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FABRICATION AND CHARACTERIZATION OF METFORMIN HYDROCHLORIDE LOADED MICROSPHERES BY INCORPORATING NATURAL AND SYNTHETIC POLYMERS FOR COMPARATIVE ANALYSIS

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ABSTRACT: The present developed method was designed to fabricate an innovative, simple, cost-effective, and compatible method for controlled delivery of Metformin hydrochloride (MF) by the use of natural and synthetic polymers for comparative analysis. For the most commonly used anti-diabetic drug Metformin hydrochloride, we have tried to formulate microspheres by incorporating natural and synthetic polymers separately in order to identify the most appropriate and safe type of controlled drug delivery system. All the fabricated microspheres formulations were characterized for various evaluating parameters like percentage yield, particle size analysis, micromeretic study, entrapment efficiency of drug, *in-vitro* drug release, FTIR study, XRD analysis, and quantitative analysis of most optimized formulation by using high-performance liquid chromatography (HPLC) method. The formulations from F1to F5 were prepared by using natural polymer sodium alginate, by ion-gelation technique, and the formulation code from F6 to F10 represents microspheres formulated by using synthetic polymer by emulsion-solvent evaporation technique. It was observed that all prepared formulations showed improved flow behavior than a pure drug; on increasing the polymer concentration, the entrapment efficiency and particle size also increased, but better results were obtained for microspheres prepared using synthetic polymer. The *in-vitro* release study indicates that the microspheres of Metformin hydrochloride exhibited controlled release of drug for up to 8 h using only natural polymer, whereas extended drug release for 12 h was achieved using synthetic polymer, following matrix diffusion mechanism. The HPLC data revealed that the present developed method was most accurate for the fabrication of Metformin hydrochloride microspheres.

INTRODUCTION: Medication is used for the treatment of acute and chronic diseases; sometimes these medicines leads to fluctuating drug levels within the body, so in order to prevent frequent drug administration and maintain therapeutic drug level in the body for treatment of chronic diseases, it is essential to administer the drug by controlled release (CR) systems [1-4].

An ideal CR system should deliver the drug at a predetermined rate, locally or systemically, for a specific period of time with enhanced bioavailability. Generally, those drugs that have short elimination half life are selected for CR

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formulations [5]. There are several preparations like, niosomes [6], microneedles [7], liposomes, micro-spheres, *etc*, that can deliver the drug in CR manner, at a predetermined rate. Microspheres are one among convenient method, it can be defined as solid, approximately spherical particles ranging from 1 to 1000 μm, consisting of the uniformly dispersed drug in either solution or microcrystalline form [8].

Disease like diabetes is one of the most prevalent chronic ailments that is characterized by hyperglycemia, and it is a major cause of public health issue now a days. Anti diabetic drugs are used to treat and control diabetes; among them Metformin Hydrochloride (MF) is one of the mo st widely preferred ant diabetic drug, having molecular formula $C_4H_{11}N_5 \bullet HCl$ (Imido dicarbo nimidic diamide, N, N-dimethyl-, mono-hydrochloride; 1,1-Dimethylbiguanide mono hydro chloride). It is an orally-administered biguanide, anti-hyperglycaemic agent, used in the management of non-insulin dependent diabetes mellitus. The molecular formula of Metformin Hydrochloride (MF) is $C_4H_{11}N_5 \bullet HCl$ (Imido dicarbo nimidic diamide, N, N-dimethyl-, mono-hydrochloride; 1,1-Dimethylbiguanide mono hydro chloride).

It is an orally-administered biguanide, anti-hyperglycaemic agent, used in the management of non-insulin dependent diabetes mellitus. Being an ant diabetic drug, Metformin Hydrochloride (MF) does not induce hypoglycemia at any reasonable dose, therefore it is called anti-hyperglycemic rather than a hypoglycemic drug. As the half life of Metformin Hydrochloride is short and it is absorbed from the upper intestine within 6 h, hence, repeated administration is required to maintain effective plasma concentration [9-14]. Most of the microspheres are prepared by incorporating the drug in a carrier system, these carrier systems are comprised of various proportions of different types of polymers. Most of the polymers are either natural or synthetic, but generally different combination of natural and synthetic polymers are selected for microspheres preparation for CR of pure drug. In present investigation, alginate, a naturally occurring, biocompatible, biodegradable and linear polysaccharide is used for Metformin Hydrochloride MF loaded microspheres preparation.

Sodium alginate is selected in this work, it is the commercially available salt form of alginate, it has a unique property of transformation from sol to hydro gel with more than 95% of water molecules physically held inside that is important for the maintenance of bioavailability by providing an aqueous environment to the entrapped substances. When alginate reacts with calcium ions, it undergoes gelation in aqueous solution due to binding of calcium ions with G-blocks of adjacent alginate chains creating ionic inter-chain bridges [15-19]. Carbopol 940 is a synthetic, cross-linked polyacrylic acid polymer, it is water soluble polymer and widely used as emulsifying, suspending, stabilizing, thickening and gelling agent.

In present research we have tried to investigate the effect of natural and synthetic polymers on release of Metformin hydrochloride microspheres. Hence, we have tried to prepare microspheres of Metformin Hydrochloride by using natural polymer that is sodium alginate by ion gelation method and by using synthetic polymer, carbopol 940 by emulsion-solvent evaporation method. We have tried to formulate microspheres of pure drug Metformin Hydrochloride (MF), as microspheres provide controlled release of drug for specified period of time, along with it solubility of drug will be enhanced due to micron level particle size and dose dumping will be avoided.

Two separate methods along with separate polymers has been selected in present work in order to estimate a comparative conclusion regarding better release of drug along with minimum harmful chemical use in preparation of Metformin Hydrochloride (MF) microspheres with simple, economic and high drug entrapment efficiency. Among various available delivery systems for sustained release formulations, here microspheres have been selected due to avoidance of dose dumping and enhanced solubility due to micron level particle size. Metformin Hydrochloride (MF) microspheres will reduce the dosing frequency and hence, it will improve patient compliance. A natural polymer alginate, which is a linear poly-saccharide, was selected due to its biocompatible and biodegradable charecteristic; it is widely used in microspheres preparations. The commercially available salt form of alginate that is sodium

alginate was taken, gelation of alginate on reacting with calcium ions forms ionic inter-chain bridges. When alginate reacts with calcium ions, it undergoes gelation in aqueous solution due to binding of calcium ions. In present work ionic gelation technique was selected in fabrication of microspheres due to its simplicity, low cost and its high entrapment efficiency. The prepared microspheres by using natural and synthetic polymers were evaluated for drug entrapment efficiency, various micromeretic properties, x-ray diffraction studies, drug-polymer interaction by FTIR, and *in-vitro* drug release pattern [20].

MATERIALS AND METHODS:

Materials: Metformin Hydrochloride (MF) was obtained as a gift sample from Hetero Healthcare Ltd. Hyderabad, India, Sodium alginate was a gift sample from Signet Chemical Co, India, Carbopol 940 was purchased from Natco, Hyderabad, India, Calcium chloride was obtained from Loba Chem Pvt. Ltd., India, HPMC-E15 obtained by Loba Chemicals, Kerala, India, buffer ingredients and Acetonitrile was obtained from Merck Chemicals, Mumbai, India. All other chemicals used were of analytical and HPLC grade reagent.

Method 1: Microsphere preparation of Metformin Hydrochloride (MF) was fabricated by ionic gelation technique using natural polymer that is sodium alginate. Initially, specified amount of sodium alginate was dissolved in sufficient quantity of distilled water to form a homogeneous polymer solution and uniformly mixed with the help of magnetic stirrer at 200 revolutions per minute speed. Finally, the drug, MF was added to the polymer solution and mixed to form a smooth viscous dispersion; lastly the resulting dispersion was added drop wise with 24 G needle in 500 ml of 5% calcium chloride solution under continuous stirring at 200 rpm. This stirring was continued for 30 min to make the dispersion as fine as possible to obtain spherical microspheres. Then the mixture was filtered and product was dried at 40 °C for 12 h [14, 15]. The prepared microspheres of MF with varying polymer coat composition are listed in **Table 1**.

TABLE 1: COMPOSITION OF METFORMIN HYDROCHLORIDE LOADED MICROSPHERES INCORPORATING NATURAL POLYMER

Formulation Code	Drug (g)	Sodium alginate (g)	Curing time (minutes)	Cross-linking agent ($cacl_2$ %w/v)	Stirring Speed(rpm)
F1	1.0	1.0	30	5.0	200
F2	1.0	1.5	30	5.0	200
F3	1.0	2.0	30	5.0	200
F4	1.0	2.5	30	5.0	200
F5	1.0	3.0	30	5.0	200

TABLE 2: COMPOSITION OF METFORMIN HYDROCHLORIDE LOADED MICROSPHERES INCORPORATING SYNTHETIC POLYMER

Formulation Code	Drug (g)	Carbopol 940(g)	HPMC (g)	Stirring Speed(rpm)
F6	1.0	1.0	0.5	800
F7	1.0	1.5	0.5	800
F8	1.0	2.0	0.5	800
F9	1.0	2.5	0.5	800
F10	1.0	3.0	0.5	800

Method 2: Microsphere preparation of Metformin Hydrochloride by using carbopol 940 was formulated by using emulsion-solvent evaporation technique. Initially, carbopol polymer was dissolved in distilled water to form a homogenous polymer solution and then core material Metformin Hydrochloride (MF) was added to the polymer solution and mixed thoroughly. The resulting mixture was then added in a thin stream to of alcohol mucilage of HPMC contained in a 450 ml beaker, while stirring at 800 rpm to emulsify the added dispersion as fine droplets. A Remi Lab Magnetic stirrer with speed meter was used for stirring. The solvent, alcohol was then removed by continuous stirring at room temperature for 3 h to produce spherical microspheres. The microspheres were collected by vacuum filtration and washed repeatedly with water. Finally, the prepared microspheres were dried at ambient temperature (25 ºC) for 24 h and dried in vacuum chamber at 25 ºC for 2 h to remove any residual solvent. The prepared Metformin Hydrochloride (MF) loaded

microsphere by emulsion-solvent evaporation technique is shown in **Table 2** along with various polymer compositions.

Characterization of Metformin Hydrochloride (MF) Loaded Microspheres: Percentage Yield: The percentage yields of all the designed formulations was calculated on weight basis with respect to the initial weight of material [21, 22]. The calculated data are represented in **Table 3**.

% Yield = (Weight of microspheres/ Total expected weight of drug and polymer) × 100

Micromeretic Study: The flow properties of prepared Metformin Hydrochloride loaded microspheres by natural and synthetic polymers were determined by calculating bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose.

Bulk density and tapped density were determined by using Bulk density apparatus (Electro lab India) and angle of repose was calculated by fixed base cone method [23, 24]. The values of micromeretic properties of prepared formulation are represented in **Table 3**.

Particle Size Analysis: Estimation of particle size of prepared Metformin Hydrochloride (MF) loaded microspheres was determined by optical microscopic method [25]. All readings were taken in triplicate and the data shown in **Table 3**.

TABLE 3: OPTIMIZED CHROMATOGRAPHIC CONDITIONS

S. no.	Parameter	Result
1	Column	Waters, X Terra MS, C18 (4.6 × 250 mm × 5µ)
2	Mobile phase	Buffer and Acetonitrile in the ratio of 25:75 (v/v)
3	Flow rate	1.8 ml/min
4	Detection Wavelength	233 nm
5	Injection Volume	20 µL
6	Run Time	30
7	Column temperature	30 °C

Entrapment Efficiency of Drug: The amount of drug entrapped in prepared Metformin Hydrochloride microspheres was tested by taking 100 mg of the formulation in 50 ml of phosphate buffer of pH 7.4 in a volumetric flask and then it was stirred for 30 min in sonicator at 125W (Imeco Sonifier, Imeco Ultrasonics, India). Finally, the volume was made up to 100 ml with phosphate buffer of pH 7.4 and again stirred for 1 h and kept overnight for 24 h to extract the drug from microspheres. Then it was filtered and the filtrate was collected by passing through 0.45 µ filter and required dilutions were made and the absorbance of resulting solution was measured at 233 nm UV-Visible spectrophotometer (UV- 2450 Shimadzu, Japan) against blank [26]. This study was conducted three times and the values are shown in **Table 4**. The drug entrapment was calculated by using the formula:

% Drug entrapment = (Calculated drug content/Theoretical drug content) × 100

***In-vitro* Drug Release Study:** The release of Metformin Hydrochloride drug from the prepared microspheres was studied in 0.1N HCl at pH 1.2 and in phosphate buffer pH 7.4 (900 ml) respectively using a USP six station dissolution (LAB DISSO 2000) rate testing apparatus with a rotating paddle at 50 rpm and 25 cm depth 20 by maintaining temperature of 37 ± 0.5℃. Sample of 5 ml was withdrawn at various time intervals and subsequently diluted using pH 7.4 phosphate buffer. After suitable dilutions the absorbance was measured at 206 nm for 0.1N HCl and 233 nm for phosphate buffer, using UV- visible spectrophotometer (2450 Shimadzu, Japan) against a blank [27]. The dissolution study was conducted in triplicate and the observed data represented in **Table 4**.

FTIR Study: The study of any possible interaction between the drug Metformin Hydrochloride, polymers and optimized formulation was done by analyzing FTIR spectra by using Perkin-Elmer FT-IR (spectrum RX) [28].

X-ray Diffraction Study: X-ray diffraction analysis was done to estimate the crystalline nature of drug and drug loaded microspheres [29].

Analytical Method Validation:

Instrumentation and Chromatographic Conditions: A novel, rapid, specific and stable reverse phase- high performance liquid chromatography (RP-HPLC) method was developed and validated for the most optimized formulation F9 of Metformin hydrochloride to estimate the actual drug content in designed formulation. RP-HPLC was performed on AGILENT 1100 series equipped

with auto sampler having a variable volume (1-200 µl) injector having UV detectors with Chromeleon software, column used for separation was Waters, X Terra MS, C18 (4.6 × 250 mm × 5 µ) in the present designed analytical method, the separation was carried out by using mobile phase consisting of phosphate buffer and Acetonitrile in the ratio of 25:75(v/v) isocratically.

Sample and Solution Preparation: In present developed method, the quantitative estimation of Metformin Hydrochloride by using a diluent as water, for dissolving samples during entire experiment.

The standard solution of Metformin Hydrochloride was prepared by dissolving about 20 mg of Metformin Hydrochloride in 200 ml with diluent (100 µg/ml).

Preparation of Metformin Hydrochloride Microspheres Assay Sample Preparation: The assay sample of Metformin Hydrochloride loaded microspheres was prepared by taking about 25 mg of equivalent powdered microspheres of most optimized formulation, F9 and then transferred to 10 ml volumetric flask.

To this added 4 ml of the diluent and sonicated for 30 min with intermediate shaking, the solution was then diluted to 10 ml with diluent and centrifuged at 3000 rpm for 10 min. The supernatant was collected, filtered through 0.22 µ filter and used as the sample solution to determine assay.

Linearity and Range: Linearity is the ability of an analytical method to obtain results that are directly proportional to the concentration of the analyte in the sample. The range of an analytical method is the interval between the upper and lower levels of analyte that have been determined with precision, accuracy and linearity using the method as written. Linearity was determined by preparing five standard solutions of Metformin Hydrochloride standard at concentration levels of 60% to 140% of test concentration and each solution injected to be confirmed. Peak area was recorded, for all the peaks, a calibration plot was constructed by plotting peak area *vs.* concentrations of Metformin Hydrochloride which was found to be linear in the range of 2 µg-20 µg/ml. Coefficient of correlation was determined.

Accuracy: The measurement for exactness of the analytical method is known as accuracy. Accuracy was determined by preparing solutions at different levels 50 %, 100% and 150 % of test concentration using Metformin Hydrochloride loaded microspheres standard solution and then added to placebo. Each solution was injected in triplicate; the accuracy of the method was determined by spiking known amount of Metformin Hydrochloride to placebo at 50%, 100% and 150% of test concentration in triplicate and analyzing as per the proposed method. The obtained results were represented in **Table 6** for most optimized formulation of Metformin Hydrochloride microspheres.

Precision: The degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings of a homogenous sample under the prescribed conditions is known as precision. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation.

System Suitability: The system suitability parameters were executed to verify the performance of the system, system precision was determined on six replicate injections of standard preparation, all essential chromatographic characteristics, including the relative standard deviation, peak tailing, theoretical plate number and resolution were measured. These all system suitability parameters covered the system, method and column performance [30-33].

RESULTS AND DISCUSSION:

Percentage Yield: The calculated percentage yield of all the Metformin Hydrochloride microsphere formulations prepared by using natural and synthetic polymers was found to be good.

Yield obtained from all the five batches prepared by using natural polymer by ion gelation method ranged from 90.6 ± 0.04 for F1 formulation to 96. ± 0.09 for F5 formulation and the values for MF formulations comprising of synthetic polymer formulated by solvent evaporation method ranged from 83.1 ± 0.01 for F6 formulation and 89.9 ± 0.02 for F10 formulation. The obtained data indicates a moderate increase in yield. The values are represented in **Table 4**.

Micromeretic Study: The flow properties of prepared Metformin Hydrochloride loaded microsphere formulations was determined by observing the obtained data for bulk density, tapped density, Hausner's ratio, Carr's index and angle of repose as depicted from **Table 4**.

Bulk Density and Tapped Density: As observed from the **Table 3**, it can be concluded that the prepared microspheres have better flow properties as compared to the pure drug, as seen from data. The increase in tapped density as compared with each bulk density of the formulation for different Drug: Polymers ratios indicate better flow behavior of microspheres.

The bulk density ranged from 1.40 ± 0.67 to 0.41 ± 0.31 and tapped density from 1.61 ± 0.78 to 0.44 ± 0.07 for microspheres prepared by natural polymer that is F1 to F5 and 1.69 ± 0.63 to 0.35 ± 0.07 is the data of bulk density for microspheres prepared by using synthetic polymer F6 to F10.

The values 1.71 ± .037 to 0.37 ± 0.33 are tapped density data for F6 to F10, for pure drug, the bulk density was 0.82 ± 0.41 and tapped density was 1.35 ± 0.21 respectively. All the values are represented in **Table 4**.

Hausner's Ratio: The value of Hausner's ratio for pure drug Metformin HCl was found to be 1.64 ± 0.17, it indicates poor flow, the values for all the prepared microspheres formulation ranged from 1.01 ± 0.08 to 1.15 ± 0.53, as indicated in **Table 4**.

Carr's Index: The values of all formulated microspheres from F1 to F10 exhibited enhanced flow properties ranging from 5.40 ± 0.74 to 13.04 ± 0.15, where as for the pure drug MF, the value calculated was 39.25 ± 2.01, indicating poor flow, all the values are represented in table 4.

Angle of Repose: Pure drug Metformin Hydrochloride had poor flow, as indicated by the value of angle of repose, that is 42.070, where as the formulated microspheres indicates excellent flow properties ranging from 13.070 to 23.810 as observed in **Table 4**.

Particle Size Analysis: The mean particle size of all ten formulations from F1 to F10 ranged between 29.07 ± 3.14 µm to 44.07 ± 5.38 µm. It was observed that mean particle size of prepared formulations increased with increase in polymer concentration for all the formulations, as seen in **Table 4**.

TABLE 4: MICROMERETIC, PARTICLE SIZE AND ENTRAPMENT EFFICIENCY STUDY OF METFORMIN HYDROCHLORIDE MICROSPHERES BY INCORPORATING NATURAL AND SYNTHETIC POLYMER

Formulation	% Yield	Bulk Density	Tapped Density	Hausner's ratio	Carr's Index	Angle of repose	Average particle size	Entrapment efficiency
Pure Drug	-	0.82±0.41	1.35±0.21	1.64±0.17	39.25±2.01	42.07	19.89±2.56	-
F1	90.6±0.04	1.40±0.67	1.61±0.78	1.15±0.53	13.04±0.15	23.81	29.07±3.14	68.71±0.86
F2	93.1±0.02	0.81±0.37	0.93±0.06	1.14±0.05	12.90±0.74	20.06	33.27±6.05	72.48±0.37
F3	94.5±0.07	0.68±0.11	0.77±0.42	1.13±0.17	11.68±0.27	18.17	37.08±7.04	75.91±0.03
F4	96.3±0.01	0.47±0.13	0.52±0.04	1.10±0.06	09.61±0. 03	16.11	38.58±2.06	76.68±0.46
F5	96.8±0.09	0.41±0.31	0.44±0.07	1.07±1.02	6.81±0.27	15.03	41.15±4.02	78.89±0.37
F6	83.1±0.01	1.69±0.63	1.71±.037	1.01±0.08	11.69±0.04	22.73	30.81±0.57	77.09±0.21
F7	83.9±0.04	0.87±0.23	0.99±0.53	1.13±0.02	12.12±0.97	21.09	34.11±6.07	79.42±0.06
F8	85.7±0.06	0.52±0.43	0.59±0.71	1.13±1.41	11.86±0.08	17.26	39.03±1.54	82.33±0.45
F9	89.4±0.07	0.41±1.08	0.46±0.61	1.12±0.05	10.86±0.03	15.03	42.32±0.81	88.68±0.09
F10	89.9±0.02	0.35±0.07	0.37±0.33	1.05±1.01	5.40±0.74	13.07	44.07±5.38	88.04±0.17

Entrapment Efficiency of Drug: Entrapment efficiency for formulations F1 to F5 prepared by natural polymer ranged from 68.71 ± 0.86 to 78.89 ± 0.37, whereas for formulations F6 to F10 had the values from 77.09 ± 0.21 to 88.69 ± 0.09, it is observed from the data of **Table 4** that better drug entrapment efficiency was observed for preparation comprising synthetic polymer using solvent evaporation technique, as compared from natural polymer formulation. This can be due to increased polymer concentration, leading to bigger particle size, hence higher drug entrapment.

***In-vitro* Drug Release Study:** The effect of various natural and synthetic polymer concentrations on release of Metformin hydrochloride loaded microspheres was studied. Various prepared formulations from F1 to F5 by ion-gelation using only natural polymer, the Metformin hydrochloride microspheres were able to sustain the release of

drug for around 4, 5, 6, 7 and 8 h respectively. The formulations F6 to F10 were prepared by using synthetic polymer by emulsion-solvent evaporation technique; from the dissolution data it was observed that the prepared microspheres were able to sustain the drug release up to 12 h. For F9 formulation 90.07% of drug was released after 12 h, hence it was selected as the most optimized formulation from all the prepared formulations by both the methods, as the data can be correlated to **Table 5**. The mechanism of drug release and release rate kinetics of drug from prepared Metformin hydrochloride loaded microspheres was studied from the data obtained from in-vitro dissolution studies. The observed data was obtained was fitted to various mathematical models like zero order, First order, Higuchi matrix, Korsmeyer-Peppas model and Hixson-Crowell mode by using software (PCP- Disso-V2). The obtained data values were represented in **Table 4**, the co-efficient of determination (R^2) was used as indicator of best fitting for each of the models was considered, it was observed from data that in all the cases the R values of Higuchi matrix model were close to 1. The diffusion coefficients (n) values ranged from 0.4569 to 0.5647 and the R values were very close to 1 for Higuchi matrix model, we can conclude that the release of drug from prepared formulation followed matrix diffusion kinetics and as the plot exhibited linearity, finally we can conclude that diffusion was the main mechanism of drug release from formulated microspheres.

The sustained release characteristic of these micro-particles was more prominent in pH 7.4 than pH 1.2. Since the R values of Higuchi matrix were close to 1, the drug release follows matrix diffusion kinetics and the plot revealed linearity; hence it was concluded that diffusion was the main mechanism of drug release from the mucoadhesive alginate microspheres [34-35]. For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the in-vitro drug diffusion data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, Korsmeyer- Peppas model and Hixson-Crowell model using software (PCP-Disso-V2). The values are compiled in **Table 5**.

TABLE 5: DRUG RELEASE PROFILE FOR PREPARED METFORMIN HCL LOADED MICROSPHERES BY INCORPORATING NATURAL AND SYNTHETIC POLYMERS

Formulation	Zero order (R^2)	First order (R^2)	Higuchi Matrix model (R^2)	Peppas model (R^2)	Hixson-Crowell model (R^2)	Parameters of Korsmeyer-Peppas equation (n)
F1	0.8817	0.8852	0.9968	0.9937	0.8802	0.5116
F2	0.8962	0.8990	0.9957	0.9952	0.8917	0.5198
F3	0.9303	0.9401	0.9946	0.9896	0.9098	0.5571
F4	0.9417	0.9489	0.9921	0.9814	0.9156	0.5506
F5	0.9589	0.9612	0.9904	0.9806	0.9314	0.5647
F6	0.8016	0.8110	0.9949	0.9968	0.7936	0.4569
F7	0.8457	0.8468	0.9967	0.9972	0.8214	0.4813
F8	0.8505	0.8617	0.9973	0.9979	0.8475	0.4941
F9	0.8791	0.8821	0.9981	0.9968	0.8556	0.5006
F10	0.8863	0.8916	0.9985	0.9959	0.8714	0.5378

The R values of Higuchi matrix model were close to 1. The diffusion coefficients (n) values ranged between 0.45 to 0.56, since the R values of Higuchi matrix were close to 1, the drug release follows matrix diffusion kinetics and the plot revealed linearity; hence it was concluded that diffusion was the main mechanism of drug release from the formulated microspheres. The sustained release characteristic of these microparticles was more prominent in pH 7.4 than pH 1.2.

FTIR Study: The FTIR obtained data revealed that there was no interaction between drug and the excipients used, as no additional peaks were seen in the spectrum of **Fig. 1**.

XRD Study: The XRD data reveals that pure drug Metformin Hydrochloride is crystalline in nature, natural polymer sodium alginate and synthetic polymer carbopol 940 are low crystalline in nature. Drug loaded microparticles with natural and synthetic polymers showed intermediate crystalline behavior. It is observed that for formulated microspheres, the crystallinity decreased due to presence of polymers.

Hence, there is a decrease in the peak intensity and baseline shift of diffractogram, as seen in **Fig. 2**.

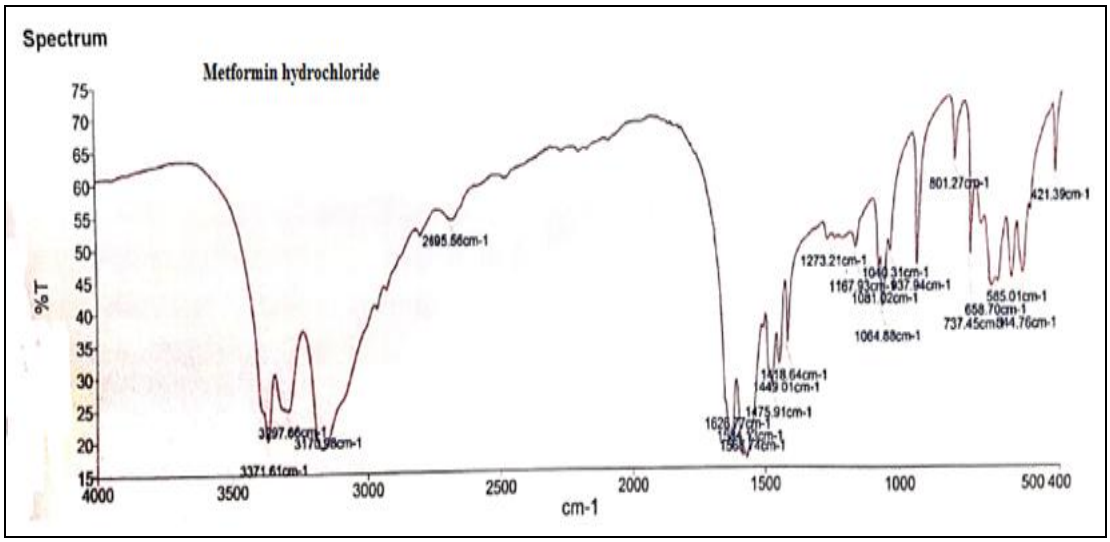

FIG. 1: FTIR SPECTRA OF PURE DRUG METFORMIN HYDROCHLORIDE

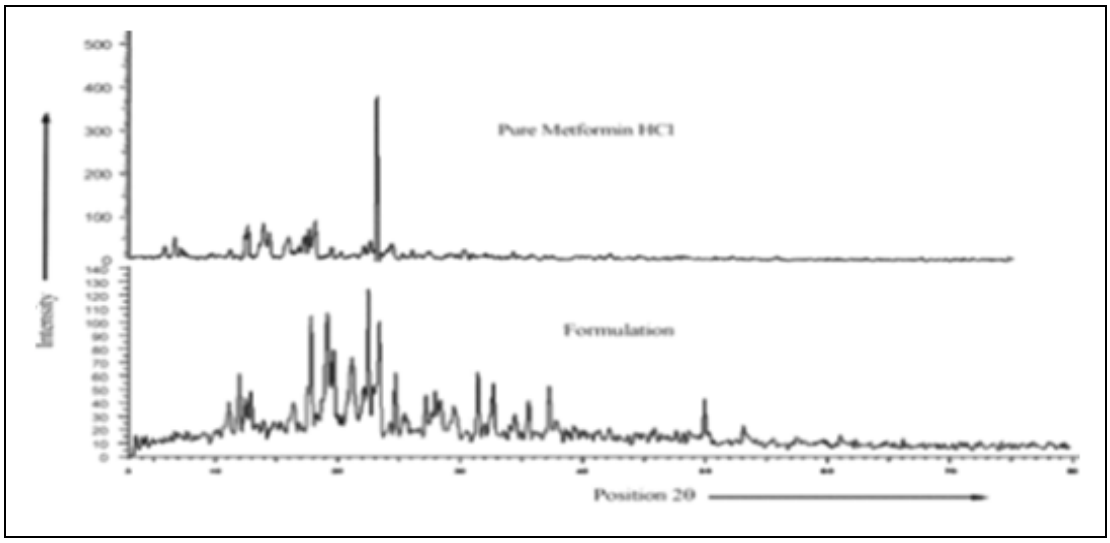

FIG. 2: X-RAY DIFFRACTION PATTERN OF PURE DRUG METFORMIN HYDROCHLORIDE AND OPTIMIZED FORMULATION

Precision: The system precision was performed by preparing standard solution as per test method and it was injected five times. The observation was shown in **Table 6**.

TABLE 6: ACCEPTANCE CRITERIA RSD SHOULD NOT BE MORE THAN 2.0 %

Conc.	Injection	Area	Statistical analysis
100%	Injection 1	1862954	Mean:1863322
	Injection 2	1870446	
	Injection 3	1863777	SD: 4486.70
	Injection 4	1861024	
	Injection 5	1858408	RSD:0.24

System Suitability: System suitability study was performed on freshly prepared standard solution of Metformin Hydrochloride formulation, under optimized chromatographic conditions and following parameters were studied to evaluate the suitability of the system.

The column efficiency, resolution and peak asymmetry was calculated for the standard solutions and the results are expressed in **Table 7**.

TABLE 7: SYSTEM SUITABILITY PARAMETERS FROM ASSAY STANDARD OF METFORMIN HYDROCHLORIDE

Name	RT*	USP Tailing	USP Plate Count	% RSD
Metformin hydrochloride	6.307	1.1	6024	0.24

* RT = Retention time

There was no blank, placebo interference in between sample and standard were seen in the chromatogram in **Fig. 4**.

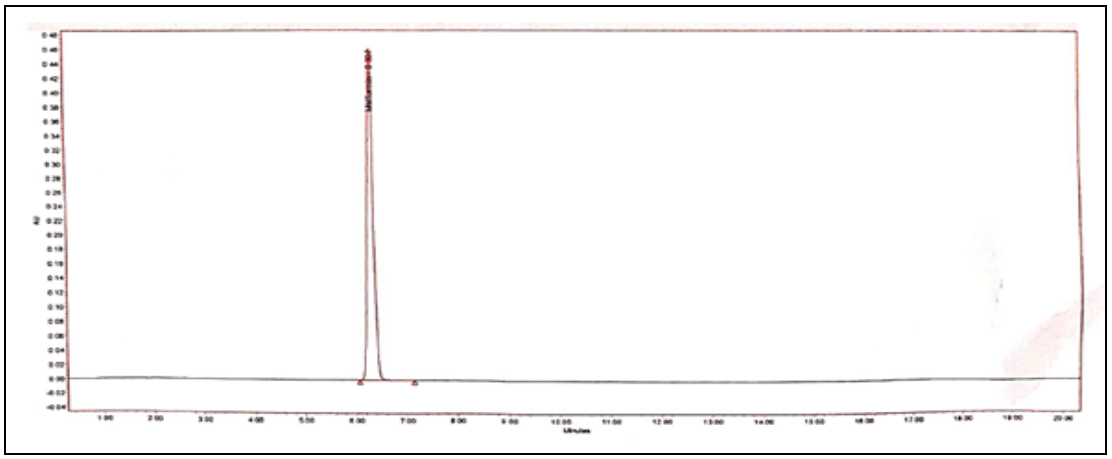

FIG. 3: CHROMATOGRAM OF STANDARD FOR METFORMIN HYDROCHLORIDE

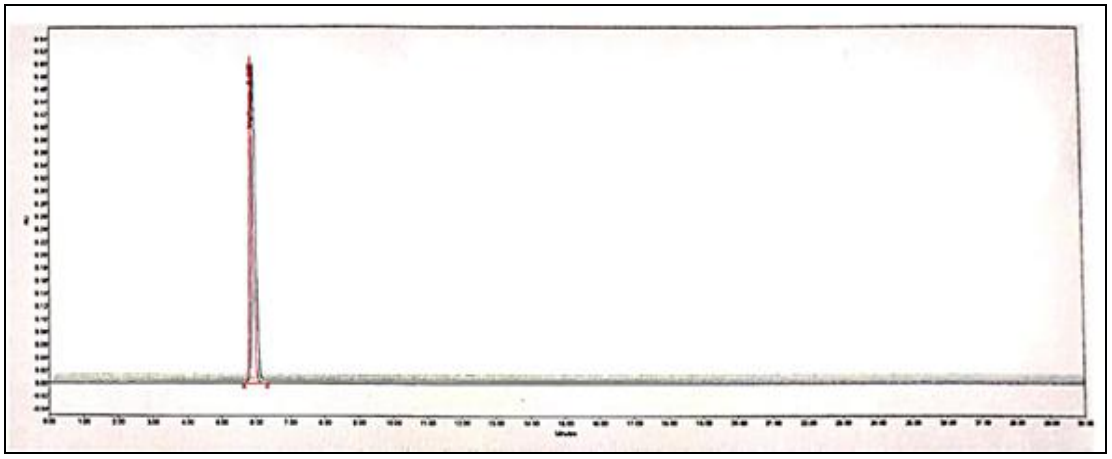

FIG. 4: OVERLAY CHROMATOGRAM OF BLANK, SAMPLE AND STANDARD FOR METFORMIN HYDROCHLORIDE

Linearity and Range: The calibration curve showed linearity over a concentration range of 60% to 140% for Metformin Hydrochloride, as shown in **Fig. 5** and was linear with a correlation coefficient of 0.999.

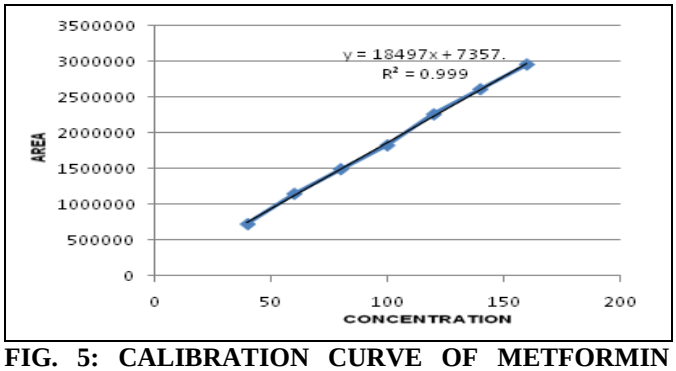

FIG. 5: CALIBRATION CURVE OF METFORMIN HYDROCHLORIDE

Accuracy: The selected analytical method meets the pre-established acceptance criteria for recovery study as per protocol, hence the method is accurate. Recovery (%) of Metformin Hydrochloride ranged from 100 to 101.0% for samples, as seen data from **Table 8**.

TABLE 8: ACCURACY DATA FOR METFORMIN HYDROCHLORIDE ASSAY

S. no.	Level (%) (n=3)	% Recovery	% RSD
1	0.5	100.41	0.56
2	1.0	100.89	0.71
3	1.5	100.05	0.39

CONCLUSION: In the above designed work for Metformin hydrochloride (MF) loaded micro-spheres formulation by incorporating natural polymer by ion-gelation method and by using synthetic polymer by emulsion solvent evaporation technique for comparative analysis, it was observed that controlled release microspheres of Metformin hydrochloride can be prepared successfully by both the methods and using both the polymers individually.

Formulation code F1 to F5 indicates data obtained for Metformin loaded microspheres containing natural polymer, the observed data reveals that these preparation have good sustained release behavior with enhanced flow properties as compared to pure drug, the drug release was sustained up to 8 h for prepared formulations by ion-gelation method using natural polymer. As we know that alginate is a naturally occurring biocompatible, biodegradable, biopolymer and is capable of rate and/or time controlled drug release, so dosing frequency can be reduced and as ion-gelation method is water based technique, so it involves total aqueous system avoiding any residual solvent in microspheres.

The percentage yield of these microspheres were higher, the FTIR study indicates there was no chemical interaction between the drug and polymers. The formulation code F6 to F10 represents data for formulated microspheres by using synthetic polymer by emulsion solvent evaporation method. It was observed that prepared microspheres were superior in flow properties as compared to pure drug, their drug entrapment efficiency was also higher as compared to preparations of natural polymer. Formulations comprising of synthetic polymer were able to provide controlled drug delivery system by controlling release of drug from microspheres up to 12 h, as concluded from dissolution data. The viscosity and increasing polymer ratio was the parameters controlling release of drug from formulation, FTIR data revealed no polymer drug interaction, XRD result indicated low-crystalline nature of microspheres. Finally, it can be concluded that among all the ten preparations from F1 to F10, the best designed formulation of Metformin hydrochloride loaded microspheres was F9 comprising of synthetic polymer, as it fulfilled entire requirements for controlled delivery of Metformin hydrochloride. For the most optimized formulation HPLC method development was done and it was observed that the assay method was precise and confirms with all pre established acceptance criteria for recovery. For future research we want to discuss a combination of natural and synthetic polymer for much enhanced results.

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