Current Trends in Biotechnology and Pharmacy
Vol. 16 (3) 308 - 315, july 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
10.5530/ctbp.2022.3.44

Formulation and Evaluation of Empagliflozin drug loaded Polymeric Nanoparticles for the Treatment of type 2 Diabetes Mellitus (T2DM)

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Abstract

Empagliflozin is an inhibitor of sodium-glucose co-transporter-2 (SGLT2). It is used in the management and treatment of diabetes mellitus (type 2). Till now the research done suggests that nano delivery systems may be the choice of drug delivery which can reduce dosing frequency and improve patient compliance. Hence, it was proposed to prepare nanoparticles of Empagliflozin. In this work, it was attempted to prepare nanoparticles of Empagliflozin using Eudragit and HPMC as polymers by solvent evaporation technique. Among the formulations, F1 and F4 have exhibited the best results. Drug loading capacity was between 13.20 to 19.96 percent. Encapsulation efficiency (%) of drug-polymer containing nanoparticles in various ratios was in-between 68.38 to 95.82. It is increased as the polymer quantity increased. For 10 hours, in vitro dissolution testing showed the drug release percentage for all formulations in the range between 89.75 and 97.93 per cent. *In vitro* studies have concluded that nanoparticles of Eudragit are superior for Empagliflozin delivery than HPMC based nanoparticles. The polymeric nano particles were evaluated for anti-diabetic Activity. All the formulations showed optimum results of which formulation containing higher concentration of Eudragit shown the better results in all the evaluated parameters. The polymeric nano particles were evaluated by *in-vitro* and *in-vivo* anti-diabetic methods and shown potential anti-diabetic activity. Thus, F1 can be concluded as the ideal batch of formulation.

Keywords: Polymeric nanoparticles, Empagliflozin, Eudragit, HPMC, Ethyl cellulose

Introduction:

In Diabetes Type 2 (T2D) known as adult-onset diabetes is a type of diabetes marked by high blood sugar levels, insulin resistance, and a shortage of insulin. Non-insulin hypoglycemic medications (non-insulin hypoglycemic agents) are routinely used to treat hyperglycaemia in people with type 2 diabetes. Because type-2 diabetes has several flaws, choosing medicines with complimentary modes of action is another sensible way to improve results. The current treatment of T2DM has the disadvantages such as lower bioavailability, less efficacy and the instant drug release, which leads to higher doses and dosing frequency. Till now the research done suggests that nano delivery systems may be the choice of drug delivery which can reduce dosing frequency and improve patient compliance [1]. Diabetes has a multifactorial pathological nature. Hence, multi-target drugs and personalized medicine may be considered as promising approaches [2].

Empagliflozin inhibits sodium glucose cotrans-

porter-2 (SGLT2). It shows actions such as lowering glucose, reduces body weight, reduces blood pressure and exhibits cardio protective benefits [3]. Sodium glucose co transporter 2 (SGLT-2) inhibitors interferes with glucose reabsorption in renal tubule of proximal section [4].

Materials and Methods

Empagliflozin was procured from Aurobindo Biotech Pvt Ltd, Hyderabad. All other analytical grade chemicals were used for analysis.

Preparation of nanoparticles

Emulsification solvent evaporation was used to synthesize Empagliflozin drug loaded polymeric nanoparticles [5]. Required amount of ethyl cellulose and Eudragit RL100 (F1, F2, F3); ethyl cellulose and HPMC- K100 polymer (F4, F5, F6) is diffused in ethanol (20 ml) and dichloromethane (1:1) mixture. The pre-determined amount of Empagliflozin was combined to the polymeric solution with magnetic stirring [6]. Then the suspension was injected quickly into light paraffin (100 ml) containing 2.5 per cent (v/v) of span 80, thus stirring to result in a w/o emulsion for 1 min at 10,000 rpm. The residue was collected. n-Hexane (50 ml) was used to wash the residue 2-3 times. Then the product is subjected to drying for 24h at a room temperature (Table 1).

Table 1: Formulation of Empagliflozin nanoparticles

S. No	Ingredients	F1	F2	F3	F4	F5	F6
1	Empagliflozin(mg)	100	100	100	100	100	100
2	Ethyl cellulose(mg)	600	675	750	600	675	750
3	Eudragit(mg)	300	225	150	-	-	-
4	HPMC(mg)	-	-	-	300	225	150

mg - Milligrams; F1, F2, F3, F4, F5, F6 - Formulations 1, 2, 3, 4, 5, 6

Characterization of nanoparticles [7]

1. Particle Size & Polydispersity Index: Nanoparticles size was calculated using Malvern Zeta Sizer. The uniformity of size in nanoparticles was measured by polydispersity Index.

2. Percentage Yield: The following formula can be used to calculate percentage yield.

Percentage Yield = (Amount of nanoparticles collected)/ (Total amount of the polymer and drug) × 100

3. Swelling property: Ethanol: methanol (1:1) was used to look over the degree of nanoparticle swelling. Sample is incubated with ethanol/ methanol solution for specified time interval (from 0.15- 12.0 hrs). By employing microscopic technique, analysis is made.

Swelling% = (Diameter of nanoparticles at time‘t’- Diameter of nanoparticles at initial time ‘0’) * 100

4. Zeta Potential: The Zeta potential of nanoparticles was adjudicated by using a Malvern Zeta sizer. All the studies were performed at 25^0C.

5. Scanning electron microscopy: Nanoparticles surface morphology was observed using SEM. The nanoparticles were placed on gold coating metal stubs and the images were taken by the Jeol Scanning electron microscope [8].

6. FTIR studies: The FTIR studies are performed to study the compatibility between drug and the polymer.

7. % Encapsulation efficiency and Drug loading

The quantity of the drug encapsulated can be calculated by a known quantity of nanoparticles (50 mg) added into ethanol and dichloromethane (50 ml each) in order to fully concentrate the drug. The filtrate (1ml) is mixed with pH 6.4 phosphate buffer (50 ml). This solution was assayed by a UV spectrophotometer at 261 nm for drug content.

EE (%) = [Actual Drug Content/Theoretical Drug Content] x 100

Drug Loading (DL) was calculated as:

DL (%) = (Actual Drug content Content/Weight of Nanoparticles) x 100

8. *In vitro* dissolution study: Empagliflozin nanoparticle (100 mg) were taken and kept in dialysis bag made up of cellulose [9]. The dialysis bag is dipped in to the recipient compartment that possesses the dissolution medium at 100 rpm at 37^0C (dissolution medium as a blank). At selected time intervals, 2 ml of sample was withdrawn and analysed at 261 nm in a double–beam UV Spectrophotometer

9. Stability Studies: Out of six formulations, F1 and F4 were evaluated for stability studies. These two Formulations were divided in to 2 sets and stored at 4 ± 1^0C; 25 ± 2^0C & 60 ± 5% RH; $37\pm2\ ^0$C & 65± 5% RH. After 30 days, the drug release of the above formulations was determined [10].

α *- amylase inhibition*

3.5-dinitrosalicylic acid (DNSA) and Empagliflozin drug loaded polymeric nanoparticles kept in phosphate buffer (pH 6.9) were used in a concentrations ranging from 50 µg/ mL to 1000 µg/mL. The drug and α-amylase solution was mixed in a quantity of 200 µL each. The concoction was kept for 15 min at 30°C. Starch (200 µL) was transferred to these tubes and kept for 3 mins. 200 mL DNSA reagent was added to stop the reaction. DNSA reagent is prepared by taking sodium potassium tartrate tetra hydrate (12 g), 2 M NaOH (10.0 mL) and 96 mM 3.5-dinitro salicylic acid solution (20 mL). The mixture is kept in a water bath at 85°C and boiled for 10 minutes. The liquid was cooled to room temperature. Then it is diluted with 5 to 6 mL distilled water before being estimated at 540 nm with a UV-Visible spectrophotometer. 200 µL of buffer was used as blank [11]. Acarbose (Bayer, Germany) acts as positive control. The reaction is performed in same way as drug does. The inhibition of amylase was stated as a percentage of inhibition.

Inhibition (%) = (absorbance of control − absorbance of control blank) − (absorbance of sample − absorbance of sample blank) / (absorbance of control − absorbance of control blank)] × 100,

The % α-amylase inhibition was plotted against the sample concentration and the IC_{50} values were obtained from the graph.

Invivo anti-diabetic study

Wistar albino rats (Male - weighing 160–200 g) were taken. The animals were placed under suitable conditions of temperature (24 ± 2°C) and at light–dark cycle of 12 hours. Animal house at Geethanjali college of Pharmacy, Telangana, India, provided albino female Wistar healthy rats weighing 120-180g. The space was adequately ventilated and kept at a constant temperature of 27°C with the relative humidity of 65% ± 10% light and dark cycles. The animals were kept in clean cages and fed rodent pellets and clean water. The animals were kept in hygienic cages and fed with rodent pellet and hygienic water. The study design was accepted by the Institutional Animal Ethical Committee (IAEC-1648/PO/Re/S/12/CPCSEA), Geethanjali College of Pharmacy, Telangana India. Four groups of albino rats (six animals each) were used.. Drugs were administrated orally.

The rats were kept fasted overnight. And then were given a single dose of freshly produced streptozotocin (STZ- 50mg/kg body weight) in Na-citrate buffer (0.1 M) pH 4.5 intraperitoneally to induce diabetes in the experimental animals. Following that, a 5% glucose solution was administered via gavage. Citrate buffer was given to control group. The other groups received streptozotocin. The induction of diabetes was validated after 72hrs by measuring glucose levels in blood. Further diabetes tests were conducted on rats with blood glucose levels more than 120 mg/dl.

Estimation of sugar in blood: The experimental animals were fasted for 12-16 hours, blood samples were taken using the “Rupturing tail vein method,” and glucose levels in blood were determined using a glucometer [12]. The glucose levels were checked on the 1st, 7th, 14th, 21st, and 28th days.

Results

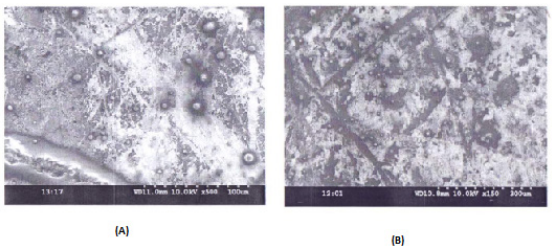
In this work, Empagliflozin nanoparticles were prepared using Eudragit and HPMC as polymers by solvent evaporation technique. Percent encapsulation efficiency and percent drug loading of nanoparticles was in between 13.20 to 19.96% and 68.38 to 95.82 % respectively and increased with a rise in polymer (Table 2). The nanoparticles were studies using SEM and FTIR respectively (Figure 1&2). The particle size range was 200-300nm. With decrease in the polymer concentration, nanoparticles size decreased significantly. The FTIR spectra are recorded for the samples over a wavelength of 4000–400 $cm^{-1.}$

Table 2: Particle size, zeta potential, Per cent encapsulation and per cent drug loading of nano particles

Formulation	Particle Size (nm)	Zeta Potential (mV)	% Drug Loading	%Encapsulation
F_1	270.03	-29.85	19.96	95.82
F_2	246.91	-27.58	17.14	82.47
F_3	192.84	-22.21	13.68	74.48
F_4	295.18	-28.04	17.72	82.36
F_5	256.87	-23.99	14.09	73.41
F_6	182.12	-20.34	13.20	68.38

nm – Nanometers; mV - Millivolts; % - Percentage

Figure 1: SEM images of Empagliflozin drug



loaded polymeric nanoparticles

(a) WD11.0mm10.0kv x500 100 µm
(b) WD10.8mm10.0kv x150 300 µm

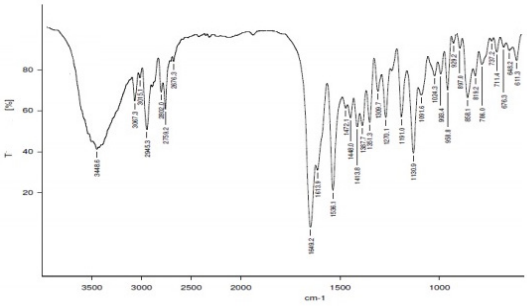


Figure 2: FTIR spectra of Empagliflozin with Eudragit

Table 3: % drug release of nanoparticles (phosphate buffer -P^H6.4)

Time (hrs)	Percentage cumulative drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	25.75	24.75	20.84	23.19	21.54	20.65
2	33.48	31.56	26.75	31.06	28.32	26.47
3	41.89	36.71	35.96	35.29	34.27	33.51
4	47.76	43.66	42.88	42.30	40.45	38.37
5	55.28	54.28	52.63	50.36	49.75	45.89
6	67.38	61.59	60.71	60.09	56.45	54.89
7	75.49	72.51	70.47	72.17	70.52	65.81
8	85.37	82.68	78.52	81.67	79.45	74.25
9	90.42	87.72	85.09	86.28	85.52	79.66
10	97.93	95.20	92.45	94.68	93.25	89.75

(FTIR spectra over a range of 4000– 400 cm^{-1})

In vitro release studies

The *in vitro* release studies of nanoparticles were carried out for a period of 10 hours in phosphate buffer of pH 7.4 as a dissolution medium. The drug release of F1, F2, F3, F4, F5 and F6 were 97.93%, 95.20%, 92.45%, 94.68%, 93.25% and 89.75 at the end of 10th hour (Table 3, Figure 3).

*Phosphate buffer of P^H 6.4; n=3

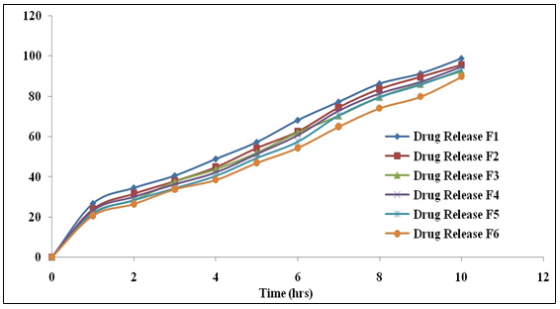


Figure 3: Percentage drug release of nanoparticles in phosphate buffer of P^H 6.4

Empagliflozin with Eudragit RL100 (F1, F2, F3)

Empagliflozin with HPMC- K100 polymer (F4, F5, F6)

Phosphate buffer of P^H 6.4; n=3

Stability studies

In Stability studies both the formulations released almost 95% percent of the drug within 10 hours of study. Formulations stored at 4±1 ^{0}C showed better results when compared to formulations stored at 25 ± 2^{0}C & 60 ± 5% RH and 37 ± 2^{0}C 65 ± 5% RH (Table 4). Hence, 4 ± 1^{0}C is ideal for storage.

Table 4: Stability studies of formulations (F_1, F_4) after 30 days of storage

Formulation	Percentage Cumulative Drug Release up to 8th hour		
	4°C ± 1°C	25 ± 2°C & 60 ± 5% RH	37 ± 2°C & 65 ± 5% RH
F_1	97.47	94.53	90.14
F_4	95.26	93.66	88.25

α- Amylase Inhibition

The results showed that similar activity with the Empagliflozin drug loaded polymeric nanoparticles and standard anti-diabetic drug Acarbose [13]. The maximum inhibition percentage of Empagliflozin nanoparticles was 60.25 ± 0.432, and the minimum percentage inhibition was 20.05 ± 0.76 (Table 5).

Table 5: in vitro alpha amylase inhibition

Concentration µg/ml	Empagliflozin nanoparticles (%)	Acarbose (%)
50	20.05 ± 0.76	21.14 ± 0.489
100	38.56 ± 0.675	39.45 ± 0.423
150	50.03 ± 0.305	52.29 ± 0.566
200	58.34 ± 0.412	60.46 ± 0.654
250	60.25 ± 0.432	62.78 ± 0.672

*n=3; µg –microgram; %-percentage

Analysis of glucose level in blood and body weight of experimental rats

Administration of STZ resulted in increased blood glucose level and an observable reduction in body weight. This situation was reversed on the treatment of diabetic rats with 100µg/kg body weight for 28 days and resulting in lower glucose level in blood [14]. In addition, the aforementioned treatment was also able to increase the weight of the experimental rats. In addition, the insulin levels were low in untreated diabetic group. Treatment with Empagliflozin drug loaded polymeric nanoparticles increased the insulin levels significantly ($p < 0.001$), similar to the untreated control group (Table 6). The animal study results indicated an increase in body weight of diabetic treated rats. Empagliflozin drug loaded polymeric nanoparticles significantly reduced glucose levels in blood, increased insulin level, and reduced insulin resistance [15].

Table 6: Plasma Insulin levels in control and experimental rats.

Groups	Plasma Insulin level (µU/ml)
I	17.03 ± 0.193
II	5.06 ± 0.223*
III	14.15 ± 0.192*
IV	15.12 ± 0.296

(Mean n+ standard deviation for 6 animals in each groups; *p<0.001)

Normal Control (0.15 M Citrate Buffer at pH 4.5) is taken as group-I. Diabetic induced control (Streptozoticin 50 mg/kg weight) is taken as group-II. Diabetic induced rats administered with Empagliflozin drug loaded polymeric nanoparticles (100 mg/Kg weight) were categorized as group-III. Diabetic induced rats administered with Glibenclamide (500 µg/kg weight) were taken as group-IV.

Discussion

In the treatment of diabetes mellitus (type 2), the drug delivery system plays crucial role as it effects the patient compliance. Nanoparticles are an efficient drug delivery systems as they increases physicochemical stability of the loaded drug and thereby its bioavailability [16]. Previously, triple fixed-dose combination with Empagliflozin was formulated using granulation methodology [17]. Empagliflozin nano particles using Eudragit as polymer have shown maximum drug loading percentage of 19.06 and 95.82% entrapment efficiency was reported. Drug release was in the range between 89.75% - 97.93% in dissolution study and increased with a rise in polymer. Scanning electron microscope pictures indicated that the nanoparticles were in a size range from 200-300 nm and spherical with a smooth surface (Figure 1). The FTIR of Empagliflozin reveals the presence of functional groups such as at 3445.98cm^{-1} (Amine (NH), 2963.28 cm^{-1}(C=N), 1730.51 cm^{-1} (Carboxylate), 1603.48 cm^{-1}(C=O) and 1066.13 cm^{-1} (C-N) respectively. In case of physical mixture of Empagliflozin with Eudragit, a band is observed at 3382.46 cm- due to N–H bond stretching. Hence, no chemical interaction was observed in physical mixture of the polymers and drug (Figure 2). *In vitro* studies have concluded that Eudragit nanoparticles are efficient for Empagliflozin delivery than HPMC based nanoparticles. It was clear from the dissolution data that there is a proportional increase in the drug release with the rise in the polymer concentration. The greater release of drug from the Eudragit nanoparticles than HPMC nanoparticles can be accredited to their higher degree of swelling which allows water penetration in to nanoparticles. Both the formulations released almost 95% per cent of the drug within 10 hours of stability study. Empagliflozin nanoparticles inhibited **α- Amylase,** reduced glucose levels in blood, increased insulin level, and reduced insulin resistance significantly.

Conclusion

By studying all the experimental results nanoparticles encapsulated with Empagliflozin can be successfully formulated by emulsification solvent evaporation method. All the formulations manifested optimum results of which formulation containing higher concentration of Eudragit showed the finest results in all the evaluated parameters. Further, Empagliflozin nanoparticles have shown significant anti diabetic activity in *in-vitro* and *in-vivo* studies.

Acknowledgements

The authors are thankful to Geethanjali College of Pharmacy; Hyderabad for providing facilities to conduct the proposed work.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no competing interests.

Statement of human and animal rights

Animals were provided by Geethanjali College of Pharmacy, Hyderabad, Telangana, India. Animal experiments and study protocols are approved by the Institutional Animal Ethics Committee of Institutional Animal Ethical Committee (IAEC) with reference number (Registration No.: (IAEC-1648/PO/Re/S/12/CPCSEA), under CPCSEA, Delhi, India. All institutional and national guidelines for the care and use of laboratory animals were followed.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

T2DM- type 2 diabetes mellitus; HPMC-Hydroxy propyl methyl cellulose; T2D- Type 2 diabetes; FTIR- Fourier transform infrared; DNSA- 3, 5-Dinitrosalicylic acid

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