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Research Article

PREPARATION OF CONTROLLED RELEASE METFORMIN HYDROCHLORIDE LOADED CHITOSAN MICROSPHERES AND EVALUATION OF FORMULATION PARAMETERS

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ABSTRACT

In this work an attempt was made for the preparation and evaluation of controlled release chitosan microspheres using anti-diabetes drug Metformin hydrochloride. The microspheres were prepared by Ionotropic gelation method using chitosan as polymer and Sodium Tripolyphosphate (TPP) as crosslinking agent. The compatibility of drug and polymer is analyzed by using FTIR and DSC method. There was no interaction detected by FTIR and DSC study. Further the prepared microspheres were evaluated for particle size, drug entrapment efficiency, surface morphology, drug content, drug loading and in vitro drug release. Amongst all the formulation batch 7 shows the best release when compared to other batch. SEM (Scanning electron microscopy) revealed that microspheres were spherical and porous. Finally it was concluded that Metformin hydrochloride loaded chitosan – TPP microspheres have been found suitable for controlled release formulation due to its bioavailability and biodegradability and thus lead to improved patient compliance.

Keywords: Microspheres, Metformin hydrochloride, Ionotropic gelation method, chitosan.

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INTRODUCTION

Over the past 30 years, greater surveillance has been focused on the development of controlled release drug delivery system to provide uniform concentration of the drug at the absorption site, and diminishing not only the side effects but also the frequency of administration.¹ There are many ways for delivering a therapeutic substance to the target site in a controlled release manner. One such way is using microspheres as carriers for drugs. Microspheres can be defined as the structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1µm to 1000 µm).² Diabetes is one of the most continual and significant issue around the world especially Indians are genetically more prone to it.³ Metformin hydrochloride is an oral anti-hyperglycemic biguanide agent used in the treatment of

non- insulin dependent diabetes mellitus (type II diabetes). Metformin hydrochloride enhances glycemic control by improving insulin sensitivity and decreasing intestinal absorption of glucose.⁴ Chitosan is most widely used as a carrier for various novel dosage forms mainly due to its characteristic to improve the solubility of poorly soluble drugs as well as to control the release of drugs by slow erosion from hydrated matrix.^{5,6}

MATERIAL AND METHODS

Metformin hydrochloride was granted from Tidal Pharmaceuticals Pvt Ltd, Gowalthai. Chitosan was obtained from LOBA. Sodium Tripolyphosphate was obtained from CDH Laboratory Reagents, New Delhi.

Preformulation Studies

Preformulation study is the initial step in judicious development of both active pharmaceutical ingredient (API) and drug product. A preformulation study is an

important mechanism for determination of physical and chemical properties of the drug before including it in formulation advancement. Preformulation studies are essential treaty for progress of safe, effective, and stable dosage form. Thus, in order to establish supreme condition for clinically useful delivery system, preformulation studies were executed. Valuable pharmaceutical dosage form development requires various preformulation guidelines. Preformulation studies include solubility, melting point, and partition coefficient.^{7,8} The drug and excipient interaction studies were carried out by FT-IR and DSC.

Determination of lambda max

UV visible spectrophotometric method was used to gather structural information about chromophoric part of Metformin hydrochloride. 10 mg of the drug was dissolved in 10 ml and further volume made up to 100 ml with saline phosphate buffer pH 7.4, suitable dilutions were made. Zero order spectra were reported in the range of 200-400 nm to establish absorption maxima of Metformin hydrochloride.⁹

Fourier Transform Infra-Red (FT-IR) Spectroscopy

IR spectroscopy is most effective system for qualitative identification of compound. The necessary information regarding the group present in specific compound has been given by IR spectroscopy. IR study was executed by using Perkin Elmer Fourier transformed infrared spectrophotometer. The potassium bromide (KBr) disk technique was applied using 100 mg of spectroscopic grade dried KBr. KBr was ground into fine powder using a mortar/pestle and coagulate into disc under a hydraulic pressure at 10,000psi. Metformin hydrochloride, chitosan, and drug excipient mixture were deposited on the KBr disc with the help of a capillary tube. Each KBr disc was examined at a resolution of 400 cm⁻¹ over the wavelength region of 4000 – 400 cm⁻¹ and characteristics bands were reported.¹⁰

Differential Scanning Calorimetry (DSC)

To analyze any possible correlation between the drug and the utilized additives, DSC thermo grams of pure drug (Metformin hydrochloride) and drug excipient mixture with additives were accomplished. The samples of 10 mg were taken and heated in open aluminium pans at a heating rate of 10°C/min in a 30°C to 300°C temperature range.¹¹

Preparation of standard curves of Metformin hydrochloride

By reading the instrument (UV-visible spectrophotometer) at 233 nm Metformin hydrochloride concentration in the solution was determined. The standard curves of Metformin hydrochloride were prepared in saline phosphate buffer pH 1.2 and, pH 7.4.

Preparation of calibration curve of Metformin hydrochloride at pH 1.2

50 ml of the potassium chloride solution (0.2M) was placed in a 200 ml volumetric flask and 95 ml hydrochloric acid solution (0.2M) was added to it and

then distilled water was added to make the volume to 200 ml.

Preparation of calibration curve of Metformin hydrochloride at pH 7.4

50 mg of Metformin hydrochloride was correctly weighed and transferred into 50 ml standard flask and then volume was made up to 50 ml. Pipette out 0.2ml, 0.4ml, 0.6ml, 0.8ml, and 1 ml from the above solution transferred into 10 ml standard flask and diluted to 10 ml with phosphate buffer 7.4.

Preparation of chitosan microspheres by Ionotropic gelation method

In this method chitosan stock solution (1% w/v) was prepared by dissolving chitosan in glacial acetic acid (1% v/v) at room temperature. The drug Metformin hydrochloride (1% w/v) was dissolved directly into the above prepared chitosan solution. Through a disposable syringe needle 10 ml of this bubble free solution was dropped into a gently agitating 100 ml of 2% (w/v) sodium tripolyphosphate solution. The dropping rate and falling distance were kept constant. The solution was magnetically stirred for half an hour followed by filtration and rinsing with distilled water. Gel like beads were achieved which air was dried for 24 hours followed by over drying for 6 hours at 50 °C.¹²

Characterization of Metformin hydrochloride loaded chitosan microspheres

Particle size determination

Particle size of microsphere formulations was measured using Zetasizer Nano Series- ZS (Malvern instrument, UK). Each sample was measured in triplicate and the average results were calculated.^{13, 14, 15}

Determination of percentage yield

The yield of microspheres was measured with concern to drug and polymer weight. After the preparation of microspheres they were collected, dried and weighed over electronic digital balance and the percentage yield was calculated by the formula¹⁶:

$$\text{Percentage yield (w/w)} = \frac{\text{weight of dried microspheres}}{\text{Weight of chitosan} + \text{weight of TPP}} \times 100$$

Determination of drug content

The drug content of Metformin hydrochloride was determined by UV spectrophotometric method. In 50ml phosphate buffer solution (pH 7.4) accurately weighed 100 mg blend of microsphere and carrier was dissolved and absorbance was measured on UV spectrophotometer at 233 nm. The method is verified for linearity, accuracy and precision.¹⁷

Determination of drug loading

Depending upon the results of drug content, drug loading was determined by the formula:

$$\text{Drug loading} = \frac{\text{weight of the drug in microspheres}}{\text{Weight of microspheres}} \times 100$$

Determination of Entrapment Efficiency

To determine entrapment efficiency firstly the chitosan microspheres were crushed to powder with the help of

mortar –pestle. 50 mg of the powder was taken for estimating the amount of drug absolutely present in microspheres. To a flask containing 10ml of 0.1 N HCl this powder was added for digestion for 24 hours. Later, the solution was filtered through Whatman filter paper no 4 and optical density of the filtrate was taken spectrophotometrically.¹⁸ The encapsulation efficiency was calculated by the following equation:

$$\% \text{ Encapsulation Efficiency} = \left(\frac{\text{Actual entrapment level}}{\text{Theoretical entrapment level}} \right) \times 100$$

In-vitro dissolution studies

USP type II apparatus (paddle type) were operated to perform *in-vitro* dissolution studies of Metformin hydrochloride. By using phosphate buffer of pH (7.4) as dissolution medium at $37 \pm 0.5^\circ\text{C}$ with 100 rpm speed the dissolution studies were conducted. From the preparation each of samples equivalent to 100 mg of microsphere were added into the dissolution medium. Periodically (0, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 10 hrs) 1ml aliquots of the sample were withdrawn and filtered through 0.45μ membrane filter. Every time the withdrawn sample was replaced with the same quantity of fresh dissolution medium. The filtered solutions were diluted suitably. By using UV spectrophotometer at wavelength of 233 nm the samples were examined for their drug content.¹⁹

Scanning Electron Microscope (SEM)

Scanning electron microscope is used to attain scanning electron micrographs of Metformin hydrochloride microspheres. The instrument used for this purpose is Hitachi S-4800 scanning electron microscope. The microspheres were assembled directly on to the SEM sample stub, using double sided sticking tape, and coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr).²⁰

Stability Studies

The main objective of stability testing is to give evidence on the changes of quality of drug product with respect to time under the influence of various environmental factors such as temperature, humidity, and light, and enables recommended storage conditions, re-test periods and shelf lives to be accomplished.

According to the ICH guidelines the optimized formulation was kept for accelerated stability for six months. Microspheres were kept in stability chamber maintained at temperature of $40^\circ\text{C} \pm 2^\circ\text{C} / 75\% \text{RH} \pm 5\% \text{RH}$. During the study period, the formulation was monitored at prearranged time intervals of 0, 15, 30, 45, 60, 75, 90, 180 days for change in physical appearance, drug content and *in-vitro* release characteristics.^[21]

RESULT AND DISCUSSION

Preformulation Studies

Metformin hydrochloride was found to be white hygroscopic crystalline powder.

Solubility

Metformin hydrochloride was found to be freely soluble in water, isopropyl alcohol, methylene chloride, slightly soluble in ethanol and practically insoluble in chloroform and acetone.

Melting Point

The calculated melting point of Metformin hydrochloride was found to be 224°C . This result is the same as reported in reference and helps in the identification and purity of the drug powder used in the study.

Partition Coefficient

The partition coefficient of Metformin hydrochloride was found to be 0.062.

Determination of absorption maxima

Absorption maxima of metformin hydrochloride in phosphate buffer 7.4 were found to be 233 nm which is similar to the pharmacopoeial standards.

Fourier Transform Infra-Red Spectroscopy

The FT-IR spectrum of Metformin hydrochloride, chitosan and their physical mixture evidenced various characteristics peaks which are showed in Fig 1, Fig 2, and Fig 3. The spectra showed that the free Metformin hydrochloride and formulated Metformin hydrochloride were in resemblance that ratified that the obtained sample of Metformin hydrochloride was pure.

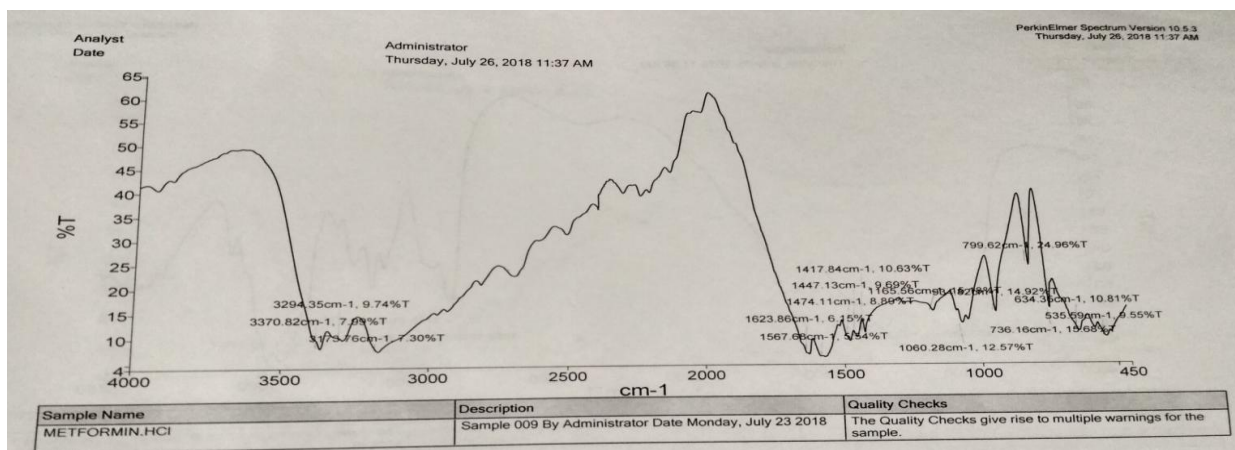


Figure 1: FT-IR spectrum of Metformin hydrochloride

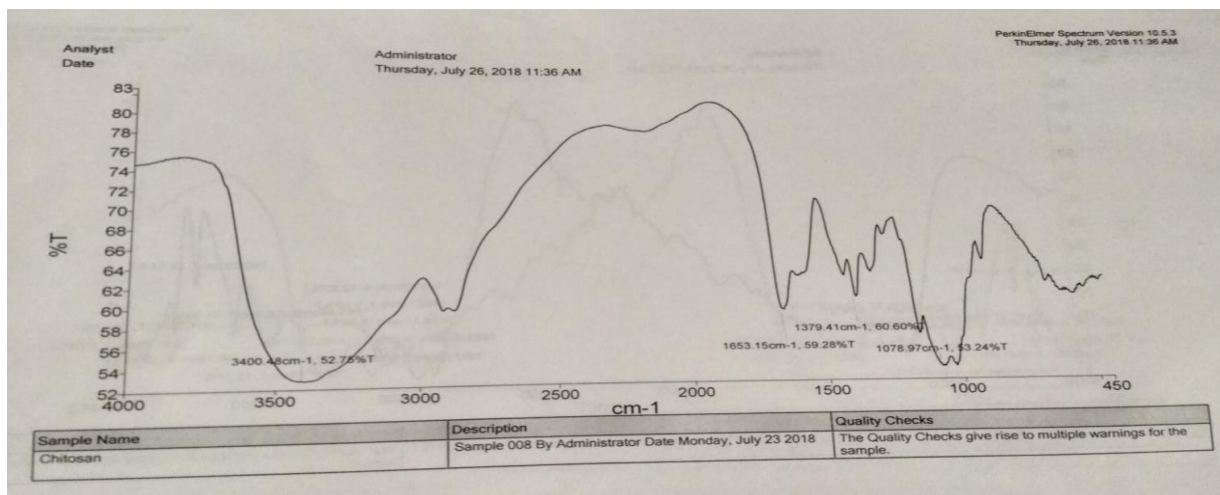


Figure 2: FT-IR spectrum of Chitosan

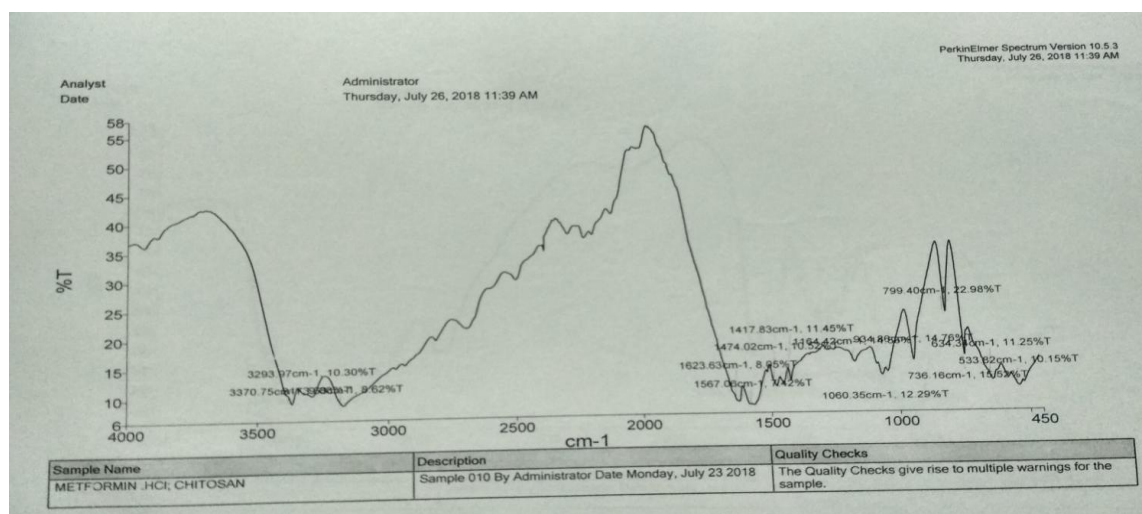


Figure 3: FT-IR spectrum of Metformin hydrochloride, chitosan.

Differential Scanning Calorimetry (DSC) studies

Thermal characteristics of pure drug, polymer, and chitosan unloaded microspheres and chitosan drug

loaded microspheres was examined by using DSC analysis which are showed in Fig 4, Fig 5, Fig 6, and Fig 7. DSC analysis revealed that there is no interaction between polymer and drug.

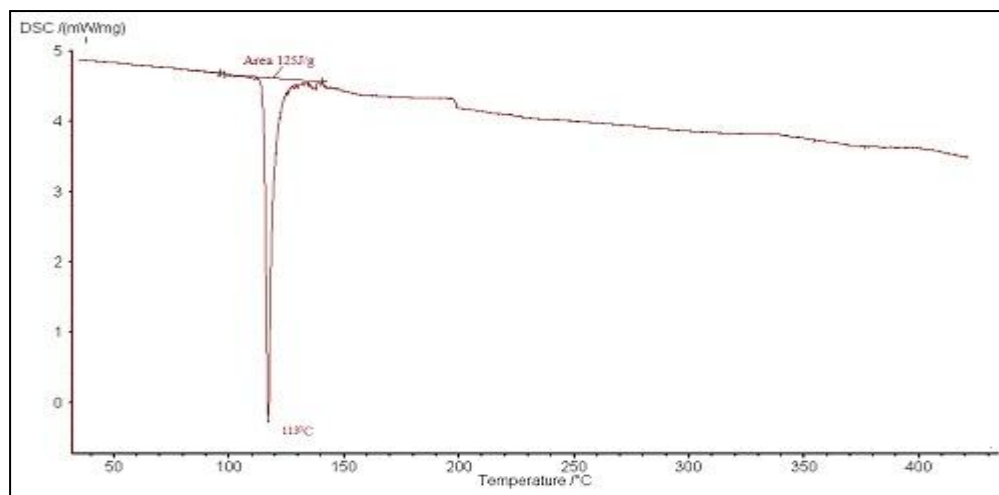


Figure 4: DSC thermogram of Chitosan

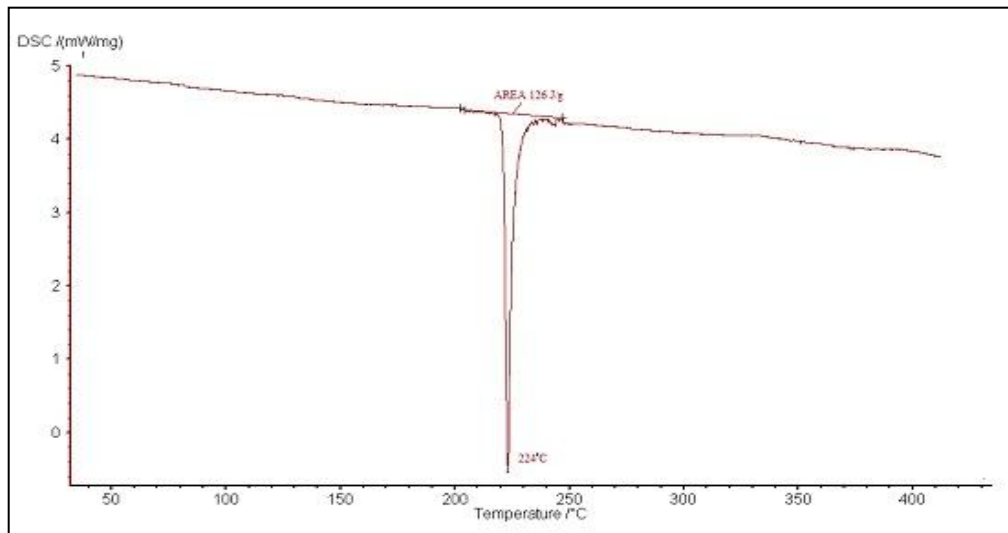


Figure 5: DSC thermogram of Metformin hydrochloride

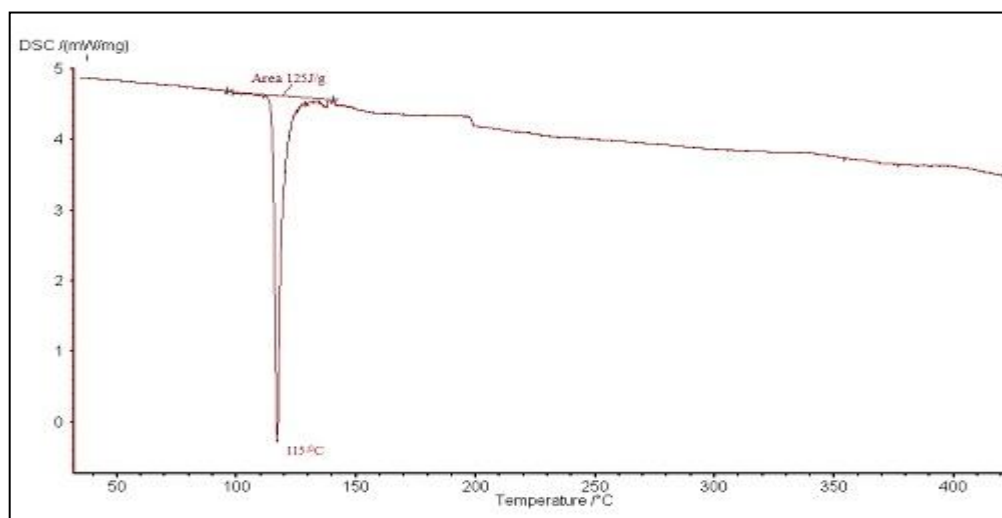


Figure 6: DSC thermogram of unloaded Chitosan microspheres

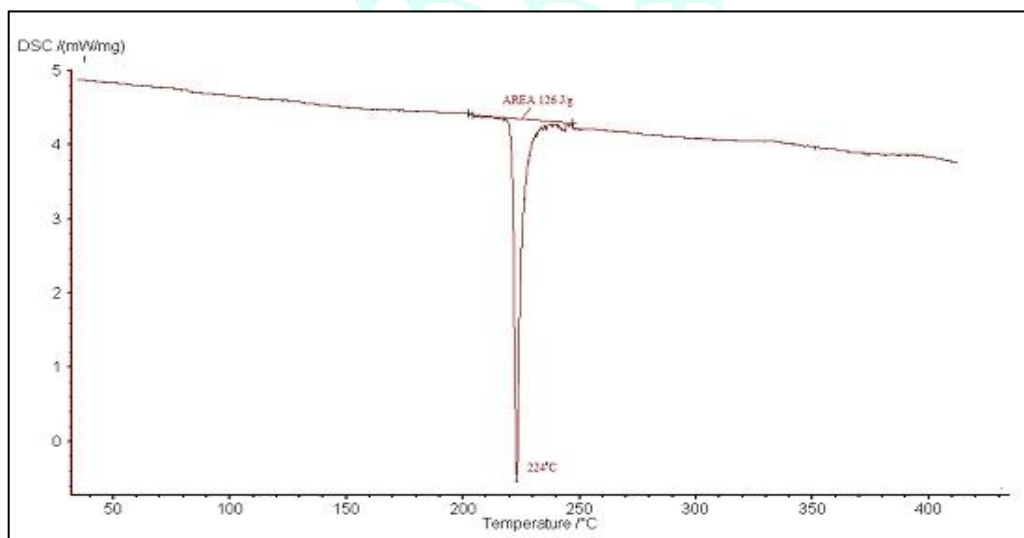


Figure 7: DSC thermogram of drug loaded chitosan microspheres.

Standard curve of Metformin hydrochloride insaline phosphate buffer pH 1.2 and pH 7.4.

Standard stock solution and standard working solutions were prepared in saline phosphate buffer pH 1.2; pH 7.4 and standard curves were obtained by plotting the data.

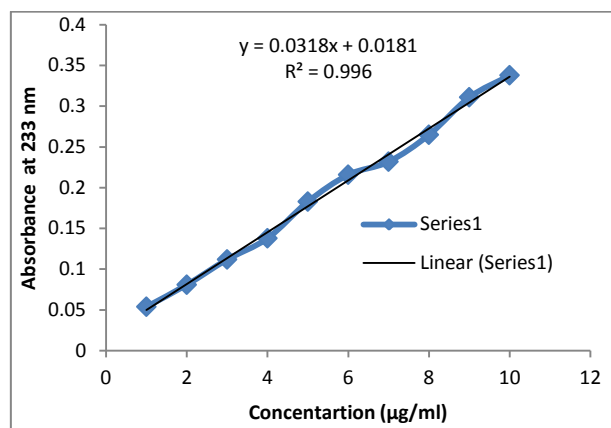


Figure 8: Standard curve of metformin hydrochloride in saline phosphate buffer pH 1.2

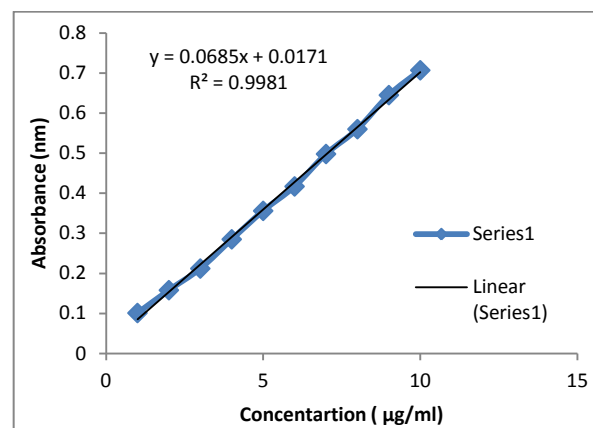


Figure 9: Standard curve of Metformin hydrochloride in saline phosphate buffer pH 7.4

Formulation and evaluation of Metformin hydrochloride loaded chitosan microspheres

Microspheres were prepared by using ionotropic gelation method. Further the prepared microspheres were evaluated for particle size, percentage yield, drug content, drug loading and entrapment efficiency.

Table 1: Variables for preparation of microspheres

S. No.	Conc. of Chitosan (%)	Conc. of TPP (%)	Stirring Speed (rpm)	Stirring time (min.)	Volume of chitosan-drug solution	Result/Sphericity
1	1	0.5	600	30	10	Spherical
2	1	1	600	30	10	Spherical
3	1	1	800	30	10	Spherical
4	1	1	800	30	10	Spherical
5	1.5	2	1000	30	10	Spherical
6	1.5	2	1000	30	10	Spherical
7	1.5	2	1200	30	10	Spherical
8	2	2	1200	30	10	Spherical
9	2	3	1400	30	10	Spherical
10	2	3	1500	30	10	Spherical

Particle size determination

Table 2: Variables for microspheres preparation

BATCH NO	Chitosan: Metformin HCl ratio	Particle size (µm)
F1	Drug free particle	600
F2	1:0.5	671
F3	1:1	595
F4	1:1.5	715
F5	1:1.75	585
F6	1:2	634
F7	1:2.5	580
F8	1:2.75	760
F9	1:3	812
F10	1:3.5	592

The microspheres prepared under stirring speed of 1200 rpm with chitosan concentration (1.5%) and TPP concentration (2%) shows best result for particle size that is 580 µm. The mean particle size of all the prepared

batches of microspheres ranged between 580µm -812µm as listed in table 2. The batch 7 having Chitosan: Metformin HCl ratio of (1:2.5) showed least particle size and was used for further experiment.

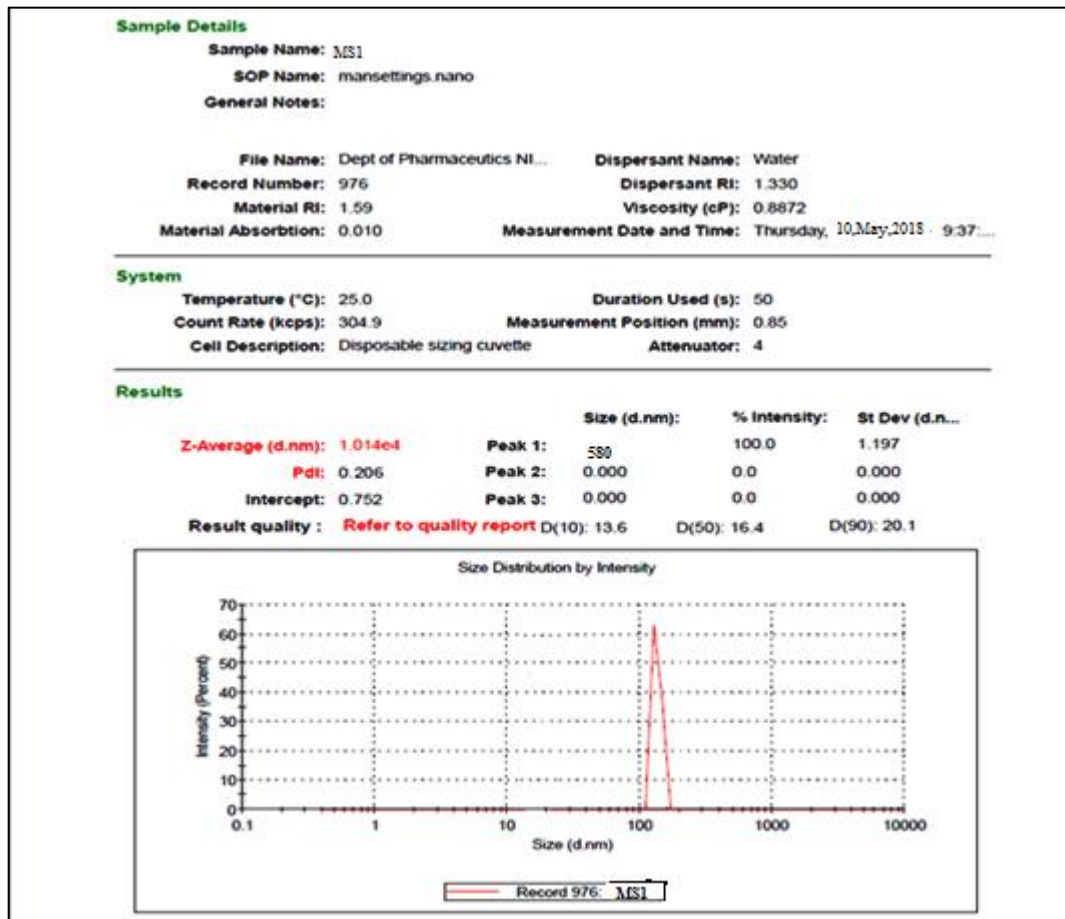


Figure 10: Particle size of final optimized formulation

Evaluation parameters of microspheres

The prepared formulations were evaluated for various parameters such as percentage yield, drug content, drug loading, and entrapment efficiency.

Table 3: Different evaluation parameters of microspheres

Batch. NO.	Percentage yield %	Drug content (%)± SD	Drug loading (%)± SD	Encapsulation Efficiency (%)
F1	23.9%	82.17±2.21	0.202±0.01	83.17
F2	19.4%	76.75±4.50	0.180±0.005	77.75
F3	23.4%	70.26±0.3	0.159±0.008	94.05
F4	15.8%	90.31±0.04	0.153±0.002	90.31
F5	15.2%	83.45±0.06	0.131±0.005	84.45
F6	26.3%	78.47±0.09	0.112±0.006	80.47
F7	26.5%	95.07±0.32	0.124±0.001	94.26
F8	25.2%	91.96±0.10	0.103±0.003	92.96
F9	24.8%	85.57±0.3	0.094±0.002	86.76
F10	22.4%	84.52±0.2	0.091±0.004	94.13

The maximum percentage yield, drug content, drug loading and entrapment efficiency was obtained from batch 7. An increase in the amount of polymer (chitosan) is mainly responsible for increase in drug content, drug loading, and entrapment efficiency. The mean particle size is directly related to the polymer concentration. The increase in polymer concentration is only the reason for increase in the particle size. From batch 7 the percentage yield was found to be 26.5%,

drug content was found to be 95.07±0.32, drug loading was found to be 0.124±0.001 and encapsulation efficiency was found to be 94.26.

In- vitro dissolution studies

The in- vitro dissolution studies of Metformin hydrochloride from microspheres was examined in saline phosphate buffer pH 7.4.

Table 4: Cumulative % drug permeated from optimized formulation

S. NO.	Time (hours)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1	0	0	0	0	0	0	0	0	0	0	0
2	0.25	4.5	5.5	5.21	5.74	6.01	4.98	6.27	2.75	4.69	5.49
3	0.5	18.23	19.02	22.4	18.2	22.26	20.52	18.7	19.07	21.1	19.19
4	1	29.36	26.09	33.24	25.52	28.46	31.99	30.84	28.05	28.99	27.69
5	2	32.82	33.25	40.65	34.63	31.86	38.66	37.98	37.09	34.18	31.88
6	3	36.33	38.63	46.89	39.72	34.96	45.69	48.32	42.98	39.81	36.35
7	4	41.75	42.64	49.95	43.98	39.03	50.21	57.07	48.11	44.67	40.94
8	5	45.65	47.36	54.06	50.48	44.25	56.59	64.2	54.8	50.92	46.09
9	6	51.25	54.25	57.32	57.26	48.64	60.18	70.56	59.27	54.79	50.68
10	8	56.37	59.87	65.14	63.59	56.83	67.84	81.26	66.78	61.57	58.05
11	10	63.32	65.39	71.93	75.41	62.6	75.54	89.98	73.44	69.57	65.23

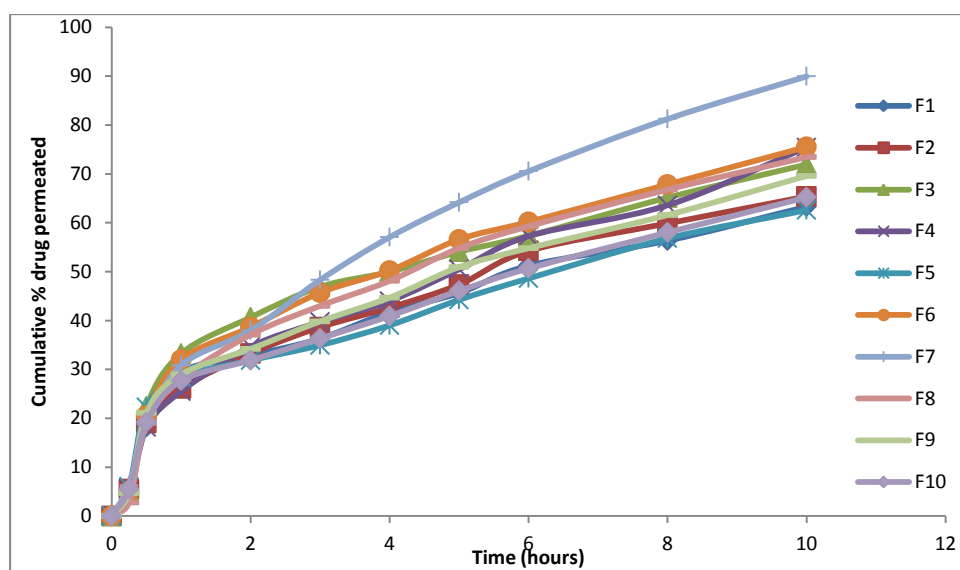


Figure 11: *In-vitro* drug release profile of different chitosan microspheres

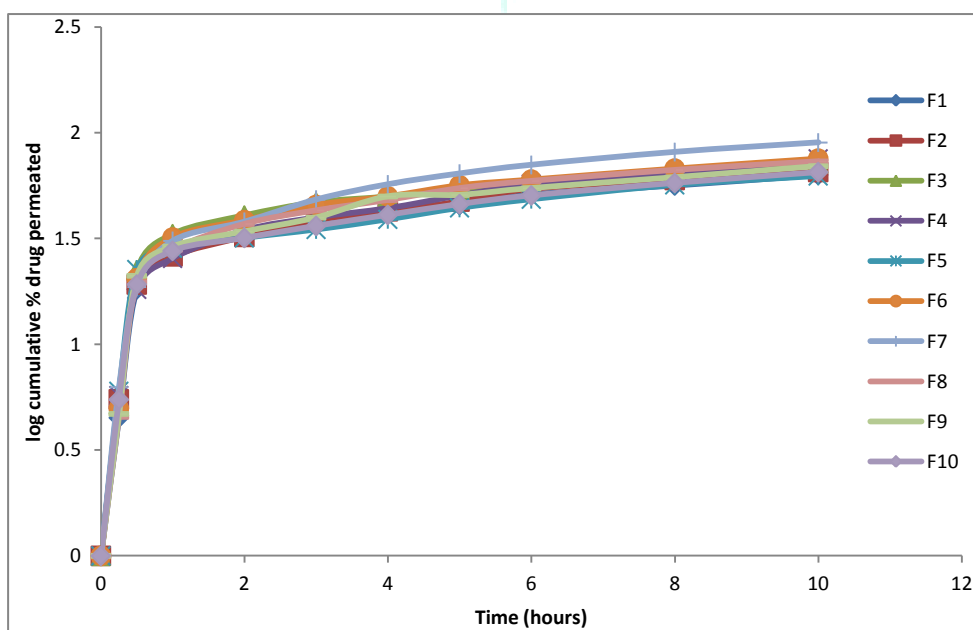


Figure 12: Drug Release Kinetics of Optimized Formulation (FirstOrder Kinetics

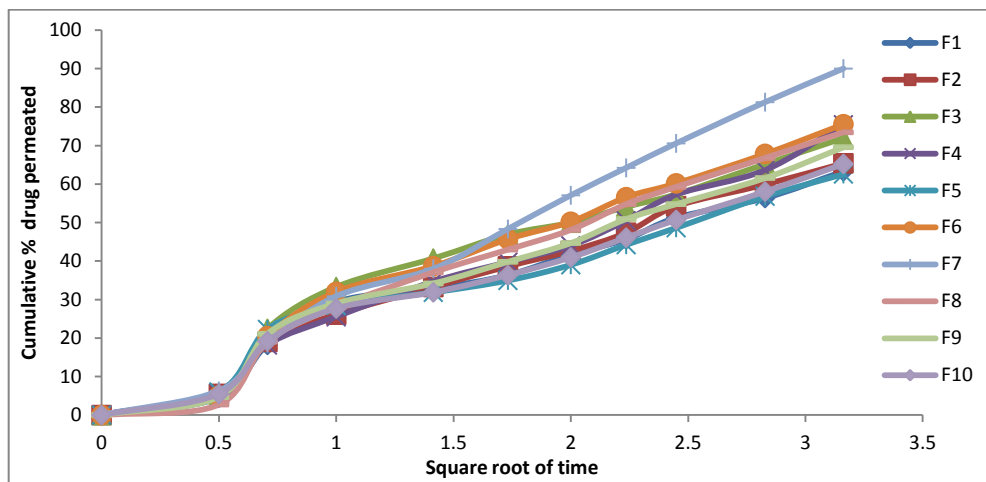


Figure 13: Drug Release Kinetics of Optimized Formulation (Higuchi Model)

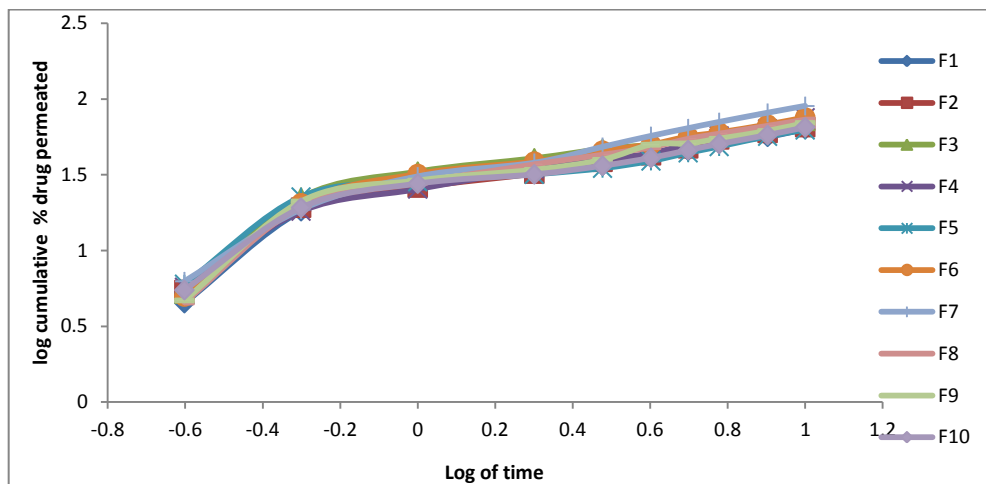


Figure 14: Drug Release Kinetics of Optimized Formulation (Korsmeyer – Peppas Model)

Scanning Electron Microscopy (SEM)

Scanning electron microscopy revealed that the microspheres were spherical and porous. The surface of the microspheres was rough and exhibited the presence of pores in the drug loaded microspheres. The initial burst release of the drug during dissolution is mainly due to the presence of drug particles on the surface. Surface study of the microspheres after dissolution showed bigger pores indicating that pores are responsible for the drug release and the mechanism of the drug may be diffusion controlled.

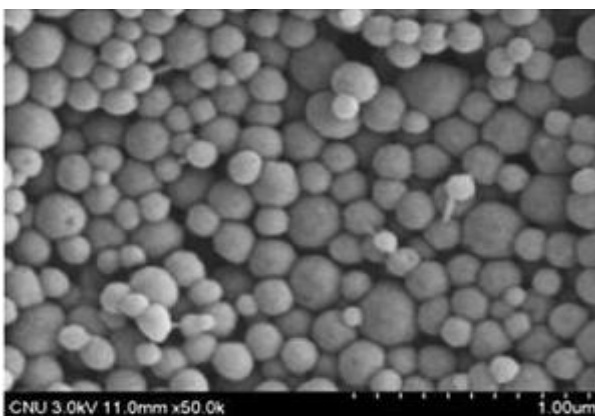


Figure 15: Scanning electron microscopy of chitosan microspheres

Stability studies of Metformin hydrochloride loaded chitosan microspheres

Optimum microspheres were exposed to accelerated storage conditions, $40^{\circ}\text{C} \pm 2^{\circ}\text{C}/75\% \text{RH} \pm 5\%$ for six months according to ICH guidelines for stability testing of new drug substances and products. Microspheres were characterized for physical appearance, drug content and *in-vitro* drug release at regular intervals. There was no change in the physical appearance of microspheres. Additionally there was no great difference in the drug content and *in vitro* release characteristics of drug. Thus result implies good stability of the formulation.

CONCLUSION

This research affirmed that the controlled release of Metformin hydrochloride loaded chitosan microspheres can be attained by ionotropic gelation technique using chitosan as polymer. DSC and FTIR studies exposed that there is no interaction between the drug and polymer which implies that drug is congenial with polymer. The ten batches were evaluated for particle size, percentage yield, drug content, drug loading and entrapment efficiency. The batch 7 (B7) with the drug and polymer ratio (1:2.5) was considered to be superior which showed least particle size, and maximum percentage yield, drug content and entrapment efficiency and

prolonged release of drug. The drug release from microspheres was affected by various chitosan concentrations. Scanning Electron Microscopy (SEM) studies revealed that the microspheres were spherical and porous. The drug release was found to be in controlled manner from batch (B7). Therefore, it was concluded that Metformin hydrochloride loaded chitosan microspheres were found to be best in the treatment of

diabetes with reduced dosing frequency and better diagnosis in a controlled release mode.

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