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Research Article **Novel drug delivery system**

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FABRICATION AND *IN VITRO* EVALUATION OF METFORMIN ENTRAPPED SUSTAINED RELEASE AMMONIO METHACRYLATE COPOLYMER MICROPARTICLES PREPARED BY NON-AQUEOUS EMULSION SOLVENT EVAPORATION TECHNIQUE

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ABSTRACT

Metformin hydrochloride is an orally administered first line drug which commonly used in the management of type-2 diabetes. To overcome the side effects of it due to high/repeated dose and patient inconvenience, the sustained release microparticles of metformin loaded RS100 were developed. The method used to prepared microparticles was novel oil in oil technique. Obtained microparticles were characterized by the different tests like Size, Encapsulation efficiency, Drug content, Percent yield determination, FE-SEM, FTIR, XRD, Dissolution study and Drug release kinetics. The obtained particles were from 236 to 313 µm in size, above 90% encapsulation efficiency and percentage yield was observed, drug and polymer not interacted, microparticles were in amorphous nature and drug-polymer influenced on dissolution profile. The more sustained batch followed Higuchi model. The statistical significance of the difference were tested by one-way ANOVA where p≤ 0.05.

KEYWORDS: **Eudragit® RS100, Disperse phase viscosity, Porous surface, Burst release, Release kinetics**

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INTRODUCTION

Diabetes mellitus is a sign of group of diseases characterized by chronic hyperglycemia and other metabolic abnormalities, which are due to deficiency of insulin effect. After a long period of metabolic derangement, specific complications of diabetes like retinopathy, nephropathy, and neuropathy may occur. Arteriosclerosis is also accelerated. Depending on the severity of the metabolic abnormality, diabetes may be asymptomatic, or may be associated with symptoms like thirst, polyuria, and weight loss or may progress to ketoacidosis and coma [1]. According to data collected more than 90% of adult diabetes diagnoses are type 2 diabetes mellitus. It associated with micro and macrovascular complications with increased morbidity and mortality among the elderly population because it adds to the burden of serious comorbidities like heart disease, stroke, and renal dysfunction which increase with age [2-4]. Metformin hydrochloride is an orally administered first line biguanide class drug which widely used in the management of type-2 diabetes [5]. Although metformin's exact mechanism of action is not completely understood, its main blood glucose–lowering activity appears to be primarily through suppression of hepatic glucose output. Its therapeutic blood glucose–normalizing action is dependent on the presence of circulating insulin. Metformin reduces gluconeogenesis by 0.6 mg/kg per minute in effect leading to a 75% reduction in hepatic glucose output. In isolated hepatocytes, metformin enhances insulin's suppression of gluconeogenesis and reduces glucagon-stimulated gluconeogenesis. Metformin may also act in the liver by activation of adenosine monophosphate–activated protein kinase, resulting in inhibition of the genes that regulate lipid genesis and enhancement of lipolysis in hepatocytes [6,7]. It is hydrophilic drug, having absolute bioavailability of 40-60% and relatively short biological half life of 0.9-2.6 hrs [8-9]. To maintain plasma level and control the blood glucose level metformin is generally given in divided doses two to three times a day. The usual starting dose is 500 mg twice daily. The dose should be increased or decreased by 250–500 mg/d every 2 weeks until the desired level of glycemic control is achieved or a maximum dose of 2000 mg/d is reached [10]. This high dose may cause adverse effects like diarrhea, nausea, abdominal pain, abdominal bloating, flatulence, dyspepsia, and anorexia, occur in up to 50% of patients receiving metformin therapy [11]. The most clinically important risk associated with the use of metformin is the potential for development of lactic acidosis characterized by blood lactate concentration >45 mg/dL, decreased blood pH, electrolyte disturbances with an increased anion gap, and an increased lactate–pyruvate ratio [12,13].

To overcome these problems, a sustained release dosage form is designed to maintain constant levels of a drug in the patient's blood stream by releasing the drug over an extended period. This mechanism enhances the therapeutic effectiveness of the drug and reduces the required number of doses. An additional advantage of this controlled release formulation is added economic value by enhancing the patient compliance. Eudragit RS polymer is widely used to develop sustained release micro or nanoparticles [14-18]. It is pH independent polymer, inert to digestive tract, impermeable to water, capable of swelling and release active ingredient by diffusion [19]. In present work, an attempt has been made to formulate sustained release microparticles of metformin HCl and characterized by particle size, encapsulation efficiency, drug content, drug-polymer interaction, X-ray diffraction, in-vitro drug release, drug release kinetics and stability study.

MATERIALS AND METHODS

Metformin HCl and Eudragit® RS100 (MW approx. 32,000 g/mol) was kindly gifted by Aarti Drugs Pvt. Ltd., Mumbai and Evonik Degussa India Pvt. Ltd., Mumbai, India respectively.

Methanol and SPAN 80 was purchased from Merck, Mumbai and Ozone International, Mumbai respectively. Liquid Paraffin Light procured from Himedia Lab Pvt. Ltd. Mumbai. Dibasic potassium hydrogen phosphate (KH_2PO_4) and sodium hydroxide purchased from Sigma chemical Co. Ltd. (Mumbai). Dissolution medium was prepared by using triple distilled water filtered with 0.22 µ membrane filter.

i. Preparation of Microparticles

Metformin HCl and RS100 were clearly dissolved in common solvent methanol by magnetic lab stirrer (Remi, India). The ratio of drug and polymer was 1:3, 1:5, 1:8 and 1:10. This organic phase was slowly added (3ml/min) to the Light Liquid Paraffin while stirring containing 0.5% v/v Span 80 as surfactant and 20 ml n-hexane as hardening agent. The speed of rotation was maintained 1000 rpm for 2 hrs (Remi Elektrotechnik Ltd. Mumbai). 10 ml n-hexane was added at the end and continues the stirring for the next 1 hr. After evaporation of methanol, the formed microspheres were collected by filtration in vacuum, washed 4-5 times with 20 ml n-hexane each and dried at room temperature for 24 hrs. This method of preparation was a novel o/o emulsion solvent evaporation method [20,21].

ii. Viscosity measurement

Viscosities of internal phases at different ratios of all polymers were measured by Brookfield rotational digital viscometer DVLV II at 25°C.

iii. Microparticle size and surface morphology

Microparticle size analysis was measured by sieving using a nest of standard BSS sieves [22] as well as by optical microscopy using Motic microscope. The sample was placed on glass slide; adjust the platform and resolution power (10x and 40x) of microscope in a such way that the image was clearly observed on computer screen. After capturing the image particle size of at least 100 microspheres was measured. The shape and surface characteristics of microparticles were investigated and photographed using Field Emission- Scanning Electron Microscopy (FE- SEM) (S4800, Hitachi, Japan).

iv. Percentage yield, encapsulation efficiency and drug content

The percentage yields of dried microparticles were calculated by using Eq. (1)

$$\text{Percentage yield } (\%) = \frac{\text{Mass of microparticles recovered}}{\text{Mass of polymers,drug and formulation excipients}} \times 100 \qquad (1)$$

For determination of encapsulation efficiency and drug content, accurately weighed microparticles were added in small volume of dichloromethane. This mixture was sonicated to dissolved polymer and added 100 ml of phosphate buffer (pH 6.8) to extract metformin from matrix. Subsequently this solution was stirred for 10 min by a magnetic stirrer (Remi, India). After evaporation of dichloromethane and removed precipitated polymer by filtration, the remained aqueous dispersion was centrifuged at 18,000 rpm for 15 min. Amount of drug in phosphate buffer was determined by using Ultraviolet spectroscopy (U2900, Hitachi, Japan) at 233 nm. Encapsulation efficiency (EE %) and drug content (DC %) were represented by Eqs. (2) and (3) respectively.

$$\text{Encapsulation Efficiency (EE \%)} = \frac{\text{Mass of drug in microparticles}}{\text{Mass of drug used in formulations}} \times 100 \qquad (2)$$

$$\text{Drug content (DC \%)} = \frac{\text{Mass of drug in microparticles}}{\text{Mass of microparticles recovered}} \times 100 \qquad (3)$$

v. Fourier Transform Infrared Spectroscopy

To determine the interaction between drug and polymer metformin HCl, RS100 and microparticles were separately mixed with potassium bromide and infrared spectrums were recorded in region of 4000-400 cm^{-1} by using an infrared spectrophotometer (IR- 8400, Shimadzu Co. Ltd., Singapore).

vi. X-ray diffraction analysis

X-ray diffraction of samples was carried out using Model-D8 Advance, Brucker AXS GmbH, Germany diffractometer. A Cu Kα source operation (40 kV, 40 mA) was employed. The diffraction pattern were recorded over a 2θ angular range of 3-50^o with a step size of 0.02^o in 2θ and a 1 sec counting per step at room temperature.

vii. In vitro dissolution study

In vitro dissolution studies were performed using USP Type II dissolution apparatus (Paddle) (Electrolab, India). Accurately weighed samples were suspended in 1000 ml phosphate buffer saline (pH 6.8). The solution was stirred at 50 rpm with temperature adjusted to 37±1 ^oC. At predetermined time intervals 5 ml samples were withdrawn and centrifuged at 10,000 rpm for 10 min. Aliquots of supernatant were analyzed by UV spectrophotometer at 233 nm. The settled microparticles in centrifuge tube were re-dispersed in 5 ml fresh phosphate buffer saline (pH 6.8) and returned to the dissolution media.

viii. In vitro release kinetic

The dissolution data of each batch was fitted to various kinetic equations and mechanism of drug release investigated. Equation (4), (5), (6) and (7) are Zero order, First order, Higuchi model and Hixon-Crowell respectively.

$Q_t = K_0 t$ (4)

$\ln Q_t = \ln Q_0 - K_1 t$ (5)

$Q_t = K_h t^{1/2}$ (6)

$Q_0^{1/3} - Q_t^{1/3} = K_{HC} t$ (7)

Where, Q_t is the percentage of drug released at time t, Q_0 is initial amount of drug present in the formulation and K_0, K_1, K_h and K_{HC} are the constants of equations. Regression coefficient (r^2) was determined from slope of the following plots: Cumulative % drug release Vs Time (Zero order kinetic model), Log cumulative of % drug remaining Vs Time (First order kinetic model), Cumulative % of drug release Vs Square root of Time (Higuchi model), Cube root of % drug remaining in matrix Vs Time (Hixon-Crowell model). Mechanism of drug release according to Korsmeyer- Peppas model was evaluated by fitted first 60% of drug release in following equation and release exponent "n" was calculated form plot Log cumulative % drug release Vs Log time [23,24].

$M_t / M_\infty = k_p t^n$ (8)

Where, M_t/M_∞ is the fraction of drug release at time t, n is the release exponent and k_p is rate constant.

ix. Statistical analysis

The statistical significance of the difference in viscosities, particle size, %EE between the different microparticle formulations were tested by one-way analysis of variance (ANOVA) Graphpad Instat® Version 3.06 software. Differences were considered to be statistically significant at a level of $p \leq 0.05$.

RESULTS

1. Internal phase viscosity

Viscosity of internal phase increased as the concentration of polymer increased. Bracketed value represents viscosities of respective drug-polymer ratios. 1:3 (1.0941±0.34), 1:5(1.2336±0.012), 1:8 (1.7456±0.024), 1:10 (2.4897±0.061).

2. Morphology, size, encapsulation efficiency

The obtained microparticles were spherical in shape and have uniform size in respective batches. The surface of particles was observed rough, grimy and porous in nature (Fig. 1).

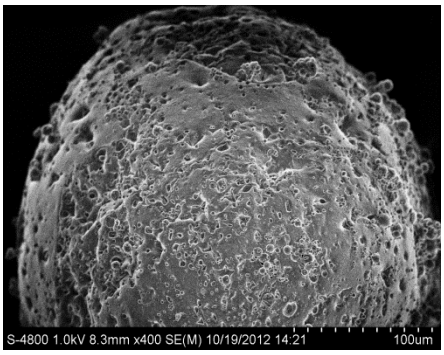

Figure 1
Surface morphology of more sustained microparticle

The size, encapsulation efficiency, drug content and percentage yield of each ratio was given in Table 1. Polymer concentration increased the encapsulation efficiency and percentage yield of the product.
Table 1. Mean particle size, Encapsulation efficiency, Drug content and percent yield of different formulations (mean ± SD, n=5)

3. Drug-polymer interaction

Table 1
Morphology, size,encapsulation efficiency

Formulations	Particle size (µm)	Encapsulation Efficiency (%)	Drug content (%)	% Yield
1:3	236.55±4.26	91.63±1.23	25.65±0.55	89.26±0.74
1:5	257.56±4.49	94.78±0.98	17.14±0.05	92.15±0.66
1:8	277.2±4.32	95.82±0.47	11.41±0.11	93.26±0.56
1:10	313.8±5.80	99.25±0.35	9.33±0.02	96.63±0.55

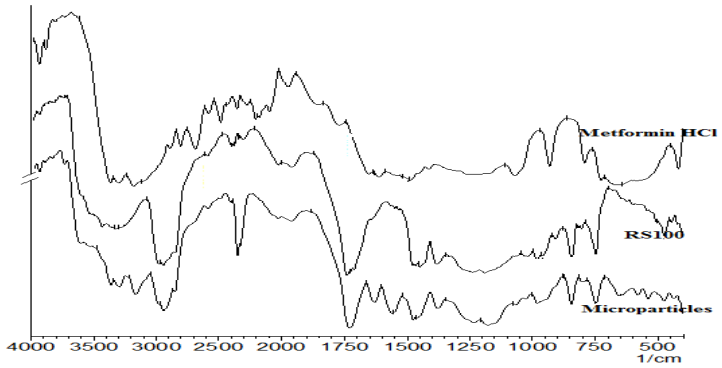

Figure 2
FTIR of Metformin HCl, RS100 and microparticles of their blend.

Fig. 2 elaborate the whether there was an interaction between metformin HCl and RS100 by interpreting the FTIR graph. Pure Metformin HCl illustrates two typical bands at 3371 cm^{-1} and 3296 cm^{-1} due to N-H primary stretching vibration and a band at 3170 cm^{-1} due to N-H secondary stretching. Characteristic bands at 1626 cm^{-1}, 1567 cm^{-1} allocate to C=N stretching. In RS100 polymer characteristic peaks of ester groups at 1190.12 cm^{-1} and 1240.27 cm^{-1} as well as C=O ester vibration at 1740 cm^{-1}. In addition CH vibration can be

discerned at 1385, 1450, 1475, and 2987 cm^{-1}. The common peaks observed in Metformin HCl and drug loaded microparticles are at 3367 cm^{-1}, 3294 cm^{-1}, 3173 cm^{-1}, 1631 cm^{-1} and 1564 cm^{-1}. Some peaks of RS100 was also observed in microparticles like 1184 cm^{-1}, 1236 cm^{-1}, 1734 cm^{-1} and in addition CH vibration also there.

4. X-ray diffraction

Percentage crystallinity of metformin HCl, RS100 and metformin HCl loaded RS100 microparticles was 98.6%, 21.7% and 25.1% respectively. Graphical presentation is in Fig 3. Metformin gives characteristic intense peaks at 2θ of 17.67°C, 22.36°C, 23.26°C, 24.63°C, 26.43°C, 27.23°C, 28.28°C, 29.53°C. RS100 and polymer coated microparticles were amorphous in nature.

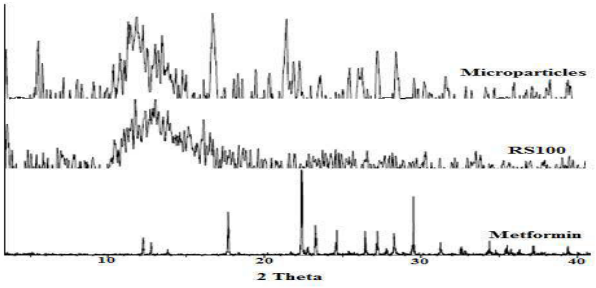

Figure 3
X-ray diffraction pattern of metformin HCl, RS100 and microparticles of their blend

5. In vitro release study

The obtained microparticle's dissolution profile at different drug-polymer ratios was illustrates in Fig. 4. 1:10 ratio formulation was the more sustained than other ratios by released 69.39 ±0.19% metformin up to 12 hrs. The burst release was 67.64 ±0.13% for lowest ratio formulation i.e 1:3 and this percent decreased up to 50.5 ±0.24% in 1:10 ratio. From obtained results it was observed that near about 20% metformin released from second hour to twelve hour.

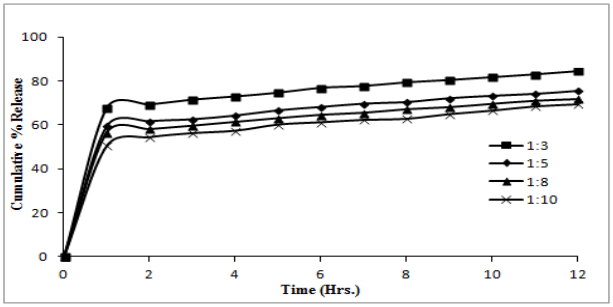

Figure 4
Dissolution profile of different drug-polymer ratio microparticle formulations

6. In vitro release kinetics

Effect of drug-polymer ratios on *In vitro* release kinetics are shown in Table 2. The formulations follow drug release kinetic model and their mechanism according to highest regression coefficient value (R^2). 1:3 followed Hixon-Crowell kinetic, 1:5 and 1:8 followed First order kinetic while 1:10 followed Higuchi model for drug release.

Table 2
In vitro release kinetic results of all formulations

Formulations	Zero order (R^2)	First order (R^2)	Higuchi (R^2)	Hixon-Crowell (R^2)	Korsmeyer-Peppas	
					(R^2)	n
1:3	0.9923	0.9977	0.9914	**0.9981**	0.9447	-
1:5	0.9888	**0.9963**	0.9895	0.9948	0.9442	-
1:8	0.9944	**0.9985**	0.9899	0.998	0.9415	-
1:10	0.9778	0.9871	**0.9886**	0.9853	0.9801	0.102

According to Korsmeyer theory, if 'n' is 0.45 then drug release will follows Fickian diffusion mechanism, for 0.45 < n < 0.89 follows Anomalous (non-Fickian) diffusion, for n = 0.89 case II transport and for n > 0.89 diffusion mechanism will super case II transport [25]. But here "n" value of 1:10 ratio formulation was beyond the limits and other formulation cannot determine by this model because of burst release more than 60% drug concentration.

DISCUSSION

Sustained release microparticles of metformin HCl was developed by non-aqueous solvent emulsion evaporation technique in which drug and polymer contained organic phase was slowly added to continuous oil phase containing surfactant with stirring. The methanol was immiscible with light liquid paraffin and having interfacial tension between them. Because of insoluble of metformin and RS100 in oil phase and evaporation of methanol from emulsion the precipitation of polymer blend takes place. Surfactant Span 80 helps to minimize the surface tension between both phases and decreased the size of organic phase droplets by moving towards the inter phase. After total evaporation of solvent, solidification of drug loaded polymer particles completed [21]. N-hexane confers hardness to the particles. In this mechanism of solidification the role of polymer concentration was significant. From obtained results it was concluded that as drug-polymer ratio increased the viscosity of internal organic phase was also significantly increased ($p<0.05$). This increased in viscosity was significantly influenced on particle size, encapsulation efficiency and percentage yield of the product ($p<0.05$). High viscous organic phase produced more size microparticles because it resist to mass transfer and breaking of droplets in continuous phase due to extra binding strength. Internal structure linkage of polymer was increased through viscosity enhancement. Inversely, in low viscous organic phase the droplets can easily split and not resist to mass transfer. Therefore lower viscous organic phase had more spreading ability in external phase leading to formation of smaller microparticles [16,27]. High viscous or more drug-polymer ratio organic phase solidified fast than other lower ratios. The fast solidification rate, concentrated organic phase and strong internal structure network was help to encapsulate maximum amount of drug [28,29]. The non-aqueous emulsion environment already facilitate to maximum drug loading. Therefore metformin leakage from polymeric globules was not occurred. More solidification or total mass transfer of organic phase confers the more percentage yield. The recovered microparticles were spherical in shape and have uniform size. The surface was porous and rough in appearance. The filthy or grimy surface may be due to irregular evaporation of methanol from emulsion.

To analyze the interaction between metformin HCl and RS100 the samples were tested by FTIR. From gained results it was revealed that the characteristic peaks of metformin HCl and RS100 was slightly shifted to lower and higher intensity than standard peaks. This shifting was negligible and within the range. So, there was not any chemical interaction between drug and polymer. To find out the molecular arrangement in recovered microparticles the sample was X-ray diffracted. Microparticles showed amorphous

characteristics because the crystalline drug may be covered by amorphous polymer. The crystalline peaks of metformin HCl were overlapped by amorphous or random arranged molecules [30]. From such results we can say that drug and polymer may fabricated with each other at molecular level. Even though the formulations encapsulated good amount of metformin means not to sustain efficiently. The Fig. 4 illustrate that the lower drug-polymer ratio had more burst release than higher ratio because in lower ratio the polymer concentration may insufficient to encapsulate metformin at the core of particles. The drug might be moved to the surface of the particle during solidification process due to decreased in surface tension between methanol and Light Liquid Paraffin through surfactant [29]. After evaporation of solvent the drug stable over there which dissolved in first hour of the dissolution test. Low polymer concentration was more soluble in organic solvent which account for slow solidification and resulted in high porous particles [31]. This may also produce burst release effect. The slowly released drug from second hour may be situated at the core of particles. So, due to strong bonding between drug and polymer, less porosity and particle size of high ratio formulation may sustain drug for more time than low ratio formulations. The drug release kinetic and mechanism by different models was illustrated in Table 2. From results it was concluded that drug-polymer ratio also affect on release kinetics. The lower ratio formulation released drug by Hixon-Crowell cube root law in which the progressive dissolution of the matrix as a function of time [32]. Both intermediate ratios were best fitted in First order kinetics which explained that drug released was depend on concentration. Last and most sustained higher ratio formulation was best fitted in Higuchi model. Higuchi model describes the release of drug from a matrix as a square root of a time-dependent process based on Fickian diffusion. Drug diffuses at slower rate as the distance for diffusion increases. Dissolution medium penetrates in porous matrix and dissolved the drug, which then diffused out. The volume and length of the opening in the matrix must be accounted [33]. To explain the mechanism of drug release Korsmeyer-Peppas model was applied for most sustained 1:10 ratio. But release exponent value was beyond the model limits may be due to influence of polymer macromolecular network and therefore difficult to predict the mechanism.

CONCLUSION

From all obtained results it was concluded that Oil in oil method was the efficient method to develop sustained release microparticles of highly water soluble drug like metformin HCl. The low leakage of drug in external phase gives more encapsulation efficiency and percentage yield of the product. Drug-polymer ratio was influenced on the physicochemical properties of the microparticles. The release profile consists of two distinctive phases; an initial burst release was followed by slow release phase. So, the developed sustained release system could be effective in management of Type II diabetes mellitus with reduced dose frequency, decreased side effects and improved patient compliance.

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