

REPORT

Formulation and *in vitro* evaluation of nateglinide microspheres using HPMC and carbopol-940 polymers by ionic gelation method

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Abstract: This study involves the design and characterization of Nateglinide (NAT) microspheres to enhance patient compliance. Ionic gelation technique was used to prepare Nateglinide Microspheres by using rate controlling polymers Carbopol-940 and Hydroxypropylmethyl cellulose (HPMC). Shape and surface were evaluated with Scanning electron microscopy (SEM). Percentage Yield, Particle size analysis, Encapsulating Efficiency, Micromeritic analysis, Fourier Transform Infra-Red Spectroscopy (FTIR), Differential Scanning Colorimetry (DSC) were done for characterization of Microspheres. Drug release studies were performed at pH 1.2 and 7.2 using USP dissolution type-II apparatus and release rates were analyzed by the application of different pharmacokinetic models. The size of microspheres was found to be varied from 781 μ m to 853 μ m. Rheological studies proved excellent flow behavior while percentage yield was found to be varied from 72% to 79%. Absence of drug-polymers interactions was confirmed from FTIR and DSC results. The microspheres prepared with sodium alginate showed cracks while microspheres obtained from blend of Carbopol-940 plus sodium alginate were smooth and spherical. Maximum entrapment efficiency (71.4%) was achieved for Microspheres with Carbopol-940. The greater retardation in drug release was observed for microspheres containing Carbopol-940 and release pattern followed Higuchi kinetics model and negligible drug release was observed at pH 1.2.

Keywords: Nateglinide, Microspheres, Carbopol-940, HPMC, Higuchi model.

INTRODUCTION

The interest in the manufacture of novel drug delivery systems has been increased because of their numerous advantages over conventional dosage forms. These drug delivery systems are not only contributing towards safety and compliance for patients but also economical production at industrial level. Novel drug delivery systems used to make drug release at desirable site at appropriate rate by use of different methodologies like microencapsulation (Santose *et al.*, 2012). There are a number of polymers used for microencapsulation depending upon the nature of drug and process employed. Microspheres are formulated with an intention to administer drug in a sustained release manner to maintain its therapeutic effects for a longer period of time by preventing fluctuations in drug plasma concentration levels. Sustained release microspheres can also lessen the probability of irritation in GIT (Widder *et al.*, 1979; Jia *et al.*, 2011).

Diabetes mellitus is one of the most common problems of these days (Arifin *et al.*, 2012) and Nateglinide (NAT) is one of the most effective drugs for its treatment (McLeod JF, 2004). Nateglinide is a non-sulfonylurea drug which blocks KATP potassium channel to perform overall

glycemic control in type-2 diabetes. Nateglinide is selective blocker of pancreatic beta-cells with a short half-life of 1.5-2.5 hrs (Norman and Rabasseda, 2001). Therefore, in order to prolong its effect in the body and to decrease oscillations in concentration level of NAT in plasma, a controlled release drug delivery system is needed for NAT.

In the present study, NAT was encapsulated by hydrophilic biodegradable polymers such as Hydroxy-PropylMethyl Cellulose (HPMC) and Carbopol-940 (acrylic acid derivative) to develop enteric coated sustained release microspheres by ionic gelation method. Shape and surface of microspheres were evaluated with SEM. These microspheres were also further analyzed for Rheological properties, entrapment efficiency, FTIR, DSC and in-vitro drug release properties.

MATERIALS AND METHODS

Materials

Nateglinide was purchased from Sigma Chemicals, USA. Sodium alginate, Carbopol 940, Hydroxy propyl methyl cellulose (HPMC) were purchased from Riedel-deHaen, China. Sodium hydroxide, Monobasic potassium phosphate, Hydrochloric acid and Calcium Chloride were obtained from Merck, Germany. All these chemicals and reagents were ensured to be of analytical grade.

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Preparation of Nateglinide microspheres

The Nateglinide loaded microspheres were formulated by Ionic gelation method and these formulations are shown in table 1. NAT microspheres were prepared by employing sodium alginate in combination with different ratios of Hydroxy Propyl Methyl Cellulose and Carbopol-940. Sodium alginate was dissolved in 100ml of distilled water in a reagent bottle by using magnetic stirrer. Nateglinide (1.0gm) was dissolved in 100ml of chloroform in a well-closed volumetric flask. Solution of drug was added to sodium alginate solution in reagent bottle and was closed with lid. Solutions were mixed with magnetic stirrer at speed of 1000 rpm for one hour in order to form a homogenous blend. Calcium chloride solution (10%w/v) was prepared by dissolving 10gm of calcium chloride in 100 ml of distilled water in a beaker (Basu and Rajendran, 2008). Then this solution was dropped manually from a hypodermic syringe through needle size number 22G into solution of calcium chloride, resultant microsphere were marked as N-1. The microspheres thus formed were allowed 30 minutes for curing in calcium chloride solution and were filtered with whatmann filter paper number 4. The distilled water was used to wash these filtered microspheres which were then air dried at room temperature for 30 minutes. The microspheres were then transferred to Petri dishes and dried in oven at $37^{\circ}\text{C} \pm 10^{\circ}\text{C}$ until a constant weight was obtained (Kumar *et al.*, 2010).

Table 1: Composition of various formulations of nateglinide loaded microspheres

Batch code	Drug (%w/v)	Sodium alginate (%w/v)	Carbopol 940 (%w/v)	HPMC
N-1	1	1	-	-
N-2	1	1	0.5	-
N-3	1	1	1	-
N-4	1	1	-	0.5
N-5	1	1	-	1

Other formulations were prepared by same method with exception that solution of drug was added to aqueous solution comprising 1.0% w/v sodium alginate and various concentrations of different polymers.

Characterization of microspheres**Production yield**

The completely dried microspheres were accurately weighed and the percentage yield (w/w) was calculated by the formula as followed (Rahman *et al.*, 2006):

$$\% \text{age yield} = \frac{\text{Amount of dried microsphere recovered}}{\text{Amount of drug + Amount of polymer}} \times 100$$

Analysis of particle size

For all formulations of microspheres, the size was determined by microscopic method (Sah, 1997). At least

100 beads were analyzed for each preparation and the mean particle size was then obtained.

Micromeritic Properties of Microspheres**Angle of repose**

The microspheres were passed from the funnel which was initially fixed in a stand. Microspheres falling from a height of 6cm (distance between top of funnel and surface) form a heap at the surface. The radius and height of the heap were calculated in order to measure angle of repose (Banker and Anderson., 1987); $\tan \theta = h/r$

Here ‘ θ ’ denotes angle of repose while ‘h’ and ‘r’ denote height and radius of heap respectively.

Angle of repose <30 shows excellent flow properties.

Bulk density

Bulk density was calculated by the use of following formula (Banker and Anderson., 1987):

$$\text{Bulk density} = \frac{\text{Sample weight}}{\text{Sample volume}}$$

Tapped density

With the help of conventional tapping methodology, the tapped density was measured. The microspheres in using 10ml measuring cylinder were subjected to 100 tapings to bring a plateau condition. It was then determined as (Shariff *et al.*, 2007):

$$\text{Tapped density} = \frac{W}{V_{t100}}$$

Here ‘W’ indicates microspheres weight and ‘ V_{t100} ’ suggests volume after 100 tapings.

Carr’s index

It was measured with the help of bulk and tapped densities. Its formula was as followed;

$$C_i = \frac{V_b - V_t}{V_t} \times 100$$

Where V_b is bulk volume and V_t is tapped volume.

Good flow behaviour could be achieved at $C_i < 15\%$ and the value above 25% indicates poor flow pattern (Shariff *et al.*, 2007).

Hausner’s ratio

Hausner’s ratio is also an indicator of flow behaviour which was calculated with the help of following formula (Shariff *et al.*, 2007); Hausner’s ratio = ρ_t / ρ_d

Where ρ_t is tapped density and ρ_d is bulk density.

Hausner’s ratio <1.2 is used to indicate free flow pattern while its value close to 1 specifies good flow characteristics.

Scanning Electron Microscopy

Shape and surface characters of microspheres were analyzed by Scanning electron microscope (SEM). Firstly,

to an aluminum stub, a double adhesive tape was attached on which microspheres were slightly scattered. By using a fine coat ion sputter, that stub was coated with gold (150-200 Å thickness) and then microspheres were examined under SEM.

Fourier transform infrared spectroscopy

The interactions between drug and rate controlling polymers were studied by FTIR spectroscopy. Disks for FTIR analysis were prepared by mixing drug/polymer/microspheres with KBr (2/mg sample in 200/mg KBr). The prepared disks were then examined in FTIR spectroscope at the resolution of 2 cm^{-1} with scanning range of $4,000\text{--}400\text{ cm}^{-1}$ (Logannathan *et al.*, 2003). Pure drug, polymers and drug-loaded microspheres were subjected to this process and their spectra were recorded.

Differential Scanning Calorimetric Analysis (DSC)

The compatibility between drug and polymer was also analyzed by Differential Scanning Calorimetry. Nateglinide and its microspheres were separately converted into finely divided powder by trituration process. The prepared samples were then heated at $40^\circ\text{C}/\text{min}$ rate up to 400°C temperature in sealed aluminium pans. The flow of nitrogen was maintained at the rate of $40\text{ ml}/\text{min}$. To verify the reproducibility of results, the process was repeated thrice for drug, each polymer and for each formulation of the microspheres (Tayade and Kale, 2004).

Percentage Encapsulation Efficiency

The formula used to calculate Percentage encapsulation efficiency was as followed (Samati *et al.*, 2006).

$$\text{Encapsulation efficiency \%} = \frac{\text{Entrapped amount of drug per gm microsphere}}{\text{Theoretical amount of drug per gm microsphere}} \times 100$$

In vitro drug release studies

For in vitro drug release studies, microspheres containing equivalent to 120 mg of NAT were packed in hard gelatin capsule shell. USP type-II apparatus with a basket stirring speed of 50 rpm at $37 \pm 0.5^\circ\text{C}$ was used for this purpose. Drug release from microspheres was evaluated first for 2 hours in 0.1 N HCl and then for eight hours in phosphate buffer of pH 7.2 (900ml). A sample of 5ml from dissolution medium was taken at definite intervals of time and analyzed by UV-VIS spectrophotometer for Nateglinide contents at λ_{max} 210-nm. After every withdrawal, 5ml of freshly prepared pre-warmed dissolution medium was added to dissolution medium in dissolution apparatus to keep its volume constant. The unknown drug concentration was calculated from standard curve which was constructed from 99.9% pure Nateglinide at a concentration range of $2\mu\text{g}/\text{mL}$ to $20\mu\text{g}/\text{mL}$.

Drug release kinetics

In this study, five kinetic models were used to evaluate the possible drug release mechanism. The kinetic models and their equations were as followed:

For zero-order model;

$$Q_t = k_0 t \dots\dots (\text{Xu and Sunada, 1995}).$$

For first-order equation;

$$\ln Q_t = \ln Q_0 - k_1 \cdot t \dots\dots (\text{Singla and Medirata, 1988}).$$

For Higuchi model equation;

$$Q = k_H \sqrt{t} \dots\dots (\text{Higuchi T., 1963}).$$

For Hixon – Crowell model;

$$A_0^{1/3} - A_t^{1/3} = kst \dots\dots (\text{Hixon and Crowel, 1931}).$$

For the model of Korsmeyer peppas;

$$M_t/M_\infty = kt.n \dots\dots (\text{Ritger and Peppas, 1987}).$$

Where, Initial amount of Nateglinide in the microsphere before release process was denoted by Q_0 or A_0 while remaining quantity of drug in the microspheres was indicated by A_t . Q or M_t denotes quantity of Nateglinide released after time t . The quantity of drug released after time $t = a$ was denoted by M_a and k specifies the rate constant for respective kinetic model in each equation. ' n ' in Korsmeyer peppas model indicates the exponent for diffusion and this exponent is used to establish drug release mechanism.

RESULTS

Sodium alginate, HPMC and Carbopol-940 were employed as release retarding polymers to prepare NAT loaded microspheres by ionic gelation method. The mean size of a microsphere was found to be varied from $781\mu\text{m} \pm 2.08$ to $842\mu\text{m} \pm 1.15$ whereas percentage yield varied from 72% to 78% in different formulations (table 2). Percentage entrapment efficiency of microspheres was found to be varied from 52% to 73% and microspheres prepared with Carbopol-940 showed maximum entrapment efficiency (table 2). All of the prepared formulations of microspheres showed good and excellent flow behavior according to angel of repose and Compressibility Index analysis (table 3). Figure 1 shows the micrographs of Nateglinide microspheres. The cracks were observed on microspheres prepared with sodium alginate alone (N-1). The microspheres of carbopol-940 was observed to be smooth and spherical. The microspheres prepared with HPMC were smooth but lack spherical shape. FTIR and DSC analysis confirmed the compatibility of drug with polymers as shown in Figures 2 and 3. In DSC analysis, Nateglinide loaded microspheres demonstrated particular thermal peaks at the melting point of Nateglinide. There is no change in the specific peaks of Nateglinide after successful encapsulation. The release profiles of NAT at pH values 1.2 and 7.2 are shown in fig. 4 and 5 respectively. The microspheres made with Sodium alginate (N-1) exhibit fast release as compared to other formulations. The microspheres prepared with HPMC or Carbopol-940

showed a significant control over drug release. Most of the formulations of microspheres followed Higuchi model of drug release.

DISCUSSION

The technique seemed to be suitable for encapsulation of hydrophobic drugs. A fact was observed in the study that percentage yield and mean size significantly increased as we increased the ratio of polymer with drug. Similar findings were also concluded and reported by Sajeev *et al.* (2002).

Table 2: Mean particle size, % Yield and Percentage Entrapment Efficiency of Microspheres

Formulation	Mean particle size (μm)	% Age Yield	% Age Entrapment
N-1	781 $\pm$ 2.08	72.23 $\pm$ 0.74	52.57 $\pm$ 0.007
N-2	824 $\pm$ 1.15	74.15 $\pm$ 0.18	62.85 $\pm$ 0.015
N-3	839 $\pm$ 1.52	75.12 $\pm$ 0.20	71.42 $\pm$ 0.007
N-4	842 $\pm$ 1.52	77.47 $\pm$ 0.44	61.44 $\pm$ 0.009
N-5	853 $\pm$ 1.17	78.92 $\pm$ 0.98	65.42 $\pm$ 0.011

All values are represented as mean $\pm$ S.E.M n =3

Percentage entrapment efficiency of microspheres was found to be in the range of 52-73%. The highest percentage entrapment (73%) was observed in microspheres made with Carbopol-940 (N-3) as shown in table 2. Similar findings regarding encapsulation efficiency were also found in the study of alginate microspheres described by researchers (Lemoine *et al.*, 1998).

Micromeritic Analysis

The comprehensive view of micromeritic analysis of all microsphere formulations is shown in table 3. The Hausner's ratio for all microsphere formulations (<1.25) suggested a good flow behaviour of microspheres (Shariff *et al.*, 2007). The outcomes of compressibility index (less than 10%) further confirmed this good flow character of all formulations of microspheres. Aulton and Wells had also reported this behavior previously (Aulton and Wells, 1998).

Scanning Electron Microscopy (SEM)

The micrographs of Nateglinide microspheres obtained by Scanning electron Microscope were presented in fig. 1. The presence of cracks on microspheres prepared with sodium alginate alone (N-1) was observed which was further confirmed from the fast release of Nateglinide from this formulation as shown in fig. 1(A). Gowda and Shivakummar also described that presence of cracks on microspheres surface increased the release rate of encapsulated drug from polymers (Gowda and Shivakumar, 2007). Uniform encapsulation of drug in the microspheres of carbopol-940 was observed because SEM micrographs of these microspheres had not shown the

presence of drug particles on their surface (Gowda and Shivakumar, 2007).

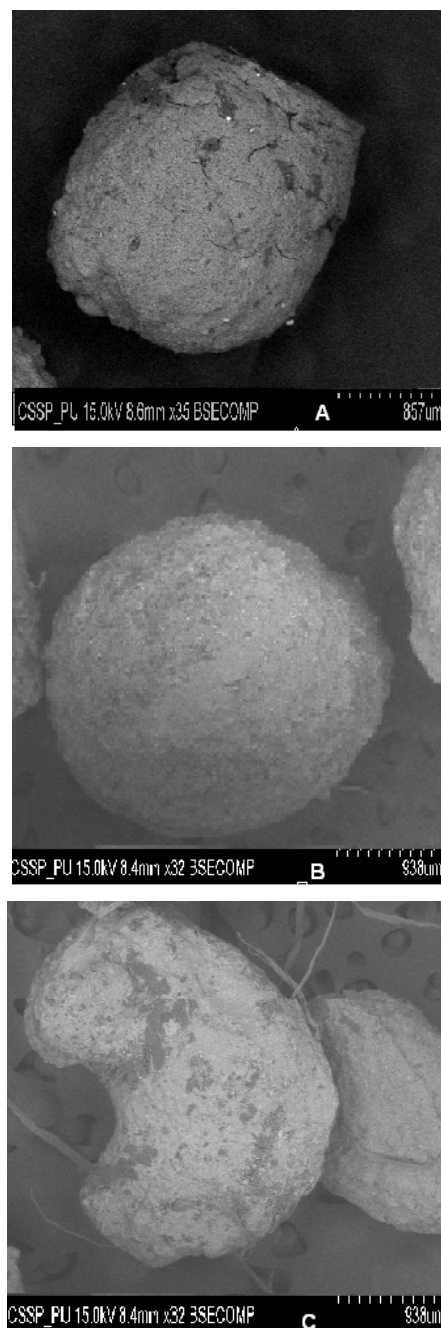


Fig. 1: SEM Photographs of Nateglinide loaded microspheres made with sodium alginate (A), Nateglinide loaded microspheres made with sodium alginate plus carbopol-940 (B), Nateglinide loaded microspheres made with sodium alginate plus HPMC (C).

HPMC produced smooth microspheres which lack spherical shape while microspheres obtained from blend of Carbopol-940 plus sodium alginate were smooth and spherical.

Fourier Transform Infrared Spectroscopy

To determine the drug and polymers incompatibilities, FTIR of the pure drug, polymers and their physical mixtures were done (fig. 2). The amino group presence in nateglinide structure was confirmed from concentrated stretching of N-H bond at a range from 3296 cm^{-1} to 3311 cm^{-1} . A prominent stretching due to C-N bond at a wave number of 1384 cm^{-1} was also observed. The principle peaks corresponding to NAT was also appeared with less intensity in the microsphere formulations. The decreased in peak intensity may be attributed to a fine dispersion of the drug in the polymers. Similar findings were also reported by Pignatello *et al.*, (2002). The absence of any change in the characteristic peaks of Nateglinide after successful encapsulation confirmed the lack of any possible interaction between Nateglinide and release controlling polymers.

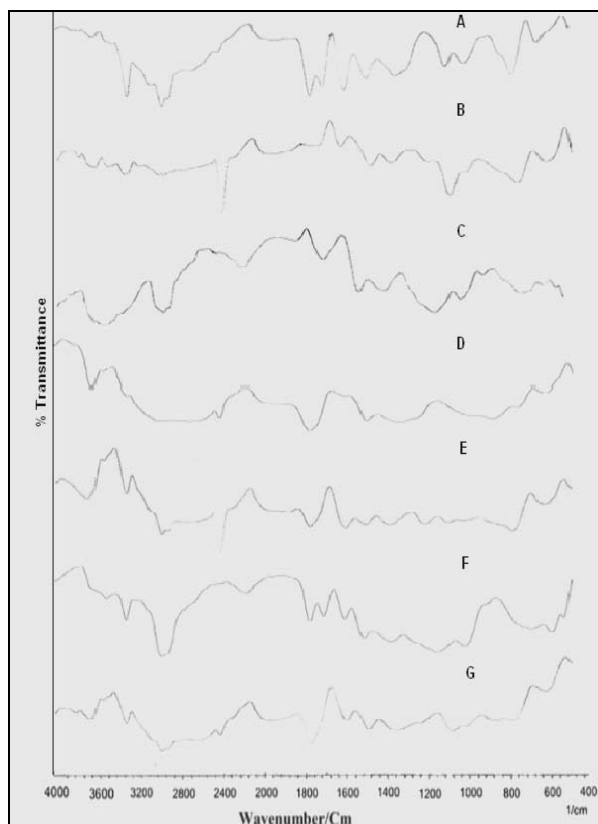


Fig. 2: FTIR Spectra of **A:** Nateglinide **B:** Sodium Alginate **C:** HPMC **D:** Carbopol-940 **E:** Nateglinide loaded microspheres made with sodium alginate **F:** Nateglinide loaded microspheres made with sodium alginate plus carbopo-940I **G:** Nateglinide loaded microspheres made with sodium alginate plus HPMC.

Differential Scanning Calorimetry (DSC)

The DSC curves of pure drug, polymers and of all prepared formulations of microspheres were shown in fig. 3. At melting point (142°C) of Nateglinide, a prominent endothermic peak was observed which was also reported by Raghupathi *et al.* (2001). In case of Sodium alginate,

HPMC and Carbopol-940, exothermic peaks corresponding to their melting points i-e at temperatures of $>300^{\circ}\text{C}$, 230°C and 94°C respectively were observed.

The DSC curves (fig. 3) of Nateglinide loaded microspheres also proved the existence of particular thermal peaks at the melting point of Nateglinide which definitely suggest the compatibility of drug with polymers used to control drug release. It was also concluded from the less sharpened DSC peak of Nateglinide in microspheres that drug lost its crystalline nature during this encapsulation process (Jones and Pearce, 1995).

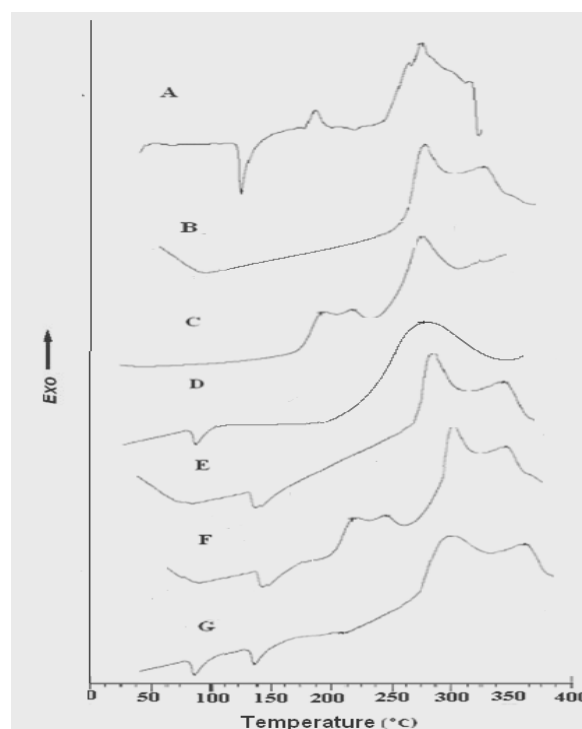


Fig. 3: DSC Thermograms of **A:** Nateglinide, **B:** Sodium Alginate, **C:** HPMC, **D:** Carbopol-940, **E:** Nateglinide loaded microspheres made with sodium alginate, **F:** Nateglinide loaded microspheres made with sodium alginate plus HPMC, **G:** Nateglinide loaded microspheres made with sodium alginate plus carbopol-940.

In vitro drug release studies

The drug release profiles at pH values 1.2 and 7.2 are presented in fig. 4 and 5 respectively. Drug release studies revealed that microspheres made with Sodium alginate (N-1) gave fast release as compared to other formulations in 10 hours which may be attributed to absence of other release retarding polymers in N-1. As we increased the ratio of HPMC or Carbopol-940 with sodium alginate, there was a decrease in the drug release because the higher polymers contents significantly lower the release of drug more effectively and similar findings were also observed in the literature (Kumar *et al.*, 2010). The microspheres showed a very less amount of drug release

Table 3: Micromeritics analysis of microspheres

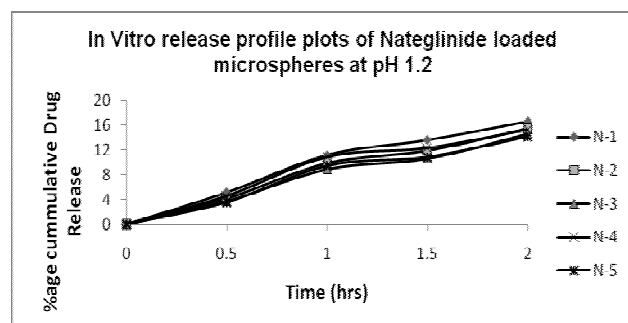
Formulation	Angle of Repose (θ)	Bulk density g/mL	Tapped density g/mL	% Carr's Index	Hausner's ratio
N-1	29.54 $\pm$ 1.21	0.472 $\pm$ 0.002	0.512 $\pm$ 0.011	7.80 $\pm$ 1.643	1.08
N-2	26.86 $\pm$ 1.45	0.414 $\pm$ 0.003	0.446 $\pm$ 0.005	7.17 $\pm$ 0.746	1.07
N-3	25.94 $\pm$ 0.60	0.382 $\pm$ 0.001	0.424 $\pm$ 0.004	9.90 $\pm$ 0.757	1.1
N-4	27.75 $\pm$ 1.07	0.429 $\pm$ 0.002	0.466 $\pm$ 0.007	7.74 $\pm$ 1.600	1.09
N-5	26.20 $\pm$ 0.30	0.382 $\pm$ 0.003	0.437 $\pm$ 0.007	8.25 $\pm$ 2.150	1.14

All values are represented as mean $\pm$ S.E.M n =3

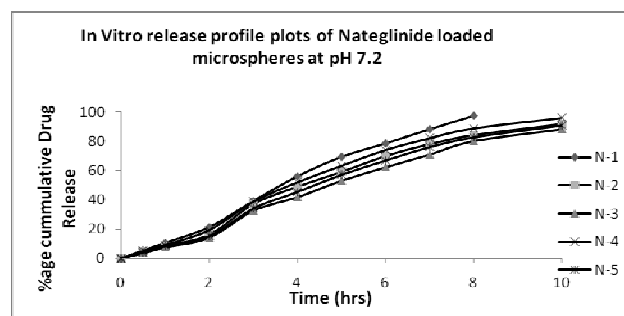
Table 4: Values of correlation coefficient for the fit of various kinetic models

Formulation	Zero order Model		First order Model		Higuchi Model		Korsmeyer Peppas Model			Hixon-Crowell Model	
	k_0	R^2	k_1	R^2	k_{HC}	R^2	k_p	N	R^2	k_H	R^2
N-1	9.537	0.994	0.369	0.830	40.21	0.987	2.057	0.894	0.998	0.242	0.940
N-2	7.779	0.994	0.273	0.877	35.36	0.987	2.085	0.832	0.832	0.197	0.941
N-3	7.365	0.987	0.218	0.951	33.71	0.992	2.057	0.815	0.994	0.188	0.919
N-4	7.415	0.991	0.231	0.932	34.82	0.992	2.061	0.812	0.984	0.195	0.927
N-5	7.124	0.988	0.203	0.947	32.66	0.993	2.011	0.849	0.990	0.186	0.911

(10 to 15%) at pH 1.2. All formulations showed a sustained release pattern of Nateglinide release at pH 7.2 as shown in fig. 5 which remained controlled over an extended period of time. The greater retardation in drug release from microspheres was offered by Carbopol-940 as compared to HPMC and these facts were further verified from the SEM photographs of microspheres in fig. 1.

**Fig. 4:** Nateglinide released from various formulations of microspheres at pH 1.2.

The release profiles of all formulations of Nateglinide microspheres were evaluated by the application of five kinetic models (table 4). The regression coefficients (R^2) were calculated by linear regression analysis as used by Mathew *et al.*, (2007). In majority of the formulations of microspheres, the R^2 values remained higher for Higuchi model suggesting diffusion mechanism of drug release from microspheres. Korsmeyer–Peppas model was also applied to know drug release mechanism from all formulations of microspheres and 'n' values was found to be in between 0.812 to 0.894 indicating non-fickian releases as shown in table 4 which is in accordance with the results obtained by Ranjha *et al.*, (2009).

**Fig. 5:** Nateglinide released from various formulations of microspheres at pH 7.2.

CONCLUSION

The study established the suitability of ionic gelation technique and these polymers to encapsulate hydrophobic drugs like Nateglinide which would be helpful in improving patient compliance by decreasing dose frequency. FTIR and DSC confirmed the absence of drug-polymer interactions and the Carbopol-940 (used in formulations N-2, N-3) was considered best to achieve study objectives. Sustained release depends not only on nature of polymer but also on its percentage used. The studies showed that polymers can successfully control the release of drug from microspheres and possible release mechanism might be following Higuchi kinetic equation.

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