venkateshwara College of Pharmacy, Madhapur
***Corresponding Author: Y. Deepika**

ABSTRACT

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers of biodegradable nature. Microspheres are particles between 0.1 and 200 µm in size. A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. A Microspheres has its drug dispersed throughout the particle i.e. the internal structure is a matrix of drug and polymeric excipients. It is the reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumour. Microspheres are spherical micro particles, and are used where consistent and predictable particle surface area is important. In microspheres drug is located centrally within the particle, where it is encased within a unique polymeric membrane. In future by combining various other strategies, microspheres will find the central place in novel drug delivery, particularly in diseased cell sorting, diagnostics, gene & genetic materials, safe, targeted and effective *in vivo* delivery.

Keywords: Microspheres, Target site, Specificity, Novel drug delivery, Controlled release.

INTRODUCTION

One of the most challenging areas of research in pharmaceutical industry is the development of novel delivery systems for the controlled release of drugs and their delivery at the targeted site in the body to minimize the side effects and enhance the therapeutic efficacy of drugs [1-3]. The basic principle behind the controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamics properties of drug in such a way that its efficacy is maximized by reducing side effects, dose frequency and cure the disease in short time by using low amount of drug administered with the most suitable route [4]. Microsphere, as carrier for drug is one of the various approaches of drug delivery which maximizes the drug concentration at the target site. Microspheres are defined as "Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles. It can also be defined as structure made up of continuous

phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level with particle size range of 1-1000 µm.

Microsphere is a homogeneous structure made of a continuous phase of one or more miscible polymers in which particulate drug is dispersed throughout the matrix, at either the macroscopic (particulates) or molecular (dissolution) level [5]. It can encapsulate many types of drugs including small molecules, proteins and nucleic acids and are easily administered through a syringe needle. They are generally biocompatible, can provide high bioavailability, and are capable of sustained release for long periods of time. Microspheres received much attention not only for prolonged release, but also for targeting of anticancer drugs to the tumour. Microspheres have played a vital role in the development of controlled or sustained release drug delivery systems. Microspheres have interest from the pharmaceutical point of view providing the possibility to achieve sustained and controlled release [6].

MATERIAL AND METHODS

Materials

Irbesartan was received as a gift sample from Smilax laboratories Pvt, Ltd., Hyderabad, India. Chitosan and sodium alginate were from Evonik India Pvt. Ltd., Mumbai, India and all solvents and other exciepients used were of best laboratory reagent grade.

Methods

Preparation of Irbesartan microspheres

Irbesartan microspheres were prepared by Ionic gelation method. The weighed amount of sodium alginate of various percentages (1.5 - 6 %) was dissolved in water, stirred for 1h using mechanical stirrer. The weighed amount of Irbesartan was dispersed into polymeric solution and stirred for 30 mins and allowed to stand in sonicator till the removal of entraped air bubbles. After sonication, this solution was added dropwise from the distance 5cm using syringe fitted with (22G) needle into coagulation fluid 200 ml consisting of calcium chloride solution (3%) and chitosan (0.5 – 5.0%) dissolved in 1% acetic acid in 250 ml beaker and stirred at room temperature using magnetic stirrer. Microspheres were left for curing for specified time and after curing, microspheres are collected by filtration and washed twice with distilled water and allowed to dry at 40°C for 24h. The dried microspheres are weighed are stored for evaluation studies.

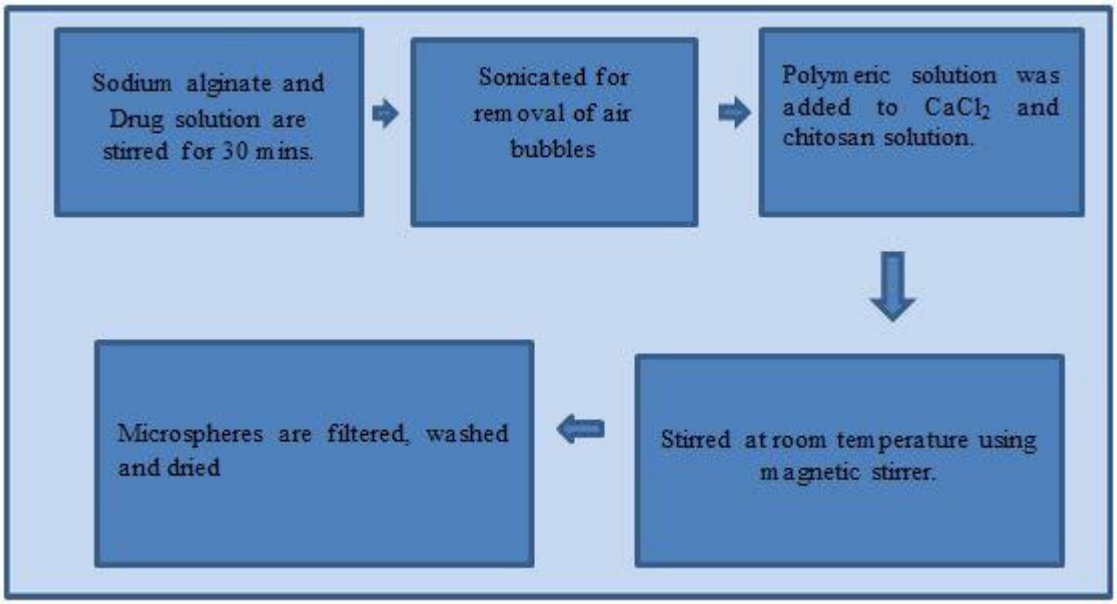


Figure 1: Flow chart for preparation of Microspheres.

Table 1: Formulation of Microspheres

Formulation	Drug (mg)	Sodium alginate (%)	Calcium chloride (%)	Chitosan
F1	150	1.5	3	0.5
F2	150	2.0	3	1.0
F3	150	2.5	3	1.5
F4	150	3.0	3	2.0
F5	150	3.5	3	2.5
F6	150	4.0	3	3.0
F7	150	4.5	3	3.5
F8	**150**	**5.0**	**3**	**4.0**
F9	150	5.5	3	4.5
F10	150	6.0	3	5.0

PREFORMULATION STUDIES

Solubility studies

The solubility of pure irbesartan was determined by taking 30 mg of Irbesartan in 10 ml of pH 1.2 buffer, in Teflon facing screw capped vials. The vials were kept at equilibrium for period of 24 h on orbital shaking incubator (CIS- 24, Remi instrument, Mumbai, India) at 37 ± 0.5^0 C and 100 rpm. The content of vials were filtered through 0.2 µm membrane filter and analysed using UV Spectrophotometer (1700, Shimadzu, Japan) at 226 nm.

Determination of melting point

The melting point of irbesartan was determined by using capillary method. A thin-walled capillary melting point tubes were used to hold melting point samples. The tube was sealed at one by inserting the tip into a Bunsen flame near the base of the flame and turning the tube in fingers. The open end was pressed gently into a small amount of the sample on a watch glass or weighing paper. To transfer the crystals from the open end to the bottom of the tube by tapping the bottom gently on the bench top. A packed capillary attached to a normal mercury thermometer. The thermometer was placed in a beaker containing liquid paraffin maintained on a water bath to provide uniform distribution of heat throughout the sample.

EVALUATION AND CHARACTERIZATION OF MICROSPHERES [7]

- Determination of percentage yield of microspheres
- Drug entrapment efficiency
- Scanning electron microscopy (SEM)
- *In vitro* drug release studies.
- Solubility studies.
- Micromeritic properties of microspheres.
- Drug content
- Conducting stability studies of optimized formulation
- Release kinetics.

Determination of percentage yield [8]

Microspheres dried at room temperature were weighed and the yield of microspheres was calculated using the formula.

Percentage yield = Practical yield (gm)/ Theoretical yield × 100

Drug entrapment efficiency [9]

The amount of drug entrapped was estimated by dissolving the 100 mg of microspheres in dichloromethane and waster in 3:1 ratio, under vigorous shaking for 1h, the resultant solution is centrifuged, both layers were separated, the drug content in aqueous layer was analysed. The amount of drug entrapped in the microspheres was calculated using the formula:

Drug entrapment efficiency (%) = Amount of drug actually present/ Theoretical drug

Load expected × 100

Particle size analysis

Determination of average particle size of irbesartan microspheres with carrier was very important characteristic. It was measured by using Malvern instruments, startech labs PVT.LTD.

Scanning electron microscopy [10]

Microspheres were observed and photographed with scanning electron microscopy (SEM) (Using

hitachi –S-3700N). Scanning electron microscopy was carried out to study the morphological characteristics of irbesartan microspheres. The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of adhesive stub. Then the microspheres were coated with gold (100 A^0) before microscopy. Finally the morphology of the microspheres was observed with the scanning electron microscopy.

Fourier Transform Infrared Spectroscopy (FT-IR) [11]

FTIR spectra was recorded by using FTIR instrument. The instrument was operated under dry air purge and scanning range was of 4000 – 400 $cm.^{-1}$ Structural changes and lack of a crystal structure can lead to changes in bonding between functional groups that can be detected by FTIR.

In vitro drug release studies [12, 13]

In vitro dissolution tests were performed according to the USP apparatus II (paddle) method at 100 rpm and 37 ± 0.5^0C containing 0.1 N HCl (pH), and phosphate buffers 6.8, 7.4 as a dissolution medium. Initially, the microspheres were treated with 900 ml of 0.1 N HCl (pH) containing 0.01% SLS for 2 hours, 25.92 g of disodium dihydrogen phosphate and 10.305 g dihydrogen potassium phosphate were added to increase the pH to 6.8 and the drug release was continued for another 4 hours. After the 6h, 2.142 g of disodiumhydrogen phosphate and 0.171 g sodium chloride were added to increase the pH up to 7.4 and the study was continued up to 24 h. the samples were withdrawn at suitable time intervals and relaced with fresh medium. The rate of drug release was analysed using UV Spectrophotometer.

STABILITY STUDIES AT DIFFERENT TEMPERATURE CONDITIONS [14]

The physical stability of the developed microspheres was carried according to ICH guidelines. The optimized formulations were stored at two different temperature ranges for 3 months. The sample containing optimized formulations were place in vials and stored at 25 ± 2º C. After 90 days the formulations were checked for physical appearance and drug content.

RESULTS AND DISCUSSIONS

Analytical method development for irbesartan

Uv scan of irbesartan

Dilution of Irbesartan was made using phosphate buffer and 0.1N HCl and scanned in UV region using UV-Visible double beam spectrophotometer [Lab India®, UV 200-400nm].

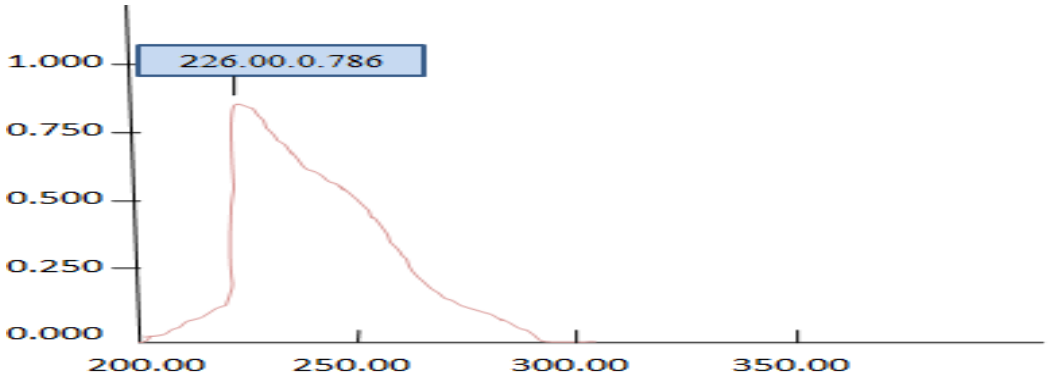


Figure: 2 UV spectrum of 10µg/ml Irbesartan in 0.1N HCl

RESULT

10µg/ml dilution of Irbesartan showed absorption maxima at 226nm in 0.1 N HCl as seen in figure 2.

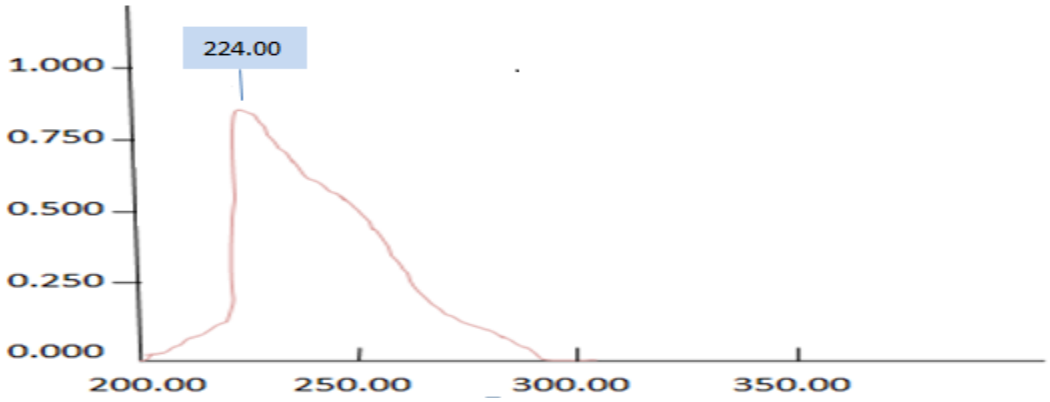


Figure: 3 UV spectrum of Irbesartan in phosphate buffer 7.4

10µg/ml dilution of Irbesartan showed absorption maxima at 224 nm in Phosphate buffer 7.4 as seen in figure 3.

Preparation of standard graph of Irbesartan in 0.1 N HCl

Standard dilutions of Irbesartan in the range 2 to 10 µg/ml were prepared and absorbance was taken at 226 nm using 0.1N HCl as blank. Standard graph of irbesartan was plotted from this data.

Table: 2 Absorbance values of Irbesartan in 0.1N HCl at 226 nm

S. No	Concentration (µg/ml)	Absorbance
1.	0	0
2.	2	0.142±0.13
3.	4	0.285±0.76
4.	6	0.468±0.21
5.	8	0.621±0.86
6.	10	0.815±0.17

Note: All the values are expressed as mean ±SD.

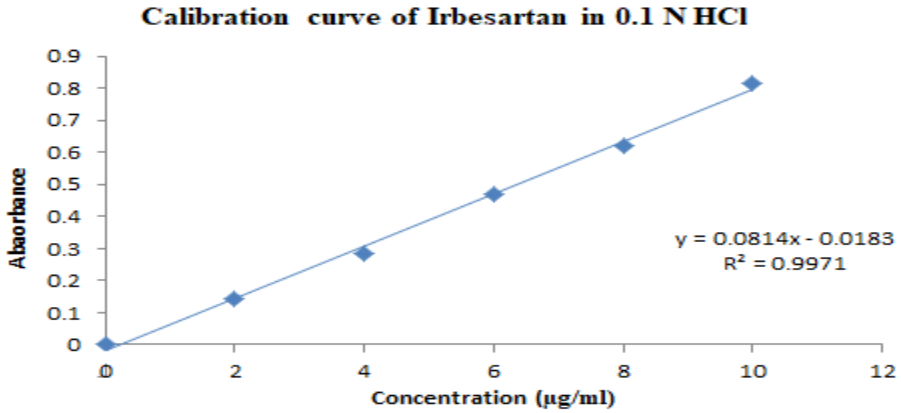


Figure: 4 Calibration curve of Irbesartan in 0.1 N HCl at 226nm.

Table: 3 Regression analysis of calibration curve of Irbesartan in 0.1N HCl

S.No	Parameters	Result
1.	Regression equation	Y = 0.0814x-0.0183
2.	Correlation coefficient	R^2=0.9971
3.	Calibration range	2 to 10µg/ml

The standard graph of Irbesartan in 0.1N HCl is linear in the range of 2 to 10µg/ml was shown in figure 4.3. The linear regression equation is y = 0.081x-0.0.183. The coefficient of determination was found to be 0.9971.

Table: 4 Absorbance values of irbesartan in phosphate buffer at 224nm.

S.No	Concentration(µg/ml)	Absorbance
1.	0	0
2.	2	0.164±0.19
3.	4	0.325±0.32
4.	6	0.486±0.68
5.	8	0.721±0.22
6.	10	0.898±0.14

Note: All the values are expressed as mean ±SD

Preparation of standard graph of irbesartan in phosphate buffer 7.4.

Standard dilutions of irbesartan in the range of 2 to10 µg/ml were prepared and absorbance was taken at 224 nm, phosphate buffer as blank. Standard curve of Irbesartan was plotted using this data.

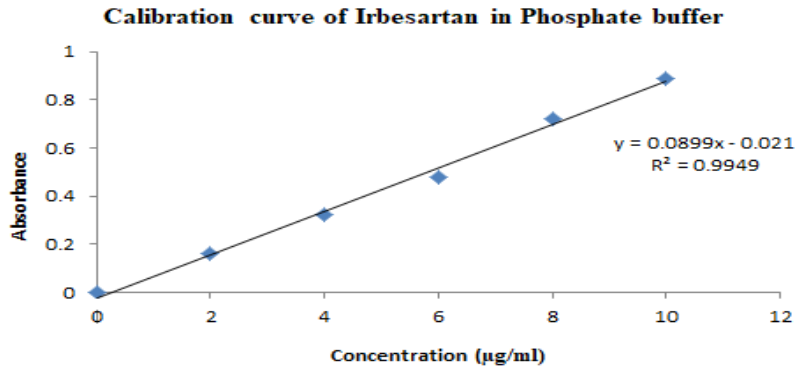


Figure: 5 Calibration curve of Irbesartan in Phosphate buffer at 224 nm.

Table: 5 Regression analysis of calibration curve of Irbesartan in Phosphate buffer.

S.No	Parameters	Result
1.	Regression equation	y = 0.0899x – 0.021
2.	Correlation coefficient	R^2=0.9949
3.	Calibration range	2 to 10 µg/ml

The standard graph of Irbesartan is shown in figure 4.4 it is linear in the range of 2 to 10 µg/ml. The linear regression equation is y = 0.0899x-0.021. The coefficient of determination (R^2) of the regression line was found to be 0.9949.

Surface Morphology

The surface morphology of the irbesartan microspheres formulated with varying concentrations of sodium alginate and chitosan was determined using Scanning Electron Microscopy (Hitachi S-3700N).

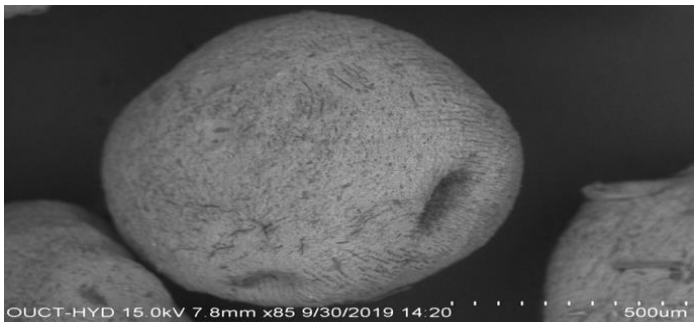


Figure No. 6 Scanning Electron Microscopic Images of Irbesartan Microspheres.

From the above Figure No.6 it was observed that the microspheres have a smooth surface and are sphere shaped. This states that the microspheres are capable of incorporating the large amounts of the Irbesartan which in turn helps in increasing the drug release**.**

Entrapment Efficiency (E.E)

Irbesartan microspheres with varying concentrations of sodium alginate and chitosan were subjected to ultra-centrifugation to determine the Entrapment Efficiency. This helps in determining the percentage amount of the drug that has been incorporated or entrapped within the microspheres.

Table No. 6 Drug Entrapment Efficiency of Irbesartan microspheres (F1-F10)

Formulation code	%Entrapment efficiency	Formulation code	%Entapment efficiency
F1	67.41 ± 1.01	F6	86.19 ± 0.77
F2	79.03 ± 1.07	F7	87.73 ± 1.19
F3	76.64 ± 0.98	F8	90.59 ± 0.46
F4	81.53 ± 1.56	F9	76.64 ± 0.76
F5	83.27 ± 1.17	F10	86.16 ± 0.61

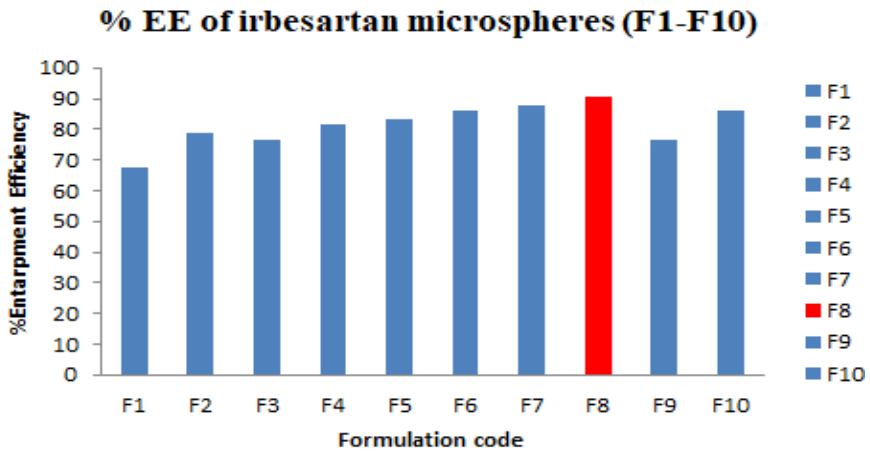


Figure No. 7 Entrapment efficiency of irbesartan microspheres (F1-F10)

From the Figure no.7 and Table no.6 it was found that among all the Irbesartan microsphere formulations with varied concentrations of sodium alginate and chitosan, the microsphere formulation

(F8) with 5% sodium alginate and 4% chitosan has the highest entrapment efficiency of 90.59% when compared with the remaining formulations.

In-vitro Drug release Studies

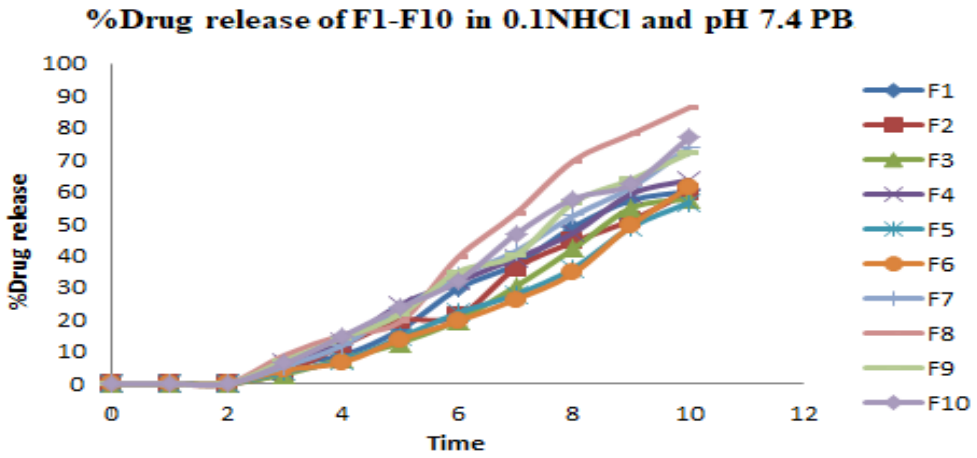
Irbesartan Microspheres with varying concentrations of sodium alginate and the chitosan were subjected to *In-vitro* Drug release. *In-vitro* drug release studies were performed for all formulations and formulation **F8** was optimized. The percentage drug release profiles of various formulations are shown in the Figure no.7 respectively.

Table No.7 *In-vitro* Drug release profile of Irbesartan Microspheres (F1-F5)

Time (hrs)	%DR of F1	%DR of F2	%DR of F3	%DR of F4	%DR of F5
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	4.68±0.29	5.38±0.11	2.93±0.38	6.54±1.01	3.79±0.45
4	10.21±0.13	11.94±1.09	7.81±1.09	13.29±1.04	7.29±1.11
5	14.09±0.06	17.23±1.00	12.78±1.02	24.68±1.03	14.47±1.03
6	29.71±0.27	23.99±1.15	22.74±1.13	30.20±1.00	18.20±1.05
7	37.29±1.14	36.09±1.01	30.39±1.03	39.17±0.99	27.88±1.09
8	**48.94±0.31**	**44.27±1.13**	**42.13±1.12**	**47.13±1.02**	**36.07±1.00**
9	57.29±1.11	51.14±1.02	54.90±1.05	54.29±1.13	48.90±1.12
10	60.07±1.01	59.53±1.04	57.76±1.00	63.65±1.02	56.53±1.04

Table No.8 *In-vitro* Drug release profile of Irbesartan Microspheres (F6-F10)

Time (hrs)	%DR of F6	%DR of F7	%DR of F8	%DR of F9	%DR of F10
0	0	0	0	0	0
1	0	0	0	0	0
2	0	0	0	0	0
3	3.84±0.51	5.49±0.99	8.98±1.28	7.29±0.76	6.34±0.86
4	6.68±1.11	11.97±1.14	15.26±1.06	13.84±0.99	14.87±1.89
5	13.87±1.00	23.19±1.05	23.81±1.16	21.64±1.17	23.79±0.38
6	19.59±1.00	33.56±1.28	39.49±1.89	34.96±1.06	32.09±1.48
7	26.31±1.02	41.38±1.03	53.22±1.06	40.16±0.84	46.68±0.93
8	34.59±1.09	52.14±1.09	69.63±0.93	56.57±1.07	57.73±1.78
9	49.47±1.11	61.29±1.28	77.97±1.32	63.81±1.05	62.27±0.47
10	61.36±1.03	73.80±1.01	86.19±1.16	71.96±1.49	76.88±0.78



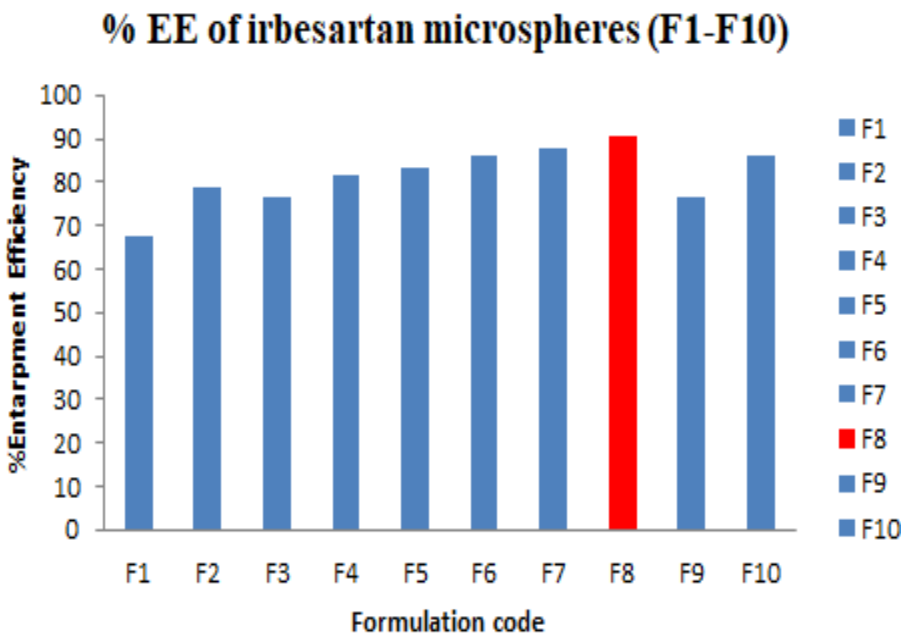


Figure No.8 *In-vitro* Drug release profile of Irbesartan microspheres F1-F10 in 0.1N HCl and Phosphate buffer pH 7.4

Figure no.8 clearly depicts that as the concentration of the sodium alginate and chitosan increases there is an increase in the solubility of the irbesartan and increase in the *In-vitro* drug release as well. From the formulations F1-F10 the concentration of the sodium alginate and chitosan is gradually increasing and the rate of the drug release is also increasing.

Solubility studies [15]

Solubility of Irbesartan was seen in various solvents. Solubility profile of Irbesartan in different solvents.

Table no: 8 Solubility studies of Irbesartan

Solvent	Category
Distilled water	Insoluble
Methylene chloride	Completely soluble
Methanol	Completely soluble

Micromeritic properties of irbesartan microspheres

Flow properties of the optimized Irbesartan microsphere formulation (F1-F10) was shown in the below Table no. 4.14. Irbesartan microspheres were subjected to Angle of Repose, Bulk Density, Tapped Density, Hausner's Ratio and Compressibility Index.

Table No.9: Flow properties of the Irbesartan microspheres.

Formulation Code	Angle of Repose	Bulk Density	Tapped Density	Hausner's Ratio	Carr's Compressibility Index
F1	26.32±0.49	0.52±0.02	0.61±0.02	1.26±0.01	14.76±0.21
F2	22.43±0.91	0.53±0.04	0.64±0.02	1.20±0.03	16.58±0.9
F3	36.52±0.81	0.46±0.02	0.34±0.04	1.11±0.02	18.79±0.7
F4	22.19±0.93	0.47±0.02	0.54±0.01	1.18±0.05	17.24±0.8
F5	19.45±0.34	0.51±0.01	0.40±0.03	1.25±0.02	18.30±0.9
F6	20.43±0.84	0.37±0.05	0.43±0.04	1.09±0.03	14.43±1.3

F7	23.89±0.45	0.49±0.03	0.57±0.02	1.23±0.03	18.72±1.01
F8	**15.98±0.93**	**0.43±0.03**	**0.53±0.03**	**1.27±0.02**	**19.46±1.03**
F9	18.39±0.48	0.39±0.06	0.45±0.04	1.25±0.03	17.29±1.1
F10	15.29±0.89	0.53±0.09	0.52±0.01	1.32±0.02	19.32±

The obtained microspheres showed good flow properties, which were evaluated in angle of repose (15.29-26.32), bulk and tapped densities (0.37-0.53 and 0.43-0.64), Carr's indices (12.94-19.14%) and Hausner's ratio (1.14-1.23).

Drug Content

Irbesartan microspheres with varying concentrations of sodium alginate and chitosan were subjected to the drug content. It helps in determining the amount of the drug present within the irbesartan microspheres.

Table No.10 Percentage drug content of the irbesartan microspheres

Formulation Code	%Drug Content	Formulation Code	%Drug Content
F1	74.63	F6	83.56
F2	83.29	F7	79.41
F3	84.21	**F8**	**89.46**
F4	78.49	F9	77.76
F5	81.31	F10	83.12

The drug content of the optimized Irbesartan microspheres formulation F8 was found to be 89.46%.

Stability Studies

Drug content and were analyzed periodically as per ICH guidelines through accelerated stability studies for optimized microsphere formulation F8.

Table No.11 Stability study data for optimized irbesartan microspheres

Stability Conditions	% Drug Content F8		
	Number of Months		
	1	2	3
30º±2ºC/65% RH	86.53	84.61	87.42

Table no.11 shows at fixed time intervals, % drug content of these formulations shows no significant change. At the first month of storage the drug content was found to be 86.53% and at the end of the third month it was found to be 87.42% at 30˚C and 65% RH. Thus we may conclude that the drug does not undergo degradation on storage.

CONCLUSION

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. The solubility of irbesartan was found to be highest in 4% of chitosan and 5% sodium alginate solutions in F8 formulation. The composition of best selected formulation consists of 4% chitosan, 5% sodium alginate, 3% calcium chloride, irbesartan 150mg. Formulation F8 showed drug content 89.46%, *in vitro* drug release 86.19%. 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. From the above results it can be concluded that the proposed objective of the present research work achieved successfully.

REFERENCES

[1]. Reddy S, Krishna KVM and Srikanth S: A review on microsphere for novel drug delivery system. *International Journal of Research in Pharmacy and Chemistry* 3(4), 2013, 763-7.

[2]. Venkatesan K, Manavalan R and Valliapan K: Microencapsulation: A vital technique in novel drug delivery system. *Journal of Pharmaceutical Sciences and Research* 1(4), 2009, 26-35.

[3]. Nagpal M, Midha K and Arora S: Microspheres: A recent update. *International Journal of Recent Scientific Research* 5(3), 2015, 543-66.

[4]. Jain NK: Controlled and novel drug delivery, New Delhi: *CBS Publishers*, 4, 2004.

[5]. Lin Y, Paschalis A. Physicochemical Aspects of Drug Delivery And Release From Polymer- Based Colloids. *Curr. Opin. Colloid Interfac. Sci.,* 5, 2000, 132-143

[6]. Vyas SP, Khar RK. Targeted and Controlled Drug Delivery Novel Carrier System. New Delhi: *CBS Publishers and Distributors*; 1, 2002, 417-441.

[7]. Mankala, Preparation and characterization of mucoadhesive microspheres of gliclazide with natural gums, *Sci. J. pharm*. 4(1), 2011, 38-48.

[8]. Shwetha S., Kamath K., Senthil Kumar SK., Design and evaluation of floating microspheres of Rabeprazole sodium. *Int. J. Pharm. Pharm. Sci.* 4(3), 2012, 357- 367.

[9]. Chowdhary KPR., Srinivasa RY., Preparation and evaluation of mucoadhesive microcapsules of indomethacin, *Ind. J. Pharm. Sci.,* 65(1), 2003, 49-52.

[10]. Dandagi PM, Mastiholimath VS, Gada AP., Iliger SR. Mucoadhesive microspheres of Propranolol hydrochloride for nasal delivery. *Ind. J. Pharm. Sci.* 69, 2007, 402-407. 20.

[11]. Rajamanikan D, Rajappan M, Varadharaan M, Srinivasan B. Formulation and Evaluation of Albumin Microspheres Containing Aceclofenac. *International Journal of Pharmaceutical Sciences and Reasearch,* 4(1), 2010, 112-117.

[12]. Ankita Garg, Prashant Upadhyay, Mucoadhesive Microspheres: A Short Review, *Asian Journal of Pharmaceutical and Clinical Research,* 5(3), 2012, 24-27.

[13]. Shiv Shankar Hardenia, Ankit Jain, Ritesh Patel, Anu Kaushal, Formulation and Evaluation of Mucoadhesive Microspheres of Ciprofloxacin. *Journal of Advanced Pharmacy Education and Research*, 1(4), 2011, 214-224.

[14]. Raghavendra Rao NG, Upendra K., Anand D., Suresh DK. Preparation and characterization of ionotropic cross-linked chitosan microparticles for controlled release of Aceclofenac. *Int. J. Pharm. Sci. Drug. Res*. 2, 2010, 107-111.

[15]. Surendranath Betala, M. Mohan Varma, K. Abbulu. Formulation and Evaluation of Sustained release microspheres of Metoprolol. *International research journal of pharmacy.* 8(11), 2017.