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Formulation and Evaluation of Metformin Engineered Polymeric Nanoparticles for Biomedical Purpose.

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ABSTRACT

The current work was intended for the formulation design and characterization of nanoparticles to obtain controlled release of the oral antidiabetic drug, Metformin. It is, however, associated with certain adversities, which can be surmounted by designing novel delivery forms for the drug. The nanoparticles of Metformin were prepared using Ethylcellulose (EC), poly (lactic-co-glycolic acid) (PLGA), poly (methyl methacrylate) (PMMA), and Chitosan as polymers. The formulated nanoparticles were subjected to in-vitro characterisation techniques for compatability studies between drug and polymer, size, surface morphology and release characteristics. The in-vitro characterisation results have shown that there were no possible chemical interaction between the drug and polymers and SEM revealed that the nanoparticles were spherical in shape with good surface morphology. The release studies were carried out up to 220 hours and, based on the release characteristics, chitosan was chosen as the best polymer. Thus the polymeric nanoparticle system can be considered as an effective delivery system for Metformin, which would deliver the drug at a controlled rate for a prolonged period of time. This can help to overcome the shortcomings of the conventional antidiabetic drugs and provide better therapeutic efficacy.

Keywords: Metformin, antidiabetic, nanoparticles, polymers, formulation, drugs

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INRODUCTION

In the recent past, substantial scientific and technological advancements have been made in the research and development of rate controlled oral drug delivery systems to counter the adversities of conventional drugs and its administration [1]. The rate controlled oral drug delivery system has given impetus to significant advancements in the engineering of novel dosage forms such as nanoparticles, which are solid colloidal polymeric carriers less than 1 µm in size [2,3]. Several attempts have been made towards developing biodegradable polymeric nanoparticles as potential drug delivery devices. In addition to the inherent property of reduced cytotoxicity, biodegradable polymeric nanoparticles have been found to be extremely effective in controlled and targeted drug release, and time controlled drug delivery system, notwithstanding the fact that the administration is oral [4,5]. Initial promise for nanoparticles was dampened by the fact that the therapeutic effect of drug loaded nanoparticles was relatively poor due to rapid clearance of the particles by phagocytosis. In recent years, this problem has been solved by designing surface modified nanoparticles [6].

Diabetes mellitus is a major and growing public health problem throughout the world and is associated with increased cardiovascular mortality; hence the current exercise is focused towards diabetes treatment. Metformin(M) improves hyperglycemia primarily through its suppression of hepatic glucose production. Many novel drug delivery systems, exhibiting extended release, slow release and sustained release, have been developed by pharmaceutical industries, but are not much significant. Hence formulating nanoparticles for Metformin could be a promising one. Nano drug delivery system enclosing antidiabetic drug may improve the therapeutic efficacy of the drug and can also control the release for a prolonged effect. Thus the adverse effects of the conventional dosage can be surmounted. Also, many biodegradable polymers have a major advantage: they do not require removal after application.

There is a pressing need for improvement in diabetes care. The prevalence of diabetes is increasing at an alarming rate, and diabetes mellitus is presenting an enormous economic burden in terms of direct health care expenditure and cost of treating diabetic complications. Although there are a number of relatively new types of therapeutic agents, none is optimal and none of the drugs available in market achieves satisfactory sustained glycemic control when given alone. The antidiabetic drugs, though potent, are associated with adverse reactions and problem of non compliance. These reasons encouraged to develop and evaluate stable nanoparticle based biodegradable delivery system using different polymers, which would deliver antidiabetic drug at a controlled rate for a prolonged period of time. These delivery systems are available for drugs, the plasma concentrations of which are critically related to efficacy. That is, such systems can maintain drug concentration at the therapeutic range in the body. Although orally administered prolonged release dosage forms are often useful for sustained action of drugs, they may not be available for maintenance of drug effects when the limited absorption window exists in the gastrointestinal tract. In order to achieve good drug absorption in such a case, the ability of the dosage form to exhibit prolonged drug release is very critical.

The aim of this paper is to model metformin polymeric nanoparticles and observe the aspects related to the currently used polymer and drug during formulation. This will further point out the most promising strategies in the experiments, according to the biomedical community needs. An ideal drug delivery system possesses two elements: the ability to target and to control the drug release. The reduction or prevention of side effects can also be achieved by controlled release.

MATERIALS AND METHODS

Ethylcellulose (EC), poly (lactic-co-glycolic acid) (PLGA), poly (methyl methacrylate) (PMMA) and Polyvinyl alcohol (PVA) were procured from Sigma Inc., (St. Louis, USA). Chitosan (MW 50,000) with degree of deacetylation 80% was obtained as gift sample from India sea foods, Cochin, India. Methanol, Dichloromethane (DCM) and Triton X procured from Ranbaxy Fine chemicals Ltd, New Delhi, India and Acetic acid procured from SRL, Mumbai, India. All other chemicals used were of analytical grade. The following materials were obtained from the indicated suppliers and used as such: Acetone, Petroleum ether, Ethanol (Ranbaxy Fine chemicals Ltd, New Delhi, India). Metformin hydrochloride was obtained from the Sigma Inc., (St. Louis, USA), and also received as a gift sample from Microlabs Ltd, Hosur, India.

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Preparation of polymeric nanoparticles

First the purity of drugs used in the formulation was determined by using UV and IR spectroscopy, Thermogravimetric analysis and Differential Scanning Calorimetry. Polymeric nanoparticles were prepared by solvent evaporation method using polymer as the coating material and drug as core material. For the preparation of PMMA and PLGA coated polymeric nanoparticles, known quantity of drug and polymer were dissolved in organic solvent, dichloromethane (organic phase). This solution was added dropwise to the aqueous phase of PVA and homogenized using IKA T 25 Digital Ultra turrax homogenizer, at 18,000rpm followed by magnetic stirring for 3h. The formed nanoparticles were recovered by centrifugation (Sigma centrifuge 3K 30) at 25,000rpm for 15min followed by washing thrice with petroleum ether and lyophilized [3]. For the preparation of chitosan coated nanoparticles, weighed quantity of drug and polymer were dissolved in suitable organic solvent (acetone) and 2% acetic acid (organic phase). For the preparation of EC polymer coated nanoparticle, the suitable solvent is acetone. All the nanoparticle formulations with different ratios of drug and polymer were prepared in triplicate to get the reproducibility and reliability [7]. The best method was selected and utilized for further characterization and optimized method was repeated in triplicate to get the reliability and desired amount of product for analysis. Nanoparticles prepared were washed thoroughly to remove the residual organic solvents. Since there is no therapeutic benefit from the residual solvents, all the solvents should be removed to the extent possible, to meet product specifications and good manufacturing practices. Then the nanoparticles were lyophilized using trehalose as cryoprotectant and stored in air tight container at 25°C until used.

Determination of Drug Incorporation Efficiency

The recovery of nanoparticles is defined as the weight ratio of freeze dried nanoparticles to the initial loadings of polymer, excipients and drug. The nanoparticle recovery, drug content and entrapment in the nanoparticles were calculated using the following equations.

$$\text{Percentage of Nanoparticle Recovery} = \frac{\text{Mass of nanoparticles recovered}}{\text{Mass of polymeric nanoparticles, drug and any formulation excipients}} \text{X } 100$$

$$\$\$\text{Percentage Drug content } \left(\frac{w}{w}\right) = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles recovered}} \text{X } 100$$

$$\text{Percentage Drug Entrapment} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug used in formulation}} \text{X } 100$$

Particle Size Analysis

Particle size was determined using Photon Correlation Spectroscopy (PCS) (Malvern S4700 PCS System, Malvern UK). Nanoparticles were first suspended in 100ml of filtered water (0.2µm filter, Ministart, Germany) and subjected to sonication for 30 seconds and vortex mixing for 10 seconds before analysis.

Scanning Electron Microscopy

The shape and surface topography of nanoparticles were examined using Scanning Electron Microscopy (SEM) (JSM-T20. Tokyo, Japan). An appropriate sample of polymeric nanoparticles was mounted on metal stubs, using double- sided adhesive tapes. Samples were gold coated and observed for morphology, at acceleration voltage of 15KV.

Fourier Transform Infrared Spectroscopy, Thermo Gravimetric Analysis, Differential Scanning Calorimetry, X-Ray Diffraction Analysis

Infrared spectroscopy was conducted using Avatar 320-FT IR UK, spectrophotometer and the spectrum was recorded in the region of 400-4000cm^{-1}. TGA data were obtained using a thermo gravimetric analyzer (TGA/SDTA851, Mettler, Switzerland). Differential Scanning Calorimetry (DSC) was performed using DSC-60. X-ray diffraction (XRD) analysis was performed on the polymer, drug and formulated nanoparticles.

High Performance Thin layer Chromatography

Silica gel 60mesh, F 254 TLC glass plates (20×20cm, layer thickness 0.2mm, E. Merck, Germany) were used as stationary phase and the mobile phase comprised of Toluene: Methanol: Aqueous ammonia (7: 3: 0.1). A camag HPTLC system (Switzerland), Camag CATS4 software and TLC scanner were used during the study. Standard and sample solutions were prepared and applied on TLC plates under nitrogen stream using semiautomatic spotter. Photometric measurements were performed in absorbance and reflectance mode.

***In-vitro* Release Study**

In-vitro release was evaluated using a dialysis bag technique [9]. The *in- vitro* release of nanoparticles was carried out thrice in stirred dissolution cells at 37.4°C by suspending nanoparticulate suspension into a beaker containing 100ml of release media: phosphate buffer saline pH 7.4. The correct *in-vitro* conditions required to study the release behavior of a hydrophobic drug were maintained [10]. Drug release was assessed by intermittently sampling the receptor media (5ml) at predetermined time intervals. Each time 5ml of fresh reservoir fluid was replaced. The amount of drug released in the buffer solution was quantified by a UV spectrophotometer [8].

Evaluation of *in-vitro* release kinetics (Theoretical calculation from % Cumulative drug release)

To study the release kinetics, data obtained from *in-vitro* release were plotted in various kinetic models.

Zero order equation- The graph was plotted as % drug released versus time in days. $\mathbf{C=K_o\ t}$ Where, K_0=Zero order rate constant in concentration/time; T=Time in days. The graph would yield a straight line with a slope equal to K_0 and intercept the origin of the axis.

First order equation- The graph was plotted as log cumulative % drug remaining versus time in days. $\mathbf{Log\ C=log\ C_0-Kt\ /2.303.}$ Where, C_0=Initial concentration of drug; K=First order constant; t=Time.

Higuchi kinetics- The graph was plotted as cumulative % drug released versus square root of time. $\mathbf{Q=Kt^{½}}$. Where, K=Constant reflecting design variable of system; t=Time in days Hence, drug release rate is proportional to the reciprocal of square root of time. If the plot yields a straight line, and the slope is one, then the particular dosage form is considered to follow Higuchi kinetics of drug release.

Hixson Crowell erosion equation- To evaluate the drug release with changes in the surface area and the diameter of particles, the data were plotted using the Hixson Crowell rate equation. The graph was plotted by cube root of % drug remaining versus time in days. $\mathbf{Q_O^{1/3} - Qt^{1/3} = K_{HC}\ x\ t}$

Where, Q_t=Amount of drug released in time t; Q_O=Initial amount of drug; K_{HC}=Rate constant for Hixson Crowell equation.

Korsmeyer -Peppas equation- To evaluate the mechanism of drug release, it was further plotted in Peppas equation as log cumulative % of drug released versus time. $\mathbf{M_t\ /M\alpha=Kt^n}$ and $\mathbf{log\ M_t\ /M\alpha\ =log\ K + n\ log\ t.}$ Where M_t /Mα=fraction of drug released at time t; T=release time;

K=Kinetic constant (incorporating structural and geometric characteristics of preparation); n= diffusional exponent indicative of the mechanism drug release [11].

RESULTS

Spectroscopic Validation

The standard solution of Metformin was prepared in phosphate buffer saline pH 7.4. It showed good linearity in all the solution system at the concentration range of 10- 50µg/ml at 232.5nm. The method was validated with respect to linearity and range, accuracy and precision. The prepared aliquots were scanned for absorbance at λ_{max}. The results are tabulated in the Table 1. These results confirm the purity of drug according to the pharmacopoeia standards.

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Table 1: Spectroscopic linearity validation of Metformin HCl

Parameters	Metformin
Absorbance Maxima	232.5nm
Beers Law Limit	10-50 µg /ml
Slope	0.013
Correlation Coefficient	0.998

Formation of Polymeric Nanoparticles

The polymeric nanoparticles were prepared by solvent evaporation method with different ratios of polymers. This method is comparatively easier than the other techniques. A suspension of polymer (PMMA, PLGA, CN, and EC) and drug in suitable solvent for each polymer forms the organic phase. Solvents used in these preparations rapidly partitioned into the external aqueous phase and the polymer precipitated around the drug. The subsequent evaporation of the entrapped solvent led to the formation of polymeric nanoparticles i.e., the polymer coated or covered around the shaped drug led to spherical shaped polymeric nanoparticles [1]. According to the solubility characteristics of drug and polymer, the solvent has been changed for different preparations. Each different polymer combination was considered as an individual preparation. Based on the efficiency of recovery and drug entrapment among the three different ratios, the best formulation ratio was selected (Table.2). The formed nanoparticles were recovered by centrifugation at 250,000rpm for 1 hour followed by washing thrice with water or Class III solvents based on the nature of nanoparticle. Lyophilization was carried out at high vacuum pressure. In lyophilization, aqueous solution of trehalose was added as the protective excipient to the nanoparticle dispersions after removal of organic solvents. The nanoparticle dispersions were frozen at -32°C for a minimum of 12h and lyophilized at -55°C and 0.5kPa for 24 hours. Finally freeze dried free flowing nanoparticles was obtained with this methodology.

Table 2: Percentage Drug content and Drug entrapment (EE) of Metformin loaded polymeric nanoparticles

Formulation	1:2		1:3		1:4		1:5	
	Drug content	EE	Drug Content	EE	Drug Content	EE	Drug Content	EE
	%							
M-PMMA	9.8±3	36.15±2	11.7±4	51.8±1	12.7±3	42.17±2	14.1±5	61.8±2
M-PLGA	12.5±3	45.6±1	15.6±1	56.5±3	14.8±1	45.6±5	16.6±1	67.6±1
M-CN	12.3±3	38.7±6	20.3±2	54.5±2	24.6±3	43.3±6	22.1±3	72.5±4
M-EC	14.1±5	51.6±7	17.1±7	45.2±2	20.5±5	56.3±2	21.6±2	75.9±2

All values are expressed mean ±SEM (n=3)

Effect of Drug Content and Drug Entrapment

In the nanoparticle preparation, the drug and the polymer were dissolved in the same organic phase. Hence, there were no chances of diffusion of the drug away from the polymer. The percentage of drug entrapment in all the formulations was found to be good at all levels of drug loading. In Metformin polymeric nanoparticles, the highest drug loading of 24.6% and 22.1% was showed by chitosan polymer. Metformin PMMA polymeric nanoparticles showed very low drug content when compared with all other polymers. The reason for this is unknown whereas Metformin EC showed drug loading in the range of 20-21% and there is not much more difference in loading characteristics between chitosan and EC. High nanoparticle recovery is required for reduction of manufacturing cost. Metformin-EC preparation showed 21.6% drug content, 75.9% drug entrapment.

Morphological Characterization of Polymeric Nanoparticles

The particle morphology of the drug materials was diverse and after formulation they were close to spherical shape. In these preparations, the polymer was fully saturated and the diffusion rate of solvent was minimal leading to the formation of smooth, spherical and individually homogeneously distributed particles and has no evidence of collapsed particles. Smooth surface reveals complete removal of solvent from the

formulated nanoparticles and is the indication of good quality. Literature shows, in freeze-dried nanoparticle coated with higher concentrations of chitosan, needle or plate like aggregates were obtained. To avoid this aggregation, the concentration of chitosan was maintained at optimum. Metformin nanoparticles appeared to be spherical and rather homogenous in size. The NP batches showed different sizes depending on the nature of polymer and solvent used. Compared with other polymers, the surfaces of EC NP exhibit fluffy nature (Figure.1 (A3 and A4). NP obtained from PMMA polymer had mostly a spherical shape, but appear to be a little aggregated as shown in Figure.2 (A7 and A8). In contrast, NP obtained from Chitosan were well defined, non aggregated and spherical.

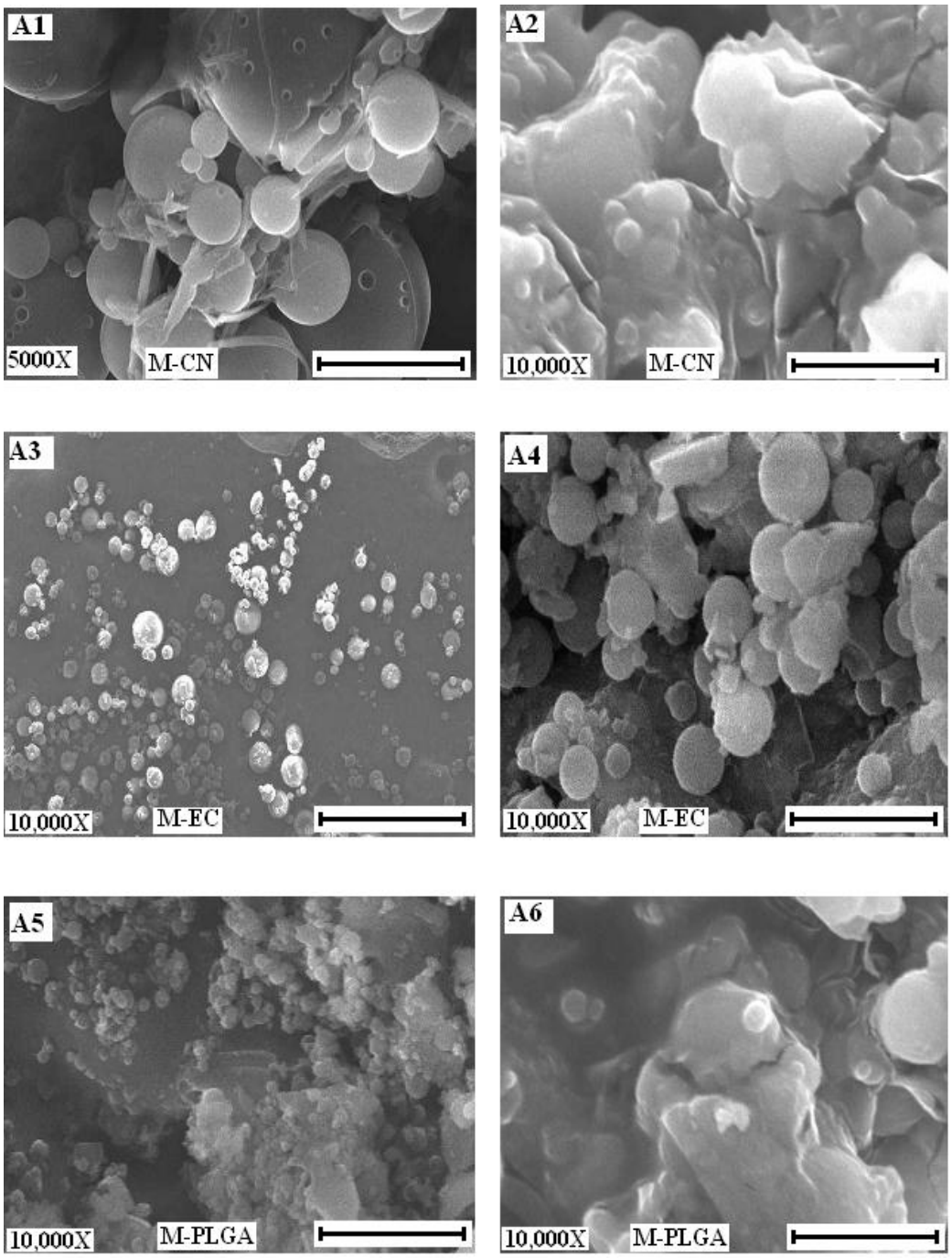

Figure 1: A1- A6 was SEM images of Metformin polymeric nanoparticle. Scale bar indicates 500nm

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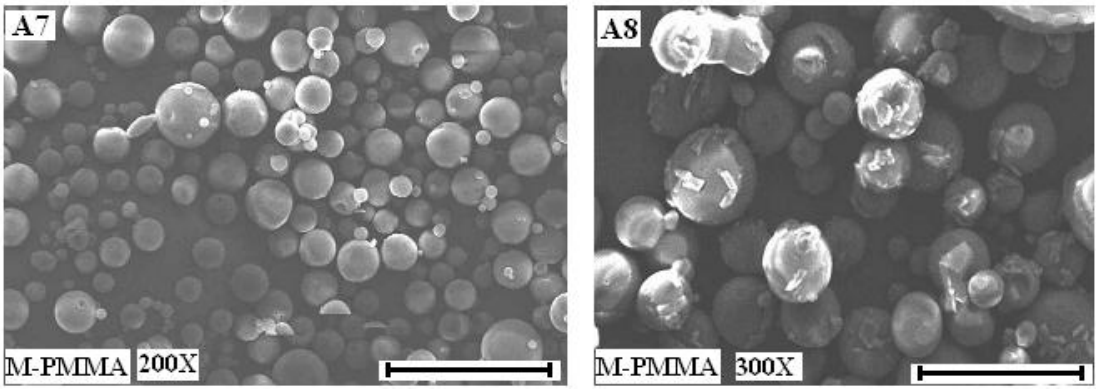

Figure 2: A7- A8 was SEM images of Metformin polymeric nanoparticle. Scale bar indicates 500nm.

Particle Size and Poly Dispersity Index

Nanoparticle size distributions determined by PCS are shown in Table 3. The particle sizes of nanoparticles were larger than those obtained by the quantitative analysis of the SEM. The contrast of the Electron Microscope (EM) pictures allows only the visualization of the nanoparticle core and gives the direct information on the particle shape whereas the hydrodynamic radius of the particles was measured by PCS. In SEM, there is no brownian motion and it scans exactly the particular nanomaterials in the splutter. Hence, some variations in particle size occur between SEM and PCS. The particle size data showed that nanoparticles produced were of sub micron size and had low poly dispersity, which indicates relatively narrow particle size distribution for preparations. In the present result, the nature and size of the particles vary depending upon the nature of the solvent and other parameters. These results showed that most of the drug and polymer with 1:5 ratio is in the optimum range with good surface property and poly dispersity index and it was further confirmed by release and other parameters like drug loading and encapsulation efficiency. The mean diameter and poly dispersity index (PI) of optimized formulations of three different polymeric nanoparticles using Chitosan, EC and PMMA are tabulated in Table 3.

Table 3: Effective size distribution and zeta potential of polymeric nanoparticles

Formulation	Size Distribution (nm)	Polydispersity index	Zeta potential
M-PMMA 1:2	65±12	1.200±0.001	-5±3
1:3	439±78	0.98±0.01	-12±5
1:4	203±89	0.922±0.003	-10±3
1:5	114±23	0.876±0.06	-2±8
M-CN 1:2	567±43	0.987± 0.09	+32±1
1:3	124±56	0.785±0.09	+12±3
1:4	260±76	0.345±0.1	+16±3
1:5	700±55	0.750±0.12	+21±7
M-EC 1:2	100±50	0.120±0.08	+22±2
1:3	120±20	1.230±0.008	+12±2
1:4	250±15	0.110±0.3	+10±2
1:5	700±25	0.675±0.1	+2±0.9
M-PLGA 1:2	64±12	0.546±0.1	+3±0.09
1:3	71±6	0.876±0.2	+23±1
1:4	250±12	0.987±0.09	+12±7
1:5	600±5	0.545±0.2	+17±1

FT-Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Thermo Gravimetric Analysis (TGA), X- Ray Diffraction analysis (XRD)

The interaction study between drugs and polymers were evaluated. A complete vibrational band assignment has been made available in Table 4, for drugs and polymers used in the formulation. The

characteristic band peak in drugs and polymers are slightly decreased or increased in the formulation due to the intermolecular hydrogen bonding between the end group of polymer and the drug. The FTIR bands of pure drug and its formulations confirmed that there is no significant interaction between drug and polymers and has good chemical stability.

Table 4: FTIR interpretation results

Description	Wave number(cm^{-1})	Band Assignment
Pure Metformin	3372 3297 2964 1626 1508 1205	N-H asymmetric stretching N-H symmetric stretching C-H stretching C=N stretching N-H plane deformation C-N stretching
Chitosan	1637 1568 3435	C=O amide I vibration N-H amide II bending N-H stretching vibration
Ethyl cellulose	3750 2500 and 2900 3350	Alkyl C-H stretching C-H stretching vibration O-H stretching vibration
PMMA	2947 1142 1727 1450	corresponds to the C-H stretching saturated ester group, C-O stretching C= O stretching of ester carbonyl C- O bond stretching
PLGA	2850-3000 1700-1800 1050-1250 3200	C-H, CH_2, CH_3 stretching C= O stretching C –O stretching O-H stretching
M +Chitosan	3354 2924 1626 1508 1205	N-H asymmetric stretching C-H stretching vibration C=O amide I vibration, C=N stretching N-H plane deformation C-N stretching
M +EC	3754 3372 3255 2944 1626	N-H,O-H stretching N-H symmetric stretching C-H stretching C-H stretching vibration C=N stretching
M +PLGA	3297 2964 1626 1508 1205	N-H symmetric, OH stretching C-H stretching C=N stretching, C= O stretching N-H plane deformation C-N stretching, C –O stretching
M+ PMMA	3352 2964 1626 1742 1508 1205	N-H asymmetric stretching C-H stretching C=N stretching C= O stretching NH plane deformation C-N stretching

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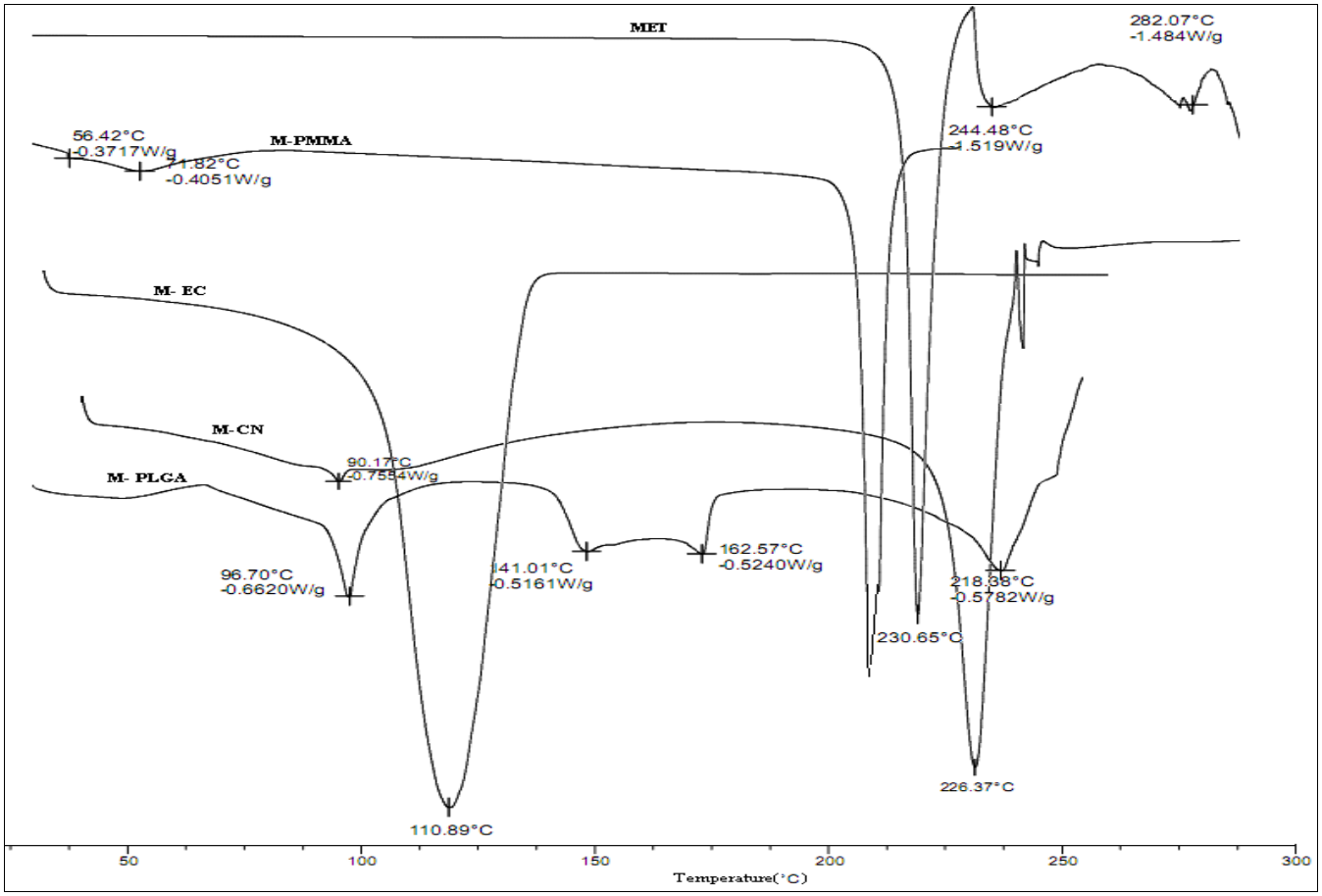

Figure 3: DSC thermograms of Metformin drug with different polymer combination

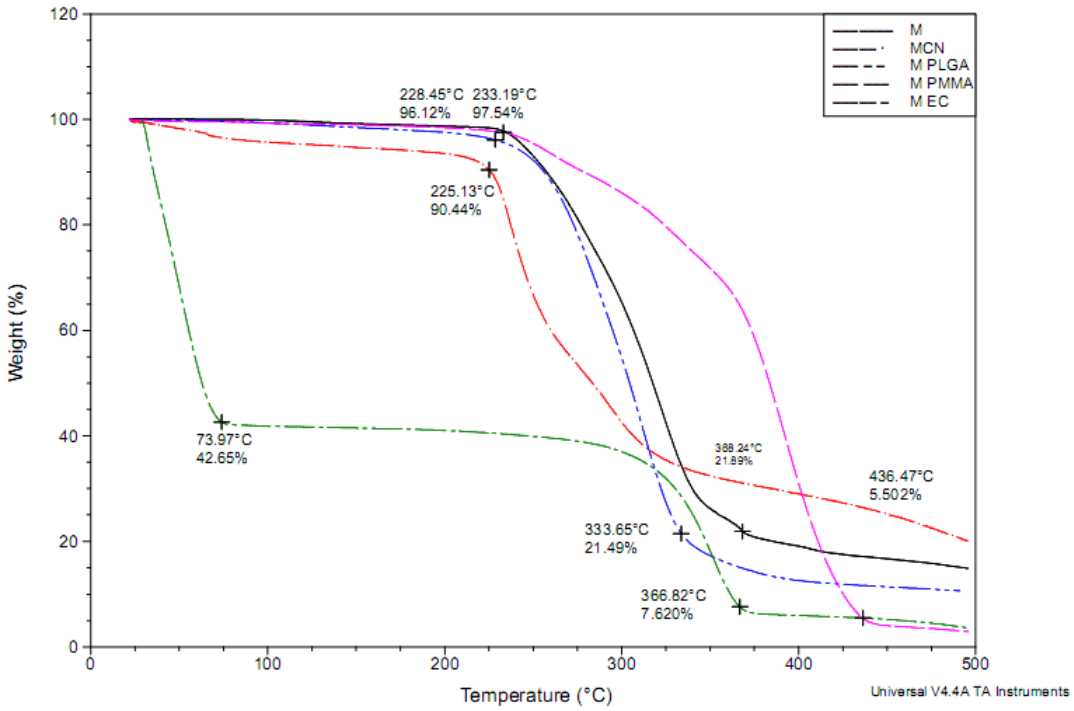

Figure 4: TGA thermograms of Metformin drug with different polymer combination

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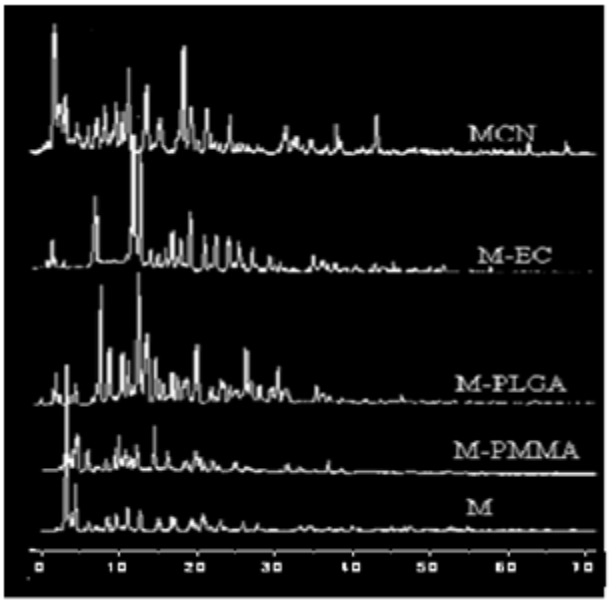
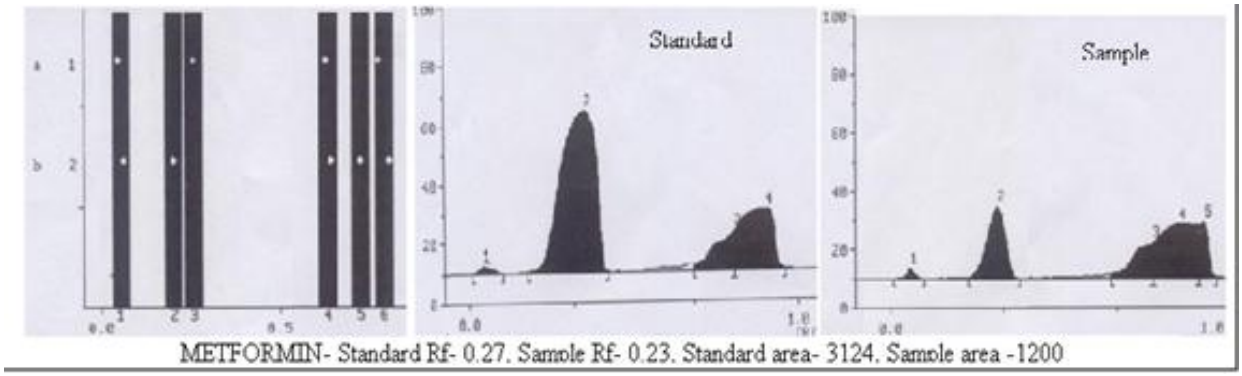


Figure 5: XRD of polymeric nanoparticle, polymer and drug



DSC examination was conducted for pure drug, polymers and for the drug loaded polymeric nanoparticles. In the present work, pure chitosan shows an endothermic peak that corresponds to the thermal decomposition with maxima at 111.66°C. DSC curves obtained are shown in Figure 3. The pure PLGA exhibit an endothermic event at approximately 152.63°C and deep at 160.51°C. The DSC curves of all formulations showed peaks resulting from simple superposition and slight changes that does not seem to be significant, because decomposition refers to both polymer and drug after formulation. This minute shift in melting point could be taken as an indication of the inclusion of drug in the polymer without any structural changes. Within the selected temperature thermal event occurred. In M-CN formulation high intense endothermic peak of drug was observed at 226.37°C and for chitosan at 90.17°C. The thermal decompositions of pure polymer, drug and formulation were studied by using thermogravimetry. TGA thermograms of Metformin formulations are elicited in Figure 4. These formulations have not shown any characteristic interaction, and there are no observable changes in the thermal decomposition temperature. XRD analysis of drug, polymer and drug loaded polymeric nanoparticle was performed and is illustrated in Figure 5.

High Performance Thin layer Chromatography (HPTLC)

The R_f value of the drug and the nanoparticle formulation was compared to check the purity and confirmation of drug loading. HPTLC data results are shown in Figure 6. R_f value of all the formulations matches with the R_f value of their respective standard drug. The lambda maximum wavelength of all the drugs was selected for evaluation, because at this wavelength there was a maximum of the absorption spectrum of drug. The peak area for the pure drug (standard) and formulation were recorded in the graph and they are mentioned below each figure. This observation confirms qualitatively the encapsulation of drug inside the

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polymer and they showed that process during formulation does not affect the drug stability. The densitometry responses from the standard and sample were used to calculate the amounts of the drug in the nanoparticle formulation. However, there are virtually no published data on these aspects by any researchers.

Figure 6: Typical HPTLC chromatogram showing the standard drug and formulation of Polymeric nanoparticle

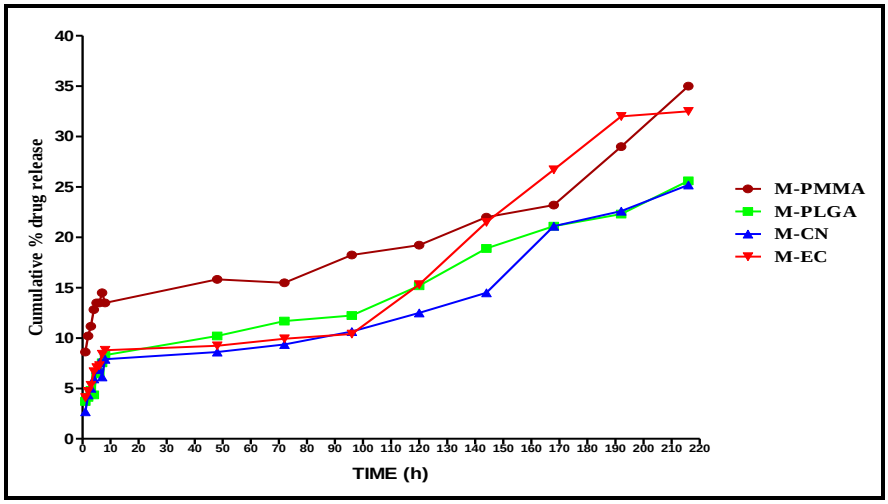
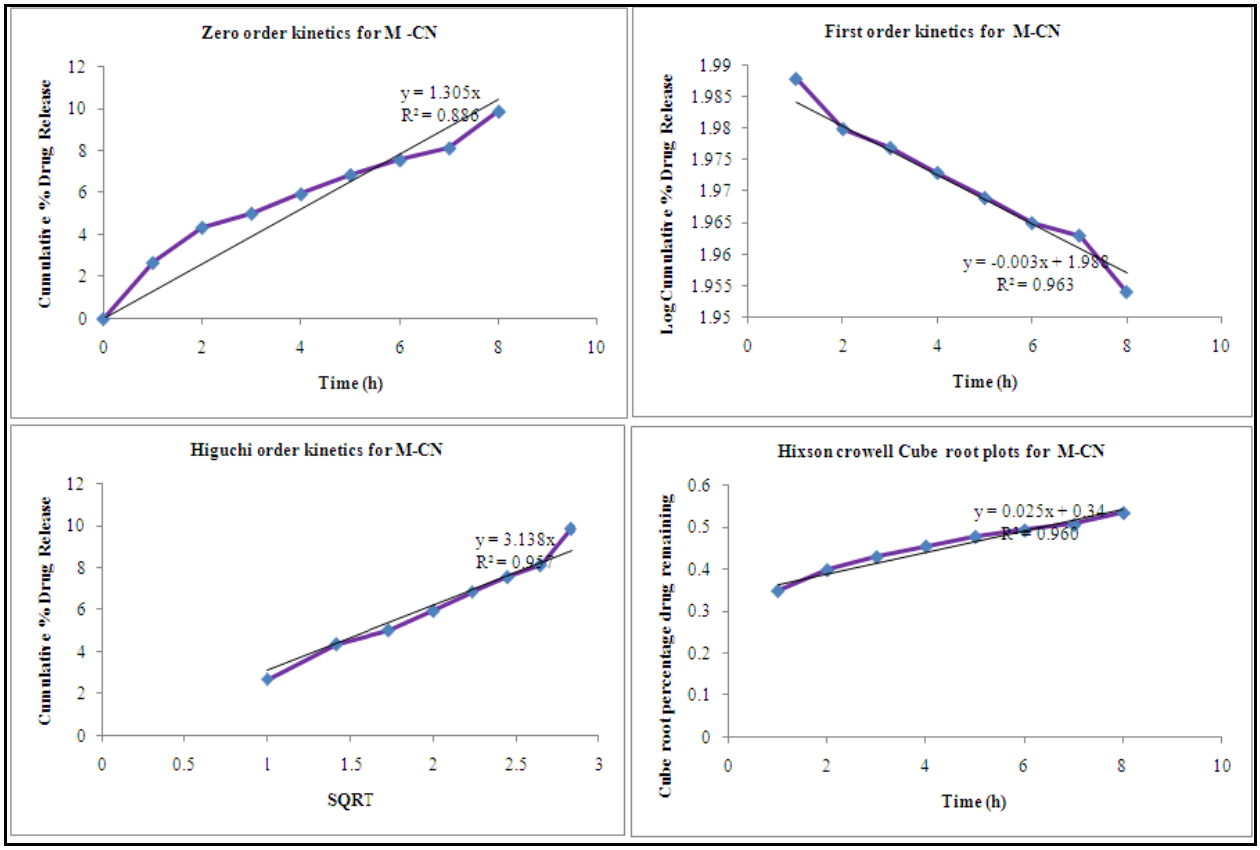


Figure 7: *In-vitro* release of Metformin from polymeric nanoparticles of 1:5 ratio



***In-vitro* Release Study**

In-vitro release profile of Metformin from each formulation, in pH 7.4 phosphate buffer, is represented in Figure 7. This shows that the cumulative % of drug release was higher in M-PMMA and a more controlled release of drug was achieved with M-CN and M-PLGA. Release studies were carried out up to 220 hours and the amount of drug released was calculated and the best formulation characteristics were selected.

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***In-vitro* Release Kinetics Study**

In order to determine the release model which best describes the pattern of drug release, the *in-vitro* release data was substituted in zero order, first order and diffusion controlled release. The zero order rate describes the systems where the drug release rate is independent of its concentration. The release constant was calculated from the slope of the appropriate plots and the regression co- efficient (r^2) was determined. In M-CN, the highest linearity of 0.963 is showed by Higuchi equation. In Korsemeyer equation r^2 value was found to be 0.987 and n value found to be 0.592 which appears that release follows mass transfer. Results are showed in Figure 8.

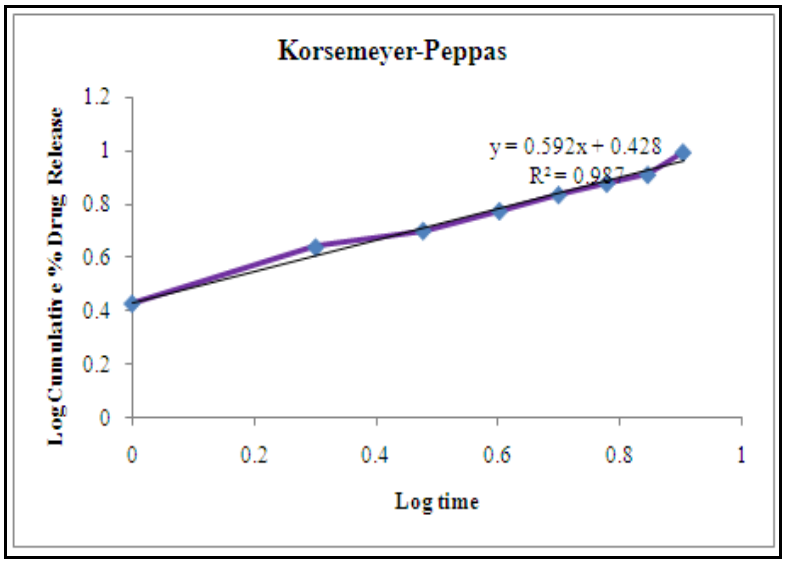

Figure 8: Release kinetics data of M-CN nanoparticle preparation

DISCUSSION

Some challenges prompted to design a nanoparticulate delivery system using low cost polymers like EC and chitosan and biodegradable polymers like PLGA and PMMA and with appropriate control of the process parameters that may develop a carrier with small size, low PI, and maximum entrapment efficiency (EE), which can effectively deliver antidiabetic drugs with fewer or no side effects. Therefore, in the present investigation an attempt was made to develop an alternative delivery system that may overcome the severe side effects of existing formulations by reducing dosage, improving patient compliance by controlled delivery, and providing safe and effective delivery at lower cost. Polymeric nanoparticles were successfully formulated by solvent evaporation method. The choice of a particular method of encapsulation is usually determined by the solubility characteristics of the drug and high pressure homogenization process allowed to reduce the mean particle size and width i.e., reduce the polydispersity index. An optimal formulation is the one, which provides good morphology, increased drug loading, high EE and prolonged release. Interestingly, the prepared nanoparticles illustrate an effective way, to prolong drug release. Trehalose was found to protect the nanoparticles, and it enhances the appearance of the dried material as a white powder, eligible for freeze dried formulation. Tween 80 improved the freeze drying result, as it acted as a steric stabilizer and increased the hydrophilicity of the nanoparticles. A hydrophilic surface enhances the redispersion properties of the freeze dried nanoparticles [12]. Hence, in this study trehalose was used as the cryoprotectant and PVA as stabilizer. The drug loading and entrapment efficiency were mainly affected by the polymer and drug ratios. However, encapsulation efficiency was proportional to the polymer ratio. This improved encapsulation efficiency was due to the greater proportion of polymer with respect to the amount of drug [13]. The researchers [14] attributed the decreased drug entrapment with increasing theoretical drug loadings to an enhanced drug leakage into the aqueous phase (if drug is water soluble) or into the organic phase (if drug is water insoluble) at high loadings. This would also lead to an enhanced drug loss. The drug content which is closer to the surface of the nanoparticle is responsible for an increased initial burst and the drug in the core of nanoparticles is responsible for a prolonged drug release from the polymer [10].

Drug loading expresses the percent weight of active ingredient encapsulated to the weight of nanoparticles, drug loading efficiency is the ratio of the experimentally determined percentage of drug content compared with actual, or theoretical mass of drug used for preparation of the nanoparticles. Hydrophobic polymers encapsulate larger amounts of hydrophobic drugs, whereas hydrophilic polymers entrap greater amounts of more hydrophilic drugs. Several formulation parameters, such as emulsifier type, weight ratio of polymer to drug, and organic to aqueous phase ratio, will influence the extent of drug loading [15].

Good entrapment efficiency of 75% was showed by 1:5 ratio and hence only 25% drug wastage was observed in that formulation. The high entrapment efficiency is believed to be due to its poor aqueous solubility or otherwise good solubility in the same solvent. This was achieved by increasing polymer drug ratio. Among the different drug polymer ratios investigated, 1:5 ratio had the optimum capacity for drug encapsulation. In these experimental modeling the volume of the processing medium and other parameters such as stirring speed, stabilizers concentration was kept constant. The ratio 1:5 was optimum, as drug wastage during nanoparticle preparation was found to be minimum. The ratios 1:2 and 1:3 delivered low yield owing to the high drug wastage and a large quantity of carrier was required to achieve sufficient amount of drug at a target site.

A number of reports have shown that EE increases with an increase in chitosan concentration [16]. The incorporation of chitosan in the carrier matrix produced a more pronounced increase in drug content. It was found that the end groups have a significant effect on the drug incorporation and its release behavior from the nanoparticles. The importance of the end groups has previously been suggested for PLGA microspheres [16, 17].

Particle size is often used to characterize nanoparticles, because it facilitates the understanding of the dispersion and aggregation [18]. The zeta potential value is an important particle characteristic as it can influence both particle characteristic as well as particle mucoadhesion. In theory, more pronounce zeta potential values, being positive or negative, tend to stabilize particle suspension. Fourier Transform Infrared spectroscopy (FTIR) has been a workhorse technique. Surprisingly, all the drugs and polymers are compatible and there is no chemical interaction except hydrogen bonding. The interactions that occur between a drug and a polymer can be identified, and in some cases quantified using Fourier transform infrared spectroscopy. In DSC analyses, there was no appreciable change in the melting endotherms of the formulation, compared to pure drugs, but slight decrease in the melting temperature had been noticed, which might be due to minor physical and morphological changes that were taking place in the drug and polymer after the formulation [1]. The difference in endotherms of polymer and formulation confirmed that there were no evidence of chemical reaction taking place between polymer and the drug. The purpose of these analyses in our experiment was to detect possible modification in the structure and nature of drug due to their organization in the form of nanoparticle. The DSC curve of formulated drug shows broad but low intensity peak accompanied by endothermic peaks, and is due to the Tg relaxation and enthalpy of polymer. These results, taken together, suggest that the encapsulation process produce a marked decrease in crystallinity of drug and /or confers to this drug a nearly amorphous state [19]. Small changes in the thermogram was attributed to the small size of the nanoparticle and their high specific area, there may be a chance of change in the melting point degrees [20]. In conclusion, no strong chemical interaction that alters the chemical structure and drug structural integrity between drug and polymer was observed. In XRD, there were ill defined peaks observed in the polymer matrix. Generally, XRD peaks depend upon the crystal size. In this formulation, the characteristic peak of drug overlapped with the noise of coated polymer. EC polymer showed intense peaks at 12.07º and 18.54º. There were ill defined peaks observed in the polymer matrix [13].

The *in-vitro* release of drugs from nanoparticles may approximate the drug release profile inside the body, although the rate is usually faster in *in-vivo* due to the presence of enzymes and surfactants in biological fluids [21]. For all the formulations, an initial burst release occurred for first few hours. Results obtained in the present study are elicited in Figure 7. The burst release can be explained by the imperfect encapsulation of the drug inside nanoparticles, resulting from the unstable nature of the emulsion droplets during the solvent removal step. Drug release process is controlled by dual mechanism; the liquid enters the polymer matrix, dissolves the drug and enable the drug to diffuse out through the liquid located in the polymer matrix. This release is attributed to the physical and chemical properties, particularly on the pKa and solubility profile of the drug. Release profile of drug molecules, irrespective of their chemical nature, was almost linear with time. But when married with the principles, it is clear that polymer forms a more compact wall around the drug.

Group of researchers also considered the corresponding "n" values as the indication of anamalous release mechanism [22,23]. Generally, the release rate observed is a cumulative effect of drugs solubility (influenced by its structure, molecular weight and pKa) polymer property (hydrophilicity/ lipophilicity molecular weight) and the relative ratio of drug and polymer. The value of n for a formulation = 0.45 for Fickian (case I) release,> 0.45but < 0.89 for non fickian (anomalous) release and 0.89 for case II (zero order) release and > 0.89 for super case II type of release (Peppas). Case II transport generally refers to the dissolution of the polymeric matrix due to the relaxation of the polymer chain and anomalous transport (Non Fickian) refers to the summation of both diffusion and dissolution controlled drug release. The Higuchi model of optimized formulation show linear regression and it can be found that release follows diffusion kinetics mechanism. The 'n' value of Peppas equation was found to be less than 0.5, from this it was concluded that the drug release follows Fickian diffusion. The high nanoparticle recovery could reduce the manufacturing costs and its size and morphology could improve the bio distribution and large surface to volume ratio. Therefore, the bioavailability of drug may be improved which may help to reduce the dose of the drug and frequency of administration. Before polymeric nanoparticles will be used in human applications, it will be necessary to carry out consistent and comprehensive studies to ensure their innocuousness. In Metformin loaded nanoparticle, chitosan was selected as the best polymer for pharmaceutical preparation based on in-vitro pharmaceutical characters. Despite many handicaps, several repetitions have been carried out to get the reproducibility of results. A successful system should not only protect the drugs from enzymatic degradation but also increase the drug permeability within the gastrointestinal barriers. The formulated nanoparticles can be used as an alternative drug delivery system for the treatment of highly prevalent and chronic disease like type II diabetes mellitus. To optimize this drug delivery system, and for deeper understanding of different mechanisms, further studies are still required. Accordingly, the next step of this work has been planned to optimize various parameters which influence efficacy and bioavailability *in-vivo*. Long before one of these compounds can reach the market; it needs to be formulated for the pharmacological activity tests and for the preclinical studies.

CONCLUSION

The finding of this investigation has convincingly introduced a novel delivery system for the oral antidiabetic drug, Metformin. Nanoparticles are known to provide controlled release of drug to ensure a prolonged effect. Thus the encapsulation of Metformin into polymeric nanoparticles improves its bioavailability, which may help to reduce the dose of the drug and frequency of administration. This can lead to significantly improved therapeutic response, considerably reduced adverse symptoms and enhanced patient compliance. Therefore, it was concluded that this formulae could be very promising alternative to the available conventional medications, for the treatment of type 2 diabetes. However, the optimization of this system can only be settled after preclinical and clinical studies.

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