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Research Article

Formulation and Evaluation of Calcium Alginate Beads from Plant Extract

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ABSTRACT

One of the advanced research areas of herbals includes use of advanced formulation techniques for delivering herbal actives. Various herbal drugs become less utilized due to their poor absorption and poor bioavailability after oral administration. The problem can be resolved by opting a suitable delivery system which can enhance the rate and extent of drug solubilising into aqueous body fluids as well as its ability to go through lipophilic biomemebranes. Here we have formulated calcium alginate beads loaded with petroleum ether extract of *Murraya koenigii* by using Orifice Gelation Technique for improving their therapeutic indices and their efficacy. A series of batches was prepared to optimize the polymer: drug ratio and the beads were evaluated for physical characteristics like percentage yield, entrapment efficiency, moisture content, micromeretic properties, scanning electron microscopy (SEM), swelling ratio, compatibility studies, X-Ray Diffraction (X-RD) and in vitro drug release studies. The beads showed the mean particle size of 994-1290µm. SEM studies revealed that the beads were spherical with nearly smooth surface. FT-IR studies revealed that there was no polymer drug interaction. Application of in vitro drug release data to various kinetic equations indicated first order release.

INTRODUCTION

Absorption of polymeric particles after oral administration has been widely documented in the literature. Part of the limitations in the development of oral particles may be technological, i.e. low rate of encapsulation in a given polymer to reach a targeted size. On the other hand, one may also raise some concern when the dosing drug is a plant extract which has low oral bioavailability. However the pharmacokinetic data are generally quite erratic and the mechanism of uptake are still not well defined. The studies presented here show that the polymeric particles, primarily because of their stability, may improve the oral bioavailability of poorly absorbed drugs. The presence of a polymeric wall around the drug will provide a good shield against the attack from the various and widespread enzymes existing in the G. I. Tract.

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Because encapsulation enables a slow release profile into degradable polymeric particles; controlled release of compounds may be achieved. Furthermore, the preferential uptake of particles by the Peyer's, part of the GALT (gut associated lymphoid system) makes them very attractive for inducing mucosal immunity .Oral administration of short half-life drugs having low bioavailability is a challenge to the formulators [1].So in this respect many strategies are employed to improve the bioavailability and prolong the half-life of the drugs. The use of natural biocompatible polymers in the design of drug delivery devices for improving bioavailability of drugs is gaining importance[2]. In the formulation of many oral multiunit dosage forms like microcapsules/microspheres, the residence time of the device is increased by incorporation of mucoadhesive polymers which enhances the bioavailability of the drugs[3].Some widely used polymers like Sodium alginate, Gelatin, Guar gum, Acacia have been reported to have bio adhesive properties[4].

Natural hydrophilic polymers, owing to their characteristic biocompatibility and biodegradability properties, are widely exploited in the pharmaceutical industry for the development of novel drug delivery systems. Among these polymers, alginate is one that has been widely used in numerous biomedical applications, processed in various dosage forms (e.g., tablets, capsules, beads, rafts, liquid suspensions), and used in sutures and dressing materials with characteristic features such as mucoadhesion, bioadhesion, and modifying drug release profile. Chemically, alginates are linear,anionic block copolymer heteropolysaccharides consisting of monomers of (-d-mannuronic acid) (M) and its C-5 epimer, (-l-guluronic acid) (G), residues joined together by 1,4- glycosidic linkages. The simple, mild, aqueous-based gel formation is achieved by the ionotropic gelling, on the addition of bivalent alkaline earth metals Ca^{2+}, Sr^{2+}, and Ba^{2+} or trivalent Fe^{3+} and Al^{3+}, due to an ionic interaction and intramolecular bonding between the carboxylic acid groups located on the polymer backbone and these cations. Calcium-induced alginate gel beads have been developed in recent years as a unique vehicle for drug delivery system. These beads have been used in formulations as single or multiple units, with or without the addition of other hydrogels or polymers, intrapenetrating networks (ipns), nanospheres,polycations, and many more dosage forms for achieving temporal and spatial drug release[5].

MATERIALS AND METHODS

Material

Sodium alginate was purchased from S.D. Fine Chemicals (Mumbai ,India) having G /M ratio 40% to 45%, 55% to 60% and viscosity of 1% wt / wt aqueous solution as 200 to 400 mega Pascal seconds.

The plant *Murraya koenigii* (Family Rutaceae) has been selected for preparation of the alginate beads. The plant commonly known as curry patta in Hindi and widely used as spice and condiment in India and other tropical countries. It belongs to the family Rutaceae and is well known for various activities. Traditionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhea, dysentery; insect bites and also used to allay heat of body [6]. Previous phytochemical investigations on this plant revealed the occurrence of carbazole alkaloids [7]. Anti-oxidant, anti-tumor, anti-microbial, anti-inflammatory, anti-trypanocidal and mosquitocidal activities have been indicated for some of these alkaloids [8]. The petroleum ether extract of the plant was used for the preparation of the alginate beads. All other chemicals and solvents were of laboratory grade.

Preparation of extract –loaded alginate beads

Extract loaded calcium alginate beads were prepared using Orifice Gelatin Technique. The formulations were prepared using different concentrations of the polymer dissolved in purified water to form a homogenous drug–polymer mixture which was kept untouched for 20 -30 minutes to make it bubble free. This was passed through 24 /22 gauge syringe drop wise into 100 ml of 1% w /v. calcium chloride solution containing 10 % glacial acetic acid so that the drops congealed into spheres .The calcium chloride solution was maintained at ambient temperature by constant stirring with magnetic stirrer. The falling distance was kept constant at 6 cm .The beads were retained in the calcium chloride solution for 30 minutes for the completion of the curing reaction and rigidization. The beads so formed were collected by decantation and washed with purified water repeatedly for three times to remove any traces of calcium chloride and then dried at room temperature for 24 hours.

Evaluation of Plant Extract loaded beads

Determination of yield and entrapment efficiency

The microcapsules equivalent to the total amount of drug loaded in the formulations was powdered using a mortar and pestle and taken in a beaker containing phosphate buffer of pH 6.8 and stirred in a magnetic stirrer for 1 hour at 300rpm.The solution was filtered and estimated for the drug content spectrophotometrically at 258m.The determination for drug entrapment efficiency the following formulas were used:

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug content}}{\text{Theoretical drug content}} \times 100$$

Determination of Moisture Content

Moisture content of the batches was determined by Karl Fisher method using Karl Fisher titrator (Veego Matic D,Veego Instruments Corp, Mumbai, India). It involved titration of the moisture present in methanolic solution with Karl Fisher reagent and the end point was detected visually.

Micromeritic properties

Angle of repose

Angle of repose of different formulations was measured according to fixed funnel standing method as given in Table 2.

$$\theta = \tan^{-1} H/R,$$

where θ is the angle of repose, R and H are the radius and height of the pile respectively.

Bulk Density

Bulk and tapped densities were measured by using 10ml graduated cylinder. The sample poured in the cylinder was tapped mechanically for 100 times, then the tapped volume was noted down and bulk density and tapped density were calculated.

Carr's index

Compressibilty index (Ci) or Carr's index value of microcapsules was computed according to the following equation and reported in Table 2.

$$\text{Carr's Index (\%)} = \frac{\text{(Tapped Density-Bulk Density)} \times 100}{\text{Tapped Density}}$$

Hausner's ratio

Hausner's ratio of microcapsules as reported in Table 2 was determined by comparing the tapped density to the bulk density using the equation:

$$\text{Hausner's Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}$$

Bead Size

Dried beads of different formulation and placebo were measured using vernier caliper with an accuracy of 0.01 mm. The test was repeated 3 times for all formulations.

Surface Topography

The surface morphology of the optimized batches of beads were determined by Scanning Electron Microscopy (SEM) using a SEM sample stub using double sided sticking tape and coated the microcapsules with gold film (thickness 200nm) under reduced pressure (0.001 mm) of Hg (JEOL-JSM6480LV).

Swelling Studies

Swelling rate of the microcapsules was measured as a function of percent of water uptake by the beads. The beads were incubated in phosphate buffer of pH 6.8 at 37˚C and at different time intervals the beads were removed and excess water was removed by using the filter paper (reported in Table 3 and graphically represented in Fig 2). The swelling rate and the extent of swelling were determined by using the following formula below.

$$\text{Swelling Ratio} = \frac{\text{Weight of wet beads}}{\text{Weight of dry beads}}$$

Compatibility of Drug and Polymer

Drug polymer compatibility studies manually

Films were prepared by the film casting method of specially designed glass molds with the plastic transparent sheet. Placebo polymer and drug – loaded polymeric films were prepared by casting the 6% polymer solution in water and drug incorporated in 6% polymer solution, obtained by stirring on magnetic stirrer. Polymeric solution was poured within a glass bangle placed on glass mould. The rate of evaporation of solvent was controlled by inverting cup funnel [9]. The films were dried for 48 hours at room temperature and 24 hours in vacuum –drying .The dried films were renewed from the glass mould and stored in desiccator until a constant weight was reached.

The freshly prepared films and films stored at room temperature for 1.5 years were then observed under a polarized microscope to investigate possible crystallization of the drug in the films [10].

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

The drug-polymer interaction were studied by FTIR spectroscopy where the spectroscopic studies of Plant extract and the polymer used were done individually first and then compared with the spectra of the formulations (in which the drug was matrixes with different concentration of polymers). Fourier Transform Infrared Analysis (FTIR) measurements of the plant extract, sodium alginate, and extract - loaded calcium alginate beads were obtained on JASCO V5300 FT-IR (Tokyo, Japan). The pellets were prepared on KBr-press (Spectra Lab, Pune, India) under hydraulic pressure of 150 kg/cm2. The spectra were scanned over the wave number range of 3600 to 400 cm−1 at the ambient temperature.

Powder X-ray Diffraction (XRD)

X-ray powder diffraction patterns of plant extract, sodium alginate, unloaded calcium alginate beads, and plant extract -loaded calcium alginate beads of different batches were recorded by using a Philips PW3042 X-ray diffractometer (Philips, Amsterdam, The Netherlands). Samples were irradiated with monochromatized Cu Kα radiation (1.542 Å) and analyzed between 2°C and 60°C. The voltage and current used were 30 kV and 30 mA, respectively. The range and the chart speed were 2 × 104 cps and 10 mm/2θ, respectively.

***In-vitro* Drug Release Studies**

Release of the crude drugs from the beads of herbal petroleum ether extract of *Murraya koenigii* was studied at 1.2 pH (900 ml) for the first 2 hours and followed by in phosphate buffer pH 7.4 (900ml) up to 8 hours using an USP Dissolution Apparatus Type-II with a basket assembly at 50 rpm. 100 mg of beads were properly weighed and taken in a basket and was placed in 900 ml dissolution fluid containing 1.2pH and maintained at 37 ± 0.2 °C. At appropriate intervals (15, 30, 60, 90, 120 minutes), 5ml of sample was taken and filtered. The dissolution media was then replaced by 5 ml of fresh dissolution fluid to maintain a constant volume. After 2 hours the basket was again immersed in 900 ml of dissolution fluid containing phosphate buffer (pH 7.4) and samples are collected at an appropriate intervals (150, 180, 300, 480 mins) as mentioned above. The samples of both the dissolution fluid are then analysed at 258 nm using Elico UV- Visible Double beam spectrophotometer. In order to investigate the mode of release from beads, release data are analysed using following mathematical equation: Zero Order Equation (Equation- 1), First Order Equation (Equation- 2) and Higuchi Equation (Equation- 3).

Equation- 1: $Q = k_o t$

Equation- 2: $\ln (100- Q) = \ln Q_0 - k_1 t$

Equation- 3: $Q = k_H t$

Where Q is Percent drug released, Q_0 is the Percent drug remaining to be released

RESULT AND DISCUSSION

The microparticulate drug delivery system is designed to enhance the dissolution of poorly soluble plant extract, and the polymer used has a retarding action or is meant for prolonging in – vivo action or to increase its bioavailability .The present investigation aims to develop petroleum ether extract loaded –calcium alginate beads to enhance its solubility within the G.I.tract for a sustained action.

The beads so prepared by ionic - gelation Technique was evaluated for Percentage yield, micromeritics studies such as particle size determination ,tapped density ,bulk density , Carr's index , Hausner's ratio and angle of repose . Along with this the drug entrapment, swelling studies, compatibility studies by preparing films and FTIR studies, *in –vitro* drug release and release kinetics were determined Advance studies like SEM and XRD were done to know its morphology.

Percentage Yield, Entrapment efficiency and Moisture content of Beads

The prepared drug loaded beads gives good percentage yield, entrapment efficiency and least moisture content in F6 as compared to F7 and F8 (Table 1) The prepared bead gives good percentage yield. The percentage yield was determined by weighing after drying. The percentage yield can be arranged as F6>F7>F8. The maximum percentage yield was 82.67%. The drug entrapment efficiency was in the range of 60-70%. F6 showed an entrapment of 68.45% while F7 and F8 showed lesser entrapment. The moisture content was found to be around 4.0%w/w.

Table 1: Percentage Yield, Entrapment efficiency and Moisture content of Beads

Formulation code	% Yield	Entrapment Efficiency (%)	Moisture Content (w/w)
F6	82.67	68.45	4.0
F7	73.33	65.32	4.1
F8	35.29	64.24	4.2

*n=3; each samples was analysed in triplicate

Micromeretics Properties

The various formulations have the average particle size in the range of 994 – 1290 µm. The tapped density value ranged from 0.4-0.5 g/cm^3, bulk density in the range of 0.4 – 0.42 g/cm3, carr`s index is in between 5-15% and Hausner's ratio is in the range from 1.11- 1.14. All the formulation showed excellent flowability as expressed in terms of angle of repose which was found in between 19°- 35°.

Table 2: Micromeretic Properties of Beads

Sl. No.	Formulation Code	Average particle Size(µm) Mean S.D.	Tapped density (g / cm 2)	Bulk density (g / cmg^2)	% Compressibility Index	Hausner's Ratio	Angle of repose, θ
1.	F6	994	0.45 ± 0.001	0.40 ± 0.001	11.11	1.12	33.92
2.	F7	997.5	0.47 ± 0.002	0.42 ± 0.002	10.64	1.11	27.93
3.	F8	1290.5	0.48 ± 0.02	0.42 ± 0.02	12.5	1.14	19.38

Table 3: Swelling Ratio at Different Time Interval

Sl. No.	Formulation Code	Swelling Ratio at different time interval		
		60 Min	120 Min	180 Min
1.	F6	3.3	4.2	5.1
2.	F7	3.0	5.3	6.2
3.	F8	3.1	3.9	4.3

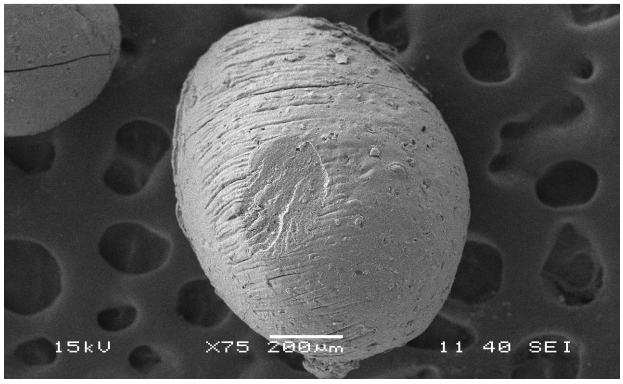


Figure 1: SEM of drug loaded Ca-alginate Beads

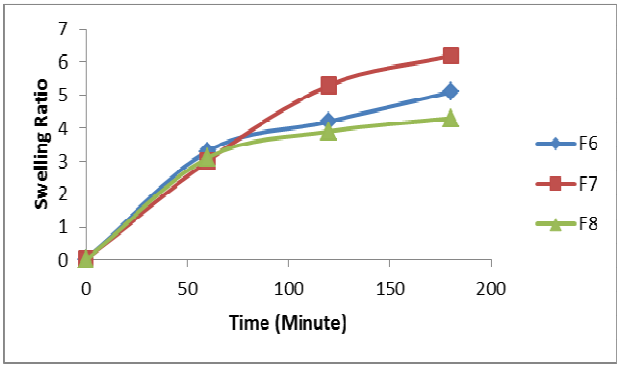


Figure 2: Graph showing Swelling Ratio in phosphate buffer (pH 6.8) Vs time relationship of extract loaded Ca-alginate beads

Surface Topography

The morphology of Ca-alginate beads was examined by scanning electron microscopy (SEM). The smooth surface of such beads as seen in Figure 1 shows the complete homogeneity of drug and polymer. The outer surface of beads was smooth and dense and showed good spherical geometry as shown in the figure.

Swelling Studies

Beads of all the batches showed swelling as given data in Table 3 and Figure 2, with rapid hydration and the swelling increased with time. This result may be because of maximum extent of cross linking that yielded compact beads.

Compatibility studies

From the manual compatibility studies it showed that a transparent film is formed which indicates that there is a good compatibility of drug and the polymer. Even after keeping the film for1.5 years, a non-crystalline state was identified which indicates the absence of birefringence of drug crystals.

The FTIR studies showed that there was no drug-polymer interaction as the spectroscopy of the drug (extract), polymer and formulation did not show any dissimilar spectra when compared and this is shown in Figure 3.

X-RD Studies

The X-ray diffraction spectra were recorded for plant extract, placebo Ca-alginate beads and drug loaded beads of formulation (F6) for investigating the dispersion of the drug in polymeric beads (Figure 4).

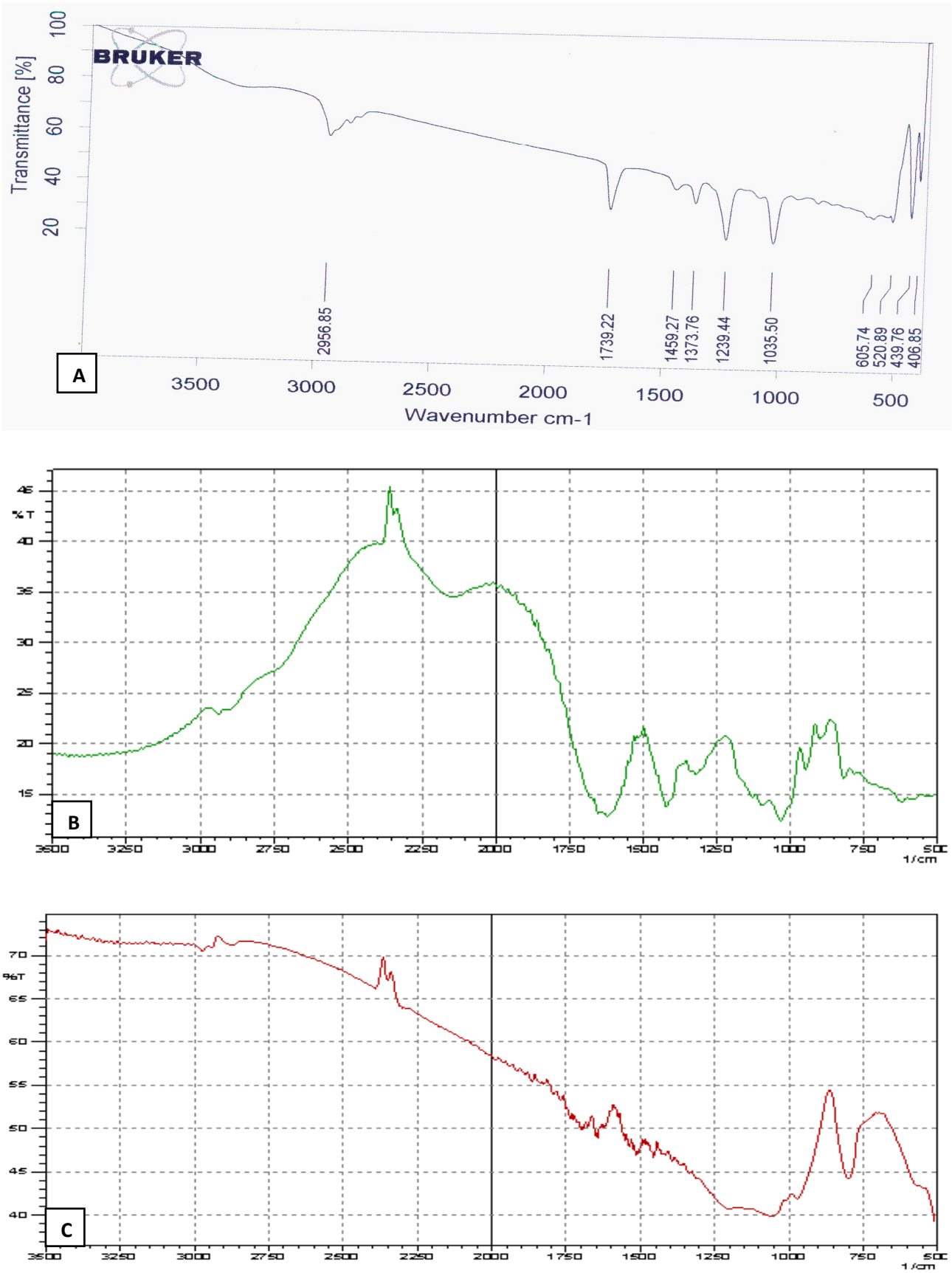


Figure 3: FT-IR Spectra of (A) Plant Extract, (B) Polymer, (C) Drug Loaded Beads

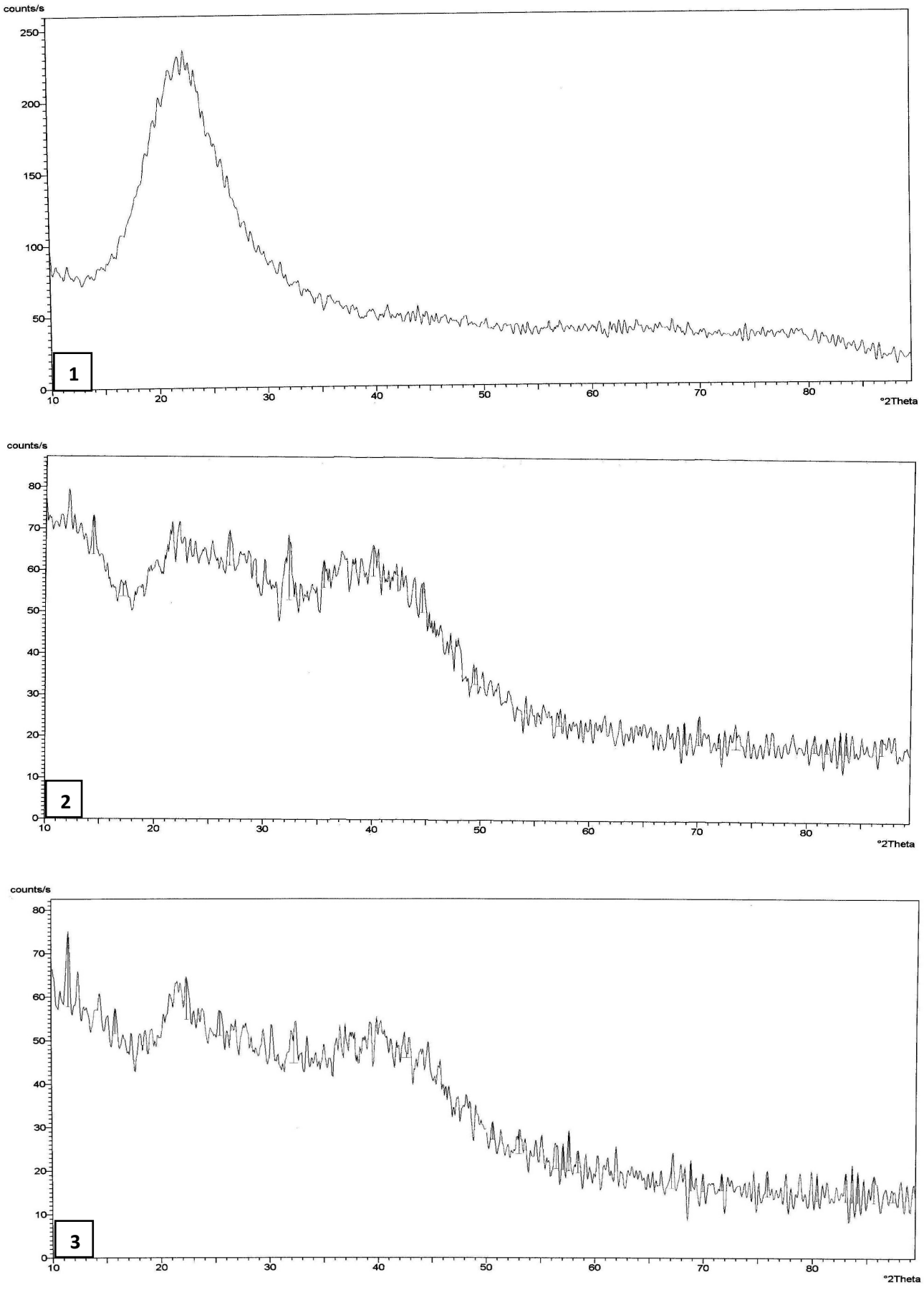
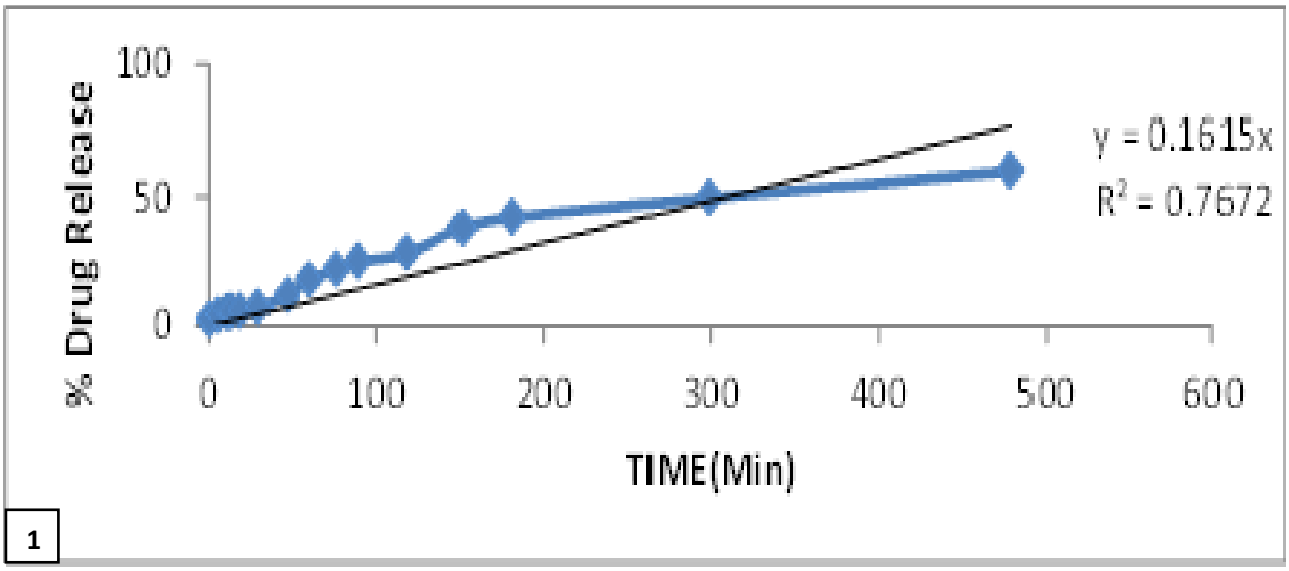
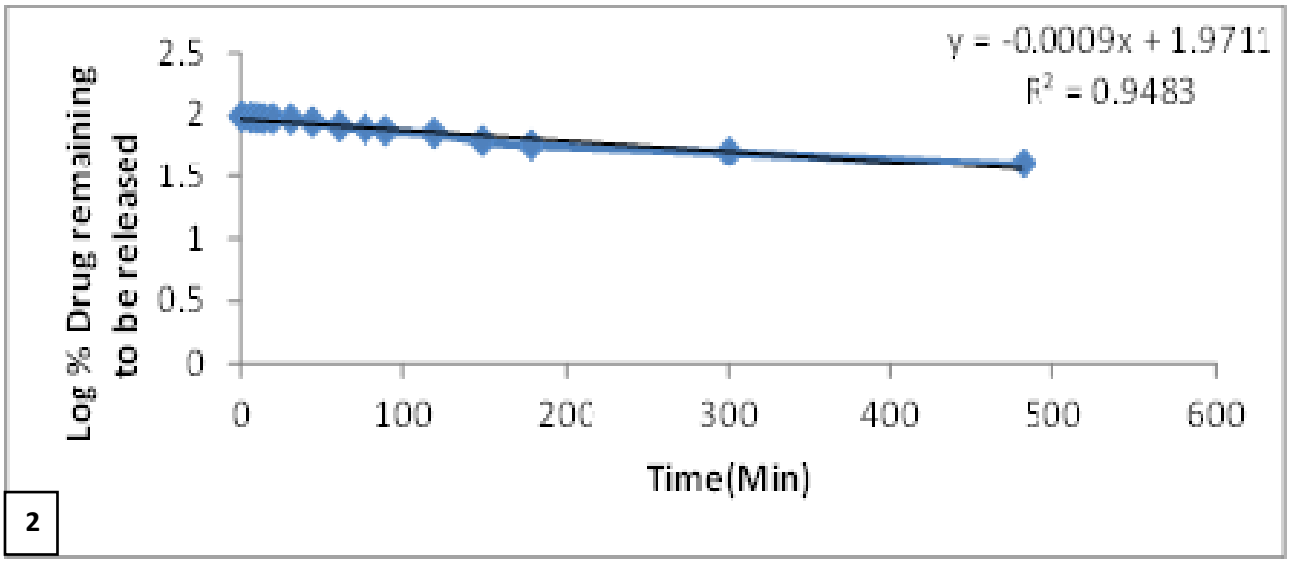


Figure 4: Powder X-RD Graph of (1) Plant extract, (2) Placebo Ca-alginate beads, (3) Drug loaded beads





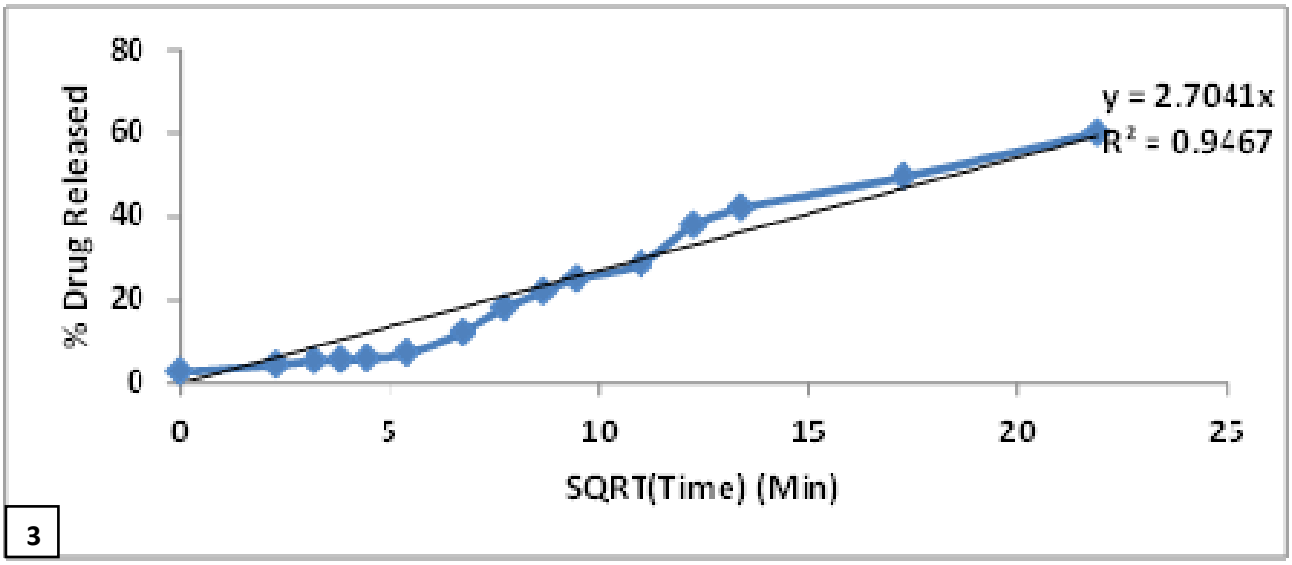


Figure 5: *In vitro* Dissolution Studies Showing (1) Zero Order Kinetics, (2) first-order Kinetics, (3) Higuchi Equation

The characteristic peak of the drug appeared at a diffraction angle 2θ at 21.9°due to its crystalline nature which disappears in drug loaded microsphere. The peaks of placebo Ca-alginate beads are similar to that of drug loaded beads. In the present study the characteristic peaks of drug overlapped with the noise of the coated polymer. This further confirms that the drug is molecularly dispersed in the polymer matrix and hence no crystals are found in the drug loaded matrix.

In-vitro Drug Release studies

Drug release from the beads was studied at pH-1.2 for 2 hrs followed by in Phosphate buffer at pH 7.4 for 6 hrs. The release profiles of beads are graphically presented in Figure 5. Result revealed that the *In-vitro* drug release of drug (Extract) at pH-1.2 for first two hours was very slow followed by instant release at pH 7.4 with negligible or no burst effect.

Release Kinetic

To investigate the drug release the data were fitted to models representing Zero order, First order and Higuchi's equation. The examination of coefficient of determination values indicated that the drug release follows First Order Kinetics followed by Zero order kinetics. The Higuchi's Plots showed that the correlation coefficient greater than Zero order kinetics and corresponds to first order kinetics indicating the drug release mechanism was diffusion controlled and follow first order kinetics.

CONCLUSION

Results obtained during this study have shown that petroleum ether extract of *Murraya koenigii* can be encapsulated in calcium alginate beads with good yields. The calcium alginate beads have also demonstrated their capability to control the release of the drug. Drug release in acidic medium is governed by the leaching of the drug due to its solubility and not by its gel properties of calcium alginate whereas the drug released in basic medium is controlled by swollen gel. Further studies are in progress in order to establish the pharmacological activity of the beads.

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