ISSN 1996-5044

Research Journal of

# Nanoscience and Nanotechnology

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

Research Journal of Nanoscience and Nanotechnology 1 (1): 12-24, 2011
ISSN 1996-5044 / DOI: 10.3923/rjnn.2011.12.24


# Preparation and Characterization of Repaglinide Loaded Chitosan Polymeric Nanoparticles

[1,2]G. Poovi, [1]U.M. Dhana lekshmi, [2]N. Narayanan and [1]P. Neelakanta Reddy
[1]Bio-Organic Chemistry Laboratory, Central Leather Research Institute (Council of Scientific and Industrial Research) Adyar, Chennai-600020, India
[2]Madras Medical College, Chennai-03, Tamil Nadu, India

*Corresponding Author: G. Poovi, Bio-Organic Chemistry Laboratory, Central Leather Research Institute, (Council of Scientific and Industrial Research), Adyar, Chennai -600020, India Tel: 091-44-24911386/09963610801 Fax: +91-44-24911589*

## ABSTRACT

The aim of this study was to formulate and optimize repaglinide (Rg) loaded chitosan (CN) nanoparticles as a sustained release. Repaglinide is a hypoglycemic agent of the meglitinide analog. In present study repaglinide loaded chitosan nanoparticles were prepared by solvent evaporation method in three different ratios. In this method weighed quantity of drug and polymer were dissolved in suitable organic solvent acetone and 2% acetic acid (organic phase). This solution was added drop by drop to the aqueous phase of PVA and homogenized using homogenizer at 18000 rpm followed by magnetic stirring for 2-3 h. The formed Rg-CN nanoparticles were recovered by centrifugation at 25,000 rpm for 15 min followed by washing thrice with petroleum ether and lyophilized. The prepared nanoparticles were evaluated for particle size, Scanning Electron Microscopy (SEM), Fourier Transform Infra Red spectroscopy study (FT-IR), percentage yield, drug entrapment and for *in vitro* release kinetics. Among the three different ratio 1:4 ratio shown high drug loading (11.22% w/w) and encapsulation efficiencies (97.0%) and nanoparticle recovery (86.40%) with nanosize. Scanning electron microscopy exposed that nanoparticles were spherical in shape with a nearly smooth surface morphology. Particle size was analyzed by Malvern particle size analyzer and shown 48-100 nm range. FT-IR study reveals that, there was no interaction between repaglinide and polymers. Based on the *in vitro* study, replaglinide released from prepared formulation was slow and sustained over 15 days. Application of the *in vitro* drug release data to various kinetic equations indicated first order release, swelling and diffusion mechanism from repaglinide nanoparticles.

**Key words:** Chitosan, repaglinide, diabetic disease, polymeric nanoparticles, nanotechnology, poly vinyl alcohol

## INTRODUCTION

In the last decade, significant effort was taken to develop nanoparticles, drug delivery (Fessi *et al*., 1992; Galindo-Rodriguez *et al*., 2004; Oppenheim, 1981; Alonso, 1996; Brigger *et al*., 2002). Nanoparticles are submicron sized colloidal polymeric systems. The micro/nanoparticulate drug delivery systems offer numerous advantages over the conventional dosage forms. These include improved efficacy, reduced toxicity and improved patient compliance (Soppimath *et al*., 2001; Kreuter, 1994; Brannon-Peppas, 1995; Couvreur *et al*., 1986). Compared to the traditional

micron-sized supports used in separation process, nanosized carriers possess quite good performance due to high specific surface area and the absence of internal diffusion resistance (Chang *et al*., 2006). In particular, nanoparticles are able to protect drugs from degradation, to improve permeation/penetration of the drugs across mucosal surfaces and also to control the release of the encapsulated or adsorbed drug (Florence *et al*., 1995; Takeuchi *et al*., 2001).

Nanotechnology is now frequently used for various applications in fiber and textiles (Perelshtein *et al*., 2008), agriculture (Speiser, 2008; Lai *et al*., 2006), electronics (Huang *et al*., 2003), forensic science (Choi *et al*., 2008), space (Liu *et al*., 2007) and medical therapeutics (Bender *et al*., 1996; Bonduelle and Foucher, 1992; Jahanshahi and Babaei, 2008; Kawashima *et al*., 2000; Rieux *et al*., 2006). These nanoparticle drug formulation reduces the patient expenses and risks of toxicity (Glen, 2005). Polymeric nanoparticles have been synthesized using various methods (Reis *et al*., 2006) according to needs of its application and type of drugs to been capsulated. These nanoparticles are extensively used for the nanoencapsulation of various useful bioactive molecules and medicinal drugs to develop nanomedicine (Panyam and Labhasetwar, 2003). These nanomedicines have many advantages in the protection of premature degradation and interaction with the biological environment, enhancement of absorption into a selected tissue, bioavailability, retention time and improvement of intracellular penetration (Alexis *et al*., 2008).

However, biodegradable nanoparticles are highly preferred and frequently used to improve the therapeutic value of various water soluble/ insoluble medicinal drugs and bioactive molecules by improving bioavailability, solubility and retention time (Shenoy and Amiji, 2005). Such nanoparticles show promise in drug delivery system and provide controlled/sustained release property, sub cellular size and biocompatibility with tissue and cells (Panyam and Labhasetwa, 2003).

Among the various polymers used for the development of sustained release formulations, one of the most widely used polysaccharides for different pharmaceutical purposes is chitosan and its derivatives (Thanou *et al*., 2001; Morishita and Peppas, 2006; Wilson *et al*., 2009). Chitosan is a natural cationic polysaccharide derived by deacetylation of chitin, a copolymer consisting of combined units of glucosamine and N-acetyl glucosamine (Lee *et al*., 1997; Majeti, 2000). In the pharmaceutical field chitosan's advantageous biological properties have prompted its extensive study as a carrier both of drugs (Bayomi *et al*., 1998; Mi *et al*., 2001) and of proteins (Calvo *et al*., 1997). This cationic polymer has attracted a great deal of attention as a drug delivery carrier because of its unique properties, such as acceptable biocompatibility (De Campos *et al*., 2001), low toxicity (Illum *et al*., 2001) and the ability to enhance the absorption of hydrophilic molecules across the epithelium via the paracellular transport pathway (Schipper *et al*., 1999). In the drug delivery field, the vesicles based on chitosan and derivatives can be used for transdermal, nasal, ocular, oral and parenteral administration and other application (Thanou *et al*., 2001; Thein-Han and Stevens, 2004).

Diabetes mellitus is a major and growing public health problem throughout the world, with estimated world wide prevalence in 2000 of 150 million people, expected to increase to 220 million people by 2010. Recent estimates project that the number of patient's diaganosed with type II diabetes will more than double to 300 million before 2025 (Nagappa, 2008). Diabetes Mellitus (DM) is defined as a group of metabolic diseases the common feature of which is an elevated blood glucose level (hyperglycaemia). Chronic hyperglycaemia is associated with the long-term consequences of diabetes that include damage and dysfunction of the cardiovascular system, eyes, kidneys and

nerves. The complications of diabetes are often divided into two groups: microvascular (retinopathy, nephropathy and neuropathy) and macrovascular (ischaemic heart disease, stroke, peripheral vascular disease). Together, these make diabetes the seventh most common cause of death in the developed world (McGinity and O'Donnell, 1997). Hence, we have focused the attention on anti diabetic treatments.

Repaglinide (Rg), a fast and short acting meglitinide analog with a very short half-life (1 h) and low bioavailability (50%) (Jain *et al.*, 2005) was chosen as the drug to overcome the problem due to the conventional dosage form. In the present study, an attempt has been made to formulate Repaglinide-loaded CN nanoparticles, which may provide prolonged drug delivery in the treatment of diabetic disorders and decreases the related side-effects.

## MATERIALS AND METHODS

**Materials:** The research project was performed at Bio Organic Chemistry Laboratory, Central Leather Research Institute, Chennai during June 2008 to February 2009. Repaglinide (Rg) was received from Sigma Aldrich, Germany. Chitosan was received from India sea foods, Cochin. The following materials were obtained from the indicated suppliers and used as received: Poly Vinyl Alcohol (PVA) (Sigma Aldrich, Germany), acetone (Ranbaxy Fine chemicals Ltd, New Delhi), glacial acetic acid (S.R.L,Mumbai) and all other chemicals used were of analytical grade.

**Preparation of polymeric nanoparticles:** Polymeric nanoparticles were prepared by solvent evaporation method using CN as coating material and Repaglinide used as core material. Weighed quantity of drug and polymer were dissolved in suitable organic solvent Acetone and 2% Acetic acid (organic phase). This solution was added drop by drop to the aqueous phase of PVA and homogenized using IKA T 25 Digital Ultra turrax homogenizer, Germany at 18000 rpm followed by magnetic stirring for 2-3 h. The formed Rg-CN nanoparticles were recovered by centrifugation (Sigma centrifuge 3K 30) at 25,000 rpm for 15 min followed by washing thrice with petroleum ether and lyophilized (McGinity and O'Donnell, 1997; Govender *et al.*, 1999).

**Nanoparticle recovery:** The nanoparticle (NP) recovery, which is also referred to as nanoparticle yield in the literature, calculated using Eq. 1. The individual values were determined (Govender *et al.*, 1999).

$$\text{Nanoparticle recovery (\%)} = \left(\frac{\text{Mass of nanoparticles recovered}}{\text{Mass of polymeric nanoparticles, drug and any formulation excipient used in formulation}}\right)\times 100 \quad (1)$$

**Determination of drug incorporation efficiency:** Freeze-dried nanoparticles were dissolved in suitable solvent (50 mL) (a common solvent for polymer and the drug). The amount of drug in the solution was measured by ultra violet spectroscopy at 243 nm (Perkin-Elmer Spectrophotometer). Drug content (% w/w) and Drug entrapment (%) were represented by Eq. 2 and 3, respectively.

$$\text{Drug content (\%)} = \left(\frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles recovered}}\right)\times 100 \quad (2)$$

$$\text{Drug entrapment (\%)} = \left(\frac{\text{Mass of drug in nanoparticles}}{\text{Mass of drug used in formulation}}\right)\times 100 \quad (3)$$

**Particle size analysis:** Particle size was determined using Photon Correlation Spectroscopy (PCS) (Malvern S4700 PCS System, Malvern UK). For particle size analysis Rg-CN nanoparticles were first suspended in 100 mL of filtered water (0.2 μm filter, Ministart, Germany) and subjected to sonication for 30 sec and vortex mixing for 10 sec before analysis.

**Scanning electron microscopy:** The shape and surface morphology of the Rg-CN nanoparticles were examined using Scanning Electron Microscopy (SEM) (JSM-T20. Tokyo, Japan). Appropriate samples of polymeric nanoparticles were mounted on metal stubs, using double-sided adhesive taps. Samples were gold coated and observed for morphology, at acceleration voltage of 15 KV.

**Fourier transform infrared spectroscopy:** Infrared spectroscopy was conducted using a Avatar 320-FT IR spectrophotometer and the spectrum was recorded in the region of 4000-400 $cm^{-1}$. The procedure consist of dispersing a sample (drug, polymer and Rg-CN nanoparticle preparation) in potassium bromide pellet (200-400 mg) and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained.

***In vitro* release study:** The *in vitro* release of Rg-CN nanoparticles was carried out in triplicate in stirred dissolution cells at 37.4°C by suspending 2 mL of Rg-CN nanoparticle suspension into a beaker containing 100 mL of release media (phosphate buffer saline pH 7.4). The correct *in vitro* conditions to study the release behavior of a hydrophobic drug were maintained (Avinash *et al.*, 2007). Drug release was assessed by intermittently sampling the receptor media (5 mL) at predetermined time intervals, each time 5 mL of fresh phosphate buffer saline pH 7.4 was replaced. The amount of repaglinide released in the buffer solution was quantified by a UV spectrophotometer at 243 nm.

**Evaluation of *in vitro* release kinetics:** In order to investigate the mechanism of release the data were analyzed with the following mathematical models: zero-order kinetic Eq. 4, first-order kinetic Eq. 5 and Higuchi kinetic Eq. 6.

$$Q_t = k_0 t \quad (4)$$

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

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

**The following plots were made:** $Q_t$ vs. t (zero order kinetic model), ln ($Q_0$ - Qt) vs. t (first-order kinetic model) and $Q_t$ vs. $t^{1/2}$ (Higuchi model), where $Q_t$ is the percent of drug released at time t, $Q_0$ is the initial amount of drug present in the microspheres and $K_0$, $K_1$ and $K_h$ are the constants of the equations. Further, to confirm the mechanism of drug release, the first 60% of drug release was fitted in Korsmeyer- Peppas model Eq. 7:

$$M_t/M_\infty = K_p t^n \quad (7)$$

where $M_t/M_\infty$ are the fraction of the drug release at time t, $K_p$ is the rate constant and n is the release exponent. The n value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released ($M_t/M_\infty$) vs. log of time (Costa and Lobo, 2001).

## RESULTS AND DISCUSSION

**Formation of polymeric nanoparticles:** The Repaglinide loaded chitosan polymeric nanoparticles were prepared by solvent evaporation method according to Jain *et al.* (2005) in three different ratio of polymer (1: 2, 1: 3 and 1: 4). Solvent evaporation method is one of the easiest method when compare to other techniques. A suspension of chitosan polymer and Repaglinide drug in suitable solvent acetone and 2% acetic acid forms the organic phase. This organic phase was poured into an aqueous phase containing PVA. The organic solvents used in these preparations rapidly partitioned into the external aqueous phase and the polymer precipitated around the drug. The subsequent evaporation of the entrapped solvent led to the formation of Repaglinide loaded chitosan polymeric nanoparticles. Specifically the polymer coated or covered around the shaped drug led to spherical shaped polymeric nanoparticles.

**Effect of drug content and drug entrapment:** The percentage entrapment efficiency was varied by varying the characteristics of polymer, drug, surfactant and cross linking agent etc., Normally the low entrapment efficiency was due to high affinity of drug and polymer in different solvents (drug in organic solvent and polymer in aqueous solvent and vice-versa) during the nanoparticle preparation and the drug loading content and entrapment efficiency were mainly affected by the polymer and drug ratios. Peng *et al.* ( 2007) also reported that the improved encapsulation efficiency may be due to the greater proportion of polymer with respect to the amount of drug.

In our Rg-CN nanoparticle preparation, the drug and the polymer were dissolved in organic phase and greater proportion of polymer were added to the drug. Hence, there was no chance in the diffusion of drug away from the polymer. The percentage drug entrapment of Repaglinide in the formulations was found to be good at all levels of drug loading. The high entrapment efficiency of Repaglinide is believed to be due to its poor aqueous solubility, high affinity of drug and polymer in the same solvent (organic solvent) and increased polymer ratio. Present report was found to be similar to that of early findings (Jain *et al.*, 2005).

The researchers (Niwa *et al.*, 1994) attributed the decreased drug entrapment with increasing theoretical drug loadings to an enhanced drug leakage into the aqueous phase (if drug is water soluble) or into the organic phase (if drug is water insoluble) at high loadings. This would also lead to an enhanced drug loss. Compared to 1:3, 1:4 ratios the 1:2 ratio shown high drug content and it produced an enhanced drug leakage which influences the absolute release profiles and responsible for an increased initial burst. Avinash *et al.* (2007) have reported that increase in drug content in the particles influences the absolute release profiles such as the cumulative amount of drug released at any time and the induction period increases. The increase in drug content increased the amount of drug close to the surface which is responsible for an increased initial burst. The increase in drug in the core of nanoparticles is responsible for a prolonged drug release from the polymer.

In the Rg-CN nanoparticles preparation, according to the result of efficiency of recovery and drug entrapment of nanoparticles among the three different ratios, 1:4 ratio was selected as the best ratio compare to 1:2 and 1:3 because these ratios leads to a low drug entrapment which implied high drug wastage during the preparation and 1:4 ratio shown low drug wastage. These polymeric nanoparticles were prepared at three consecutive times for reproducibility and result elicited in Table 1.

Rg-CN preparation of 1:4 ratio shown 11.22% w/w drug content, 97.0% drug entrapment and 86.40% nanoparticle recovery which revealed increased drug entrapment, nanoparticle recovery and particle size. Douglas *et al.* (1987) have reported that high nanoparticle recovery is required for reducing manufacturing costs and its size and morphology important for quality control and bio distribution.

**Morphological characterization of polymeric nanoparticles:** Figure 1 shown that the Rg-CN preparation has smooth spherical shaped appearance. The surface of formulated nanoparticles depends on two factors according to Fessi *et al.* (1992) and Galindo-Rodriueg *et al.* (2004). A saturated solution of polymer produced smooth and high yield nanoparticles. The undissolved polymer produced irregular and rod shaped particles. The diffusion rate of solvent is too fast and the solvent may diffuse into the aqueous phase before stable nanoparticles are developed or formed causing the aggregation of nanoparticle preparation. In this study a portion of the CN preparations possessed sparingly soluble property; the addition of 2% acetic acid to acetone in the CN preparation reduces the fast diffusion rate. This condition is suitable for spherical shaped nanoparticle formation. Due to the solubility and diffusion rate the Rg-CN preparation exposed good spherical appearance (Fig. 1).

Table 1: Percentage of nano particle recovery, drug content, entrapment and wastage for three ratios (1:2, 1:3, 1:4)

| Drug-polymer preparation (Rg-CN) | Nano particle recovery (%) | Drug content (%) | Drug entrapment (%) | Drug wastage (%) |
|---|---|---|---|---|
| 1:2 | 64.43±0.52 | 12.33±0.31 | 63.6±0.6 | 36.4±0.12 |
| 1:3 | 73.42±0.314 | 12.10±0.27 | 80.2±0.481 | 19.8±0.273 |
| 1:4 | 86.40±0.012 | 11.22±0.08 | 97.0±0.018 | 03.0±0.001 |

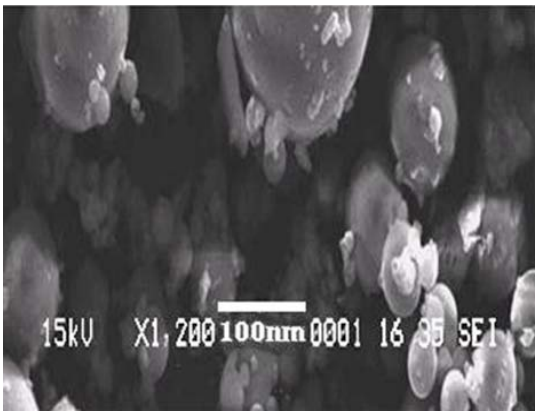


Fig. 1: Scanning electron microscopy photograph of Rg-CN (1:4)

**Particle size and poly dispersity index:** Nanoparticle size determined by PCS was shown in Fig. 2. Birnbaum *et al.* (2000) have reported that particle size of nanoparticles was larger than those obtained by the quantitative analysis of the SEM. The contrast of the Electron Microscope (EM) pictures allows only the visualization of the nanoparticle core, whereas the hydrodynamic radius of the particles is measured by PCS. Particle size is often used to characterize nanoparticles, because it facilitates the understanding of the dispersion and aggregation. Due to large surface area and attractive force between the particles more chance of aggregation is possible in small sized particles. The surfactant may aid in reducing aggregation of the particles once the nanoprecipitates are formed. The addition of PVA in Rg-CN preparation could reduces the aggregation formation which is conformed by result of low poly dispersity index. Birnbaum *et al.* (2000) have reported that PVA appeared as the most suitable surfactants in reducing aggregation of the particles which suspends immediately after formation. The particle size data shown that nanoparticles produced were of sub micron size and of low poly dispersity index ( 48-100 nm and 0. 280PI) which indicated a relatively narrow particle size distribution for Rg-CN preparation.

**FT-Infrared Spectroscopy (FTIR):** FTIR spectra of pure repaglinide, polymer and repaglinide loaded polymeric nanoparticle were shown in Fig. 3. Infrared spectra of chitosan sample studied displayed several characteristic vibration properties in the region. A band at 3419 $cm^{-1}$ corresponds to the combined peaks of the $NH_2$ and OH group stretching vibration in chitosan. The band at 1657 $cm^{-1}$ is attributed to the $CONH_2$ group. The 1598 $cm^{-1}$ peak of the c ($NH_2$) bending vibration is sharper than the peak at 1657 $cm^{-1}$, which shows the high degree of deacetylation of the chitosan. A shift from 3419 to 3427 $cm^{-1}$ is shown and the peak is sharper in the chitosan nanoparticles, which indicates that the hydrogen bonding is enhanced. The intensities of ($CONH_2$) band at 1657 $cm^{-1}$ and ($NH_2$) band at 1598 $cm^{-1}$, which can be observed clearly in pure chitosan, FTIR of repaglinide shown peaks at 3320 (NH stretching), 2947(CH stretching), 1728 (C = O), 1604 (C = C), CH deformation at 1460-1438 $cm^{-1}$. Similar peaks were seen in repaglinide loaded CN nanoparticle preparation. Hence, the study confirmed that there is no interaction between drug and polymer.

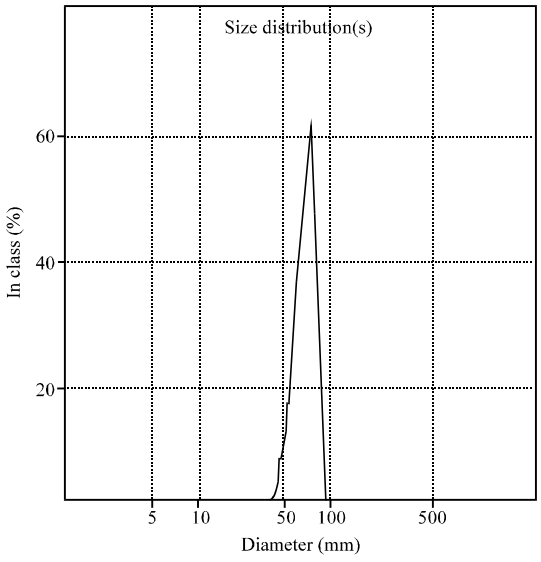


Fig. 2: PCS of Rg-CN nanoparticle

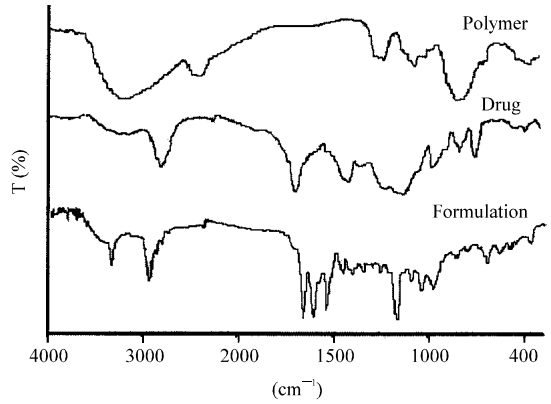


Fig. 3: FTIR of polymer, drug and Rg-CN nanoparticle

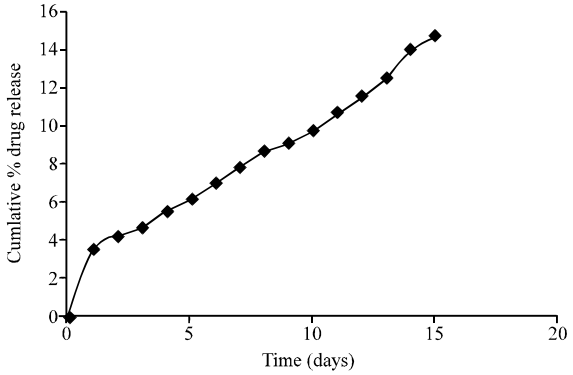


Fig. 4: *In vitro* release of Repaglinide from polymeric nanoparticles of 1:4 ratio

Table 2: Release kinetics data of Rg-CN polymeric nanoparticle

| Equation | Zero order | First order | Higuchi | Korse meyer | Hixson-crowell |
|---|---|---|---|---|---|
| $R^2$ | 0.976 | 0.987 | 0.946 | 0.968 | 0.990 |

***In vitro* release study:** The *in vitro* release of repaglinide from CN polymeric nanoparticles were shown in Fig. 4. The drug released from CN polymeric nanoparticle preparations were 14.80% upto15 days. The release of replaglinide mainly depended upon the polymer concentration. The release rate of the drug from the nanoparticles was found to decrease drastically on increasing the polymer concentration. The decreased percentage of drug release indicates that this polymer may form a more compact wall and it indicates that they have sustained drug release for a prolonged period of time.

***In vitro* release kinetics study:** The data obtained for *in vitro* release was fitted into equations for zero order, First order, Korse meyer , Hixson-crowell and the Higuchi release model (Fig. 5-9). The interpretation of data was based on the value of the resulting regression coefficient (Table 2). The zero order rate describes the systems where the drug release rate is independent of its concentration (Fig. 5). The first order which describes the concentration dependant release (Fig. 6). Higuchi model describes the release of drugs from an insoluble matrix as a square root of a time

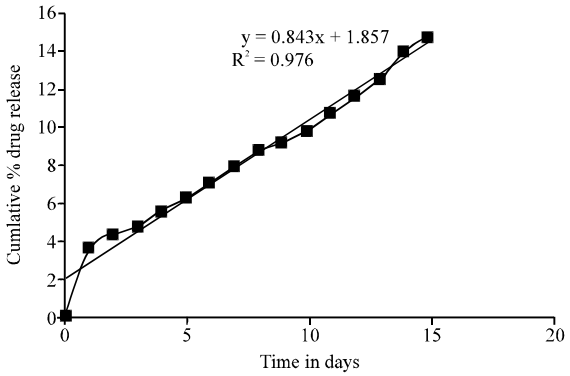


Fig. 5: Zero order kinetics data of Rg-CN nanoparticle preparation

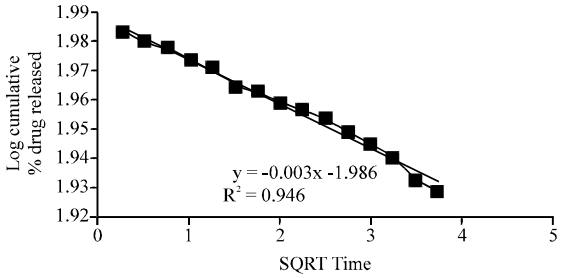


Fig. 6: First order kinetics data of Rg-CN nanoparticle preparation

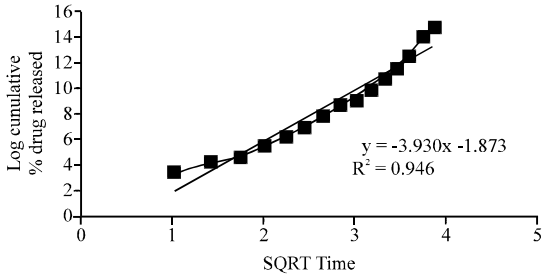


Fig. 7: Higuchi equation data of Rg-CN nanoparticle preparation

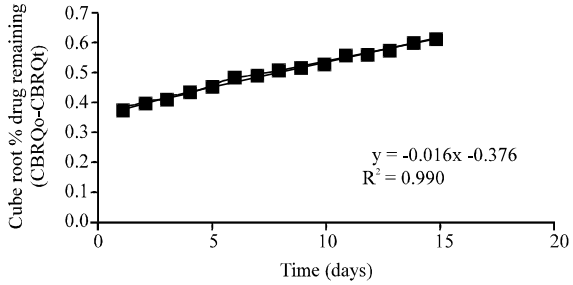


Fig. 8: Hixson-Crowell equation data of Rg-CN nanoparticle preparation

dependant process based on Fickian diffusion. Figure 7 exposed the higuchi kinetics (Sood and Pachangnula, 1998; Merchant *et al.*, 2006). The release constant was calculated from the slope of

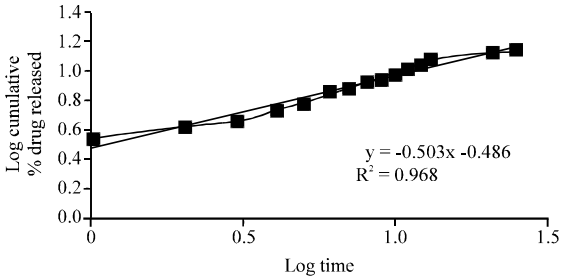


Fig. 9: Korsmeyer-Peppas eqation data of Rg-CN nanoparticle preparation

the appropriate plots and the regression co efficient ($r^2$) was determined and results tabulated in Table 2.

In this Rg-CN preparation, release profile of the repaglinide was fit into various kinetic models to find out the mechanism of drug release. Among this highest correlation coefficient was shown in first order followed by Higuchi and Hixson Crowell (Fig. 8) equations. The release rates were calculated from the slope of respective plots. The data obtained was also fit in to the Korsmeyer-Peppas (Fig. 9) in order to find out the n value, to describe the drug release mechanism. The n value of 0.503 for Rg-CN preparation indicates the mass transfer. It follows a non-Fickian model or anomalous transport,indicating the drug release is controlled by more than one process. That is superposition of both phenomenon, the diffusion-controlled and swelling- controlled release. Merchant *et al.* (2006) have reported the similar findings. In Rg-CN there was significant difference in the $R^2$ values of zero-order equation and Hixson-Crowell eqation. Hence the erosion mechanism was not involved in the release pattern. From these results we concluded that the release of Repaglinide from the CN matrix was predominently controlled by First order, diffusion and swelling mechanism.

## CONCLUSIONS

Repaglinide loaded chitosan nanoparticles were successfully prepared by solvent evaporation method. The surface morphology of these Rg-CN preparations was found to be smooth. These Rg-CN preparations shown high drug loading and encapsulation efficiency with nanosize. Rg loaded CN polymeric nanoparticles with a small size and a narrow size distribution were obtained. *In vitro* release kinetics studies shown that Rg loaded CN nanoparticles were capable of releasing the drug in a slow sustained manner. The nanoparticles may improve the oral absorption of repaglinide due to high surface to volume ratio, high bio distribution. Therefore, the bioavailability of drug may be improved and may help to reduce the dose of the drug and frequency of administration. From the present investigation it may be concluded that the repaglinide loaded chitosan nanoparticles is an effective carrier for the design of a controlled drug delivery system of poorly water soluble drugs like repaglinide.

## REFERENCES

Alexis, F., E. Pridgen, L.K. Molnar and O.C. Farokhzad, 2008. Factors affecting the clearance and biodistribution of polymeric nanoparticles. Mol. Pharm., 5: 505-515.

Alonso, M.J., 1996. Nanoparticulate Drug Carrier Technology. In: Micro Particulate Systems for the Delivery of Proteins and Vaccines, Cohen, S. and H. Bernstein (Eds.). Marcel Dekker, New York, pp: 203–242.

Avinash, B., J.S. Steven and I. Karen, 2007. Controlling the *in vitro* release profiles for a system of Haloperidol-Loaded PLGA nanoparticles. Int. J. Pharm., 87: 92-92.

Bayomi, M.A., S.A. Al-Suwayeh, A.M. El-Helw and A.F. Mesnad, 1998. Preparation of casein-chitosan microspheres containing diltiazem hydrochloride by an aqueous co-acervation technique. Pharm. Acta. Helv., 73: 187-192.

Bender, A.R., H. von Briesen, J. Kreuter, I.B. Duncan, H. Rubsamen-Waigmann, 1996. Eficiency of nanoparticles as a carrier system for Antiviral agents in human immune deficiency virus-infected human monocytes /macrophages *in vitro* antimicrob. Agents Chemother, 40: 1467-1471.

Birnbaum, D.T., J.D. Kosmala and L. Brannon-Peppas, 2000. Optimization of preparation techniques for poly (lactic acid –co-glycolic acid) nanoparticles. J. Nanoparticle Res., 2: 173-181.

Bonduelle, S. and C. Foucher, 1992. Association of cyclosporine to isohexyl cyano acrylate nanospheres and subsequent release in human plasma *in vitro*. J. Microencapsul., 9: 173-182.

Brannon-Peppas, L., 1995. Recent advances on the use of Biodegradable microparticles and nanoparticles in the controlled drug delivery. Int. J. Pharm., 116: 1-9.

Brigger, I., C. Dubernet and P. Couvreur, 2002. Nanoparticles in cancer therapy and diagnosis. Adv. Drug Deliv. Rev., 54: 631-651.

Calvo, P., C. Remunan-Lopez, J.L. Vila-Jato and M.J. Alonso, 1997. Novel hydrophilic chitosan polyethylene oxide nanoparticles as protein carriers. J. Applied Polym. Sci., 63: 125-132.

Chang, Y.C., S.W. Chang and D.H. Chen, 2006. Magnetic chitosan nanoparticles: Studies on chitosan binding and adsorption of Co (II)ions. Reactive Functional Polymers, 66: 335-341.

Choi, M.J., A.M. McDonagh, P. Maynard and C. Roux, 2008. Metal-containing nanoparticles and nano-structured particles in ?ngermark detection. Forensic Sci. Int., 178: 87-97.

Costa, P. and J.M.S. Lobo, 2001. Modelling and comparison of dissolution profiles. Eur. J. Pharm. Sci., 13: 123-133.

Couvreur, P., L. Grislain, V. Lenaerts, F. Brasseur, P. Guiot, P. Guiot and P. Couvreur, 1986. Polymeric Nanoparticles and Microspheres. CRC Press, Boca Raton, FL.

De Campos, A.M., A. Sanchez and M.J. Alonso, 2001. Chitosan nanoparticles: A new vehicle for the improvement of the delivery of drugs to the ocular surface Application to cyclosporine A. Int. J. Pharm., 224: 159-168.

Douglas, S.J., S.S. Davis and L. Illum, 1987. Nanoparticles in drug delivery. CRC Crit, Rev. Ther. Drug Carrier Syst., 3: 233-261.

Fessi, C., J.P.D. Saguet, F. Puisie and T. Curt, 1992. Process for the preparation of dispersible colloidal systems of a substance in the form of nanoparticles. US Patent, 5118 528, 2 Jun. http://www.freepatentsonline.com/5118528.html.

Florence, A.T., A.M. Hillery, N. Hussain and P.U. Jani, 1995. Nanoparticles as carriers for oral peptide absorption: studies on particle uptake and fate. J. Controlled Release, 36: 39-46.

Galindo-Rodriguez, S., E. Allemann, H. Fessi and E. Doelker, 2004. Physicochemical parameters associated with nanoparticle formation in the salting-out, emulsification-diffusion and nanoprecipitation methods. Pharm. Res., 21: 1428-1439.

Glen, A., 2005. The impact of nanotechnology in drug delivery: Global developments, market analysis and future prospects. http://www.nanomarkets.com.

Govender, T., S. Stolnik, M.C. Garnett, L. Illum and S.S. Davis, 1999. PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. J. Controlled Release, 57: 171-185.

Huang, D., F. Liao, S. Molesa, D. Redinger and V. Subramanian, 2003. Plastic compatible low resistance printable gold nanoparticle conductors for ?exible electronics. J. Electrochem. Soc., 150: 412-417.

Illum, L., I. Jabbal-GilI, M. Hinchcliffe, A.N. Fisher and S.S. Davis, 2001. Chitosan as a novel nasal delivery system for vaccines. Adv. Drug Delivery Rev., 51: 81-96.

Jahanshahi, M. and Z. Babaei, 2008. Protein nanoparticle: A unique stem as drug delivery vehicles. Afr. J. Biotech., 7: 4926-4934.

Jain, S.K., A.M. Awasthi, N.K. Jain and G.P. Agrawal, 2005. Calcium silicate based microspheres of repaglinide for gastro retentive floating drug delivery: Preparation and *in vitro* characterization. J. Controlled Release, 107: 300-309.

Kawashima, Y., H. Yamamoto, H. Takeuchi and Y. Kuno, 2000. Mucoadhesive d,l-lactide/glycolide copolymer nanospheres coated with chitosan to improve oral delivery of elcatonin. Pharm. Dev. Technol., 5: 77-85.

Kreuter, J., 1994. Nanoparticles. In: Colloidal Drug Delivery Systems, Kreuter, J. (Ed.). Marcel Dekker, New York, pp: 219-342.

Lai, F., S.A. Wissing, R.H. Mller and A.M. Fadda, 2006. *Artemisia arborescens* L essential oil loaded, solid lipid nanoparticles for potential agricultural application: Preparation and characterization. AAPS Pharm. Sci. Tech., 7: 1-2.

Lee, O.S., B.J. Ha, S.N. Park and Y.S. Lee, 1997. Studies on the pH-dependent swelling properties and morphologies of chitosan/calcium-alginate complexed beads. Macromol. Chem. Phys., 198: 2971-2976.

Liu, T.M., L.D. Musinski, P.R. Patel, A.D. Gallimore, B.E. Gilchrist and M. Keidar, 2007. Nanoparticle electric propulsion for space exploration in space. Technology and Applications International Forum, STAIF. http://aerospace.engin.umich.edu/spacelab/pdf/STAIF_2007.pdf.

Majeti, N.V.R.K., 2000. A review of chitin and chitosan applications. Reactive Functional Polymers, 46: 1-27.

McGinity, J.W. and P.B. O'Donnell, 1997. Preparation of microspheres by the solvent evaporation technique. Adv. Drug Delivery Rev., 28: 25-42.

Merchant, H.A., H.M. Shoaib, J. Tazeen and R.I. Yousuf, 2006. Once-daily formulation and *in vitro* evaluation of cefopodoxime using hydroxypropyl methyl cellulose: A technical note. AAPS Pharm. Sci. Tech., 7: 178-183.

Mi, F.L., H.W. Sung and S.S. Shyu, 2001. Release of indomethacin from a novel chitosan microsphere prepared by naturally occurring cross linker: Examination of crosslinking and polycation/anionic drug interaction. J. Applied Polymer Sci., 81: 1700-1711.

Morishita, M. and N.A. Peppas, 2006. Is the oral route possible for peptide and protein drug delivery. Drug Discovery Today, 11: 905-910.

Nagappa, A.N.D., 2008. Novel strategies for the therapeutic management of type II diabetes. Health Administrator, 20: 58-68.

Niwa, T., H. Takeuchi, T. Hino, N. Kunuo and Y. Kawashima, 1994. *In vitro* drug release behaviour of DL-Lactide/Glycolide co polymer (PLGA) nanospheres with nafarelin acetate prepared by a novel spontaneous emulsification solvent diffusion method. J. Pharm. Sci., 83: 727-732.

Oppenheim, R., 1981. Solid colloidal drug delivery systems: nanoparticles. Int. J. Pharm., 8: 217-234.

Panyam, J. and V. Labhasetwar, 2003. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. Adv. Drug Delivery Rev., 55: 329-347.

Peng, D., K. Huang, Y. Liu and S. Lin, 2007. Preparation of novel polymeric microspheres for controlled release of finasteride. Int. J. Pharm., 342: 82-86.

Perelshtein, I., G. Applerot, N. Perkas, G. Guibert, S. Mikhailov and A. Gedanken, 2008. Sono chemical coating of silver nanoparticleson textile fabrics (nylon, polyes terand cotton) and their anti bacterial activity. Nanotechnology, 19: 245705-245705.

Reis, C.P., R.J. Neufeld, A.J. Ribeiro and F. Veiga, 2006. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine, 2: 8-21.

Rieux, A.D., V. Fievez, M. Garinot, Y.J. Schneider and V. Preat, 2006. Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach. J. Controlled Release., 116: 1-27.

Schipper, N.G., K.M. Varum, P. Stenberg, G. Ocklind, H. Lennernas and P. Artursson, 1999. Chitosan as absorption enhancers for poorly absorbable drugs 3: Influence of mucus on absorption enhancement. Eur. J. Pharm. Sci., 8: 335-343.

Shenoy, D.B. and M.M. Amiji, 2005. Poly (ethyleneoxide)-modi?ed poly (epsilon caprolactone) nanoparticles for targeted delivery of tamoxifenin breast cancer. Int. J. Pharm., 293: 261-270.

Sood, A. and R. Pachangnula, 1998. Drug release evaluation of dilitizem CR prepartions. Int. J. Pharm., 175: 95-107.

Soppimath, K.S., T.M. Aminabhavi, A.R. Kulkarni and W.E. Rudzinski, 2001. Biodegradable polymeric nanoparticles as drug delivery devices. J. Controlled Release, 70: 1-20.

Speiser, B., 2008. Nanoparticles inorganic production: In Issues and opinions. Proccdings of the 16th IFOAM Organic World Congress, June 18-20, Modena, Italy, http://www.ifoam.org/events/ifoam_conferences/owc/modules/abstracts_pdfs/Speiser_proc_GMO.pdf.

Takeuchi, H., H. Yamamoto and Y. Kawashima, 2001. Muco adhesive nanoparticulate systems for peptide drug delivery. Adv. Drug Delivery Rev., 47: 39-54.

Thanou, M., J.C. Verhoef and H.E. Junginger, 2001. Oral drug absorption enhancement by chitosan and its derivatives. Adv. Drug Delivery Rev., 52: 117-126.

Thein-Han, W.W and W.F. Stevens, 2004. Transdermal delivery controlled by a chitosan membrane. Drug Dev. Ind. Pharmacy, 30: 397-404.

Wilson, B., M.K. Samanta, K. Santhi, K.P. Kumar, M. Ramasamy and B. Suresh, 2009. Chitosan nanoparticles as a new delivery system for anti-Alzheimer's drug tacrine. Nanomedicine, 6: 144-152.