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Preparation And Characterization Of Gelatin Nanoparticles Loaded With Repaglinide

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ABSTRACT :

Nanoparticles loaded with Repaglinide were prepared using gelatin as the polymeric matrix, pluronic F127 as the emulsifier and glutaraldehyde as the cross linking agents. A method of nanoprecipation was used for obtaining the nanoparticles. All the formulations (F1 to F4) were characterized for yield, entrapment efficiency, particle size and in vitro drug release. The pharmacokinetic release profile of drug from all the formulations was determined using zero order, Higuchi equation and Korsemeyer-Peppas release model. The highest particles size was obtained in F1 (822.75 nm) while the lowest was in F4 (437.24 nm). The ability of the gelatin particles to entrap itself in the hydrophobic core of pluruonic was responsible for the lower particle size obtained in formulation using higher concentration of the emulsifier. The yield of the nanoparticles was affected by the concentration of Pluronic F127 and ranged from 26-44%. The drug entrapment efficiencies of different formulations were in the range of 28.18 to 37.61 %. As already discussed, higher concentration of the emulsifier were able to entrap the drug in efficient amounts. The release of drug from the nanoparticles obeyed Korsemeyer-Peppas model suggesting an initial Fickian release due to swelling of the gelatin matrix and later non-Fickian release due to erosion of the swollen matrix.

Keywords Repaglinide, nanoprecipitation, gelatin, nanoparticles, pluronic

Introduction :

Chronic diseases with slower but steady progression are called as non-communicable diseases (NCD). Diabetes is a NCD that is expected to increase globally from 171 million in 2000 to 366 million in 2030 (ADA 2006). The main goal of the therapy of diabetes is to maintain blood glucose homeostasis, prevent ketosis and other secondary complications. Diet and exercise, insulin replacement therapy and the use of oral hypoglycemic agents are the major methods to control diabetes. An ideal drug delivery system should be capable of transferring the desired dose of the drug to a specific disease site. But due to the inherent physiological metabolic mechanisms, this cannot be practically achieved (Brahmankar 2005). The toxic side effects of therapeutics can be minimized by critical control of drug delivery site dosage and frequency of drug administration (Nagavarma et al. 2012). Nano drug delivery systems offer the best set of characteristics to achieve drastically improved drug delivery with reduced side effects. Nanoparticles have the potential to help the movement of drug substances from the systemic circulation to the brain by overcoming the Blood-Brain Barrier (BBB) which is a common problem in many conventional drug systems. Nano particles can be easily customized to achieve both passive and active drug targeting (Mohanraj VJ & Chen Y 2006) (Hans ML & Lowman AM 2002). Relatively high drug loading can be achieved on nanoparticle systems without any chemical reaction, thereby preserving the drug activity (Mohd & Amar 2014).

Repaglinide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM) (drugbank, 2024).

Chitosan, gelatin and alginate nanoparticles are easy to prepare and customize. In addition to their excellent drug binding capacity, they have been demonstrated to possess biocompatibility and proved to be safe for oral consumption. The chitosan and alginate nanoparticles have been studied previously for repaglinide with promising results (Wu et al., 2019; Gautam & Mishra, 2018; Sharma et al., 2017; Shinde et al., 2015; Ebrahimi et al., 2015. Hence it was envisioned to produce gelatin nanoparticles loaded with repaglinide and study the effect on drug release. The commonly prescribed dose of repaglinide in adults with type 2 diabetes is 1mg tablets 4 times a day and it exhibits bioavailability of 56%. Hence an attempt has been made to provide sustained release of repaglinide from the alginate nanoparticles to minimize the dosing frequency.

Material and Methods

Repaglinide was obtained as gift sample from Torrent Pharmaceuticals Limited, Ahmedabad. Gelatin was procured form Oxford Lab fine chemicals, Mumbai and was used as obtained. All other chemicals and reagents used were of analytical grade and used as obtained.

Formulation of gelatin nanoparticles

Four batches of gelatin nanoparticles of Repaglinide were prepared using four different concentrations of the emulsifier and a fixed amount of gelatin using the nanoprecipitation method (Table 1)

Table 1 Composition of different batches of gelatin nanoparticles

S.No.	Ingredient	Batch Code			
		F1	F2	F3	F4
1	Repaglinide (mg)	10	10	10	10
2	Gelatin (mg)	1.25	1.25	1.25	1.25
3	Glutaraldehyde (5% w/v) (µL)	30	30	30	30
4	Pluronic F 127 (% w/v)	0.5	1.0	1.5	2.0
7	Deionized water	qs	qs	qs	qs

Gelatin (125 mg) was dissolved in 100 ml de-ionized water at 60°C. 1 ml (1.25 mg gelatin) of this solution was added drop-wise to 20 ml ethanol solution containing 40 mg of Pluronic F-127 and the required amount of the drug as per formula in table 5.1, under continuous stirring (emulsifier/polymer mass ratio = 32:1). After 15 min, 30 µl glutaraldehyde solution (5%, w/v) was added, and the solution was stirred for 12 h to allow cross-linking of the particles. The particles were purified by 15 min centrifugation at 12 000 ×g and re-dispersion in de-ionized water (Lee et al., 2011). The particles were finally redispersed in 3 ml water, sonicated for 20 minutes using a bath sonicator and lyophilized.

Characterization and Evaluation of Nanoparticles

Determination of Yield

The lyophilized nanoparticles were collected and weighed accurately. The percentage yield was then calculated using formulae given below:

$$\% \text{ yield} = \frac{\text{Mass of the lyophilized nanoparticles} * 100}{\text{Total weight of drug and gelatin}}$$

Determination of particle size of microspheres

The particle size of each formulation batch was determined by preparing a suspension of the nanoparticles and analyzing it using a particle size analyzer.

Determination of drug entrapment in the nanoparticles

The various formulations of the nanoparticles were subjected to drug content analysis. 50 mg of the microspheres of each batch were accurately and dispersed in 10 mL methanol and sonicated at 125 Watts power for 2 min. The suspension was then centrifuged at 6000 rpm for 2 min. The supernatant was diluted appropriately in water and analyzed using UV visible spectrophotometer at 245 nm. The percentage drug entrapment was calculated as follows:

$$\% \text{ Drug Entrapment} = \frac{\text{Calculated Drug Concentration} * 100}{\text{Theoretical Drug Concentration}}$$

In vitro release study

USP type II dissolution apparatus (paddle type) was performed at 50 rpm in 900 mL 0.1N HCl, maintained at 37 ± 0.5°C. 50 mg of nanoparticles were placed in the dissolution vessel for studying the rate and extent and dissolution. 5 mL of the sample was withdrawn at a predetermined interval and the volume of dissolution medium was maintained by adding equal volume of fresh dissolution medium. The absorption of the withdrawn sample was

measured spectrophotometrically with suitable dilution and the corresponding concentration was determined from the calibration curve. The percentage of drug released at various time intervals was calculated and plotted against time.

Kinetics of drug release

To examine the drug release kinetics and mechanism, the cumulative release data were fitted to models representing zero order (Q v/s t), first order [Log(Q0-Q) v/s t], Higuchi's square root of time (Q v/s √T) and KorsemeyerPeppas double log plot (log Q v/s log t) respectively, where Q is the cumulative percentage of drug released at time t and (Q0-Q) is the cumulative percentage of drug remaining after time t.

Results and Discussion

FTIR of Repaglinide

FTIR of pure repaglinide showed peaks at 3611 cm^{-1} (NH stretching), 2836 cm^{-1} (CH stretching), and 1699 cm^{-1} (C = O stretching). The bands at 1046 cm^{-1} and 1200 cm^{-1} are related to C–O stretching in phenyl alkyl ether structure. The bands at 1575 cm^{-1} and 1610 cm^{-1} are due to aromatic C = C and N–H bending respectively (Figure 1).

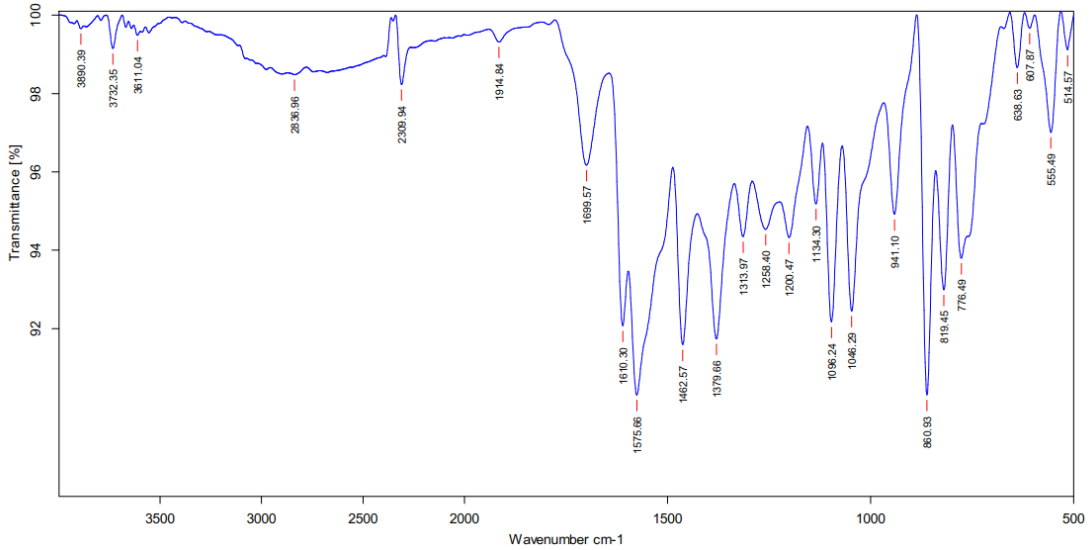

Figure 1 FTIR Spectra of Repaglinide

Preparation of nanoparticles

The nanoparticles of Repaglinide were prepared using gelatin as the polymer and by varying the concentration of the emulsifier (Pluronic F127) and glutaraldehyde as the cross-linking agent that affects the ability of the particles to remain stable and keep the drug entrapped. Nanoprecipitation method was used for precipitating the nanosized particles. The success of formulation depends upon its percentage yield, entrapment efficiency, particle size and drug release duration. The highest particles size was obtained in F1 (822.75 nm) while the lowest was in F4 (437.24 nm). The ability of the gelatin particles to entrap itself in the hydrophobic core of pluruonic was responsible for the lower particle size obtained in formulation using higher concentration of the emulsifier.

Particle Size analysis

The particle size of various formulations was determined Malvern particle size analyzer (Figure 2). The particles size was widely influenced by the concentration of the emulsifier. The lowest particles size was obtained when the emulsifier concentration of 2%.

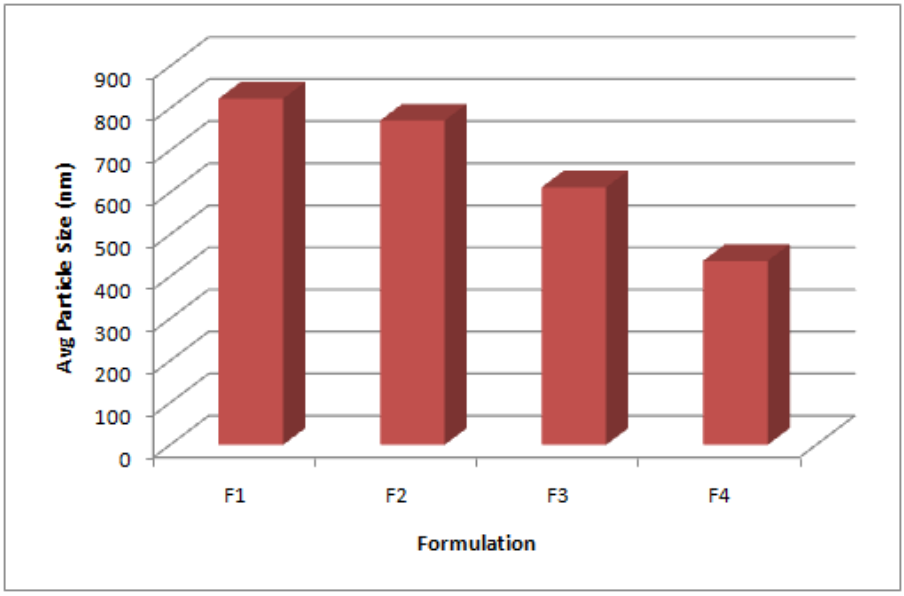

Figure 2 Particle size obtained in various formulations

Percentage Yield of nanoparticles

The various formulations of the prepared nanoparticles were evaluated for the percentage process yield. The percentage yield varied from 26-44% (Table 2). The yield of the nanoparticles was affected by the concentration of the emulsifier. It was visible from the result that increasing the emulsifier concentration decreased the yield of the nanoparticles. Nevertheless at low concentrations it was found that the particles formed coagulates and were not very stable.

Determination of entrapment of drug in the nanoparticles

The drug entrapment of various formulations of Repaglinide was carried out as per the procedure and performed in triplicate. The drug entrapment efficiencies of different formulations were in the range of 28.18 to 37.61 % (Table 2). At lower emulsifier concentration, the entrapment was also low and increased with increasing the emulsifier. This might be attributed the ability of gelatin to enclose itself in the hydrophobic core of the emulsifier.

Table 2 Percent yield and drug entrapment

Formulation	Percent Yield (%)	Drug entrapment (%)
F1	44.19	28.18
F2	39.68	31.86
F3	32.54	35.29
F4	26.27	37.61

In vitro drug release study

The *in vitro* drug release study of the nanoparticles was evaluated in 0.1N HCl. The % release, % cumulative drug release calculated (Table 3) and the cumulative release data was subjected to kinetic modeling studies.

Table 6.7 *In vitro* release of Repaglinide from nanoparticles

Time (h)	Cumulative Drug Release (%)			
	F1	F2	F3	F4
0	0	0	0	0
1	16.58	15.96	14.28	13.93
2	31.22	29.35	27.27	24.35

4	45.67	43.33	40.91	36.69
6	56.29	54.74	54.11	53.23
8	63.27	61.93	61.89	60.57
10	76.19	76.85	74.38	71.78
12	87.72	86.34	84.71	78.56
24	98.26	95.79	94.33	93.42

From the release data it could be inferred that all the formulations were able to sustain the release of repaglinide for a period of 24 h. All the formulations presented excellent sustained relase property for the entrapped drug.

Release kinetic Study

The release kinetic was mathematically modeled to determine the type of release behavior exhibited by the drug from the nanoparticles. The zero order, first order, Higuchi and Korsemeyer-Peppas model were studied. The plots were used to determine the release behavior (Figure 3a-3d).

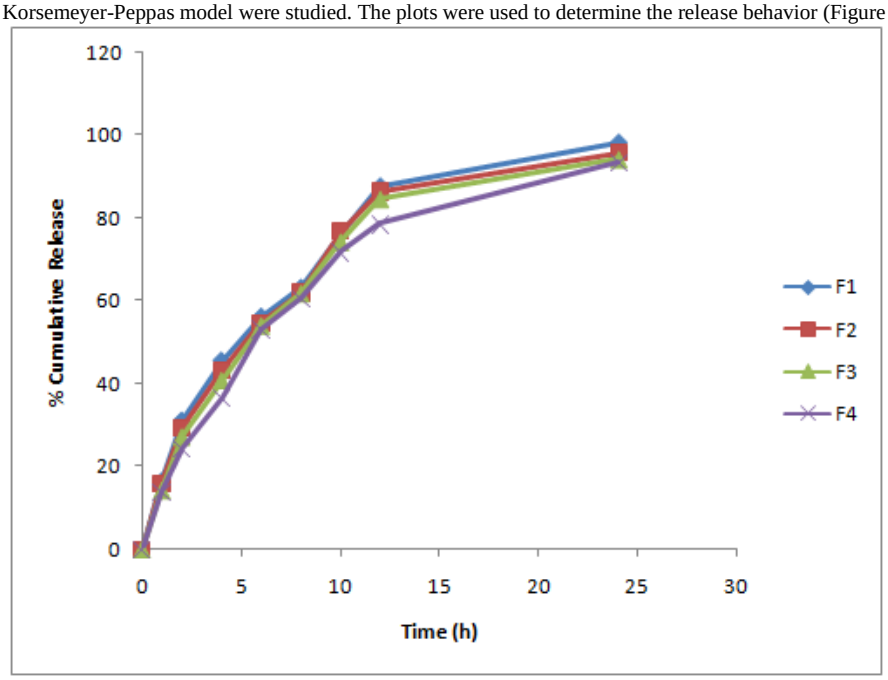

Figure 3a Zero order of Repaglinide from nanoparticles

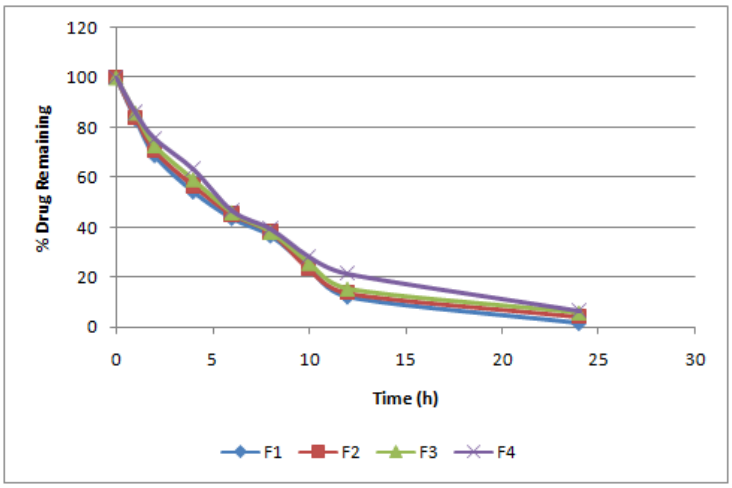

Figure 3b First order of Repaglinide from nanoparticles

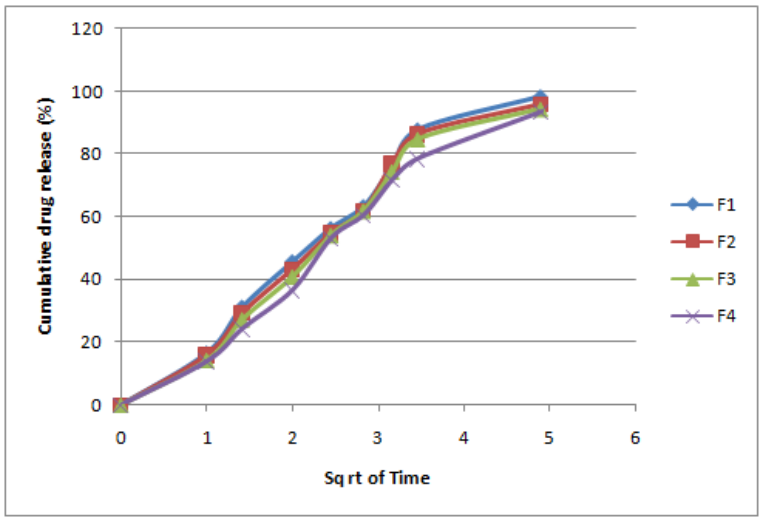

Figure 3c Higuchi plot of release of Repaglinide from nanoparticles

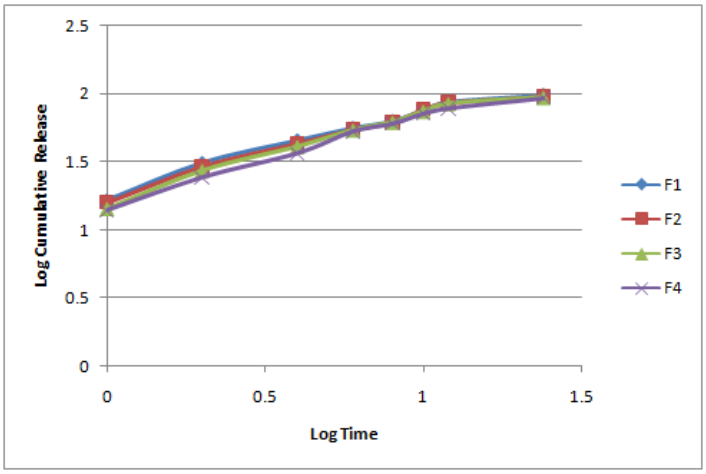

Figure 3d Korsemeyer-Peppas plot of release of Repaglinide from nanoparticles

The linear regression coefficients obtained from the release kinetic curves revealed that the release of drug from the nanoparticle matrix followed primarily the Korsemeyer-Peppas model (Table 4).

Table 4 Linear regression coefficients of release kinetic models

Formulation	Linear Regression coefficient			
	Zero order	First Order	Higuchi	Korsemeyer-Peppas
F1	0.799	0.799	0.965	0.965
F2	0.794	0.794	0.959	0.966
F3	0.799	0.799	0.959	0.964
F4	0.823	0.823	0.969	0.973

The release of drug from the nanoparticles obeyed Korsemeyer-Peppas model suggesting an initial Fickian release due to swelling of the gelatin matrix and later non-Fickian release due to erosion of the swollen matrix.

Conclusion

In the present study, nanoparticles loaded with Repaglinide were prepared by nanoprecipitation method using gelatin as the polymer, pluronic F127 as the emulsifier and glutaraldehyde as the cross lining agent. The results obtained showed that this methodology was able to produce reproducible nanoparticles suitable for sustained release of drug from the formulations. Additionally, it can be concluded that the nanoparticles produced from gelatin using pluronic F127 (2%w/v) is an excellent delivery system that has higher ability to sustain the release behavior of the drug for our 24 h, providing a once a day administration possibility of the drug.

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