International Journal of Drug Development & Research
| January-March 2013 | Vol. 5 | Issue 1 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands
SJR Impact Value 0.13 & H index 2

Full Length Research Manuscript

Development & Optimization of Pectin microsphere of Metformin HCL

Konar Sanjoy[1], Dutta Roy Swagata [2], Sahoo Himanshu Bhusan[3], Nandy Subhangkar[3]
[1]Dept. of Pharmaceutical Chemistry, SRLT College of Pharmaceutical Science & Technology, Ekdil, Etawah.
[2]Dept. of Pharmaceutics, SRLT College of Pharmaceutical Science & Technology, Ekdil, Etawah.
[3]Dept. of Pharmacology, Vedica College of Pharmacy, RKDF Group, Bhopal, MP, India.

Abstract

To develop & optimize the pectin microspheres of Metformin hydrochloride. Solvent evaporation method used in the microencapsulation process. Concisely the polymer ethyl cellulose (500mg) was dissolve in 40 ml of ethanol to get a clear solution. The drug Metformin (500mg) was added and dissolved in the polymer solution. The resultant mixture was then stirred at 900 r.p.m for 2 hr to evaporate the volatile substance. The formed microspheres were collected and air dried for 3hr and stored in desiccators for further use.

The percentage yield of all the formulations was found to be satisfactory. It can be due to the involvement of process parameters. Drug entrapment efficiency (DEE) of F2 formulation found to be high because, the drug is fully dispersed in the polymer phase by continuous stirring for a longer period. The particle size of all the formulations found to be satisfactory and within the range of (34.56 to71.34µm). Particle sizes of the formulations prepared by W/O emulsion solvent evaporation method are within the range of 34.02 µm and that formulation prepared by solvent evaporation method were within the range of 39.64 to 48.21 µm. FT-IR spectra for the drug were recorded and it was compared against the FT-IR spectra of the formulated drug along with the polymer. Metformin gives characteristics peaks at wave number 1254,1473,1620,3270 and 3288. The peak at 1254 corresponds to the C-N stretching, 1473 to C-H stretching 1620 to c=c stretching, where as the peak at 3270 and 3288 corresponds to –NH stretching. Thus FT-IR studies revealed that there was no shift in peaks of the formulation, thus indicating there was no interaction between drug and other polymers used. Pectin microspheres of metformin hydrochloride prepared by solvent evaporation method showed good entrapment property with well release characteristics and there no interaction drug and excipients is observed.

*Corresponding author, Mailing address:
Subhangkar Nandy,
Asst. Professor in Pharmacology,
Vedica College of Pharmacy, Bhopal, MP
E-mail: subhangkarnandy@gmail.com

Key words:
microencapsulation, microsphere, Drug entrapment efficiency, stretching

How to Cite this Paper:
Konar Sanjoy, Dutta Roy Swagata, Sahoo Himanshu Bhusan, Nandy Subhangkar "Development & Optimization of Pectin microsphere of Metformin HCL" Int. J. Drug Dev. & Res., January-March 2013, 5(1): 339-356.

Article History:----------
Date of Submission: 01-02-2013
Date of Acceptance: 18-02-2013
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION

The novel system of drug delivery offer a means of improving the therapeutic effectiveness of incorporated drugs by providing sustained, controlled delivery and targeting the drug to desired site. The goal of any drug delivery system is to

provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then maintain the desired drug concentration. The history of controlled-release technology can be divided roughly into three time periods. From 1950-1970 is the period of sustained drug release. A number of system containing hydrophobic polymers and waxes were fabricated with drugs into dosage form with the aim of sustaining drug levels and hence drug action for an extended period of time. However, a lack of understandings of anatomical and physiological barriers imposed impediments on the development of efficient delivery system. The period 1970 to 1990 was involved in the determination of the needs in controlled drug delivery and to understand the barriers for various routes of administration [1, 2 and 3]. Post 1990 is the modern era of controlled release technology and represents the period in which an attempt at drug optimization is emphasized. The drug delivery system should deliver a drug at a rate dictated by the needs of the body over a specified period of treatment [4, 5 and 6]**.** This idealized objective points to the two aspects most important to the drug delivery namely,

1. Relates to targeting a drug to a specific organ or tissue.
2. To controlling the rate of drug delivery to the target tissue.

The objective of the proposed work is to evaluate the efficacy of a naturally obtained herbal gums and exudates of some selected plants to qualify them as polymers for designing an oral microsphere for the delivery of selected oral anti-diabetic drugs. Formulation of the microspheres employing the polymer, or a polymer-copolymer blend will also be a part of this endeavor. Subsequent evaluation of the different formulations for its different physical parameters as well as its *in-vitro* release profiles will be undertaken. Characterization of the polymer remains another key target. Statistical interpretation will be applied wherever possible. Attempts will also cover the possibility of establishing efficacy of the formulated dosage form by different *in-vivo* animal studies.

MATERIALS AND METHODS

Materials

Metformin HCl (Zydus health care, Sikkim), Ethyl cellulose, Liquid paraffin light, Hydrochloric acid, Sodium hydroxide, Span-80 (Sd-Fine chemicals, Mumbai), HPMC (Lova Chem, Mumbai), Hexane, Methanol, Acetone (Universal laboratory, Mumbai), Acrylcoat S 100 (Corel Pharma, Ahmedabad), Pectin (Lova Chem, Mumbai). All other instruments were used analytical grade.

Methods

Varieties of methods were studied literature survey and fabricated according to the investigating Drug and Polymer. Numerous methods were tried in laboratory and finally it was finding that with these three methods it was possible to prepare microsphere with good physical texture.

Methods implemented:

Solvent evaporation method

This is the method widely used in the microencapsulation process. Concisely the polymer ethyl cellulose (500mg) was dissolve in 40 ml. of ethanol to get a clear solution. The drug Metformin (500mg) was added and dissolved in the polymer solution. The resultant mixture was then stirred at 900 r.p.m for 2 hr to evaporate the volatile substance. The formed microspheres were collected and air dried for 3hr. and stored in desiccators for further use [7].

W/O emulsion solvent evaporation method

Microsphere was prepared by the water in oil (w/o) emulsification solvent evaporation technique. The drug is dissolved in polymeric aqueous solutions.The solution was poured into 200ml. of paraffin liquid containing 0.5% span 80 as an emulsifying agent. The aqueous phase was emulsified into the oily phase by stirring the system in a 500ml. beaker and it's content were heated by a hot plate at 80^{o}C.Stirring

and heating were maintained for 2.5hr. until the aqueous phase was completely removed by evaporation. The light oil was decanted and collected microsphere washed three times with 10ml. hexane, filtered through whatman filter paper, dried in an oven at 500 for 2hr. and stored in desiccators at room temperature [8].

O/W/O emulsion solvent technique

Briefly ethyl cellulose (200 mg.) was dissolve in 5 ml. of toluene. Similarly the drug 100 mg was dissolve in 5 ml. of distilled water separately. The organic phase was added to the aqueous phase with stirring at 1000 r.p.m to produce O/W type emulsion. The resultant O/W was poured drop wise to liquefied Parafin 50 ml. with span 80.Then allowed to stir continuously at 900 r.p.m for 3 hrs. The temperature was bringing down to to 4^{o}C with using ice bath & then the microsphere was removed by centrifugation and washed with n-hexane and dried at room temperature. [9]

Preformulation Study

Evaluation of Metformin HCl. loaded microaphere

Microsphere prepared with particular method was evaluated for parameters such as (1) percentage yield,(2)drug content estimation,(3)drug entrapment efficiency,(4)particle size measurement,(5)percentage of moisture loss,(6)drug polymer inter action study,(7)*in vitro* drug release profile,(8)*in vitro* drug release kinetics .The ratio of drug and polymer concentration was change in every formulation.[10]

Evaluation Study

Determination of λmax by scanning and preparation of standard calibration curve of Metformin HCl : A weight of accurately 100 mg. of Metformin HCl. power was taken and dissolved in 100 ml. of distilled water(solution A).From the solution A,1ml. was pipette out and diluted in a 100 ml.volumetric flask with distilled water(solution B).From solution B different volumes of 2,4,6,8,10 ml. was taken and diluted up to 10ml. with distilled water. The absorbance was measured at 233nm in a UV-visible spectrophotometer (UV-1700, Shimadzu, Japan) against a blank. (Figure 1)

Percentage yield(% yield)

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer total amount of that particular batch multiply by 100.

Drug content estimation

The loaded microspheres (100mg) were powdered and suspended in 100ml. methanolic:water(1:99v/v) solvent. The resultant dispersion were kept for 20 minis for complete mixing with continuous agitation and filtered through a 0.45µm membrane filter. The drug content was determined spectrophotometrically (UV-1700, Shimadzu, Japan) at 233nm using a regression equation from the standard graph [10].

Drug entrapment Study

The drug entrapment efficiency was calculated by the equation:

DEE= (Pc/Tc)×100

Here, Pc is the practical content, Tc is the theoretical content, and all the experimental unites were analyzed in triplicate [10].

Determination of size distribution of microspheres

The microspheres were sized and photographed in normal saline containing 0.1% Tween 80 to prevent aggregation under a light microscope (Olympus C 011, Japan) equipped with an ocular micrometer and a light camera (Seagull DF-1, China). Two hundred microspheres were sized by the above mentioned method and the mean diameter as well as size distribution of microspheres were determined [11]. (Figure 2)

Percent of moisture loss

The Metformin HCl. loaded microspheres of different polymers were evaluated for percentage of moisture loss which sharing an idea about its hydrophilic nature**.** The microspheres weighed initially and kept

in a desiccators containing calcium chloride at 37° C for 24 hour. The final weight was noted when no further change in weight of sample.

% of *moisture loss= Initial wt.- Final wt./ Initial wt.×100*

Loose surface crystals study

The Metformin HCl. loaded microspheres prepared by various techniques were evaluated by loose surface crystal study to observe the excess drug present on the surface of microspheres. From each batch,100 mg of microspheres was shaken in 20ml. of distilled water for 5 mins. and then filtered through what man filter paper 41.The amt. of drug loss in filtrate was determined spectroscopycally and calculated as a percentage of total drug content. The loose surface crystal study helps to estimate the excess amount of drug attached on the surface of microspheres and tabulated in table 5.

Accelerated stability studies

Stability studies were performed according to ICH guidelines. The formulation were stored in room temperature (RT) at 25±1°,in oven at 45±1° and in refrigeration condition at 4±1° for a period of 6 weeks. The samples were analyzed lastly for drug content by spectrophotometer at 233nm.This stability study is done on the basic of drug content.[12, 13 and 14]

Calculate the absorption of final product and then determine the absorption of this product after the stability study. Accelerated stability studies of prepared Metformin HCl. loaded microspheres were done according to ICH guidelines and found to be satisfactory.

Drug polymer Interaction Study

Fourier Transform infrared radiation Measurement (FT-IR)

The FTIR spectral measurements were taken at ambient temperature using IR spectrophotometer (shimadzu,model 840, Japan).Two mg of pure drug, empty microspheres and drug loaded microspheres were selected separately. [12]

UV visible spectrophotometer measurement

The UV Visible spectrophotometer (UV-1700, shimadzu, japan)was used for measuring the wavelength(λmax)for the determination of drug and polymer interaction. About 10µg/ml. concentration of drug and formulations were scanned for measuring the wavelength.

In-vitro drug release

In vitro drug release study was carried out in USP XXI basket type dissolution test apparatus using 6.8 pH phosphate buffers as a dissolution medium. Volume of dissolution medium was 900 ml.Bath temperature was maintained at 37±1°C throughout the study. Basket speed was adjusted to 50 rpm. Samples were withdrawn(5 ml.) in 5min,10min,15min,20 min,30 min,45 min,60 min and then an interval of 1 hr. up to 9 hr. with replacement of 5 ml. fresh medium and analyzed for Metformin HCl. content by UV –Visible spectrophotometer at233nm.all the experimental units were analyzed in triplicate(n=3).

The in vitro dissolution data is summarized in table 3.5 to 3.12 for F1 to F8 for all the formulations found to release Metformin HCl. in a controlled manner.

HPLC Assay

The determination of drug in plasma was performed by HPLC assay using methanol and phosphate buffers, pH 4.3 (75:25 vol/vol) mixtures as mobile phase delivered at 1.0 ml/min by HPLC pump (LC-20 AT) and the detector (SPD-20A). Twenty micro liters of injection volume was eluted in column at room temperature. The column eluent was monitored at 236 nm. [13]

Scanning electron microscopy

The microspheres are studied under photomicroscope RXL-5T (carton optical industries Ltd., Burdwan University) and observed for the distribution of drug and polymer in the patches. The surface morphologies of the microspheres were investigated by using scanning electron microscope, sample were gold coated to make them electrically conductive.

Statistical analysis

All the results obtain during evaluation, were verified with different statistical analysis like one way ANOVA, standard deviation and probability log scale plotting (For measurement of particle size).

RESULT

The percentage yield of all the formulations was found to be satisfactory. It can be due to the involvement of process parameters. Drug entrapment efficiency (DEE) of F2 formulation found to be high because, the drug is fully dispersed in the polymer phase by continuous stirring for a longer period. The particle size of all the formulations found to be satisfactory and within the range of (34.56 to71.34µm). Particle sizes of the formulations prepared by W/O emulsion solvent evaporation method are within the range of 34.02 µm. and that formulation prepared by solvent evaporation method were within the range of 39.64 to 48.21 µm. This narrow range of particle size can be attributed to the effect of stirring time and stirring speed during preparation of microspheres. The percentage of moisture loss was found to be minimum in all the formulation but the F3, F6, F7 and F8 loose very large amount of water.F2 and F1 lose a very minimum amount of water. This leads to draw a conclusion that F1 and F2 are stable for prolonged storage due to less loss of water.

The formulations F2 and F3 shows a constant and high release in the dissolution profile, so among from this two formulation I optimized the formulation F2, due to the better result of other evaluation parameter.

The FT-IR study, the spectrum peak of Metformin HCl. was identified with the polymer pectin to confirm the safety profile of all the formulation for ensuring of the absence of any drug polymer interaction.

The UV scanning of pure drug done and found to be 233 nm which is almost same for all the formulations prepared.

Result of the present study suggest that the formulation F2 was adopted on the basis of smaller particle size, higher drug entrapment efficiency, less percentage of moisture loss high yield with constant drug release profile. The diabetics are a generic and lifelong disease now a day's. So, these research projects are helpful to those diabetics' patients who are consuming Metformin HCl. in a large amount of dosage form due to controlled release mechanism.

The polymer is a biodegradable polymer, so it does not make any harmful effect in our biological system also.

From the preformulation stage, FT-IR spectroscopy study of the pure drug (Metformin HCl) alone and the combination of drug with polymer pectin under study was carried out. The FT-IR spectrum of Metformin HCl. revealed the result was based on the matching the main peak of pure drug with selected formulations. FT-IR spectra for the drug were recorded and it was compared against the FT-IR spectra of the formulated drug along with the polymer. Metformin gives characteristics peaks at wave number 1254,1473,1620,3270 and 3288.The peak at 1254 corresponds to the C-N stretching (range of C-N stretching :1000-1400),1473 to C-H stretching (range of C-H stretching :1300-1500),1620 to c=c stretching (range of c=c stretching :1450 – 1650),where as the peak at 3270 and 3288 corresponds to –NH stretching (range of –NH stretching:3220-3500).Thus FT-IR studies revealed that there was no shift in peaks of the formulation ,thus indicating there was no interaction between drug and polymer used.

Preparation of microspheres

In reference to section 4.2 of chapter 4, microspheres are prepared with two methods. Solvent evaporation method and W/O emulsion solvent evaporation

method in a single ratio 1:1 by using four different polymers.

Evaluation of Metformin loaded microspheres

Microsphere prepared with particular method was evaluated for parameters such as (1) percentage yield,(2)drug content estimation,(3)drug entrapment efficiency,(4)particle size measurement,(5)percentage of moisture loss,(6)drug polymer inter action study,(7)*in vitro* drug release profile,(8)*in vitro* drug release kinetics .The ratio of drug and polymer concentration was change in evry formulation.

Determination of λmax by scanning and preparation of standard calibration curve of Metformin HCl .

A weight of accurately 100 mg of Metformin HCl power was taken and dissolved in 100 ml. of distilled water (solution A). From the solution A,1ml. was pipette out and diluted in a 100 ml.volumetric flask with distilled water(solution).From solution B different volumes of 2,4,6,8,10 ml. was taken and diluted up to 10ml. with distilled water. The absorbance was measured at 233nm in a UV-visible spectrophotometer (UV-1700, Shimadzo, Japan) against a blank. A graph was plotted by taking concentration vs. absorbance. The slope and regression value was calculated from the graph. (Figure 1)

Percentage yield (% yield)

The yield was calculated as the weight of the microspheres recovered from each batch divided by total weight of drug and polymer total amount of that particular batch multiply by 100.

Drug content estimation

The loaded microspheres (100mg) were powdered and suspended in 100ml. methanolic:water(1:99v/v) solvent. The resultant dispersion were kept for 20 minis for complete mixing with continuous agitation and filtered through a 0.45µm membrane filter. The drug content was determined spectrophotometrically (UV-1700, Shimadzu, Japan) at 233nm using a regression equation from the standard graph.

Drug entrapment Study

The drug entrapment efficiency was calculated by the equation:

DEE= (Pc/Tc)×100

Here, Pc is the practical content,Tc is the theoretical content, All the experimental unites were analyzed in triplicate.

The drug entrapment efficiency obtained best in F2 (67.34%) and other four formulations F1, F3, F4 and F5 have poor drug entrapment efficiency(less than 60%). The percentage yield of all the formulation was found to be satisfactory. It can be due to the involvement of process parameters. Drug entrapment efficiency (DEE) of F2 formulation found to be high because, the drug is fully dispersed in the polymer phase by cont. stirring for a longer period. The percentage of yield of all the formulation was found satisfactory (more than 80%) and summarized in table 2.The drug content estimation is also satisfactory. (Figure 2)

Determination of size distribution of microspheres

The microspheres were sized and photographed in normal saline containing 0.1% Tween 80 to prevent aggregation under a light microscope (Olympus C 011, Japan) equipped with an ocular micrometer and a light camera (Seagull DF-1, China). Two hundred microspheres were seized by the above mentioned method and the mean diameter as well as size distribution of microspheres were determined.

In-vitro drug release

In vitro drug release study was carried out in USP XXI basket type dissolution test apparatus using 6.8 pH phosphate buffers as a dissolution medium. Volume of dissolution medium was 900 ml.bath temperature was maintained at 37±1° throughout the study. Basket speed was adjusted to 50 rpm.Samples were withdrawn (5 ml.) in 5min, 10min, 15min, 20

min, 30 min, 45 min, 60 min and then an interval of 1 hr. up to 9 hr. with replacement of 5 ml. fresh medium and analyzed for Metformin HCl. content by UV –Visible spectrophotometer at233nm.all the experimental units were analyzed in triplicate (n=3). (Table 4 to 11)

In vitro drug release kinetics

In order to study the exact mechanism of drug release from microspheres, drug release data was analyzed according to zero order[7], first order[7], higuchi square root[8].The criterion for selecting the most appropriate model was chosen on the basis of goodness of fit test.

The calibration curve of Metformin was presented in figure 5.1.The particle size distribution for all the sample was represented in figure 2 .The in vitro drug release Metformin HCl. loaded microspheres were represented in table 4 to table 11. The FTIR peaks for the formulations with pure Metformin HCl. and the polymer pectin represented in figure 6 to 9.

The formulation F2 and F3 shows a constant and high release in the dissolution profile, so among from this two formulation I optimized the formulation F1, due to the better result of other evaluation parameter.

The drug release kinetics are described in (zero order, First order and higuchi model) table3.13.From above drug release kinetics it can be observed that the optimized formulation are truly followed the zero order release which is the major part of the micro particulate drug delivery system.

All the formulations found to release Metformin in a controlled manner. To describe the kinetics of drug release from microspheres, release data was analyzed according to different kinetic equations. Release data of F9, F10, F11 and F12 obeys zero order kinetic as well as higuchi square root equations. .The *in-vitro* drug releases from Metformin loaded microspheres were represented in Table 4 to 11. The formulations F9 prepared by W/O emulsion solvent evaporation method was found the release maximum in phosphate buffer pH 6.8. Putting all the data's in different release kinetics model and comparing the the coefficient of determination (r^2).it was found that F9,F11,F12 tends to fit with fickian diffusion model given by Higuchi confirming drug release by diffusion mechanism,wheres F9,F10,F11 and F12 fits with zero order kinetic model. (Table 12 and 13)

In-Vivo Evaluation

Rabbits (New Zealand, white) of either sex weighing (2.8-3.2 KG.) are divided into three groups, each consisting of one animal. First group received an oral dose (25mg) pure Metformin HCl., second group received placebo and third group received an oral dose of 25mg formulated drug. The drugs are put behind the tongue to avoid their destruction due to biting. Food was withdrawn from the rabbits 12 hrs. before drug administration. All rabbits had free access to water throughout the study. The institutional Animal Ethical Committee approved the protocol for this study.

Blood samples were collected from the marginal ear vein of rabbits. Blood sample are centrifuged at 2000 r.p.m for 10 minis. (Remi Equipment,Mumbai,India) and drug concentration after deproteinization with mobile phase was determined by HPLC assay.(Shimadzu,Koyato corporation,Japan).

Procedure for separation of plasma

Firstly take 10 ml. iodine free saline solution and 1ml. of Heparin into a beaker to wash the syringe properly. Then blood sample are taken from the marginal ear vein of the rabbits. The blood sample was then subjected into the centrifuge tube along with 5%TCA solution (anti-coagulating agent). The cooling centrifuge was operated for 10 mins. and then it was passed through the column of silica gel to absorb the impurities

HPLC Assay

it has been found that the test samples and the standard sample does not show any significant fluctuation in relation to their retention time. Thus it can be inferred that the test sample (F9) showed significant presence within the plasma. (Figure 22-26).

Scanning electron micrscopy

To detect the surface morphology of the microspheres, SEM of the microspheres done under lower and higher resolutions. Scanning electron microphotographs of microspheres prepared by W/O emulsion solvent evaporation method under lower resolution and higher resolutions are represented. (Figure 27, 28 and 29)

DISCUSSIONS

The percentage yield of all the formulation was found to be satisfactory except F11. It can be due to the minimum involvement of process parameters and smaller amount of drug loss during manufacturing. Drug entrapment efficiency (DEE) of F9 formulation found to be high because, the drug is fully dispersed in the polymer phase by cont. stirring for a longer period. The particle size of all the formulations found to be satisfactory and within the range of (34.56 to 71.34µm).Particle sizes of the formulations prepared by W/o emulsion solvent evaporation method are within the range of 34.02 µm and that formulation prepared by solvent evaporation method were within the range of 39.64 to 48.21 µm. This narrow range of particle size can be attributed to the effect of stirring time and stirring speed during preparation of microspheres. The percentage of moisture loss was found to be minimum in all the formulation but the F9 and F12 loose very large amount of water.F8 and F10 lose a very minimum amount of water. This leads to draw a conclusion that F8 and F10 are stable for prolonged storage due to less loss of water. The loose crystal surface study helps to estimate the excess amount of drug attached on the surface of the microspheres after a successful drug entrapment, the least value obtained indicates the proper encapsulation and minimum drug loss.

The accelerated stability studies were performed according to ICH guidelines for a period of one week and the results met the terms of the ICH guidelines providing a safety profile of storage of Metformin loaded microspheres in varying temperature.

The formulations F9 prepared by W/O emulsion solvent evaporation method was found the release maximum in phosphate buffer pH 6.8. Putting all the data's in different release kinetics model and comparing the coefficient of determination (r^2).it was found that F9,F11,F12 tends to fit with fickian diffusion model given by Higuchi confirming drug release by diffusion mechanism, wheres F9,F10,F11 and F12 fits with zero order kinetic model.

Determination of interaction between drug and polymer were performed using FT-IR,UV spectroscopy as well as in HPLC.FT-IR spectra study showed no change in the fingerprint of pure drug spectra, thus confirming absence of drug to polymer interaction. It was further confirmed by UV-Visible spectroscopy. From the final formulation stage, FT-IR spectroscopy study of the pure drug (Metformin HCl) alone and the combination of drug with various polymers ethyl cellulose, HPMC and Acrylcoat S100 under study was carried out. The FT-IR spectrum of Metformin HCl. revealed the result was based on the matching the main peak of pure drug with selected formulations. FT-IR spectra for the drug was recorded and it was compared against the FT-IR spectra of the formulated drug along with the polymer. Metformin gives characteristics peaks at wave number 1254,1473,1620,3270 and 3288.The peak at 1254 corresponds to the C-N stretching (range of C-N stretching :1000-1400),1473 to C-H stretching (range of C-H stretching :1300-1500),1620 to c=c stretching (range of c=c stretching :1450 – 1650),where as the peak at 3270 and 3288 corresponds to –NH stretching (range of –NH stretching:3220-3500).Thus FT-IR studies revealed that there was no shift in peaks of the formulation ,thus indicating there was no interaction between drug and other polymers used. To detect the surface morphology of the microspheres, SEM of the microspheres done under lower and higher resolutions. Scanning electron microphotographs of microspheres prepared by W/O emulsion solvent evaporation method under lower resolution and

higher resolutions were represented in (Figure 6 and 7).

Standard Curve of Metformin HCL

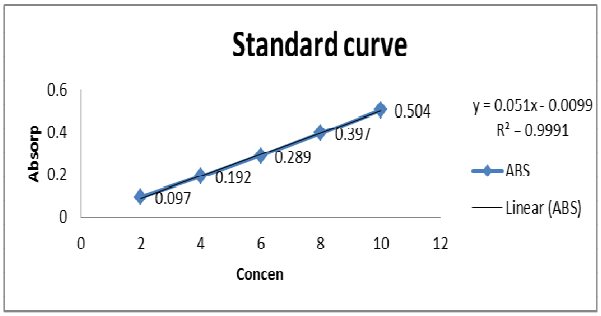

Figure 1: The Metformin loaded microsphere were prepared by W/O emulsion solvent evaporation method by using pectin as a natural polymer in a various ratio(1:.5,1:1,1:1.5,1:2,1:2.5,1:3,1:3.5 and 1:4).The calibration data for Metformin HCl.was presented in table 3.1.The standard curve is represented in figure 1.

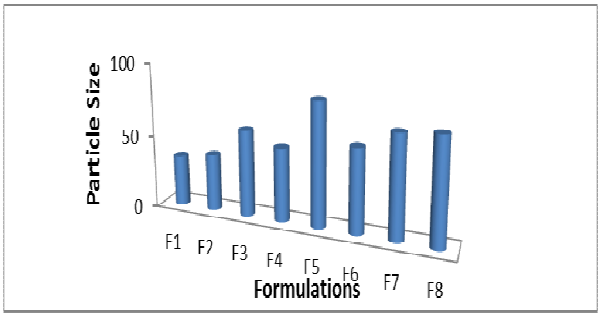

Figure 2: Figure showing the the particle size were found in a good range (34.56 to 83.66).

Table 1: Determination of percentage of moisture loss of prepared Metformin HCl. microsphere

Formulation	Initial Weight(mg.)	Final Weight(mg.)	Moisture Loss	% Moisture loss
F1	639.45±0.234	628.71±0.342	10.74±0.056	1.70
F2	854.32±0.164	845.18±0.367	9.14±0.045	1.08
F3	1080.12±0.324	1056.31±0.287	23.81±0.041	2.25
F4	1272.89±0.235	1258.61±0.317	14.28±0.147	1.13
F5	1379.67±0.189	1362.94±0.328	16.73±0.089	1.22
F6	1696.34±0.215	1671.42±0.371	24.92±0.178	1.49
F7	1982.92±0.253	1954.87±0.179	28.05±0.294	1.43
F8	2167.58±0.143	2134.52±0.193	33.26±0.139	1.55

Values expressed in mean±Standared deviation (n=3)

Table 2: Loose surface crystal study of Metformin loaded microspheres:

Formulation	Drug content in filtrate(mg.)	Loaded drug content(mg.)	%Total drug content
F9	2.46	39.66	6.20
F10	2.17	20.80	10.43
F11	1.81	14.91	12.13
F12	2.54	16.34	15.54

Values are expressed in mean±standared deviation (n=3)

Table 3: Accelerated stability studies of Prepared Metformin HCL. loaded microspheres according to ICH guidelines:

Weeks	Atmospheric condition(Temperature)	F9	F10	F11	F12
0 (Initial)	25±2°C	100	100	100	100
3	25±2°C	99.09	98.61	98.79	99.02
	45±2°C	99.09	99.39	99.16	97.72
	04±1°C	99.49	99.23	98.62	99.03
6	25±2°C	98.24	98.73	98.07	97.88
	45±2°C	98.41	98.60	97.89	97.06
	04±1°C	98.94	98.31	98.07	98.37

Values are expressed in mean±standared deviation(n=3)

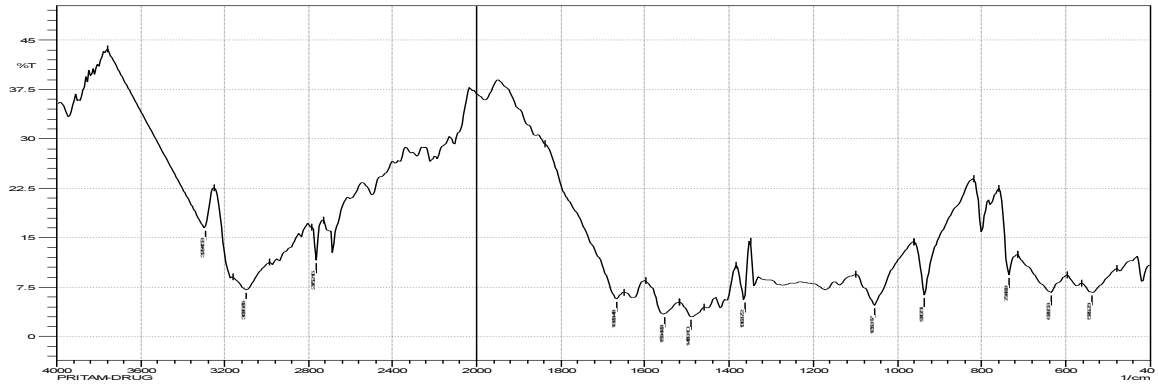

Figure 3: FT-IR Spectrum of Metformin HCL

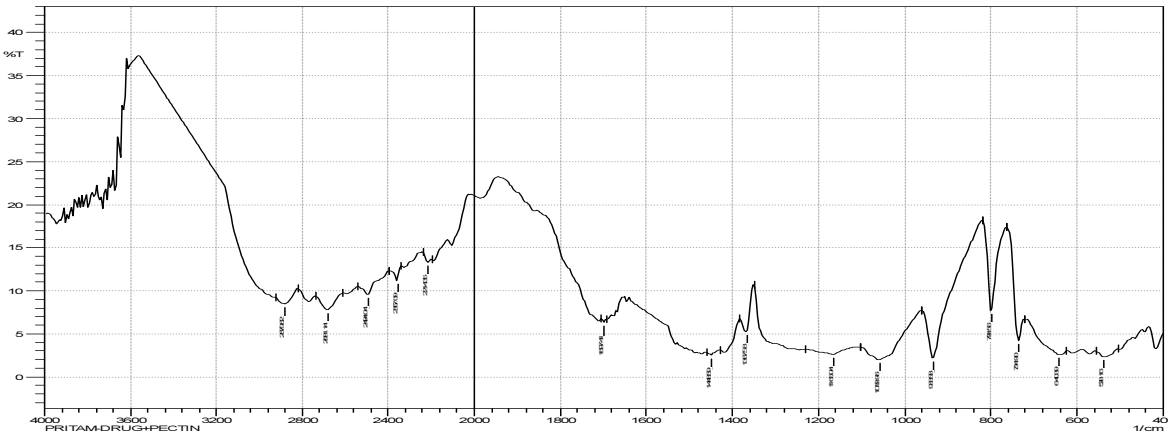

Figure 4: FT-IR Spectrum of Pectin with Metformin HCL

Table 4: In vitro release profile of F1

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.5	.214	.755	.037	6.795	6.795	37.79
1	.223	.786	.039	7.074	7.16	39.82
2	.236	.832	.041	7.488	7.23	40.21
3	.238	.839	.0419	7.55	7.34	40.82
4	.241	.850	.042	7.65	7.49	41.65
5	.246	.868	.043	7.81	7.69	42.76
6	.255	.899	.044	8.09	7.93	44.10
7	.259	.913	.045	8.21	8.22	45.71
8	.265	.935	.046	8.41	8.59	47.77
9	.301	1.062	.053	8.83	9.31	51.77

Table 5: In-vitro release profile of F2

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.5	.189	.666	.033	5.994	5.994	30.24
1	.196	.691	.034	6.219	6.061	30.56
2	.208	.733	.036	6.597	6.16	31.06
3	.216	.762	.038	6.858	6.99	35.24
4	.221	.779	.038	7.011	7.18	36.20
5	.236	.832	.041	7.488	7.73	38.83
6	.296	1.044	.052	9.396	9.76	48.91
7	.375	1.32	.066	11.88	12.21	61.57
8	.435	1.53	.076	13.770	14.18	74.68
9	.510	1.79	.089	16.11	16.613	83.77

Table 6: In vitro release profile of F3

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.5	.245	.864	.043	7.776	7.776	34.61
1	.251	.885	.044	7.96	8.003	35.67
2	.270	.952	.047	8.56	8.69	38.74
3	.281	.991	.049	8.91	9.09	40.52
4	.296	1.044	.052	9.39	9.62	42.88
5	.298	1.051	.052	9.45	9.73	43.37
6	.307	1.083	.054	9.74	10.52	46.90
7	.379	1.33	.066	11.97	12.44	55.46
8	.413	1.45	.072	13.05	13.52	60.31
9	.489	1.72	.086	15.48	15.48	65.04

Table 7: In vitro release profile of F4

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.5	.112	.395	.019	3.55	3.55	19.95
1	.137	.483	.024	4.34	4.38	24.62
2	.165	.582	.029	5.23	5.30	29.79
3	.189	.666	.033	5.99	6.09	34.23
4	.202	.712	.035	6.40	6.54	36.76
5	.261	.920	.046	8.28	8.46	47.55
6	.326	1.15	.057	10.35	10.59	59.52
7	.339	1.19	.059	10.71	11.01	61.88
8	.358	1.26	.069	11.34	11. 70	64.76
9	.364	1.28	.064	11.52	11.94	67.11

Table 8: In vitro release profile of formulation F5

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.5	.062	.218	.010	1.96	1.96	14.19
1	.071	.250	.012	2.25	2.28	16.50
2	.081	.285	.014	2.56	2.59	18.78
3	.089	.314	.015	2.82	2.87	20.78
4	.112	.395	.019	3.55	3.62	26.21
5	.127	.448	.02	4.03	4.12	29.83
6	.139	.490	.024	4.41	4.52	32.72
7	.154	.543	.027	4.88	5.02	36.35
8	.159	.561	.028	5.04	5.23	37.87
9	.163	.575	.028	5.17	5.31	38.45

Table 9: In vitro release profile of formulation F6

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.5	.049	.172	.080	1.54	1.54	8.42
1	.071	.250	.012	2.25	2.33	12.75
2	.093	.328	.016	2.95	3.05	16.69
3	.096	.338	.016	3.04	3.15	17.24
4	.097	.342	.017	3.07	3.19	17.46
5	.127	.448	.022	4.03	4.17	22.82
6	.147	.518	.025	4.66	4.74	25.95
7	.156	.550	.027	4.95	5.05	27.64
8	.165	.582	.029	5.23	5.34	29.22
9	.167	.589	.029	5.30	5.42	29.66

Table 10: In vitro release profile of formulation F7

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.5	.057	.20	.01	1.8	1.81	11.77
1	.078	.27	.01	2.43	2.45	15.94
2	.092	.32	.01	2.88	2.91	18.93
3	.112	.32	.01	2.88	2.92	18.99
4	.126	.44	.02	3.96	4.02	26.15
5	.139	.49	.02	4.41	4.49	26.61
6	.152	.53	.02	4.77	4.87	31.68
7	.176	.62	.03	5.58	5.71	37.15
8	.181	.63	.03	5.59	5.75	37.41
9	.186	.65	.03	5.61	5.8	37.73

Table 11: In vitro release profile of formulation F8

Time (hr.)	Absorbance	Concentration (µg/ml.)	Amount pipette out (mg/5ml.)	Amount in (mg/900ml.)	Cumulative drug release(CR)	% CR
.3	.096	.338	.016	3.04	19.37	19.37
1	.132	.465	.023	4.21	26.83	26.83
2	.149	.525	.026	4.78	30.46	30.46
3	.161	.568	.028	5.20	33.14	33.14
4	.178	.628	.031	5.77	36.77	36.77
5	.192	.677	.033	6.24	39.77	39.77
6	.196	.691	.034	6.40	40.79	40.79
7	.199	.702	.035	6.51	41.49	41.49
8	.208	.733	.036	6.80	43.33	43.33
9	.213	.751	.037	6.97	44.42	44.42

Table 12: In vitro Drug release kinetics

Formulation	Zero order release	First order release	Higuchi square root equation
F1	0.954	0.899	0.844
F2	0.926	0.769	0.942
F3	0.931	0.850	0.917
F4	0.969	0.964	0.947
F5	0.919	0.977	0.968
F6	0.952	0.959	0.961
F7	0.962	0.966	0.953
F8	0.890	0.920	0.931

Table 13: Formulation Design

Formulation Code	Drug: Polymer	Polymers Used	Method of Preparation
F9	1:1	Pectin	W/O emulsion solvent evaporation
F10	1:1	Ethyl Cellulose	Solvent Evaporation
F11	1:1	HPMC	Solvent Evaporation
F12	1:1	Acryl Coat S100	Solvent Evaporation

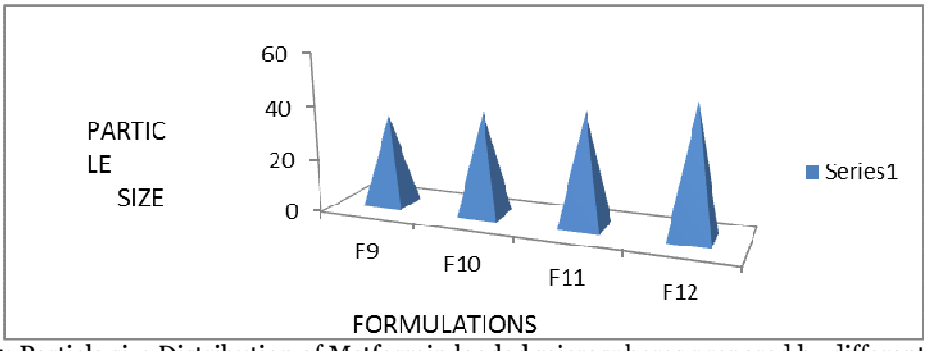

Figure 5: Particle size Distribution of Metformin loaded microspheres prepared by different methods

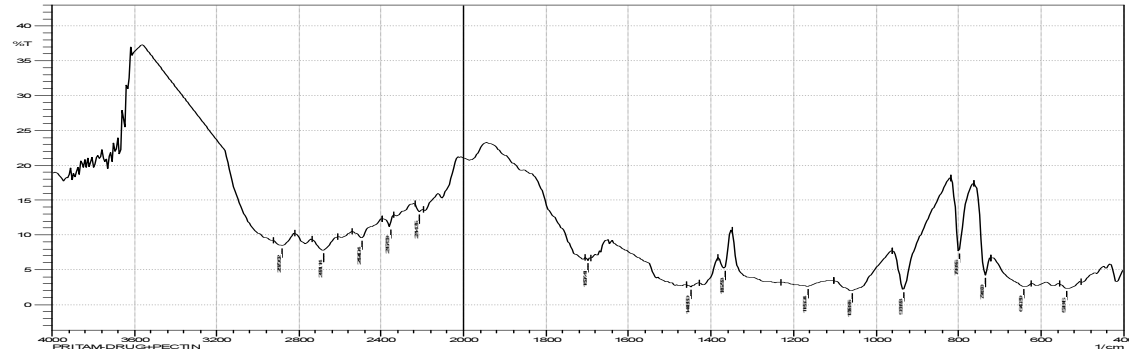

Figure 6: FT-IR Spectrum of Pectin with Metformin HCL

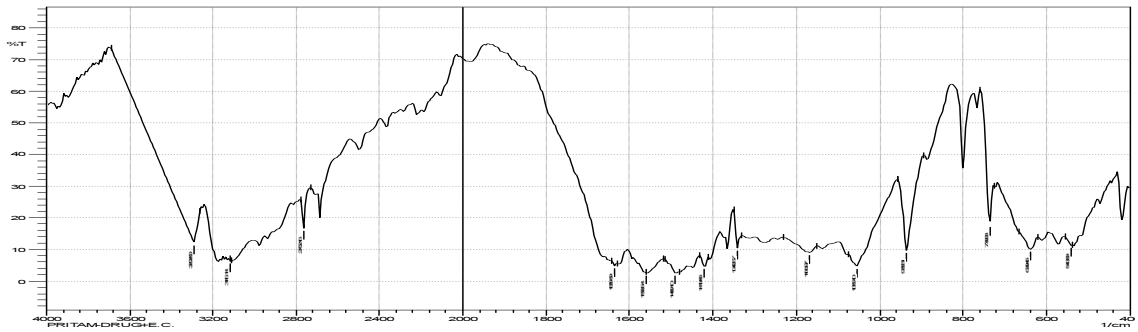

Figure 7: FT-IR Spectrum of Ethyl cellulose with Metformin HCL

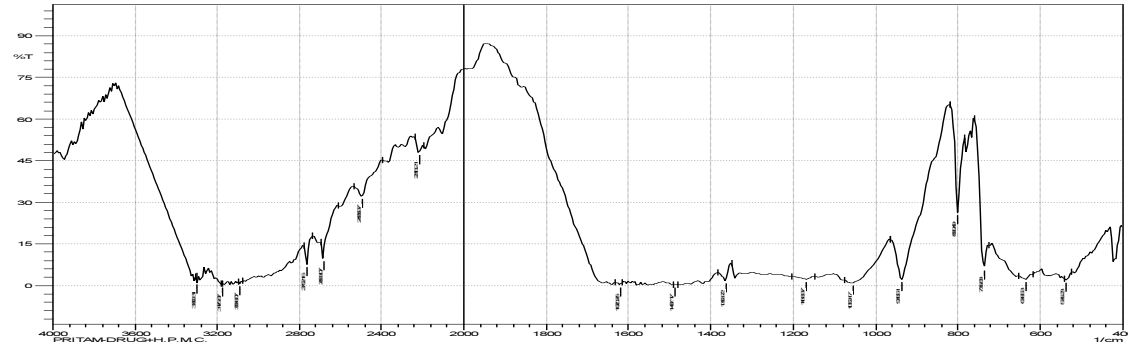

Figure 8: FT-IR Spectrum of HPMC with Metformin HCL

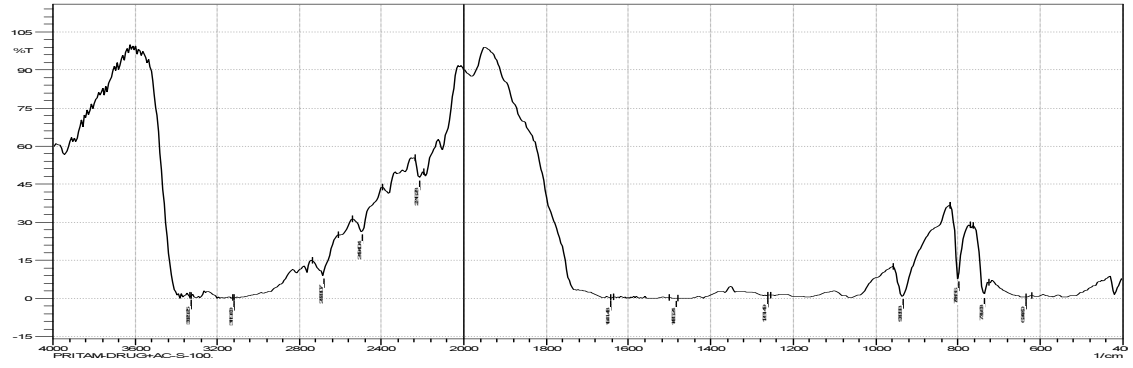

Figure 9: FT-IR Spectrum of Acryl Coat S-100 with Metformin HCL.

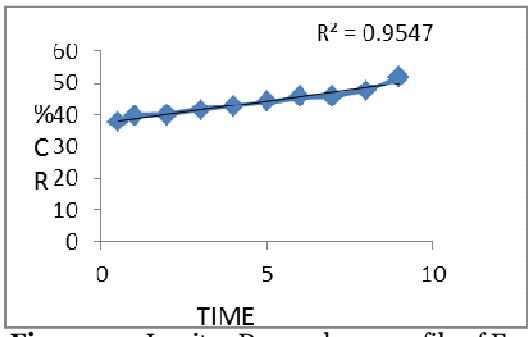

Figure 10: In-vitro Drug release profile of F9.

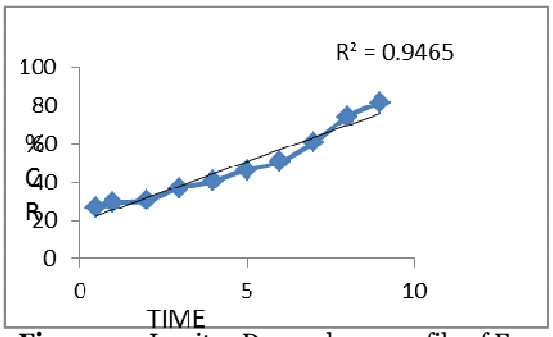

Figure 11: In-vitro Drug release profile of F10.

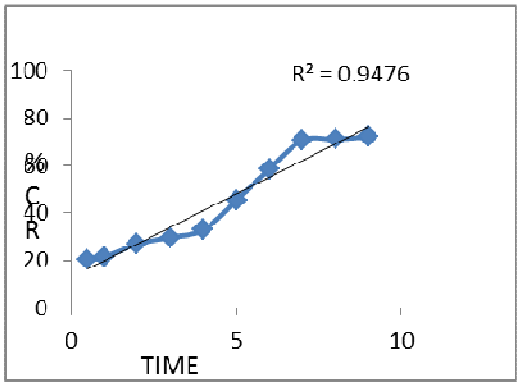

Figure 12: In-vitro Drug release profile of F11.

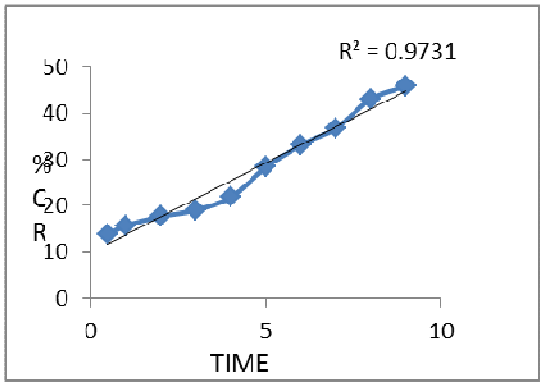

Figure 13: In-vitro Drug release profile of F12.

In-vitro first-order drug release profile of all the Formulations

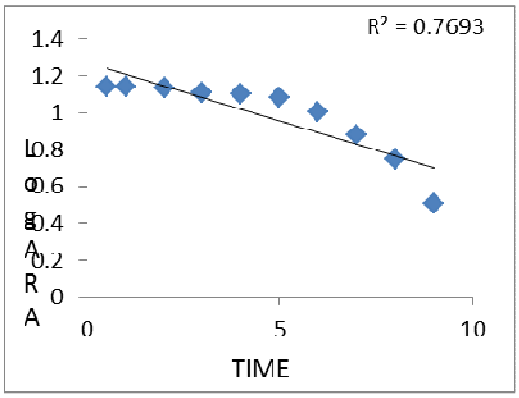

Fig. 14: In-vitro first-order drug release profile of F9.

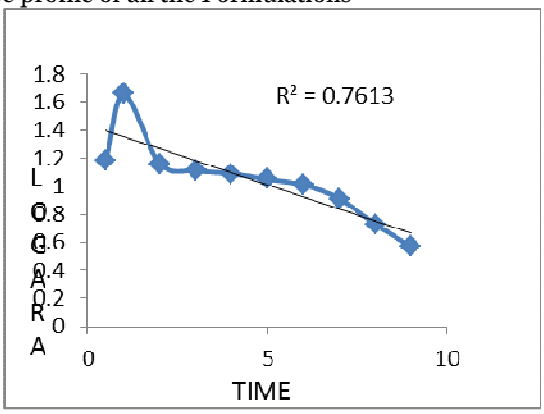

Fig. 15: In-vitro first-order drug release profile of F10

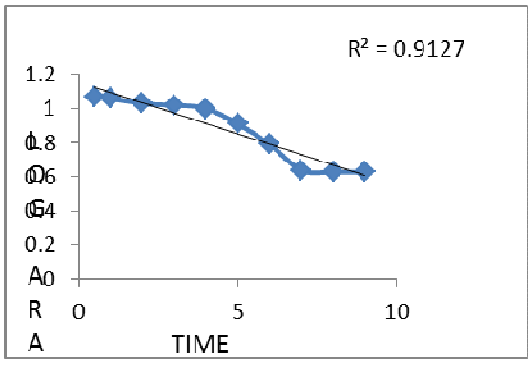

Fig. 16: In-vitro first-order drug release profile of F11.

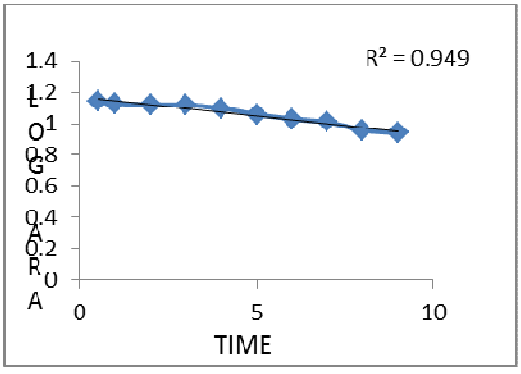

Fig. 17: In-vitro first-order drug release profile of F12

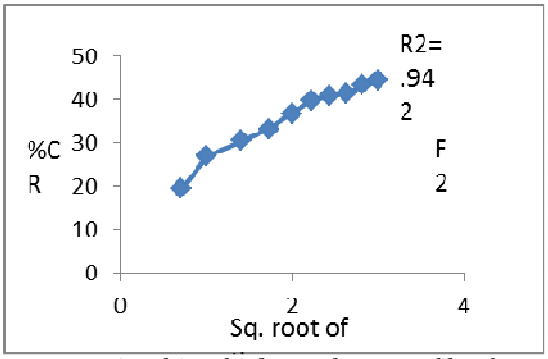

Figure 18: In-vitro higuchi drug release profile of F9.

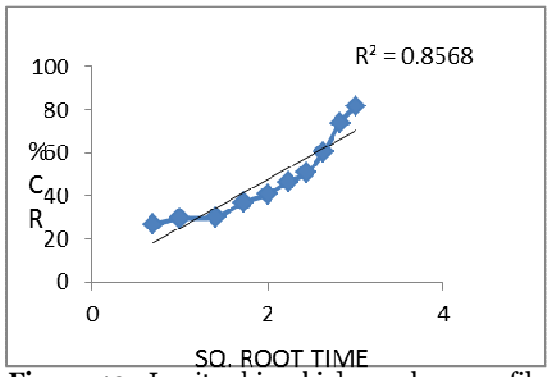

Figure 19: In-vitro higuchi drug release profile of F10

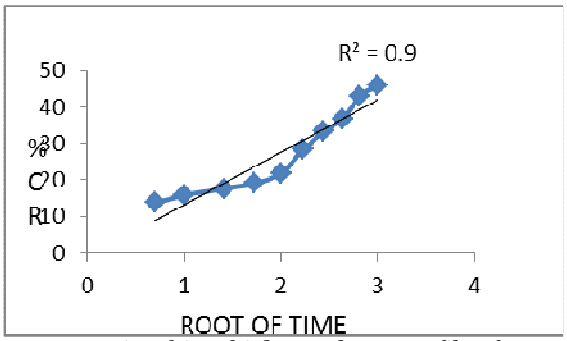

Figure 20: In-vitro higuchi drug release profile of F11.

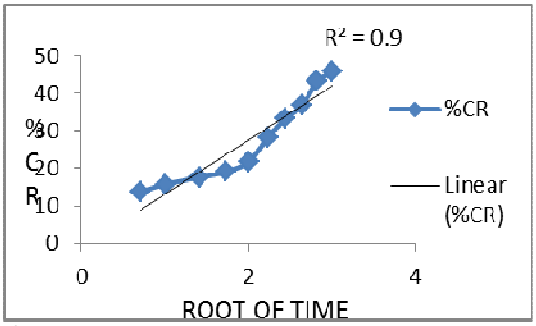

Figure 21: In-vitro higuchi drug release profile of F12.

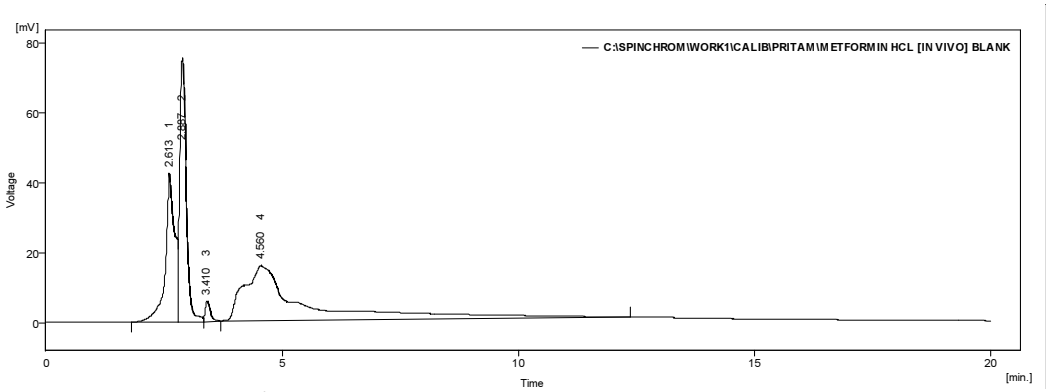

Figure 22: HPLC chromatogram of Blank Sample

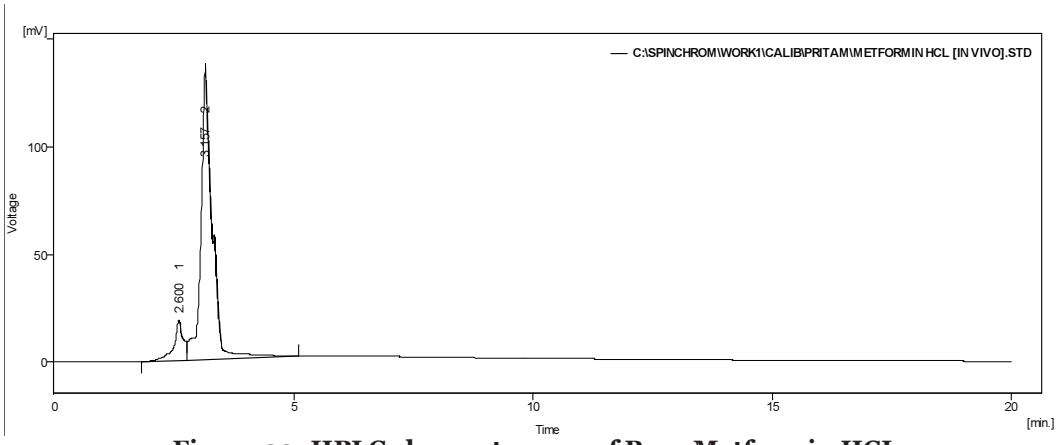

Figure 23: HPLC chromatogram of Pure Metformin HCL

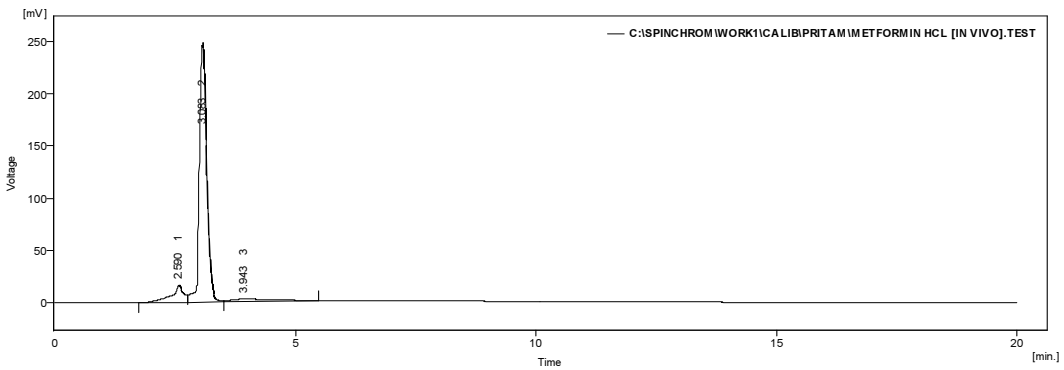

Figure 24: HPLC Chromatogram of Formulated sample (F9)

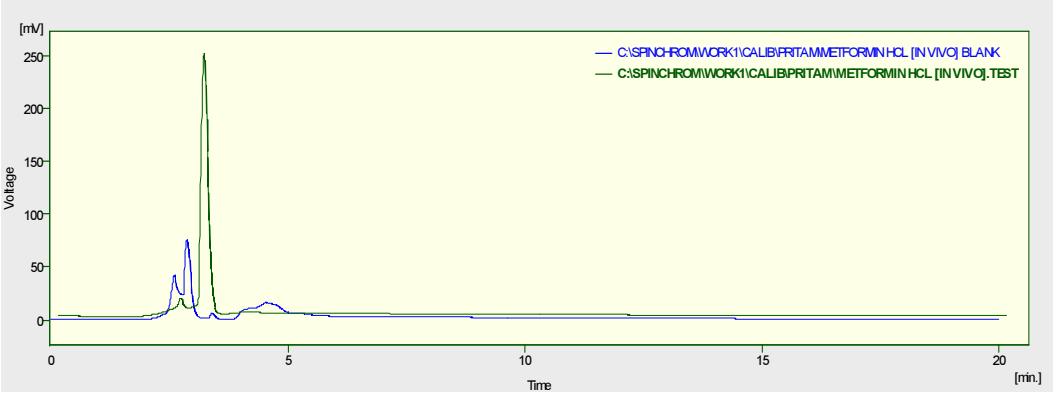

Figure 25: Overlap of HPLC chromatogram between Blank and Test

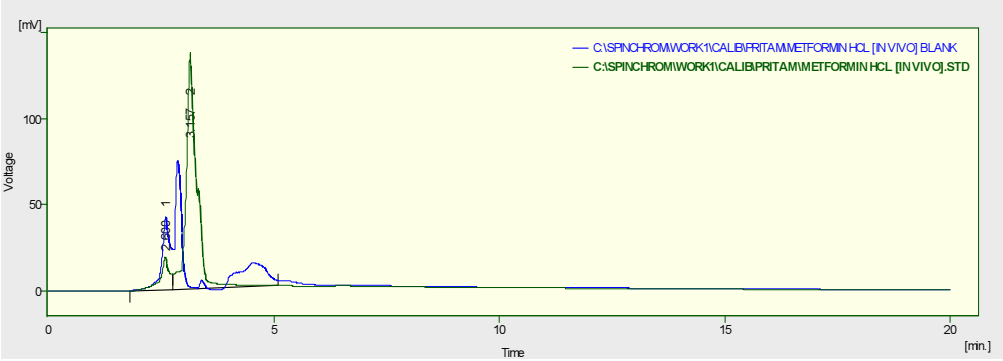

Figure 26: Overlap of HPLC chromatogram between Blank and Standard

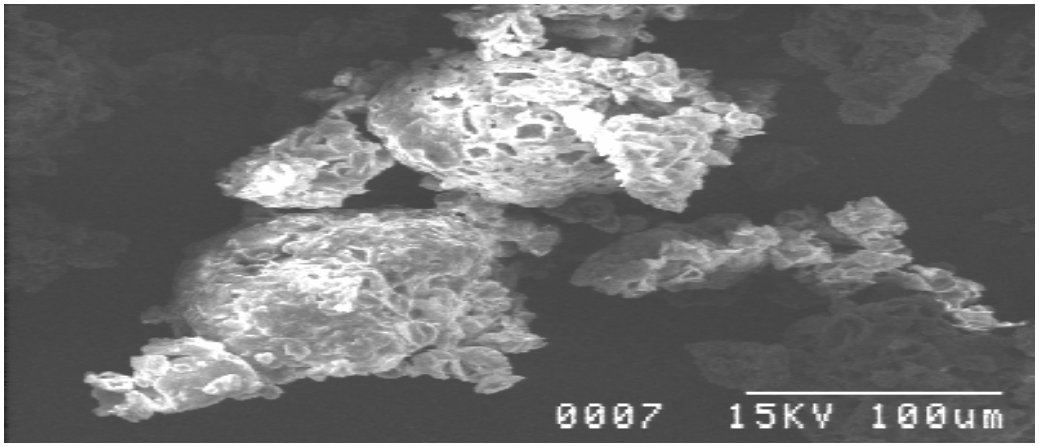

Figure 27: Scanning electron micrograph of microspheres prepared by W/O emulsion solvent evaporation method under lower resolution.

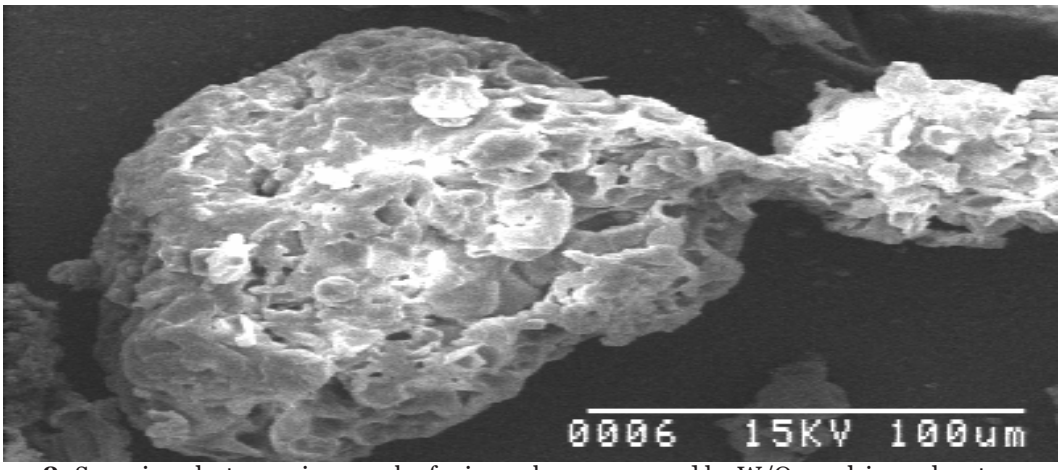

Figure 28: Scanning electron micrograph of microspheres prepared by W/O emulsion solvent evaporation method under higher resolution.

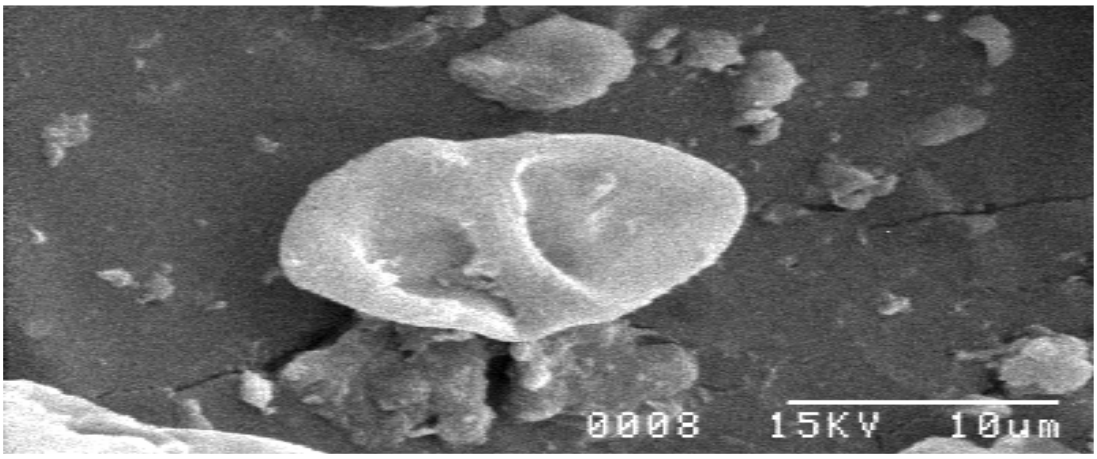

Figure 29: Scanning electron micrograph of microspheres prepared by W/O emulsion solvent evaporation method under higher resolution.

REFERENCE

1) J.M.Gwen, J.R. Robinson. Sustained and controlled release drug delivery systems, In Modern pharmaceutics. Marcel Dekker, 2010; 76(3): 589.
2) W.Y. Thomas, J.R Robinson. Controlled release drug delivery system. *In The science and Practice of Pharmacy* 2011; 2(20): 902.
3) V.H.K. Li,J.R Robinson, V.H.L. Lee. In controlled drug delivery. Macel Dekkaer. 2010; 2: 4. L.Lachman,P.Deluca,M.J.Akers. Kinetic principle and stability testing. In L.Lachman, H.A.Liberman: kanig J L(Eds). *The theory and practice of Industrial Pharmacy* 2011; 5: 765-812.
4) N.Rammurthy, P.Srinivasan. ECD-Based film coating for controlled drug delivery. *J.Pharmacy & research* 2011; 41: 1115.
5) G.M. Jantzen, J. R.Robinson. Sustained and controlled- release drug delivery systems, In Modern pharmaceutic. Marcel Dekker 2010; 73(4): 585-594.
6) P.G.Welling, M.R.Dobrinska, J.R.Robinson. In controlled drug delivery, Marcel Dekkaer 2009; 4: 257.
7) Kunal Pal, Allan T. Paulson, Dérick Rousseau. Biopolymers in Controlled-Release Delivery Systems. *Modern biopolymer science* 2009; 1016(10): 519-557.
8) M.R.Goyal, R.G.Nayak, A.Rangaswami. Preparation and formulation evaluation of diclofenac sodium. *Indian J.Pharm Sci.* 2008; 70(8): 547-554.
9) D.H.Kenneth, M.C.Kathleen, H. L.Danny,A.P.Claude,T.Schmidta. PEGylated insulin in PLGA microparticles. In vivo and In vitro

analysis. *Journal of Controlled Release* 2008; 104(4): 447-460.

10) M.C.Gohel, R.K.Patrik, A.surati. Preparation and formulation evaluation of terbinafine hydrochloride. *Indian J.Pharm Sci.* 2007; 67(8): 575-581.

11) G.T.Kulkarni, K.Gowthamarajan, B.Suresh. Stability testing of Pharmaceutical Products:An overview. *Indian J.Pharm. Educ.Res.* 2008; 40(11) 184-198.

12) D.R.Bhumkar, M.M.Patil. Studies on effect of variabilities by response methodology for naproxane microspheres. *Indian drugs* 2008; 40(8): 455-461.