ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Novel Bio-wall material from *Cicer arietinum* for formulation of Repaglinide nanoparticles.

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ABSTRACT

The current aim of our research work is to formulate repaglinide nanoparticles using *cicer arietinum* (CA) biomaterial as a wall material. The CA biomaterial from *cicer aritinum* seeds was isolated as per our earlier published method. *Cicer arietinum* belongs to family fabaceae. The seeds of *cicer arietinum* composed of protein, carbohydrates, fat, crude fiber, ash and minerals like phosphorus, calcium, magnesium, iron and zinc. Five formulations of repaglinide nanoparticles were formulated by using *cicer arietinum* biomaterial as a wall material in various concentrations ranges, repaglinide as a model drug by non-solvent evaporation method. The formulated nanoparticles were subjected for various evaluation parameters like particle size, polydispersity index, particle shape, drug loading capacity and in-vitro drug release. Our experimental results revealed that all formulation showed uniform shape, diameter ranging from 289-723 nm, and polydispersity index ranging from 0.2 to 0.3. The *in-vitro* drug release showed in a controlled manner for period of 12 hours. Formulation FN3 was selected as the best formulation by comparing the above parameters. Finally conclusion was drawn that *cicer arietinum* can serve as a wall material for formulating various drug loaded nanoparticles.
Keywords: Repaglinide, *Cicer arietinum,* polydispersity index, release kinetic study.

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ISSN: 0975-8585

INTRODUCTION

Nanotechnology is now frequently used for various applications in several fields including medical fields [1-5]. Nanoparticles vary in size from 10 to 1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. Nanoparticles offer unique properties as compared to micro or Macroparticles like Small size, High surface area, Easy to suspend in liquids, Deep access to cells and organelles, Variable optical and magnetic properties, Particles smaller than 200nm can be easily sterilized by filtration with a 0.22-mm filter. Biodegradable nanoparticles are frequently used to improve the therapeutic value of various water soluble/insoluble medicinal drugs and bioactive molecules by improving bioavailability, solubility and retention time [6]. When the particles are of nanometer length scale, surface irregularities can play an important role in adhesion, as the irregularities may be of the same order as the particles [7]. Nanoparticles can show a strong adhesion because of the increased contact area for van der Waals attraction [8]. Repaglinide, [9,10] a non-sulfonyl urea oral hypoglycemic agent of the meglitinide class, is mainly used in the management of type II diabetes mellitus. Chemically, repaglinide is a *S*(+)2-ethoxy-4-(2-((3-methyl-1-(2-(1-piperidinyl)phenyl)butyl)amino)-2-oxoethyl) benzoic acid. Repaglinide having bioavailability 56%, $t_{1/2}$ 1 hour and more than 98% protein binding capacity. Repaglinide lowers blood glucose by stimulating the release of insulin from the pancreas. It achieves this by closing ATP-dependent potassium channels in the membrane of the beta cells. This depolarizes the beta cells, opening the cells' calcium channels, and the resulting calcium influx induces insulin secretion. Repaglinide, a BCS class II compound for treatment of type II diabetes,[11-14] BCS class II compounds are poorly soluble but highly permeable, and they exhibit bioavailability that is limited by dissolution rate[15].

Cicer arietinum is an edible legume of the family Fabaceae, subfamily Faboideae. The seeds of cicer arietinum are high in protein content, enriched source of zinc, folate and protein.[11][12] They contain dietary fiber and hence a healthy source of carbohydrates for persons with insulin sensitivity or diabetes. *Cicer arietinum* are a nutrient-dense food, providing rich content like protein, dietary fibre, folate, and certain dietary minerals such as iron and phosphorous along with thiamine vitamin B6, magnesium and zinc [16,17].

The nanoparticles are formulated by using synthetic or natural biopolymer as a retardant or encapsulating agent. In this paper an attempt was made to formulate bionanoparticle using repaglinide and CA biomaterial as a novel wall material, which can serve as a retardant. The current aim of our research work is to formulate and evaluate repaglinide nanoparticles using *cicer arientinum* CA biomaterial as a wall material.

MATERIALS AND METHODS

Repaglinide (assigned purity, 99.8%) was a gift sample from M/S Torrent Pharmaceuticals Ltd., Ahmedabad, India. *Cicer arietinum collected from local market of Dehradun U.K.*

Experimental:

Isolation of CA bio material:

Flour of *Cicer arietinum* was procured from the local market. 100g of *Cicer arietinum* seeds were powdered and treated with 750 ml of distilled water and mixture stirred on magnetic stirrer at 4000 rpm for 1 hr. the mixture was subjected for centrifugation (Remi) at 4000rpm for 30 minutes. The supernatant liquid was pooled and treated with thrice the volume of acetone and the mixture was kept in a refrigerator for 12 hours. The CA biomaterial was recovered by subjecting the mixture for centrifugation at 5000 rpm for 30 minutes. The supernatant liquid was discorded and the CA biomaterial was collected and dried in a vaccum desicator for a period of 12 hours. The bio material was purified by hot dialysis method using dialysis membrane and ORCHID scientific dialysis apparatus for complete removal of impurities like Chlorides and sulfates. The procedure was optimized by repeating the procedure for 6 times. The purified bio material was screened through 200# and stored for further research study.

Physicochemical properties of the CA biomaterial extracted from *Cicer arietinum:*

The isolated CA biomaterial was subjected for various physicochemical properties like color, texture, solubility, color changing point, IR spectra along with the presence of proteins and carbohydrates.

Formulation:

Nanoparticles were prepared by nano precipitation and solvent evaporation method [18, 19]. For the formulation of nanoparticles drug solution was prepared by dissolving 10 mg of repaglinide in 5 ml of methanol. CA biomaterial dispersion was prepared by dissolving CA biomaterial in 20 ml of double distilled water and sonicated for 5 minutes at 30°C. Drug solution was incorporated into the CA biomaterial solution under stirring mode in incremental manner. The stirring was continued for 30 minutes at 40°C until complete evaporation of solvent. The mixture was subjected for sonication and micro centrifugation and the nanoparticles were recovered and evaporated and the same for all three formulations i.e. FN1, FN2, FN3, FN4 and FN5 (Table No.1).

Evaluation of the nanoparticles

Particle size

Particle size analysis was performed by Back Man Coulter, Delsa™ Nano. Nanoparticles were diluted 100 times with double distilled water. The mean particle size of each sample was determined three times and the average values were calculated [20].

Determination of drug loading and entrapment efficiency:

One ml of formulation was taken and dissolved in a minimum quantity of methanol. This solution was centrifuged at 13,000 rpm for 20 minutes. One milliliter of supernatant was taken and adjusted to 10 mL with methanol:water (1:1, vol/vol) system. From this stock solution, again 1 mL solution was withdrawn and adjusted to 10 mL. The solution was analyzed spectrometrically at 238 nm. Each experiment was repeated in triplicate. Percentage drug entrapment was determined by the following formula [21]:

$$\frac{\text{Amount of Repaglinide actually present in nanoparticle}}{\text{Amount of Repaglinide actually used}} \times 100$$

Nanoparticle size, zeta potential and Polydispersity index:

The prepared NPs were prepared for quick and better absorption. Thus, particle size and particle size distribution are crucial parameters for safe administration of such a formulation [20].Polydispersity index of prepared nanoparticles was carried out by using Malvern Zetasizer.[22]

***In-vitro* drug release profile**

In-vitro drug release from NPs was evaluated using 8-stations Franz diffusion cell (Orchid Scientifics). The receptor compartment was filled with 7ml of methanol and phosphate buffer of pH 7.4 in the ratio of 3:7. Egg shell membrane as a dialysis membrane was placed on receptor compartment and removed air bubbles. Finally the donor compartment was fitted over egg shell membrane with the help of clamp. 2mg drug equivalent of nanoparticle was placed in donor compartment. The assembly was set on stirring mode at 500rpm and temperature was maintained at 37 ± 0.5°C. At regular time intervals 1 ml sample were withdrawn from the receptor compartment and replaced by the same volume of fresh mixture of methanol and phosphate buffer of pH 7.4. The samples were analyzed by UV-1800 Shimadzu spectrometer at λmax 238 nm. [23].

Release kinetic study: For estimation of the kinetic and mechanism of drug release, the result of in vitro drug release study of nanoparticles were fitted with various kinetic equation like zero order (cumulative % release vs. time), first order (log % drug remaining vs time), Higuchi's model (cumulative % drug release vs. square root

of time). R^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.[24]

RESULT AND DISCUSSION

Physicochemical properties of the *Cicer arietinum* biomaterial

The isolated *Cicer arietinum* was reddish brown in color, of smooth texture, amorphous in nature, slightly soluble in water and the color changing point was found to be 327.3±1ºC. The *Cicer arietinum* showed negative for benedicts test and positive for Ninhydrin which showed that carbohydrate was absent and protein was present in *Cicer arietinum*. The yield of *Cicer arietinum* was found to be 3.5±0.01/100g. Its λmax was found to be 343.5nm. The IR-spectral data showed the presence of –NH (3259.47cm^{-1}) and –C=O (1660cm^{-1}) groups suggest that the *Cicer arietinum* may posses mucoadhesive property due to presence of mucophilic groups (Figure No.1).

Particle size

The effect of drug:polymer ratio has an immense effect on particle size and distribution. All batches showed a small mean size. The mean particle size for drug loaded formulations varied from 289-723 nm (Figure No. 2). Increase in drug: polymer ratio first decreases the particle size after that increases. Thus, larger particle size was obtained for formulations containing more polymers.

Drug loading capacity

The effect of drug:polymer ratio has an immense effect on drug loading capacity. The percent drugs loading capacity for the formulations FN1, FN2, FN3, FN4 and FN5 were found to be 71.3, 75.7, 79.6, 74.7 and 72.5% respectively (Figure No.3).

Size and Zeta potential

All repaglinide containing formulations and blank formulations showed a positive zeta potential value. Increase in drug polymer ratio first increases the drug loading capacity and then slightly decreased.

Polydispersity Index

The polydispersity index of the formulations was ranging from 0.2 to 0.3 which confirms that the formulated nanoparticles were nanorange with significant polydispersity index (Figure No.2).

in-vitro release study

The in-vitro release study revealed that all the formulations showed release in controlled manner for the period of 12 hours (Table No. 2), (Figure No.4). Finally the release data was fitted with various models like zero order, first order Higuchi model and Hix.Crow. models. The r^2, t_{50} and t_{90} values were correlated and FN3 was selected as the best formulation by comparing the above parameters. The r^2, t_{50} and t_{90} values for FN3 formulation was found to be 0.9901, 9.63 hrs and 31.98 hrs respectively. The drug release was found in first order manner(R^2=0.9901) and Hix.Crow. mechanism (R^2=0.9910) (Figure No.5).

DISCUSSION

A novel biopolymer was isolated and screened for its retardability by formulating Nanoparticle using *Cicer arietinum* as a wall material. Our experimental results revealed that the isolated biomaterial possess a novel inbuilt retardability which was confirmed by formulating nanoparticles and studying its release behavior which was so promising and significant. Finally conclusion was drawn that this biopolymeric material can serve as a bioretardant for formulating various controlled release formulations loaded with APIs.

CONCLUSION

ISSN: 0975-8585

A novel biopolymer was isolated and screened for its retardability. Our experimental results revealed that the isolated biomaterial possess a novel inbuilt retardability which was confirmed by formulating nanoparticles and studying its release behavior which was so promising and significant. Finally conclusion was drawn that this biopolymeric material can serve as a bioretardant for formulating various controlled release formulations loaded with APIs.

Table 1: Formulation table of Repaglinide loaded nanoparticle

S.no.	Ingredients	FN1 (1:0.5)	FN2 (1:1)	FN3 (1:1.5)	FN4 (1:2)	FN5 (1:3)
1	Repaglinide (mg)	10	10	10	10	10
2	CA biomaterial (mg)	05	10	15	20	30
3	PVA (1%) ml	0.1	0.1	0.1	0.1	0.1
4	Methanol (ml) q.s.	5	5	5	5	5
5	Double distilled Water (ml)	20	20	20	20	20

Table 2: Drug release of Repaglinide loaded nanoparticles

Time(hrs)	Formulations				
	FN1(1:0.5)	FN2(1:1)	FN3(1:1.5)	FN4(1:2)	FN5(1:3)
0	0	0	0	0	0
1	17.428	15.44	10.86	12.75	15.63
2	21.142	18.87	13.44	16.92	20.83
3	24.57	23.3	16.87	18.51	23.74
4	27.71	25.45	20.3	23.91	26.82
6	38.47	37.51	33.58	35.81	39.81
8	49.28	48.87	44.83	47.69	49.73
10	61.84	55.71	51.65	55.72	58.63
12	83.143	69.71	57.71	71.41	78.42

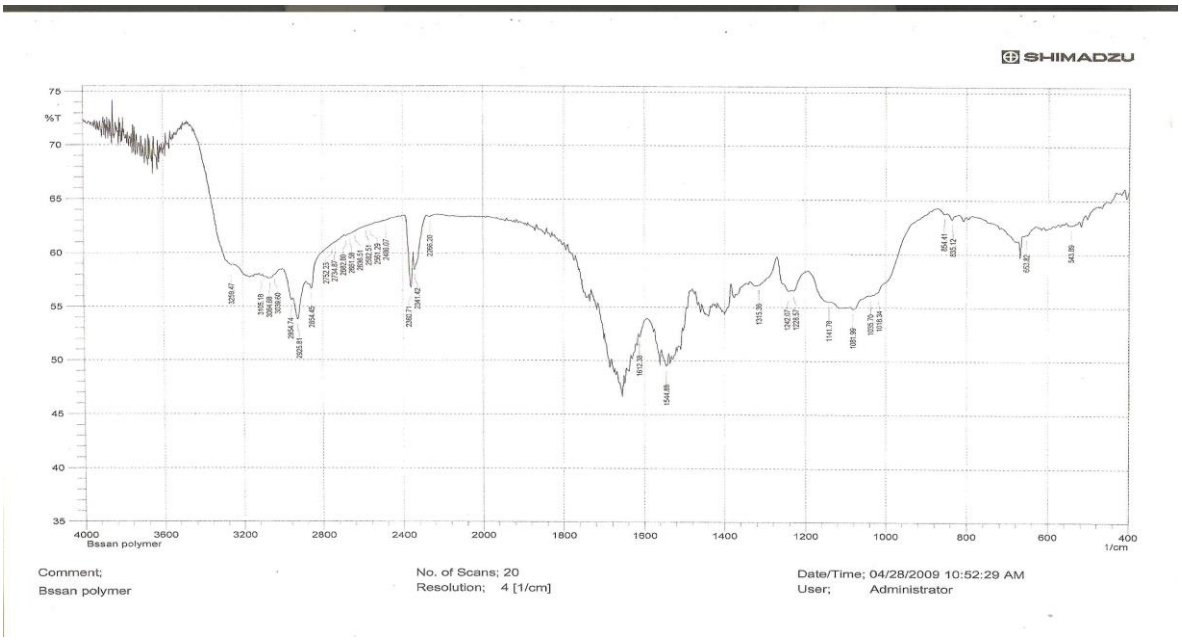
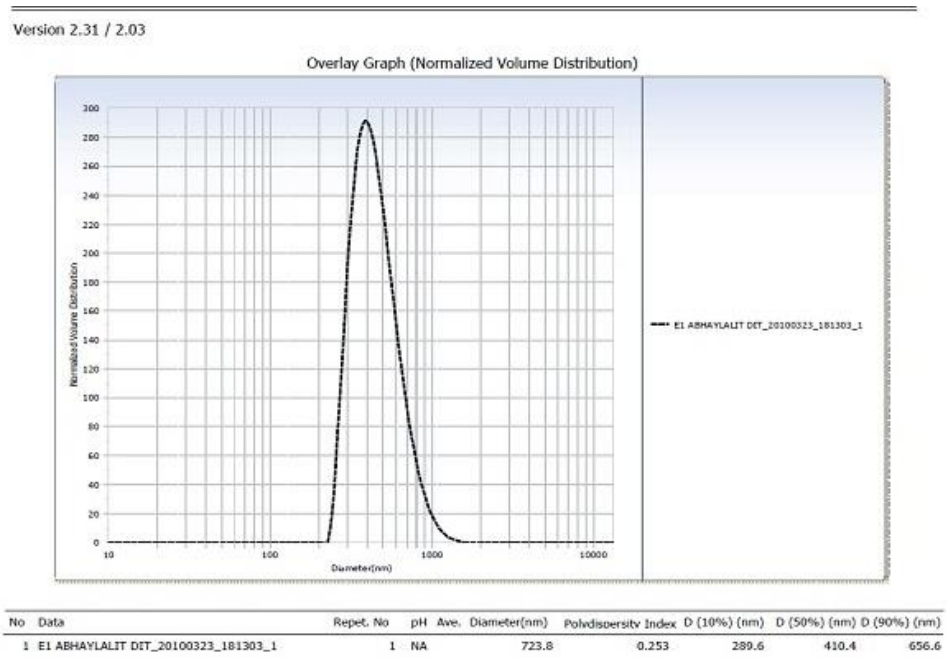


Figure 1: IR-spectra of the CA biomaterial

ISSN: 0975-8585



No	Data	Repet. No	pH	Ave. Diameter(nm)	Polydispersity Index	D (10%) (nm)	D (50%) (nm)	D (90%) (nm)
1	E1 ABHAYLALIT DIT_20100323_181303_1	1	NA	723.8	0.253	289.6	410.4	656.6

Figure 2: graph showing diameter and polydispersity index of best formulation (FN3)

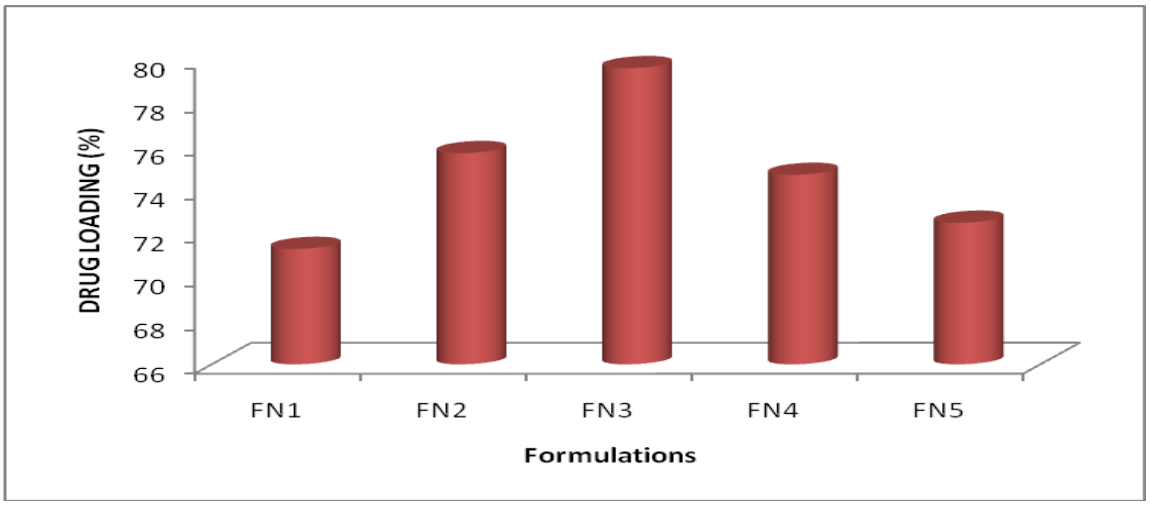


Figure 3: Drug loading capacity of different formulations

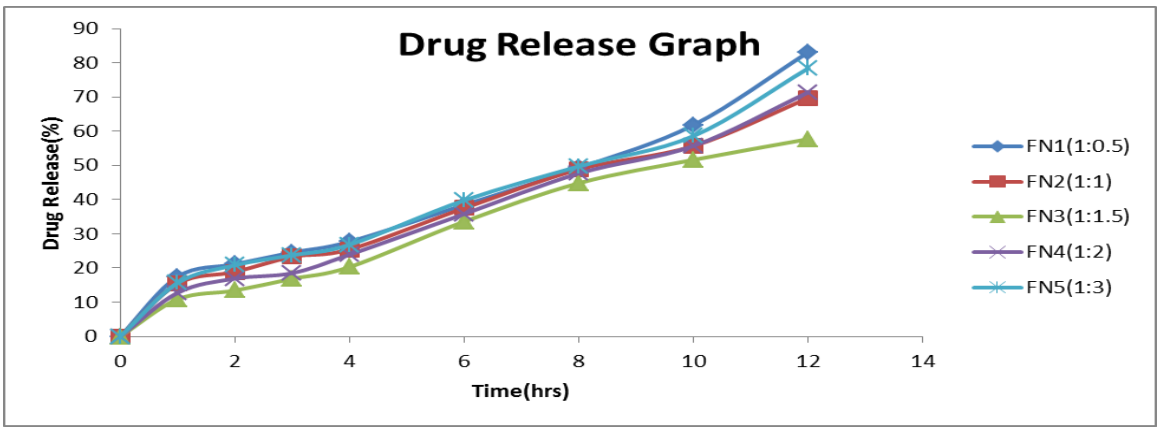


Figure 4: Drug release graph of different formulations

ISSN: 0975-8585

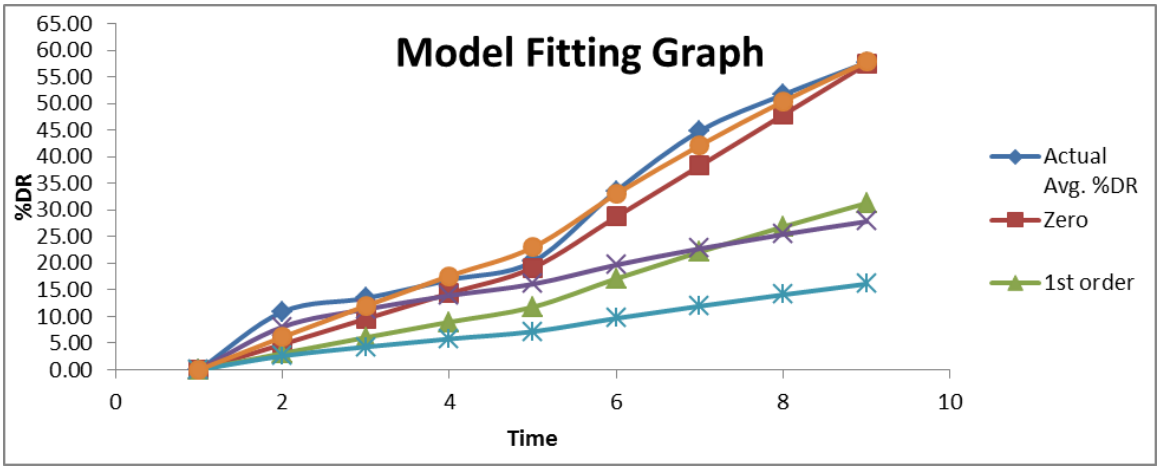


Figure 5: Model fitting graph of different formulations

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