POLYMERIC NANOPARTICULATE FORMULATION TO IMPROVE BIOAVAILABILITY OF METFORMIN IN RATS

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Metformin (MTF) is a biguanide antidiabetic drug administered orally for the treatment of type-2 diabetes mellitus. The aim of this study was to enhance MTF bioavailability through the formulation of MTF as nanoparticles utilizing Eudragit RS100 and Ethyl cellulose as matrix polymers. Nano-precipitation method was used for the preparation of MTF nanoparticles with drug to polymer ratios (1:1, 1:3, and 1:5) for both investigated polymers. The prepared formulations were characterized for particle size, zeta potential, scanning electron microscope (SEM), and in-vitro MTF diffusion. The selected formula, according to the characterization data, was subjected to in vivo Pharmacokinetic studies compared with the marketed MTF product in a randomized parallel pharmacokinetics study. MTF-EudragitRS100 formula (1:3) have shown improved drug loading and encapsulation efficiency with smaller particulate sizes compared with Ethyl cellulose formulae. SEM images shows rounded, homogeneous particle size distribution, and smooth surface for MTF-Eudragit RS100 nanoparticles. Pharmacokinetic results revealed that the selected MTF-Eudragit RS100 nanoparticulate formula improved the bioavailability of MTF by 1.4 fold compared with the marketed MTF tablets.

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1. Introduction

The oral absorption of low-permeable, high-soluble BCS Class III compounds, in particular, is often limited. Drugs which have too aqueous solubility are not able permeate the biological membrane and which have the too lipophilic are not dissolve GI aqueous environment [1]. So, the drugs should be have the both the hydrophillity and lipophillity for get good absorption. Drugs which have partition coefficient (Log P) value in the range of 1 to 3 shows good absorption through the fatty membranes, and the drugs with Log P greater than 3 or less than 1 the transport properties have a bad absorption characteristics [2]. Metformin is considered high soluble, low permeable drug according to BSC biowavier classification [3]. Many methods to increase oral bioavailability which depend on nanoparticles preparation and drug delivery technique this allow drugs to reach to site of action [4].Some people rationalize the low bioavailability of metformin due to its high polarity 99.9 % of metformin is ionized at physiological pH as cataionic species so, its permeability by passive diffusion is limited this can rationalize its bioavailability which about $55 \pm 16\%$. It is absorbed predominately from the small

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intestine [5]. Metformin is a Not well absorbed from the gastrointestinal tract. Its bioavailability is about 50-60%, although this percentage dropped to some extent if taken with food. Once absorbed by the plasma protein binding is negligible, and excreted in the urine without change. The pKa of MTF is l l .5 and it occurs as a cataion at the pH of the gastrointestinal tract (GIT) [2]. Because of water solubility and its ionization properties, metformin absorption is limited through Caco-2 cells [6]. Many trials carried out to improve metformin bioavailability through formulation metformin as floating tablet, nanoprticles by using O-Carboxymethyl chitosan nanoparticles for metformin delivery to pancreatic cancer cells [7]. The improved characteristics of the prepared biodegradable MTF nanoparticles will pave the road to investigate the prepared formulae in vivo. The realization of sustained release biodegradable MTF nanoparticles for oral or application could improve the activity of the drug for longer time with lower dosing frequency that improves patient tolerability and compliance.

2. Materials and methods

2.1 Materials

Metformin Hydrochloride, amoxicillin trihydrate and hydrochlorothiazide (used as internal standard, IS) were gifts from the Egyptian International Pharmaceutical Industries CO. (EIPICO) (Tenth of Ramadan, Egypt). Eudragit RS100, Ethyl cellulose and Polyvinyl alcohol (PVA) was agift from (DEEF Pharmaceutical Industries co., Saudi Arabia), Sodium hexane sulphonic acid and Ortho-Phosphoric acid were purchased from Sigma-Aldrich (MO, USA). HPLC grade acetonitrile, methanol, potassium dihydrogen orthophosphate and tetrahydrofuran were purchased from Fisher Scientific (NJ, USA). Millipore™ ultrapure water was used for the preparation of mobile phase and sample working standards.

2.2 Instruments and chromatographic conditions

The analysis was performed using the a HPLC instrument (1200 series, Agilent Technologies, CA, USA) that consisted of diode array detector set at a wavelength of 236 nm, with autosampler, and a quaternary HP 1200 pump. The HP thermostatted column compartment was set at 35 °C. The chromatographic separation was accomplished using Intersil® ODS-3 (250 ×4.6mm, 5µm packing) reverse phase analytical column (GL Sciences Inc, Japan). The mobile phase consisted sodium hexametaphosphate dissolved in 1 liter of potassium dihydrogen phosphate (pH 5.8 and molar concentration of 0.01 M) and acetonitrile (90:10) with an isocratic flow rate of 1.3 mL/minute. , Sonics Ultra sonicator (VC750.,Sonics.,USA), Christ freeze dryer(Alpha-1,Germany)., Buchi Rotavapor (R-210, Germany), Microtrack , Flex 10.5.4Zetasizer Scanning electron microscope(SEM) (NOVA NanoSEM 430, FEI, Czech Republic).

2.3 Preparation of Metformin/Ethyl cellulose, Metformin/Eudragit RS 100 nanoparticles

Eudragit RS100 and Ethylcellulose nanoparticles loaded with metformin were prepared by using nanoprecipitation method which described before by Fahmy [8].All formulae composition platted in table no.1. Briefly, Eudragit RS100 (50,150 and 250mg) or Ethyl cellulose (50,150 and 250mg) was dissolved in acetone. The organic phase was dropped wise into 6 mL of 1% PVA solution containing 50 mg of metformin then emulsified using ultrasonication(Sonics, VC750) for 2 min. organic solvent was evaporated by a rotary evaporator at 30°C for 2 hours, nanoparticles were centriugated at 20.000 rpm for 60 min. The nanoparticles were washed three times with distilled water. After the final washing, the nanoparticles were re-suspended in distilled water and then lyophilized overnight.

Table 1. Metformin nanoparticles composition ratios

Formula no.	MTF	Eudragit RS100	Ethyl cellulose
1	1	1	-
2	1	3	-
3	1	5	-
4	1	-	1
5	1	-	3
6	1	-	5

3. Characterization of nanoparticles

3.1 Surface morphology

The surface morphology of the nanoparticles was examined by scanning electron microscope (SEM). Surface properties can influence on the particles behavior in vivo, the lyophilized nanoparticles were mounted on metal stubs with conductive silver paint, sputtered with gold, and then SEM images were taken.

3.2 Particle size analysis and zeta potential measuring

The nanoparticles were evaluated for their particle size, and surface charge potential, by using Zetasizer. The formulations were diluted 1:100 with the aqueous phase of the formulation. Analysis was performed at 25 °C. Each determination was made in triplicate.

3.3 Encapsulation efficiency

To determine the total content of the MTF in the formed particles [9] 50 mg of the prepared formula weighed accurately and mixed with 5 ml of acetone and mixed by vortex for 5 minutes. This mixture was sonicated in a water bath sonicator for 15 minutes. Metformin extracted from the nanoparticles, and add 5 ml of phosphate buffer (pH 6.8) to this mixture and mixed by vortex for another 10 minutes. The organic layer was drained and aqueous layers contain the drug then the drug content of each sample is measured using the validated HPLC method. The calibration curve based on a range of 1-1000 ug / ml. EE% was determined from the equation:

$$Encapsulation\ Efficiency\ ({}^{W}/_{W}\,\%) = \frac{\text{amount of FIN in the nanoparticles}}{\text{weight of FIN initially added}} \quad (1)$$

4. Drug release from the nanoparticles

Incubation method was used to investigate the release of metformin from the prepared formulae, in an amber-20 ml glass sample containing 10 mg of suspended each formula in 5 ml of phosphate buffer vial (pH 6.8). the sample glass was immersed in a constant temperature (37 ° C) water bath with excitement at 50 rpm in a shaker horizontal laboratory, at different times (1.2, 4,5,6,7 and 8 hours) periods of samples (1 ml) was withdrawn from the center of the launch and replaced with a new buffer. Before analysis, all samples were centrifugated at 15,000 rpm for 40 minutes [9]. And their drug was measured by means of a validated HPLC method.

5. Pharmacokinetics study

Male Wistar rats (weight: 240 ± 20 g) were used for the pharmacokinetic study. These animals were inspected biomedically for any pathogens and were acclimated for at least 5-7 days

in their environmentally controlled cages (25 ± 1 °C and 12/12 hours dark/light cycle) with free access to standard food and water. The rats were fasted overnight while water was accessed ad libitum before the experiments. All experimental protocols were conducted after being approved by the Animal Ethics Committee of King Abdulaziz University, Jeddah KSA.

After optimization and characterization of formulae, formula no.2 chosen to be compared with brand drug (Glucophage), ten rats was divided in to two groups and only single dose (100mg/kg) was suspended in 1.5 ml of distilled water for each rat.

The blood samples in amount of 300 µl blood with anticoagulant were collected from each rat by the puncture of the retro-orbital sinus. This was performed at 0 (predose), 1, 2, 2.5,3, 5, 6, 7, 8 hr after oral administration of doses. All rats anaesethized using light ether anesthesia method, plasma was obtained by centrifugation and was stored at –20 °C until analysis.

The pharmacokinetic parameters of metformin for each rat after the two treatments were compared by the analysis of variance (ANOVA) with Tukey's pairwise comparisons. Significance was defined as ($P < 0.05$). The difference between metformin pharmacokinetic parameters for the two treatments were not significant except for C_{max} that showed significant differences ($P<0.05$).

6. Results

For formula no. 2 which composed of metformin and Eudragit RS 100 with ratio (1:3), the surface morphology of the nanoparticles was examined by scanning electron microscope (SEM), nano particles are spherical regularly shaped in nano-size range and have rounded edges as shown Fig. 1. For particle sizes, in general Ethyl cellulose formulae are larger than Eudragit RS 100 formulae. However, entrapment efficiency for Eudragit RS 100 formulae was more than Ethyl cellulose formulae as shown in Table 2.

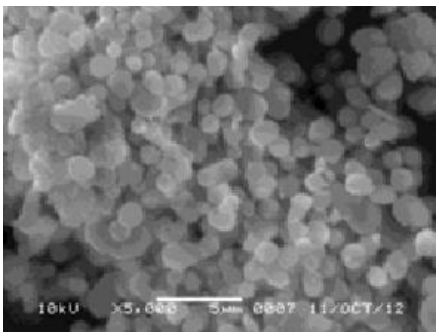

Fig. 1. A scanning electron microscope (SEM) pictures for formula no.(2).

Table 2. Particle Size (nm), Drug loading (%), Encapsulation efficiency (%), Zeta potential (mV) for all formulae.

	F1	F2	F3	F4	F5	F6
Particle size (nm)	452±23	205±53	854±76	2431±231	3241±213	5432±412
Encapsulation Efficiency (%)	12.1±2.1	16.2±2.1	11.2±1.2	8.76±2.1	6.5±1.1	5.7±0.4
Zeta potential (mV)	12.12±1.2	31.2±2.1	21.2±1.1	6.6±1.1	7.6±0.1	5.3±0.2

All prepared formula shown bi phasic release pattern which characterized with initial fast release then slow release. The pharmacokinetics study revealed that, there were significant

differences between AUC∞→0, $t_{1/2}$, k and ClT for the two treatments There were significant increase in AUC∞→0, t1/2, AUC∞→0, was 1147 ± 55.0 µg.hr/L and $t_{1/2}$ Was 6.48±0.33 hr versus 877.09 ±31.39 µg.hr/L and 3.03± 1.80 hr for metformin loaded Eudragit RS100 formula and Glucophage respectively as shown in figure no. (2).

Table 3. Mean ± SD of Pharmacokinetics data for control and Eudragit RS100 (F2) groups.

Parameter	Unit	Control	Formula no. 2
Vd/F	(L)	11.43± 0.88	11.44 ± 0.9
k	(hr^{-1})	0.2±0.04	0.03± 0.001
AUC 0→∞	(µg.hr/L)	877.09 ± 31.39	1147.71±55.04*
$t_{1/2}$	(hr)	3.03± 1.8	6.48±2.26*
t_{max}	(hr)	1.89 ± 1.22	2.2 ±0.68
C_{max}	(µg/L)	16618.14 ± 1505.11	17635.17±873
C_{lT}	(L/hr)	2.24 ± 0.45	1.21±0.1

(*)Significant difference defined as ($P < 0.05$).

7. Discussion

Increase polymer concentration leading to increase the particle size this can explained by increasing polymer amount with same volume of organic phase that mean increasing the emulsion viscosity that lead to formation of bigger particles which not easy to break by sonication. This fact is explained by the greater probability that the desolvated macromolecules (or small aggregates formed from these molecules) coalesce in a more concentrated solution, thereby forming larger particles [10]. Based on published data which confirm that there is no physical interaction between metformin and each of: Eudragit RS100 and Ethyl cellulose which confirmed by FTIR spectra and DSC diagram which revealed that there was no such interaction between the drug and the polymers used [11]. From our results it is appear that metformin: Eudragit RS100 formula (1:3) has smaller particle sizes, ,uniform shape ,more drug loading and higher encapsulation efficiency so, it was chosen as the best formula no.2(F2).

It was clearly observed that all batches have low drug loading, as mentioned in table no. (2), distilled water used for washing of PVA, so, most of all metformin particles which not entrapped in side Eudragit RS 100 molecules may be dissolved during washing of PVA.

Metformin release from Eudragit RS100 and Ethyl cellulose nanoparticle formulations is depicted in Table 2. All profiles displayed a biphasic release pattern characterized by an initial fast release (burst effect) followed by a slower release. These results agree with [12].

The amount of drug released was determined using calibration curve constructed over the range 1-1000 µg/ml. Some of the potential reasons that may lead to burst release of the drug are surface adsorption, morphology and porous structure of dry material. Another explanation for the burst effect in that some drug reside on the surface of the particle during the formulation process and is released immediately in a release medium [13]. It was clearly observed that using of formula no. 2 (metformin/eudragit loaded nanoparticles) enhance metformin bioavailability by sustaining metformin release.

8. Pharmacokinetics study

Data from table 3 revealed that, significant enhancement in metformin bioavailability, three potential mechanisms for the intestinal absorption of metformin nanoparticles are: (i) uptake and translocation via a Para cellular pathway; (ii) transcytosis or receptor-mediated transcytosis and transport via epithelial cells of the intestinal mucosa. So sustained delivery of metformin into the systemic circulation would cause significant alteration in the levels of plasma metformin

adhesion of Eudragit RS100 with mucosa [14]. There are two strategies which this research depends to increase bioavailability of metformin, the first the poorly absorbable metformin has a low bioavailability. Positively-charged polymers (such as Eudragit RS 100or Ethyl cellulose) can be used to increase its bioavailability as they may interact with the negatively charged mucus and hence open up the tight junctions of epithelial cells to allow passage through the Para cellular pathway[15].

The second strategy is sustaining metformin release by entrapping metformin in the two polymers to increase AUC to minimize peak plasma levels and reduce the risk of adverse reactions, allow for more predictable and extended duration of action, reduce the frequency of re-dosing and improve patient acceptance and compliance [16].

The use of bioadhesive polymers to prolong contact time in the various mucosal routes of drug administration, the ability to maintain a delivery system at a particular location for an extended period of time has great appeal for both local disease treatment as well as systemic drug. Positively charged polymers such as Eudragit RS100 can be used to increase metformin bioavailability as they may be interact with negatively charged mucus and hence open up the tight junctions of epithelia cells to allow passage through paracellular pathway [17].

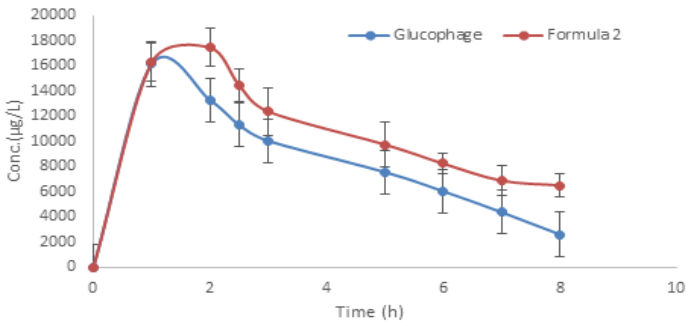

Fig. 2. The mean (±S.D.) plasma metformin concentration time curves observed after administration of a single oral dose (100 mg/kg) of metformin (Glucophage) administered to six rats .and single oral dose (100mg/kg) of metformin eudragit RS100 (formula no.2)nanoparticles administered to six rats.

9. Conclusions

The achievement of MTF nanoparticulate formulation with enhanced physical characters and sustained the release characteristics of MTF compared with raw MTF marketed tablets (Glucophage). As a result, blood glucose levels could be improved adjusted for longer time with lower dosing frequency that improves patient tolerability and compliance.

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Conflict of interest

The authors report no conflicts of interest in this work.

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