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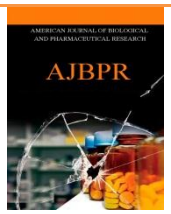

FORMULATION AND CHARACTERISATION OF FLOATING MICROSPHERES CONTAINING CINNARIZINE

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ABSTRACT

The purpose of present work was to develop floating microspheres of Cinnarizine for sustained drug delivery. The microsphere was prepared by solvent evaporation and ionic gelation technique. From the results it seem that formulation F4 was found to be satisfactory in terms of excellent micromeritic properties, yield of microsphere incorporation efficiency 82.32±1.8 and highest in vitro drug release of 100.02% in a sustained manner with constant fashion over extended period of time for 12 hrs. It was observed that concentration drug and polymer (HPMC-K 100) 1:4 and 40mg of $NaHCO_3$ and $CaCO_3$ affected all the evaluation parameter significantly.

INTRODUCTION

To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects [1]. There are various approaches in delivering a therapeutic substance to the target site in a sustained controlled release fashion. One such approach is using microspheres as carriers for drugs. The development of new delivery systems for the controlled release of drugs is one of the most interesting fields of research in pharmaceutical sciences.[1] A well designed controlled drug delivery system can overcome some of the problems of conventional therapy and enhance the therapeutic efficacy of a given drug. To obtain maximum therapeutic efficacy, it becomes necessary to deliver the agent to the target tissue in the optimal amount in the right period of time there by causing little toxicity and minimal side effects. There are various approaches in targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome, bio erodible polymer, implants, monoclonal antibodies and various particulate. One such approach is using microspheres as carriers for drugs. Microsphere can be used for the controlled release of drugs, vaccines, antibiotics, and hormones[2].

For example, by taking advantage of the characteristics of microspheres, beyond the basic benefits, the microspheres could provide a larger surface area and possess an easier estimation of diffusion and mass transfer behavior. Microspheres are defined as “Monolithic sphere or therapeutic agent distributed throughout the matrix either as a molecular dispersion of particles” (or) can be defined as structure made up of continuous phase of one or more miscible polymers in which drug particles are dispersed at the molecular or macroscopic level[4]. Microspheres are small spherical particles, with diameters in the micrometer range (typically 1 μm to 1000 μm)[5]. Microspheres are sometimes referred to as micro particles. Biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin, the synthetic

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polymer include poly lactic acid and polyglycolic acid. The solvents used to dissolve the polymeric materials chosen according to the polymer and drug solubility and stabilities, process safety and economic considerations [2]. Microspheres for oral use have been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation. In addition, multiparticulate delivery systems spread out more uniformly in the gastrointestinal tract. This results in more reproducible drug absorption and reduces local irritation when compared to single-unit dosage forms such as no disintegrating, polymeric matrix tablets. Unwanted intestinal retention of the polymeric material, which may occur with matrix tablets on chronic dosing, can also be avoided [3]. Microencapsulation is used to modify and retard drug release. Due to its small particle size, are widely distributed throughout the gastrointestinal tract which improves drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa [4].

Materials Used

Microspheres used usually are polymers. They are classified into two types.

1. Synthetic Polymers
2. Natural polymers

Synthetic polymers are divided into two types.

Non-biodegradable polymers

- Poly methyl methacrylate (PMMA)
- Acrolein
- Glycidyl methacrylate
- Epoxy polymers

i. Biodegradable polymers [5, 6]

- Lactides, Glycolides & their co polymers
- Poly alkyl cyano Acrylates
- Poly anhydrides

Natural polymers obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates [7, 8]

A) Proteins:

- Albumin
- Gelatin [9]
- Collagen

B] Carbohydrates:

- Agarose
- Carrageenan
- Chitosan [10]
- Starch

C)Chemically modified carbohydrates:

- Poly dextran [11]
- Poly starch.

TYPES OF MICROSPHERE

Bioadhesive Microspheres

Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc can be termed as bio adhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action [12-17].

Magnetic Microspheres

This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. The different types are therapeutic magnetic microspheres and diagnostic microspheres [18].

i. Therapeutic Magnetic Microspheres: It is used to deliver chemotherapeutic agent to liver tumor. Drugs like proteins and peptides can also be targeted through this system.

ii. Diagnostic Microspheres: It can be used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles supramagnetic iron oxides.

Floating microspheres

In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, if the system is floating on gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover it also reduces chances of striking and dose dumping. One another way it produces prolonged therapeutic effect and therefore reduces dosing frequencies [19, 20].

Polymeric Microspheres

The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and synthetic polymeric microspheres [21].

Biodegradable Polymeric Microspheres

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bioadhesive in nature. Biodegradable polymers prolongs the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium, results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. The main drawback is in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release [22].

ii. Synthetic Polymeric Microspheres

The interest of synthetic polymeric microspheres

are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particles drug delivery vehicles etc and proved to be safe and biocompatible. But the main disadvantage of these kinds of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

ADVANTAGES

1. Microspheres provide constant and prolonged therapeutic effect.
2. Reduces the dosing frequency and thereby improve the patient compliance.
3. They could be injected into the body due to the spherical shape and smaller size.
4. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
5. Microsphere morphology all owes a controllable variability in degradation and drug release [23]

LIMIATION

Some of the disadvantages were found to be as follows:

1. The modified release from the formulations.
2. The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
3. Differences in the release rate from one dose to another.
4. Controlled release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity.
5. Dosage forms of this kind should not be crushed or chewed.

CHARACTERISTICS OF MICROSPHERES

1. Microsphere size may be critical to the proper function of an assay, or it may be secondary to other characteristics. Considering traditional diagnostic methods, the test or assay format commonly dictates particle size, such as the use of very small spheres (~0.1- 0.4μm) to ensure satisfactory wicking in lateral flow tests, or the use of larger, cell-sized spheres (~4-10μm) for bead based flow cytometric assays.
2. Common microsphere compositions include polystyrene (PS), poly(methyl methacrylate) (PMMA), and silica. These materials possess different physical and optical properties, which may present advantages or limitations for different applications. Polymer beads are generally hydrophobic, and as such, have high protein binding abilities. However, they often require the use of some surfactant (e.g. 0.01-0.1% Tween® 20 or SDS) in the storage buffer to ensure ease of handling. During synthesis, functional monomers may be co-polymerized with styrene or methyl methacrylate to develop beads with surface reactive groups. Functional groups may be used in covalent binding reactions, and also aid in stabilizing the suspension. Silica microspheres are inherently hydrophilic and negatively charged. Consequently, aqueous silica suspensions rarely require use of surfactants or other stabilizers. Carboxyl- and amine functionalized silica spheres are available for use in common covalent coating protocols, and plain silica microspheres may be modified using a variety of silanes to generate functional groups or alter surface properties.
3. Microspheres may be coated with capture molecules, such as antibodies, oligonucleotides, peptides, etc. for use in diagnostic or separation applications. Microsphere coatings are typically optimized to achieve desired specific activity, while minimizing nonspecific interactions. Consideration should also be given to the required stability, development time frame and budget, and the specific biomolecule to be coated. These factors will aid in determining the most fitting coating strategy for both short- and long-term objectives. Standard microsphere products support three basic coating strategies: adsorption, covalent coupling, and affinity binding.
4. Many applications in the life sciences demand added properties, such as fluorescence or a visible color, or iron oxide inclusions for magnetic separations. Polymer spheres (and polymer based magnetic spheres) are often internally dyed via organic solvent swelling, and many standard products are available. Dye concentrations can be adjusted to produce beads with different intensities to meet special needs, such as QuantumPlex™ for multiplexed flow cytometric assays, or our Dragon Green or Flash Red Intensity Standards, which support imaging applications and associated instrument QC. Many surface- or internally labelled fluorescent beads are also available as specialized flow cytometry standards [24].

CRITERIA FOR MICROSPHERE PREPARATION

Incorporation of solid, liquid or gases into one or more polymeric coatings can be done by micro encapsulation technique [25]. The different methods used for various microspheres preparation depends on particle size, route of administration, duration of drug release and these above characters related to rpm, method of cross linking, drug of cross linking, evaporation time, co-precipitation etc [26]. Preparation of microspheres should satisfy certain criteria [27]

1. The ability to incorporate reasonably high concentrations of the drug.
2. Stability of the preparation after synthesis with a clinically acceptable shelf life.
3. Controlled particle size and dispersability in aqueous vehicles for injection.
4. Release of active reagent with a good control over a wide time scale.
5. Biocompatibility with a controllable biodegradability and
6. Susceptibility to chemical modification.

METHOD OF PREPERATION

The various methods of preparations are:

Emulsion Solvent Evaporation Technique

In this technique the drug is dissolved in polymer which was previously dissolved in chloroform and the resulting solution is added to aqueous phase containing 0 .2 % sodium of PVP as emulsifying agent. The above mixture was agitated at 500 rpm then the drug and polymer (eudragit) was transformed into fine droplet which solidified into rigid microspheres by solvent evaporation and then collected by filtration and washed with demineralised water and desiccated at room temperature for 24 hrs [28].

PURPOSE OF THE STUDY

Cinnarizine is a potent, highly selective antiemetic and anti vertigo drug. Cinnarizine is widely prescribed in control or prevents motion sickness, vertigo, nausea and vomiting. Cinnarizine is a BSC class-II having short biological half-life (2 to 3 hours). Moreover, the site of absorption of Cinnarizine is in the stomach.

The recommended adult oral dosage of cinnarizine is 20 mg, three to four times in day. Cinnarizine is weakly basic drug and it has good solubility in acidic pH, but significantly reduced solubility in alkaline medium. So, it is rapidly absorbed in stomach and upper part of gastrointestinal tract. Hence, the focus of present work is to prepared and evaluate gastro-retentive floating Microspheres of the cinnarizine to increase the residence time in the stomach and there by gives prolong action. Once in day cinnarizine gastro-retentive Microspheres offer better patient compliance through less frequent administration and lower the cost of total therapy. A Gastro-retentive Spheres of cinnarizine was prepared to give sustained effect for 12 hrs.

Several approaches are currently used to prolong gastric retention time. These include floating drug delivery systems, also known as hydro-dynamically balanced systems, swelling and expanding systems, polymeric bioadhesive systems, modified-shape systems, high-density systems, and other delayed gastric emptying devices. The principle of buoyant preparation offers a simple and practical approach, to achieve increased gastric residence time for the dosage form and sustained drug release.

The present investigation was carried out with following aims:

❖ To select and optimize polymer and other excipient concentration that gives best results with drug release profile as well as buoyant property of the formulations.

❖ To optimize best formula that actually effect in the development of sustained release gastro-retentive floating Microspheres by using Box-Behnken experimental design.

❖ To identify the factors affecting formulation of calcium alginate floating Microspheres of cinnarizine.

❖ To characterized the prepared Microspheres for Microspheres size, Percentage buoyancy, Incorporation efficiency, Production yield and Micromeritic properties, etc.

❖ To investigate the Kinetics of drug release for the prepared formulation by using various models like zero-order, first-order, Kosymer-Peppas Model and Higuchi kinetics.

❖ To optimized the process parameter for the preparation of Sodium alginate

Aim

The Aim of the present work is to formulate and Characterization of Cinnarizine Floating Microspheres

Objective

- Improves patient compliance by decreasing dosing frequency.
- Gastric retention time is increased because of buoyancy.
- Enhanced absorption of drugs which solubilise only in stomach
- Drug releases in controlled manner for prolonged period.
- Site-specific drug delivery.

PLAN OF WORK

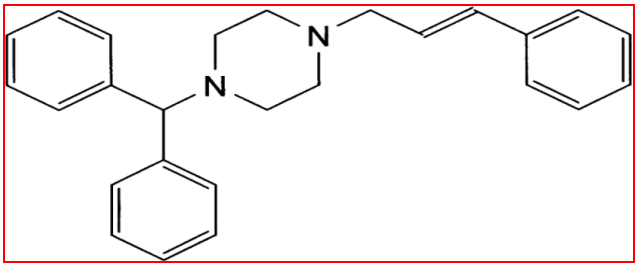
- Literature Review
- Selection of drug and polymers
- Drug and excipient compatibility studies
- Selection of suitable method based on Review
- Construction of standard graph
- Formulation and development of Floating Microspheres of Cinnarizine
- Evaluation parameters

CINNARIZINE

Category

Antiemetic Drug, Antinauseants Drug, Antivertigo Drugs, Anti Histaminic

Chemical structure



- **Chemical structure of Cinnarizine**
- **Physicochemical Property of Cinnarizine**
- **Chemical IUPAC Name** 1-benzhydryl-4-cinnamyl-piperazine
- **Chemical Formula** : C26H28N2
- **Molecular weight** : 368.514 g/mol

METHODOLOGY

Analytical method development

a) Determination of absorption maxima

100mg of Cinnarizine pure drug was dissolved in 15ml of Methanol and make up to 100ml with 0.1N HCL (stock solution-1). 10ml of above solution was taken and make up with100ml by using 0.1 N HCL (stock solution-2 i.e 100µg/ml). From this 10ml was taken and make up with 100 ml of 0.1 N HCl (10µg/ml). Scan the 10µg/ml using Double beam UV/VIS spectrophotometer in the range of 200 – 400 nm.

b) Preparation calibration curve:

100mg of Cinnarizine pure drug was dissolved in 15ml of Methanol and volume make up to 100ml with 0.1N HCL (stock solution-1). 10ml of above solution was taken and make up with100ml by using 0.1 N HCl (stock solution-2 i.e 100µg/ml). From this take 0.1, 0.2, 0.3, 0.4 and 0.5ml of solution and make up to 10ml with 0.1N HCl to obtain 2, 4, 6 ,8, and 10 µg/ml of Cinnarizine solution. The absorbance of the above dilutions was measured at 254nm by using UV-Spectrophotometer taking 0.1N HCl as blank. Then a graph was plotted by taking Concentration on X-Axis and Absorbance on Y-Axis which gives a straight line Linearity of standard curve was assessed from the square of correlation coefficient (R^2) which determined by least-square linear regression analysis. The experiment was preformed in triplicate and based on average absorbance; the equation for the best line was generated. The results of standard curve preparation are shown in table-5.1 & figure-6.3

Drug – Excipient compatibility studies

Fourier Transform Infrared (FTIR) spectroscopy

Drug excipient interaction studies are significant for the successful formulation of every dosage form. Fourier Transform Infrared (FTIR) Spectroscopy studies were used for the assessment of physicochemical compatibility and interactions, which helps in the prediction of interaction between drug and other excipients. In the current study 1:1 ratio was used for preparation of physical mixtures used for analyzing of compatibility studies. FT-IR studies were carried out with a Bruker, ATR FTIR facility using direct sample technique.

Preparation of microspheres

Microspheres are matrix systems that contains drug throughout their structure and are potential candidates for oral controlled release. Microspheres can be defined as solid.

spherical particles ranging from 1 to 1000µm in size. These particles contains of the drug which is the core material, and a coating material. The choice of methods for the preparation of microspheres depends on many factors such as the drug solubility, partition co efficient, Polymer composition, molecular weight etc.

The microsphere was prepared by solvent evaporation and ionic gelation technique. The 25 mg of cinnarizine was dispersed uniformly in aqueous mucilage of Sodium alginate. To this dispersion desired polymer was mixed in suitable proportion. Then, gas-forming agent such as Calcium carbonate and sodium bicarbonate was separately added to the solution. The resulting solution was dropped through a 26G syringe needle into 5% (w/v) glutaraldehyde/$CaCl_2$ solution which is prepared in water containing 10% (v/v) acetic acid. The process done with constant stirring (600rpm) at 60-70^{o}C. The solvent is slowly evaporated. The solution containing suspend microsphere was kept for 1.5 hr. To improve the mechanical strength of the microsphere and allowed to complete the reaction to produce gas. The fully formed microsphere were collected, washed with distilled water and subsequently air dried. The composition of floating microsphere are shown in Table 1.

Characterization of Cinnarizine Microspheres

Determination of mean particle size

The particle size was measured using an optical microscope, and the mean particle size was calculated by measuring 200 particles with the help of a calibrated ocular micrometer6. A small amount of dry microspheres was suspended in purified water (10 ml). A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing microspheres was mounted on the stage of the microscope and diameter of at least 100 particles was measured using a calibrated optical micrometer. The mean particle size was calculated and results are given in table-6.12 [10]

Incorporation Efficiency (IE)

To determine the incorporation efficiency, 10 mg microspheres were thoroughly triturated and dissolved in minimum amount of ethanol. The resulting solution was made up to 100 ml with 0.1 N HCl and filtered. Drug content was analyzed spectrophotometrically at 254 nm. The percentage incorporation efficiency percentagedrug loading were calculated using eq. 4.2 & results of Incorporation Efficiency are depicted in table-6.12 [11]

Percentage Buoyancy

The floating test was carried out to investigate the floatability of the prepared microspheres. To assess the floating properties, the microspheres were placed in 0.1 N HCl containing 0.02% v/v Tween 20 surfactant (pH 2.0, 100 ml) to simulate gastric conditions. The use of 0.02% Tween 20 was to account for the wetting effect of the natural surface-active agents, such as phospholipids in the GIT. The mixture was stirred at 100 rpm in a magnetic stirrer. After 12 h, the layer of buoyant microparticles was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in an oven at 65^{0}C until constant weight. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles. Despite the solution being stirred for 12 h, the

microspheres still floated, indicating that the microspheres exhibit an excellent buoyancy effect. Density values of the microspheres (<1.000 g/cm3) were less than that of the gastric fluid (1.004 g/cm3), further supporting the floating nature. The in vitro floating test was conducted on the drug-loaded microspheres. Results of Percentage buoyancy calculated using equation 4.3 & results of different batches is shown in table

Production yield Production yield of microspheres containing a drug was determined by the weight ratio of the dried microspheres to the loading amount of the drug and Polymer. Production yield was calculated using eq. 4.4 & results are depicted in table 6.12

In vitro drug release study

The release rate of Cinnarizine from microspheres was determined using USP dissolution testing apparatus I (Basket type). The dissolution test was performed using 900 ml of 0.1 N HCl, at 37 ± 0.5°C and 100 rpm. Microspheres equivalent to 25 mg were used for the test. Aliquots (5 ml) were withdrawn at hourly intervals for 12 hours. Samples were replaced by its equivalent volume of dissolution medium. The samples were filtered through Whatman filter paper and solutions were analyzed using UV spectrophotometer (Shimadzu 1700 UV/V is double beam Spectrophotometer Kyoto, Japan). Results of % drug release shown in table-6.12 [7]

***In vitro* drug release studies**

Apparatus --	USP-I, Basket Method
Dissolution Medium--	p H 0.1N HCl
RPM	-- 100
Sampling intervals (hrs)	--1, 2, 3, 4, 5, 6, 8, 10 & 12.
Temperature	--37°c $\pm$ 0.5°c

Micromeritic properties of microspheres

The microspheres are characterized by their micromeritic properties, such as particle size, tapped density, compressibility index, true density, and flow property. The tapping method was used to calculate tapped densities and percentage compressibility index. Tapped densities and percentage compressibility index can be calculated using equation 4.5 and 4.6

Here, V and V0 are, respectively, the volumes of the sample after and before the
standard tapping.

The angle of repose (ϕ) of the microspheres, which measures the resistance to particle flow, was measured using fixed funnel method and calculated as per following equation 4.7

Where 2H/D is the surface area of the free-standing height of the microsphere heap. that is formed after making the microspheres flow from the glass funnel.
Results of micromeritic properties like Angle of Repose, Compressibility Index, Tapped Density and True Density are shown in table-6.12

Drug Release Kinetics

In order to investigate the mechanism of drug release from microspheres of different optimized batches, the release data were analyzed with the following mathematical models

1) Zero-order equation

$$Q=Q0+k0t$$

Where, Qtis the amount of drug release in time t, Q0 is the initial amount of drug in the solution (most times, Q0= 0) and k0 is the zero-order release rate.

2) First-order equation

$$\ln Qt=\ln Q0+k1t$$

Where, Qt is the amount of drug released in time t, Q0 is the initial amount of drug in the solution and k1 is the first-order release rate constant.

3) Higuchi s equation

$$Q= kH\ t½$$

Where, Q is the amount of drug release at time t, and kH is the Higuchi diffusion rate constant.

4) Koresmeyer's peppas equation

Further, to confirm the mechanism of drug release, the first 60 % of drug release was fitted in Korsmeyer-Peppas model following equation

$$Mt= M\infty\ Kt$$

Where, Mt is the amount of drug released at time t, M∞ is the amount of drug released after infinite time, Kt is a kinetic constant and n is the diffusional exponent indicative of the drug release mechanism. and is calculated from the slope of the plot of log of fraction of drug released
(Mt / Mα) vs. log of time (t)

RESULTS AND DISCUSSION

The present work was designed to developing floating Microspheres of Cinnarizine using various polymers. All the formulations were evaluated for physicochemical properties and *in vitro* drug release studies.

Analytical Method

Standard graph of Cinnarizine in 0.1N HCL

The scanning of the 10µg/ml solution of Cinnarizine in the ultraviolet range (200-400 nm) against 0.1 N HCL the maximum peak observed at λ_{max} as 254 nm. The standard concentrations of Cinnarizine (10-50 µg/ml) was prepared in 0.1N HCL showed good linearity with R^2 value of 0.999, which suggests that it obeys the Beer--Lamberts law.

From the FTIR data it was evident that the drug and excipients doses not have any interactions. Hence, they were compatible

EVALUATION PARAMETERS

Micromeritic properties of Microspheres

The Micromeritic properties of different batch are shown in above table. The mean diameter of the CNZ-loaded Sodium alginate microspheres, the mean diameter of batch 1 to 10 ranges between 221.98±5.21- and 475.36±3.74µm. The average size of the microspheres increased slightly as the amount of polymer concentration increased. The hardening agent caused a decrease in bead size as it promoted the formation of cross-links between the alginate molecules. The tapped density of beads of different batch 1-10 ranges between 0.286±0.04- 0.361±0.07gm/ml respectively. The Compressibility Index ranges between 7.12±0.13-12.9±0.07gm/ml, shows that all the formulation preparations were good flowability. The Hausner's ratio of different batch ranges between 1.24±0.55-1.84±0.07. The Hausner's ratio result shows that all the preparations were good flowability.

Drug Entrapment Efficiency (EE) and floating property

The floating property of the microspheres was calculated from the fractional amount of drug and polymer density of the microspheres. As shown in above table the Floating efficiency of the sodium alginate microspheres. The floating agent sodium bicarbonate containing (batch 1-5) ranges from 67.33±0.054and 68.24±0.05% and floating agent Calcium Carbonate containing formulations (batch 6-10) shows from 56.24±0.65-62.76±0.25.

The % drug release of formulations (F1 to F5) containing HPMC-K 100 depends on the concentration of polymer. The concentration of HPMC-K 100 1:1, 1:2 and 1:3was unable to retard the drug release up to desired time. When the concentration of polymer increased to 1:4 was able to retard the drug up to 12 hours. In F5 formulation 1:5 ratio (drug: polymer) ratio was more retardation, maximum drug release was showed after 12 hours.

The % drug release of F6 to F10 formulations depends on polymer ratio HPMC-K 4M and the floating agent Calcium carbonate. The concentration of HPMC-K 4M 1:1 to 1:4 ratios was unable to retard the drug release up to desired time. In F10 formulations, HPMC-K 4M contain 1:5 ratio showed maximum % drug release i.e 99.90% at 12 hours.

Hence based on dissolution data of 10 formulations, F4 and F10 formulations showed better release up to 12 hours. Among these formulations F4 formulation having the low concentration of polymer compare to F10 and it showed the drug release within the Specified limits. So F4 formulation is optimized formulation.

Pharmacokinetics

Application of Release Rate Kinetics to Dissolution Data

Data of *in vitro* release studies of formulations which were showing better drug release were fit into different equations to explain the release kinetics of Tramadol Hydrochloride release from Sustained tablets. The data was fitted into various kinetic models such as zero, first order kinetics; higuchi and korsmeyer peppas mechanisms and the results were shown in below table

Based on the data above results the optimized formulation followed Higuchi release kinetics.

SUMMARY

The present study reports the development of drug-loaded floating sustained release Microspheres of cinnarizine by "Ionotropic Gelation by solvent evaporation Technique by using various polymers HPMC-K100 and HPMC-K4M. From the above result and discussion, it was concluded that batch no. F4 and F10 gives good result in micromeritic properties that required in the present work. The production yield of batch no. F4 and F10 were highest comparison to other formulation. The main important things in this study was that the floating and incorporation efficiency of microspheres. In this present work, required low floating lag time and higher entrapment efficiency, that seen in the batch no. F4 and F10 prepared by Ionic gelation and solvent evaporation technique. The Spheres having various densities due to various concentrations polymer by changing the floating agent concentration $NaHCO_3$ and $CaCO_3$ beads floated immediately and remained floating for 12 hours. Beads prepared by ionic gelation by solvent evaporation technique give good encapsulation efficiencies and micromeritic properties for formulation as single-unit dosage forms. It was found that Batch F4 with HPMC-K100 1:4 give sustained released effect for 12 hrs with more than 99% drug release. Batch no.F4 prepared by "Ionic-Gelation by solvent evaporation Technique", have lower densities, exhibits buoyancy and retain in the gastric environment for more than 10 h. Thus, major advantages of the system include: (i) Ease of preparation, (ii) Good buoyancy, (iii) High encapsulation efficiency, and (iv) Sustained drug release over several hours. Further study includes applying Box–Behnken experimental design to the optimized formula (i.e. batch no.F4) and process parameter optimization. Formula optimized batch no.F4 would be used in further study.

Table 1. Microsphere property

S.No	Property	Consideration
1.	Size Diameter	Uniformity/distribution
2.	Composition	Density, Refractive Index, Hydrophobicity /hydrophilicity Nonspecific binding Auto fluorescence
3.	Surface Chemistry	Reactive groups Level of functionalization Charge
4.	Special Properties	Visible dye/fluorophore Super-paramagnetic

Table 2. Composition of Floating Microspheres

Formulation code	Drug (mg)	Dispersing agent	Drug : Polymer		Floating agents			Cross linking agent
	Cinnarizine	Na alginate (w/v)	HPMC-K 100	HPMC-K4 M	$NaHCO_3$ (mg)	$CaCO_3$ (mg)	Citric acid	Glutaraldehyde (%)
F1	25	5	1:1	--	10	--	5	5
F2	25	5	1:2	--	20	--	5	5
F3	25	5	1:3	--	30	--	5	5
F4	25	5	1:4	--	40	--	5	5
F5	25	5	1:5	--	50	--	5	5
F6	25	5	--	1:1	--	10	5	5
F7	25	5	--	1:2	--	20	5	5
F8	25	5	--	1:3	--	30	5	5
F9	25	5	--	1:4	--	40	5	5
F10	25	5	--	1:5	--	50	5	5

Table 3. Scale for compressibility index

S.No:	Compressibility Index	Category
1.	5-15	Excellence
2.	12-16	Good
3.	18-21	Fair
4.	23-35	Poor
5.	36-38	Very Poor
6.	>40	Extremely poor

Table 4. Scale for angle of repose

S. No :	Angle of Repose	Category
1.	25-30	Excellence
2.	30-35	Good
3.	35-40	Fair
4.	40-45	Poor
5.	45-50	Very Poor

Table 5. Standard curve of cinnarizine in 0.1 N HCl

S.No	Concentration mcg/ml	Absorbance			
		I	II	III	Avg
1.	**0**	0	0	0	0
2.	**2**	0.174	0.173	0.174	0.174
3.	**4**	0.325	0.325	0.325	0.325
4.	6	0.465	0.466	0.467	0.466
5.	8	0.619	0.618	0.618	0.618
6.	10	0.777	0.779	0.778	0.778

Table 6. Evaluation of Microspheres.

Batch No:	Mean Particle size(µm)	Bulk Density (gm/ml)	Carr's Index	Hausner's ratio	Angle of repose (θ)
F1	221.98±5.21	0.286±0.04	7.12±0.13	1.24±0.55	23.16±0.31
F2	276.55±3.47	0.307±0.06	9.82±0.21	1.46±0.21	23.94±0.36
F3	320.36±4.13	0.323±0.01	10.52±0.03	1.62±0.03	24.14±0.05
F4	391.39±2.78	0.342±0.05	12.1±0.05	1.79±0.05	24.9±0.07
F5	472.96±3.77	0.354±0.03	12.9±0.07	1.84±0.07	23.16±0.08
F6	233.17±4.21	0.311±0.08	8.22±0.23	1.32±0.08	25.03±006
F7	291.16±5.22	0.323±0.07	9.48±0.12	1.44±0.04	24.16±0.08
F8	361.66±3.74	0.332±0.04	10.38±0.13	1.52±0.02	24.91±0.075

F9	401.33±4.41	0.349±0.02	11.62±0.25	1.64±0.06	23.25±0.24
F10	475.36±3.74	0.361±0.07	11.01±0.08	1.70±0.07	24.56±0.42

Table 7. Result of mean Particle Size, Buoyancy % and Encapsulation efficiency%

Batch No:	Mean Particle size(µm)	*In vitro* Buoyancy (in sec)	Encapsulation efficiency%
F1	221.98±5.21	69.2±0.07	57.6±0.03
F2	276.55±3.47	68.24±0.05	68.61±1.57
F3	320.36±4.13	67.33±0.054	77.65±0.44
F4	391.39±2.78	66.87±0.085	82.32±1.8
F5	472.96±3.77	65.78±0.45	84.6±1.3
F6	233.17±4.21	57.56±0.78	54.75±1.33
F7	291.16±5.22	56.24±0.65	65.61±2.5
F8	361.66±3.74	56.37±0.84	73.74±0.63
F9	401.33±4.41	58.96±0.75	79.39±2.7
F10	475.36±3.74	62.76±0.25	88.6±1.4

Table 8. *In vitro* drug release of Sodium Alginate HPMC-K 100 containing cinnarizine F1 to F5 formulations

TIME (hr)	CUMULATIVE PERCENT DRUG RELEASED				
	F1	**F2**	**F3**	**F4**	**F5**
0	0	0	0	0	0
1	31.3	28.6	24.3	19.4	17.7
2	43.5	35.89	30.5	26.8	24.1
3	55.6	48.77	42.6	37.4	35.9
4	69.7	64.2	55.74	49.3	41.8
5	83.4	71.8	66.3	58.7	49.4
6	97.6	79.6	73.85	64.8	56.3
8	99.9	91.63	87.6	76.2	70.6
10		99.7	97.83	89.6	84.5
12			99.13	100.02	91.3

Table 9. *In vitro* drug release of sodium Alginate HPMC-K 4 **containing cinnarizine F6 to F10 formulations**

TIME (hr)	CUMULATIVE PERCENT DRUG RELEASED				
	F6	**F7**	**F8**	**F9**	**F10**
0	0	0	0	0	0
1	38.31	32.33	27.67	23.31	20.15
2	49.91	45.54	36.88	30.55	27.25
3	61.3	55.61	45.72	41.63	38.47
4	74.3	68.74	62.16	56.74	50.68
5	89.6	79.40	72.88	67.37	59.86
6	100.0	94.65	82.65	74.85	65.72
8		99.98	93.67	88.68	77.32
10			99.75	97.83	90.70
12				99.13	99.90

Table 10. Release kinetics data for optimized formulation (F4)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	PEPPAS log Q/100	% Drug Remaining
0	**0**	0			2.000		100
19.4	**1**	1.000	1.288	0.000	1.906	-0.712	80.6
26.8	**2**	1.414	1.428	0.301	1.865	-0.572	73.2
37.4	**3**	1.732	1.573	0.477	1.797	-0.427	62.6
49.3	**4**	2.000	1.693	0.602	1.705	-0.307	50.7
58.7	**5**	2.236	1.769	0.699	1.616	-0.231	41.3
64.8	**6**	2.449	1.812	0.778	1.547	-0.188	35.2
76.2	**8**	2.828	1.882	0.903	1.377	-0.118	23.8

89.6	**10**	3.162	1.952	1.000	1.017	-0.048	10.4
100.02	**12**	3.464	2.000	1.079	#NUM!	0.000	-0.02

Fig 1. Polymers used in Microspheres Development

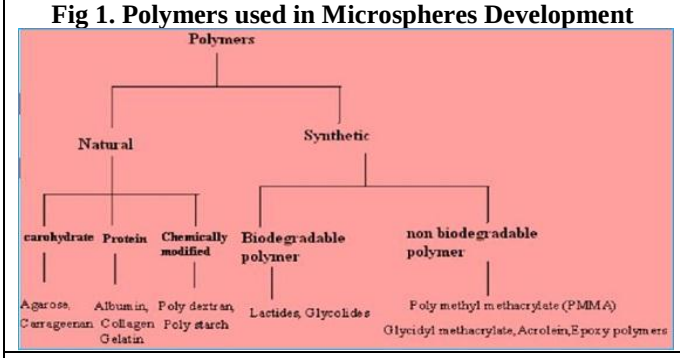


Fig 2. Microspheres by Double Emulsion Technique

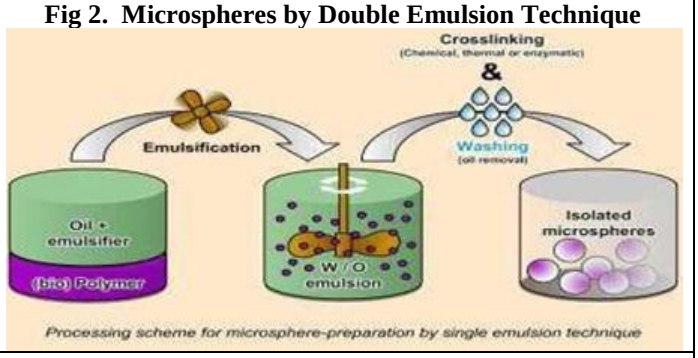


Fig 3. Microspheres by Single Emulsion Technique

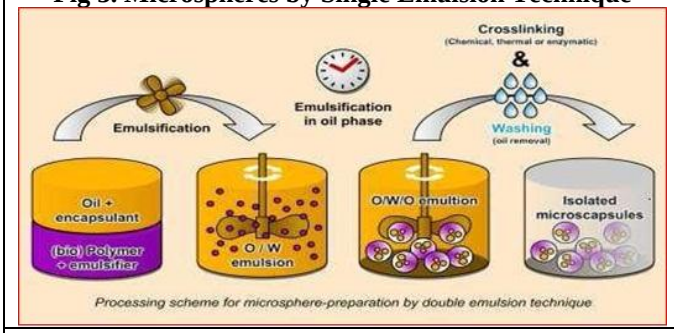


Fig 4. Microspheres by Spraying Drying Technique

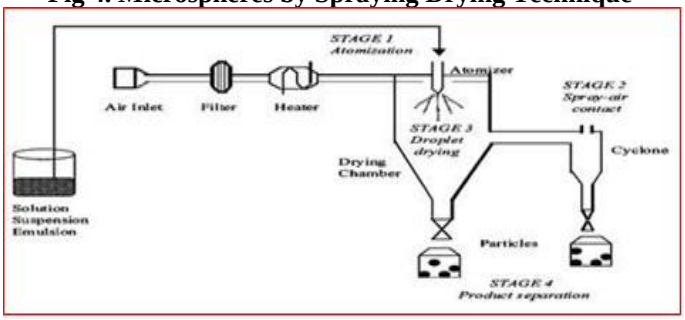


Fig 6. Calibration curve of Cinnarizine in 0.1 N HCL at 254nmDrug and Excipient Compatibility Studies

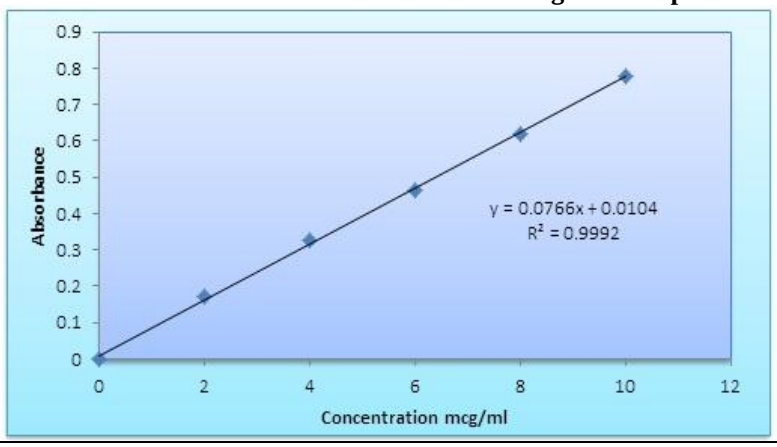


Fig 7. FTIR Graph of Pure Drug

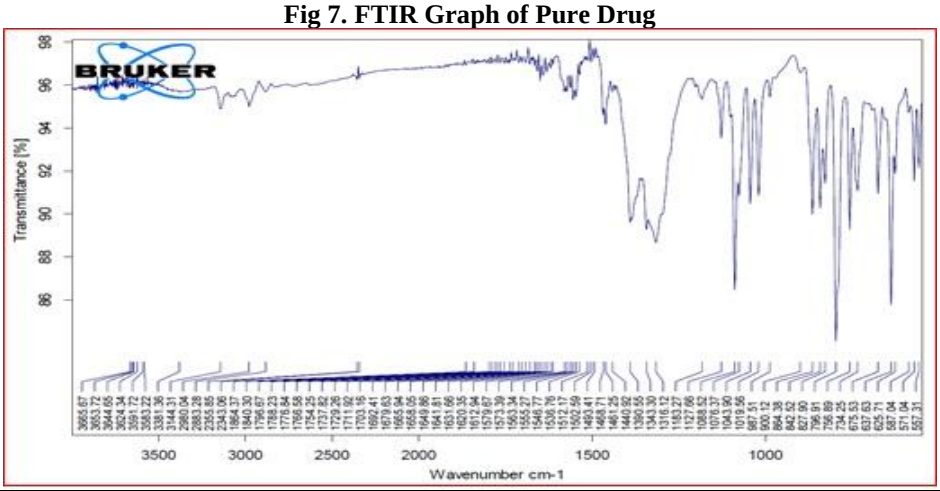


Fig 8. FTIR Graph of Optimised Formulation

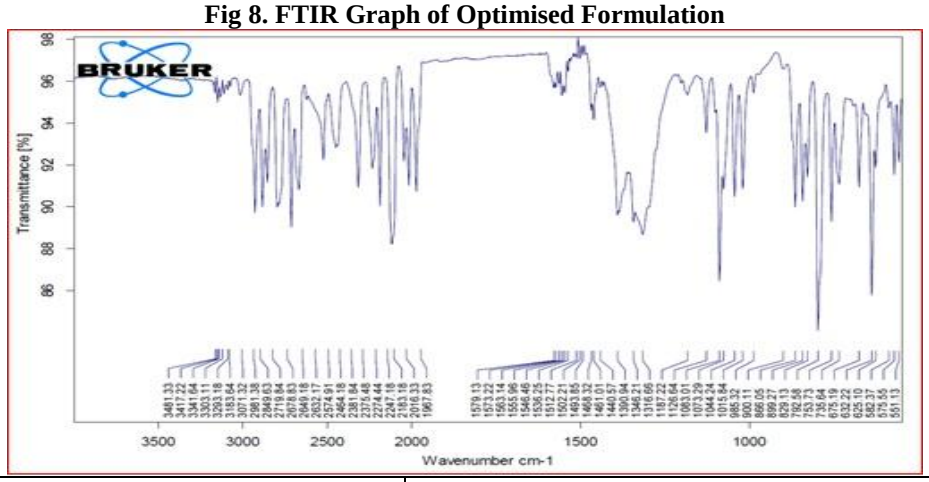


Fig 9. Dissolution study of Cinnarizine Floating Microspheres (F1 to F5) with Sodium bi carbonate

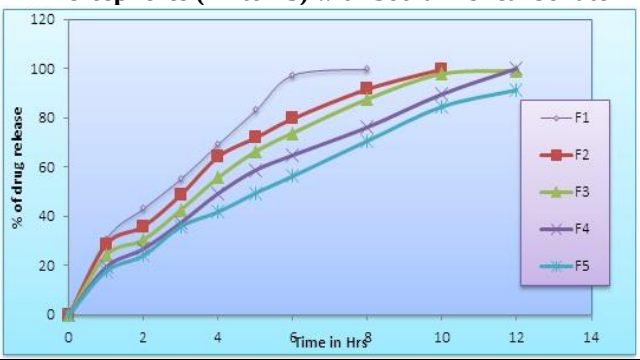


Fig 10. Dissolution study of Cinnarizine Floating Microspheres (F6 to F10) with Calcium Carbonate and HPMC-K 4M

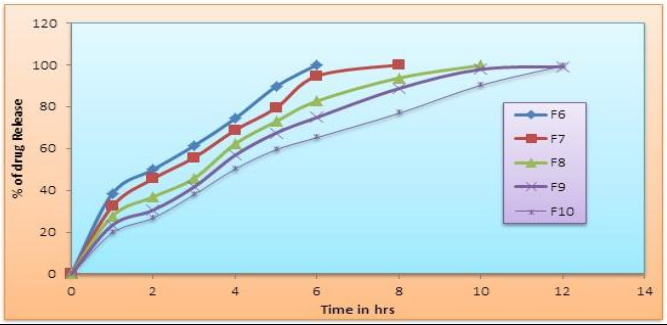


Fig 11. Graph of zero order kinetics

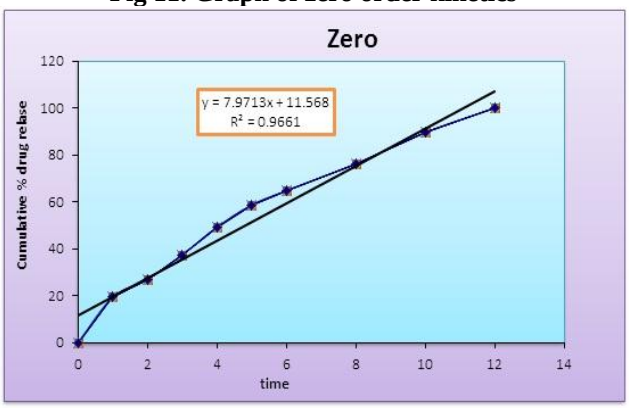


Fig 12. Graph of higuchi release kinetics

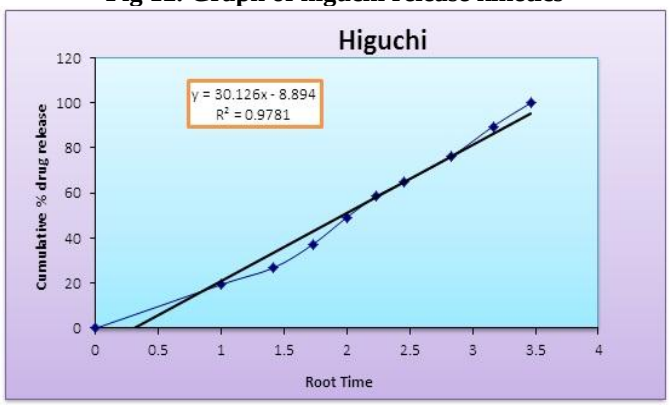


Fig 13. Graph of peppas release kinetics

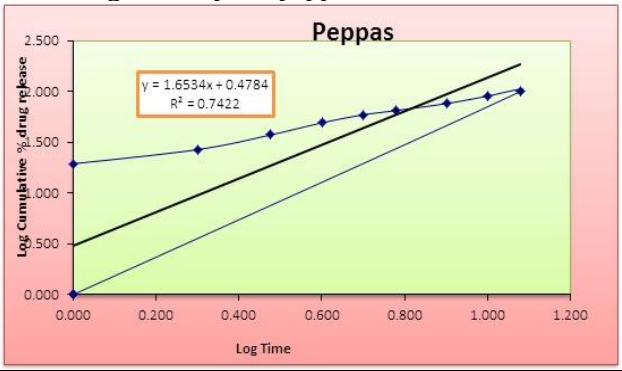
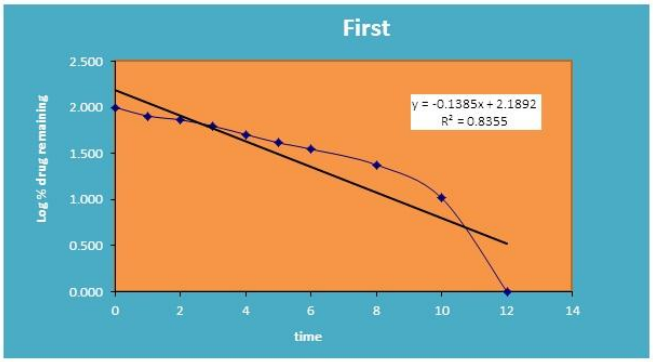


Fig 14. Graph of first order release kinetics



CONCLUSION

The purpose of present work was to develop floating microspheres of Cinnarizine for sustained drug delivery. From the results it seem that formulation F4 was found to be satisfactory in terms of excellent micromeritic properties, yield of microsphere incorporation efficiency 82.32±1.8 and highest in vitro drug release of 100.02% in a sustained manner with constant fashion over extended period of time for 12 hrs. It was observed that concentration drug and polymer (HPMC-K 100) 1:4 and 40mg of $NaHCO_3$ and $CaCO_3$ affected all the evaluation parameter significantly. Hence the prepared floating microspheres of Cinnarizine may prove to be potential candidate for safe and effective sustained drug delivery.

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