FORMULATION AND EVALUATION OF CYTARABINE MICROSPHERES FOR SUSTAINED DRUG DELIVERY

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Abstract

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. Aim of the study is to formulate Cytarabine based microspheres using different polymers by solvent evaporation technique. Six batches of formulations, of drug: polymer ratio i.e. 1:1,1:2 and 1:3, were prepared and evaluated for particle size, % yield, drug content, drug entrapment efficiency, Swelling studies, *In-vitro* mucoadhes.ion test, *in vitro* drug release and Flow property had shown satisfactory results. The IR Spectra's revealed that, there is no interaction between polymer and Cytarabine. The polymer used is compatible with the Cytarabine. The basis of release data and graphical analysis formulation C3 showed a good sustained release profile with maximum entrapment efficiency because of high polymer concentration. Hence, from all the above obtained data it can be summarized that it is possible to formulate a promising sustained release mucoadhecive microspheres of Cytarabine by solvent evaporation technique using an ideal polymer like HPMC15cps +Carbopol 934p.

Key words Solvent evaporation, sustained release, entrapment, swelling, Cytarabine, controlled rellease

1. INTRODUCTION

Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half-life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. Rational

approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site-specific manner.

One of the most challenging areas of research in pharmaceuticals is the development of novel delivery systems for the controlled release of drugs and their delivery at the targeted site in the body to minimize the side effects and enhance the therapeutic efficacy of drugs[2,3]. The basic principle behind the controlled drug delivery system is to optimize the biopharmaceutic, pharmacokinetic and pharmacodynamics properties of drug in such a way that its efficacy is maximized by reducing side effects, dose frequency and cure the disease in short time by using low amount of drug administered with the most suitable route [4,5, 6,7].

In 1997, first time microspheres were prepared for the sustained action of the drug. Since then, microparticles have proved to be good candidates for sustained and controlled release of drug and become an alternative of conventional or immediate release formulations. These particles are also a beneficial to deliver the active pharmaceutical ingredients which are pharmacologically active but are difficult to deliver due to limited solubility in water. In such type drugs, the attainment of required therapeutic concentrations of drug in the blood is problematic enabling to attain higher C_{max}, T_{max} and area under curve. Microsphere – based formulations can release a constant amount of drug in the blood or to target drugs to specific site in the body [8,9].

For many decades, medication of an acute disease or a chronic disease has been accomplished by delivering drugs to the patients via various pharmaceutical dosage forms like tablets, capsules, pills, creams, ointments, liquids, aerosols, injectables and suppositories as carriers. To achieve and then to maintain the concentration of drug administered within the therapeutically effective range needed for medication, it is often necessary to take this type of drug delivery systems several times in a day. This results in a fluctuated drug level and consequently undesirable toxicity and poor efficiency. This factor as well as other factors such as repetitive dosing and unpredictable absorption leads to the concept of controlled drug delivery systems. The word new or novel in the relation to drug delivery system is a search for something out of necessity. An appropriately designed sustained or controlled release drug delivery system can be major advance toward solving the problem associated with the existing drug delivery system.

The objective of controlled release drug delivery includes two important aspects namely spatial placement and temporal delivery of drug. Spatial placement relates to targeting a drug

to a specific organ or tissue, while Temporal delivery refers to controlling the rate of drug delivery to the target tissue.

Oral controlled release dosage forms have been developed over the past three decades due to their considerable therapeutic advantages such as ease of administration, patient compliance and flexibility in formulation. However, this approach is be filled with several physiological difficulties such as inability to restrain and locate the controlled drug delivery system within the desired region of the gastrointestinal tract (GIT) due to variable motility and relatively brief gastric emptying time (GET) in humans which normally averages 2-3 h through the major absorption zone, i.e., stomach and upper part of the intestine can result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose.[10,11]

The objective in designing a controlled release system is to deliver the drug at a rate necessary to achieve and maintain a constant drug blood level. This rate should be similar to that achieved by continuous intravenous infusion where a drug is provided to the patient at a rate just equal to its rate of elimination. This implies that the rate of delivery must be independent of the amount of drug remaining in the dosage form and constant over time, i.e release from the dosage form should follow zero-order kinetics.[12]

1.1.DEFINITION AND GENERAL DESCRIPTION:

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 µm. They are made of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats, and waxes. The natural polymers include albumin and gelatin9-10 the synthetic polymers include polylactic acid and polyglycolic acid. Fig. 1.2 shows two types of microspheres: Microcapsules, where the entrapped substance is completely surrounded by a distinct capsule wall, and micromatrices, where the entrapped substance is dispersed throughout the microsphere matrix.

Microspheres are small and have large surface to volume ratios. At the lower end of their size range they have colloidal properties. The interfacial properties of microspheres are extremely important, often dictating their activity.

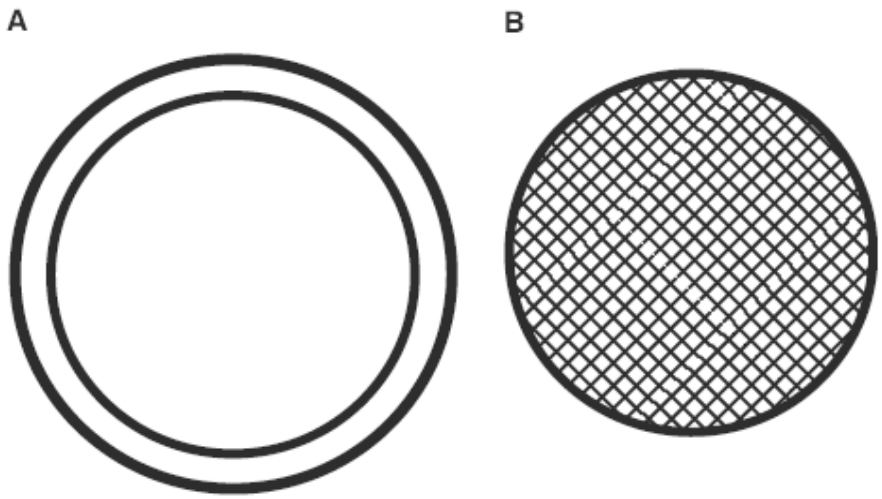

Fig : 1 : Schematic diagram illustrating microspheres. (A) Microcapsule consisting of an encapsulated core particle and (B) micromatrix consisting of homogeneous dispersion of active ingredient in particle

Advantages of microspheres [18]

1. They provide protection before after administration for unstable drug.
2. They reduced concentration of drug at site other than the tissue or the target organ.
3. Decrease dose and toxicity.
4. Particle size reduction for enhancing solubility of poorly soluble drugs.
5. Provide constant and prolonged therapeutic effect.

Limitation:[19]

Some of the disadvantages were found to be as follows

1. The costs of the materials and processing of the controlled release preparation, are substantially higher than those of standard formulations.
2. The fate of polymer matrix and its effect on the environment.
3. The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
4. Reproducibility is less.
5. Process conditions like change in temperature, pH, solvent addition, and evaporation/agitation may influence the stability of core particles to be encapsulated.
6. The environmental impact of the degradation products of the polymer matrix produced in response to heat, hydrolysis, oxidation, solar radiation or biological agents.

TYPES OF MICROSPHERES

MUCOADHESION / BIOADHESION:[20]

Mucoadhesive drug delivery system are the systems which utilizes the property of bio adhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time.

The term "mucoadhesion" was coined for the adhesion of the polymers with the surface of the mucosal layer. Bio adhesions are a phenomenon in which two materials at least one of which is biological and are held together by means of interfacial forces. In biological systems, bio adhesion can be classified into 3 types:

1. Adhesion between two biological phases, for example, platelet aggregation and wound healing
2. Adhesion of a biological phase to an artificial substrate, for example, cell adhesion to culture dishes and bio film formation on prosthetic devices and inserts
3. Adhesion of an artificial material to a biological substrate, for example, adhesion of synthetic hydrogels to soft tissues and adhesion of sealants to dental enamel.

For drug delivery purposes, the term bio adhesion implies attachment of a drug carrier system to a specified biological location. The biological surface can be epithelial tissue or the mucus coat on the surface of a tissue. If adhesive attachment is to a mucus coat, the phenomenon is referred to as mucoadhesion / mucoadhesion as the interaction between a mucin surface and a synthetic or natural polymer. In bio adhesion, the polymer is attached to the biological membrane.

Magnetic microspheres[21]

This kind of delivery system is very much important which localises 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 type are Therapeutic magnetic microspheres are used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.

Floating microspheres[22]

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, increases gastric residence and fluctuation in

plasma concentration. It also reduces chances of striking and dose dumping and produces prolonged therapeutic effect. Drug (ketoprofen) given through this form.

Radioactive microspheres [23]

Radio emobilisation therapy microspheres sized 10-30 nm are of larger than capillaries and gets tapped in first capillary bed when they come across. They are injected to the arteries that lead to tumour of interest. So these radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. It differs from drug delivery system, as radio activity is not released from microspheres but acts from within a radioisotope typical distance and the different kinds of radioactive microspheres are α emitters, β emitters, γ emitters.

Polymeric microspheres

The different types of polymeric microspheres can be classified as:

Biodegradable polymeric microspheres [24]

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.

Synthetic polymeric microspheres [25]

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 kind of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

MATERIALS AND METHODS[26]

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

- Synthetic Polymers
- Natural polymers

Synthetic polymers are divided into two types.

a) Non-biodegradable polymers

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

b) Biodegradable polymers

Lactides, Glycolides & their co polymers, Poly alkyl cyanoacrylates, Poly anhydrides

Natural polymers obtained from different sources like proteins, carbohydrates and chemically modified carbohydrates.

Proteins: Albumin, Gelatin, Collagen

Carbohydrates: Agarose, Carrageenan, Chitosan, Starch

Chemically modified carbohydrates: Poly dextran, Poly starch.

METHODS OF PREPERATION

1. Emulsion solvent evaporation technique
2. Emulsion cross linking method
3. Coacervation method
4. Spray drying technique
5. Emulsion-solvent diffusion technique
6. Multiple emulsion method
7. Ionic gelation
8. Hydroxyl appetite (HAP) microspheres in sphere morphology

Preparation of microspheres should satisfy certain criteria:

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 dispersibility 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.

Emulsion solvent evaporation technique[25]

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. Aceclofenac microspheres were prepared by this technique.

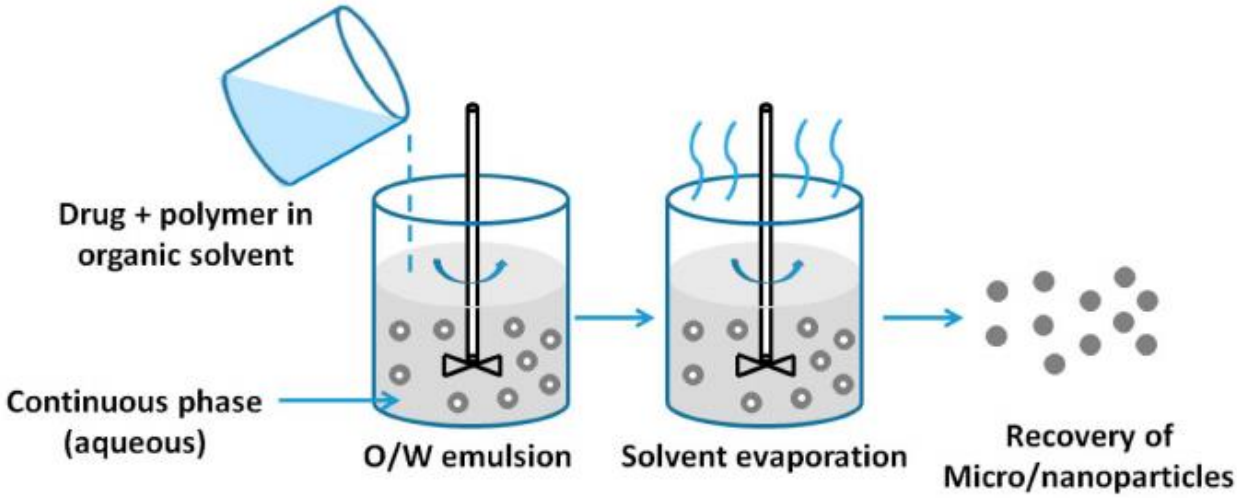

Fig 2: Emulsion solvent evaporation technique

Emulsion cross linking method[27]

In this method drug was dissolved in aqueous gelation solution which was previously heated for 1 hr at 40^{o}C. The solution was added drop wise to liquid paraffin while stirring the mixture at 1500 rpm for 10 min at 35oC, results in w/o emulsion then further stirring is done for 10 min at 15^{o}C. Thus the produced microspheres were washed respectively three times with acetone and isopropyl alcohol which then air dried and dispersed in 5mL of aqueous glutaraldehyde saturated toluene solution at room temperature for 3 hrs for cross linking and then was treated with 100mL of 10mm glyciene solution containing 0.1%w/v of tween 80 at 37^{o}C for 10 min to block un reacted glutaraldehyde. Examples for this technique is Gelatin A microspheres.

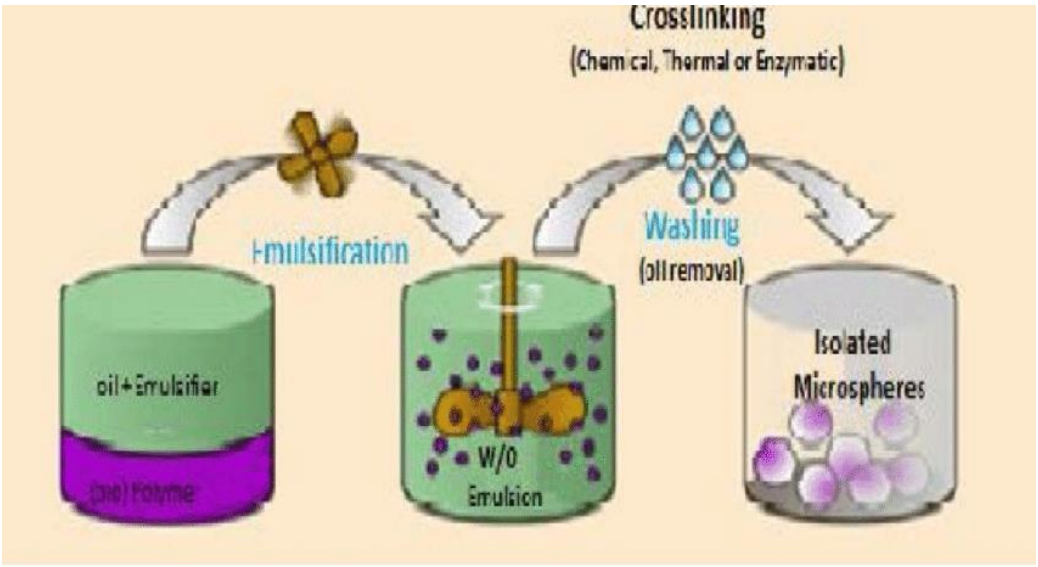

Fig 3 : Emulsion cross linking method

Coacervation method

Coacervation thermal change: Performed by weighed amount of ethyl cellulose was dissolved in cyclohexane with vigorous stirring at 80^{o}C by heating. Then the drug was finely pulverized and added with vigorous stirring on the above solution and phase separation was done by reducing temperature and using ice bath. Then above product was washed twice with cyclohexane and air dried then passed through sieve (sieve no. 40) to obtain individual microcapsule.

Coacervation non solvent addition: Developed by weighed amount of ethyl cellulose was dissolved in toluene containing propyl iso butylene in closed beaker with magnetic stirring for 6 hr at 500 rpm and the drug is dispersed in it and stirring is continued for 15mins. Then phase separation is done by petroleum benzoin.14 times with continuous stirring. After that the microcapsules were washed with n-hexane and air dried for 2 hr and then in oven at 50^{o}C for 4 hr.

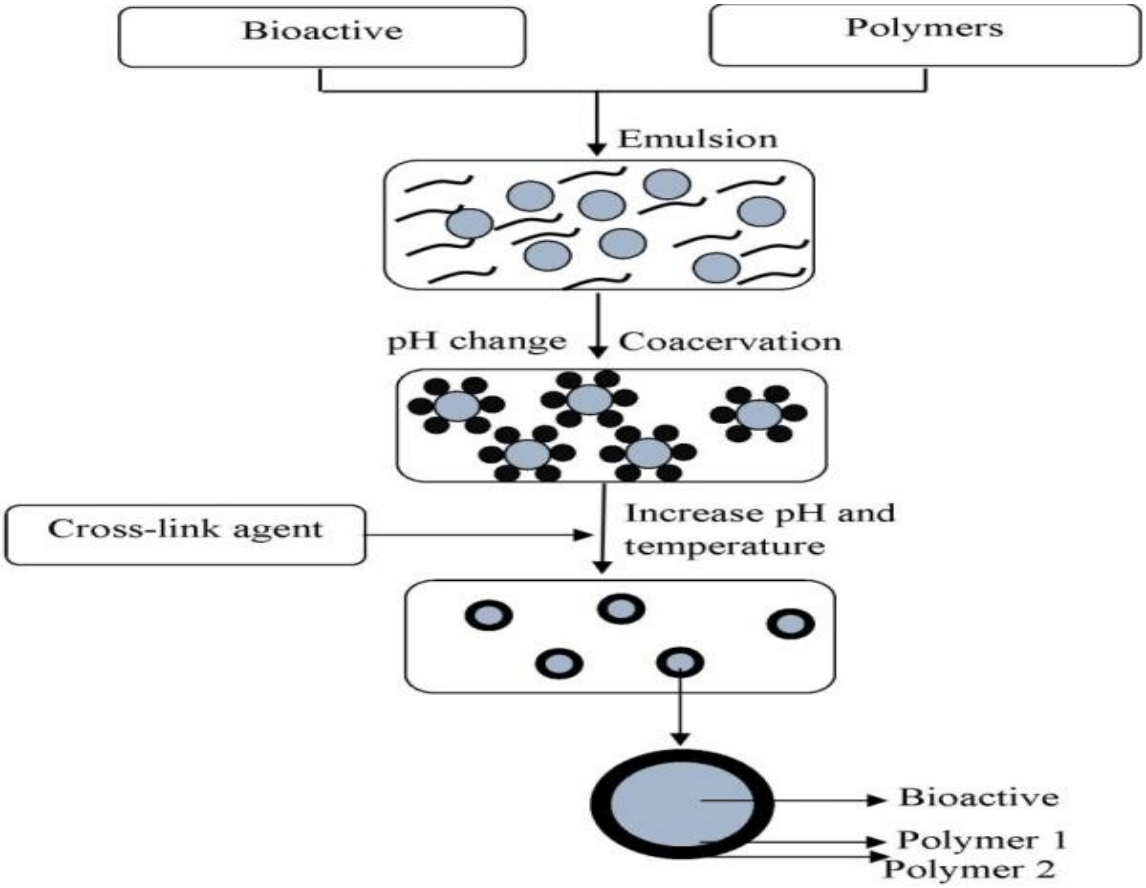

Fig 4 : Coacervation method

Spray drying technique[28]

This was used to prepare polymeric blended microsphere loaded with ketoprofen drug. It involves dispersing the core material into liquefied coating material and then spraying the

mixture in the environment for solidification of coating followed by rapid evaporation of solvent. Organic solution of poly (epsilon caprolactone) (PCL) and cellulose acetate butyrate (CAB), in different weight ratios and ketoprofen were prepared and sprayed in different experimental condition achieving drug loaded microspheres. This is rapid but may loose crystalinity due to fast drying process.

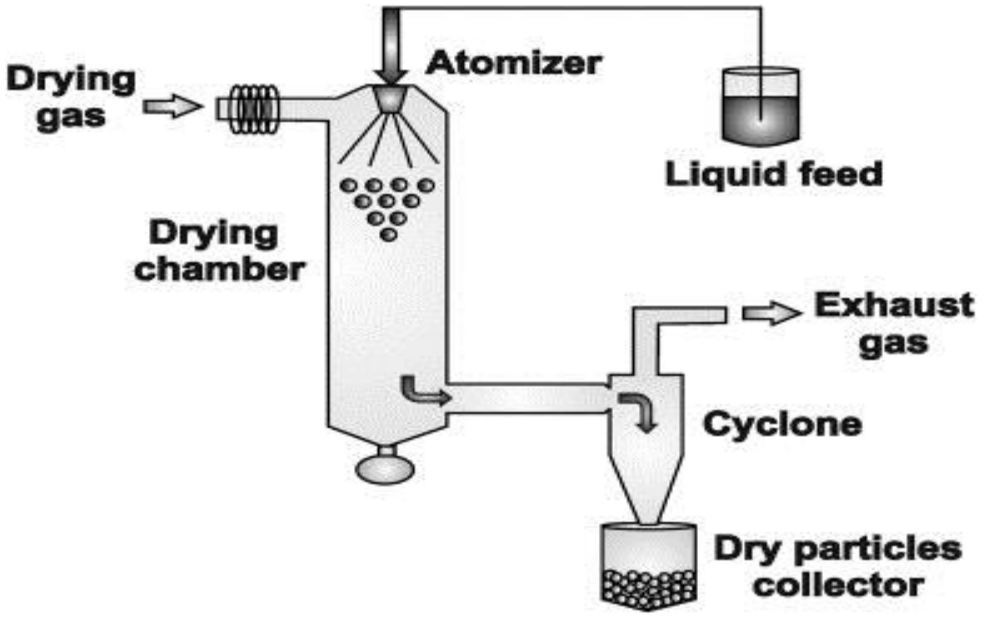

Fig 5 : Spray drying technique

Emulsion-solvent diffusion technique[28]

In order to improve the residence time in colon floating microparticles of ketoprofen were prepared using emulsion solvent diffusion technique. The drug polymer mixture was dissolved in a mixture of ethanol and dichloromethane (1:1) and then the mixture was added drop wise to sodium lauryl sulphate (SLS) solution. The solution was stirred with propeller type agitator at room temperature at 150 rpm for 1 hr. Thus the formed floating microspheres were washed and dried in a dessicator at room temperature. The following microparticles were sieved and collected.

Multiple emulsion method[28]

Oral controlled release drug delivery of indomethacin was prepared by this technique. In the beginning powder drug was dispersed in solution (methyl cellulose) followed by emulsification in ethyl cellulose solution in ethyl acetate. The primary emulsion was then re emulsified in aqueous medium. Under optimized condition discrete microspheres were formed during this phase.

Ionic gelation[28]

Alginate/chitosan particulate system for diclofenac sodium release was prepared using this technique. 25% (w/v) of diclofenac sodium was added to 1.2% (w/v) aqueous solution of sodium alginate. In order to get the complete solution stirring is continued and after that it was added drop wise to a solution containing Ca2+ /Al3+ and chitosan solution in acetic acid. Microspheres which were formed were kept in original solution for 24 hr for internal gellification followed by filteration for separation. The complete release was obtained at Ph 6.4-7.2 but the drug did not release in acidic pH.

Hydroxyl appetite (HAP) microspheres in sphere morphology[29]

This was used to prepare microspheres with peculiar spheres in sphere morphology microspheres were prepared by o/w emulsion followed by solvent evaporation. At first o/w emulsion was prepared by dispersing the organic phase (Diclofenac sodium containing 5% w/w of EVA and appropriate amount of HAP) in aqueous phase of surfactant. The organic phase was dispersed in the form of tiny droplets which were surrounded by surfactant molecules this prevented the droplets from co-solvencing and helped them to stay individual droplets .While stirring the DCM was slowly evaporated and the droplets solidify individual to become microspheres.

1.1.4. APPLICATIONS

1. Microspheres in vaccine delivery

An ideal vaccine must fulfill the requirement of efficacy, safety, convenience in application and cost. Biodegradable delivery systems for vaccines that are given by parenteral route may overcome the shortcoming of the conventional vaccines. The interest in parenteral (subcutaneous, intramuscular, intradermal) carrier lies since they offer specific advantages including:

1. Improved antigenicity by adjuvant action
2. Modulation of antigen release
3. Stabilization of antigen.

2. Targeting using microparticulate carriers

The concept of targeting, i.e. site specific drug delivery is a well established dogma, which is gaining full attention. The therapeutic efficacy of the drug relies on its access and specific interaction with its candidate receptors. The ability to leave the pool in reproducible, efficient and specific manner is center to drug action mediated by use of a carrier system.

3. Monoclonal antibodies mediated microspheres targeting

Monoclonal antibodies targeting microspheres are immune microspheres. This targeting is method used to achieve selective targeting to the specific sites. Monoclonal antibodies are extremely specific molecules. Mabs can be directly attached to the microspheres by means of covalent coupling. The Mabs can be attached to microspheres by any of the following methods

1. Non specific adsorption and Specific adsorption
2. Direct coupling
3. Coupling via reagents

4. Chemoembolisation

Chemoembolisation is an endovascular therapy, which involves the selective arterial embolisation of a tumour together with simultaneous or subsequent local delivery the chemotherapeutic agent.

5. Imaging

The particle size range of microspheres is an important factor in determining the imaging of particular sites using radio labbled microspheres. The particles injected intravenously apart from the portal vein will become entrapped in the capillary bed of the lungs. This phenomenon is exploited for the scintiographic imaging of the tumour masses in lungs using labeled human serum albumin microspheres.

6. Topical porous microspheres

Microsponges are porous microspheres having myriad of interconnected voids of particle size range 5-300µm. These microsponges having capacity to entrap wide range of active ingredients such as emollients, fragrances, essential oils etc., are used as the topical carries system.26

7. Medical application.

- ✓ Release of proteins, hormones and peptides over extended period of time.
- ✓ Gene therapy with DNA plasmids and also delivery of insulin.
- ✓ Vaccine delivery for treatment of diseases like hepatitis, influenza, pertusis, ricin toxoid, diphtheria, birth control.
- ✓ Passive targeting of leaky tumour vessels, active targeting of tumour cells, antigens, by intra arterial/ intravenous application.
- ✓ Tumour targeting with doxorubicin and also
- ✓ Treatments of leishmaniasis.

- ✓ Magnetic microspheres can be used for stem cell extraction and bone marrow purging.
- ✓ Used in isolation of antibodies, cell separation and toxin extraction by affinity chromatography.
- ✓ Used for various diagnostic tests for infectious diseases like bacterial, viral, and fungal.[30]

8. Radioactive microsphere's application.[31]

- ✓ Can be used for radio embolisation of liver and spleen tumours.
- ✓ Used for radio synvectomy of arthiritis joint, local radiotherapy, interactivity treatment.
- ✓ Imaging of liver, spleen, bone marrow, lung and even imaging of thrombus in deep vein thrombosis can be done.

MATERIALS AND METHODS

PREFORMULATION STUDIES

SPECTROSCOPIC STUDIES

PREPARATION OF 0.1N HCl (pH 1.2):

Take 8.5ml of HCl in a 1000ml volumetric flask and make up the volume with distilled water

DETERMINATION OF λ_{MAX}:

Weigh 10mg of Cytarabine and transferred into 10ml volumetric flask and dissolved in 10ml methanol (stock-I) to get concentration of 1000 µg/ml. From the stock-I take 1ml solution and make up 10ml with 0.1N HCL. From the second stock take 1ml solution and make up to 10ml with 0.1N HCL to get 10 µg/ml. Then scan from 200-400nm.

Preparation of Standard Calibration Curve of Cytarabine:

1. 10 mg of Cytarabine was accurately weighed and dissolved in 10ml of methanol (Stock Solution – I) to get a concentration of 1000 µg/ml.
2. From the stock solution- I, 1ml of aliquots was taken and suitably diluted with 0.1N HCl (Stock Solution-II) to get concentrations of 100µg/ml.
3. From the stock solution- II, aliquots were taken and suitably diluted with 0.1N HCl (pH 1.2) to get concentrations in the range of 5 to 25µg/ml. The absorbance of these samples were analyzed by using UV-Visible Spectrophotometer at 272nm against reference solution 0.1N HCl (pH 1.2). The procedure repeated to pH 6.8 phosphate buffer and pH 7.4 phosphate buffer.

METHOD OF PREPARATION

Microspheres were prepared by emulsification solvent evaporation technique. Briefly, Cytarabine and polymers were mixed in 50ml distilled water. A different polymer ratio 1:1, 1:2 and 1:3 used to prepare the different formulations. Polymeric aqueous solution was made in which the drug was dispersed and then the solution was added drop wise into 150 ml of light liquid paraffin containing 2% span- 80 as an emulsifying agent. The aqueous phase was emulsified in oily phase by stirring the system in a 500ml beaker, Constant stirring at 500 rpm was carried out using magnetic stirrer at 80ºC, stirring and heating were maintained for 4hrs, Until The aqueous phase was evaporated. The microspheres were washed 5 times with n- hexane, filtered through whattman's filter paper and dried in hot air oven at 50°C for 2 hours.

CHARACTERIZATION OF MICROSPHERES:

Table 1: Prepared formulation of Microspheres

INGREDIENTS	FORMULATION CODES					
	C1	**C2**	**C3**	**C4**	**C5**	**C6**
Cytarabine	100	100	100	100	100	100
HPMC15cps +Carbopol 934p (ratio)	1:1	1:2	1:3	-	-	-
HPMC15000cps + Carbopol 934p	-	-	-	1:1	1:2	1:3
Liquid paraffin (ml)	150	150	150	150	150	150
Span-80 (2%)	2	2	2	2	2	2

Micrometric Properties

Particle size analysis:

Samples of the micro particles were analyzed for particle size by optical microscope. The instrument was calibrated and found that 1unit of eyepiece micrometer was equal to 12.5µm. Nearly about 100 Micro particles sizes were calculated under 45 x magnifications.

The average particle size was determined by using the Edmondson's equation:

$$D_{mean} = \frac{nd}{n}$$

Where,

n – Number of microspheres observed

d – Mean size range

Micromeritic properties

The microspheres were characterized by their micromeritic properties such as particle size, bulk density, tapped density, compressibility index, Hausners ratio and angle of repose.

Bulk density

In this method microspheres are transferred to a measuring cylinder and is tapped manually till a constant volume is obtained. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres.

Tapped density

In this method microspheres were transferred to a measuring cylinder & tapped for 100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density microspheres.

Percent Compressibility index was determined by using the formula,

Carr's Index = (tapped density – bulk density) x 100 / tapped density

Hausners ratio

Hausners ratio of microspheres was determined by comparing tapped density to bulk density using the equation

Hausner ratio = tapped density / bulk density

Angle of repose

Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method4. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was measured and angle of repose was calculated using the following equation.

$\theta = \tan^{-1} h / r$

here,

θ - Angle of repose

h - Height of granules above the flat surface

r - Radius of the circle formed by the granule heap.

Percentage yield

The percentage of production yield was calculated from the weight of dried microsphere recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical mass (Microspheres)}}{\text{Theoretical mass (Polymer + Drug)}} \times 100$$

Drug entrapment efficiency:

Weighed amount of microspheres (100 mg) with phosphate buffer pH 7.4 (10 ml) was added in a vial. The solution was stirred vigorously for 24 hours with mechanical stirrer. Supernatent was collected by centrifugation and drug content in supernatent was determined by using UV spectrophotometer at wavelength 272nm. The amount of drug entrapped in the microspheres was calculated by the following formula,

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Swelling study:

Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2 .The microspheres were removed periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{(Wt - W0)}{(W0)} \times 100$$

Where W0 & Wt are initial weight and Final weight of microspheres respectively

***In vitro* drug release study:**

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus (37 ± 0.50C, 50 rpm) using the USP type – I rotating basket method in simulated gastric fluid pH 1.2 (900ml) for 2 hours then replace the media with pH 6.8 phosphate buffer for 3 hours, then replace the media with pH 7.4 Phosphate buffer. A quantity of accurately weighed microspheres equivalent to 100mg Cytarabine each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 272 nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same

volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment.

***In-vitro* drug release kinetics**

The release data obtained was fitted into various mathematical models. The parameters 'n' and time component 'k',the release rate constant and 'R',the regression coefficient were determined by Korsmeyer-Peppas equation to understand the release mechanism.

To examine the release mechanism of Cytarabine from the microspheres, the release data was fitted into Peppa's equation,

$Mt / M\infty = Ktn$

Where, $Mt / M\infty$ is the fractional release of drug, 't' denotes the release time, 'K' repr-esents a constant incorporating structural and geometrical characteristics of the device, 'n' is the diffusional exponent and characterize the type of release mechanism during the release process.

Table 2 : *In-Vitro* Drug Release Kinetics

Release exponent (n)	**Drug transport mechanism**	**Rate as a function of time**
0.5	Fickian diffusion	$t^{-0.5}$
0.5<n<1.0	Anomalous transport or non-Fickian	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

If n < 0.5, the polymer relaxation does not affect the molecular transport, hence diffus-ion is Fickian.

If n > 0.5, the solid transport will be non-fickian and will be relaxation controlled.

Other equations to study the drug release kinetics from dosage forms

a. Zero Order

% R = kt

This model represents an ideal release in order to achieve prolonged pharmacological action. This is applicable to dosage forms like transdermal systems, coated forms, osmotic systems, as well as Matrix tablets containing low soluble drugs.

b. First Order

log (fraction unreleased) = kt/2.303

The model is applicable to hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices.

c. Matrix (Higuchi Matrix)

% R = kt 0.5

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

d. Peppas Korsmeyer Equation

% R = kt n

log % R = logk + nlogt

This model is widely used when release mechanism is well known or when more than one type of release phenomenon could be involved. The 'n' values could be used to characterize different release mechanisms as.

COMPATIBILITY STUDIES

A proper design and formulation of a dosage form requires considerations of the physical, chemical and biological characteristics of both drug and excipients used in fabrication of the product. Compatibility must be established between the active ingredient and other excipients to produce a stable, efficacious, attractive and safe product. If the excipient(s) are new and if no previous literature regarding the use of that particular excipient with an active ingredient is available, then compatibility studies are of paramount importance. Hence, before producing the actual formulation, compatibility of Cytarabine with different polymers and other excipients was tested using the Fourier Transform Infrared Spectroscopy (FT-IR) technique.

FOURIER TRANSFORMS INFRARED SPECTROSCOPY (FT-IR):

In order to check the integrity (Compatibility) of drug in the formulation, FT-IR spectra of the formulations along with the drug and other excipients were obtained and compared using Shimadzu FT-IR 8400 spectrophotometer. In the present study, Potassium bromide (KBr) pellet method was employed. The samples were thoroughly blended with dry powdered potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the spectrophotometer and the spectrum was recorded. The FT-IR spectra of the formulations were compared with the FT-IR spectra of the pure drug and the polymers.

RESULTS AND DISCUSSION

8.1. PREFORMULATION STUDIES

8.1.1. SPECTROSCOPIC STUDIES

Determination of λ_{max}

A solution of 10µg/ml of Cytarabine was scanned in the range of 200 to 400nm. The drug exhibited a λ_{max} at 272 and 275nm in simulated gastric fluid pH 1.2 and pH 7.4 phosphate buffer respectively. Correlation between the concentration and absorbance was found to be near to 0.998, with a slope of 0.028 and intercept of 0.004.

Calibration curve of Cytarabine in simulated gastric fluid pH 1.2

Table 8.1 shows the calibration curve data of Cytarabine in simulated gastric fluid pH 1.2 at 272nm. Fig.8.1 shows the standard calibration curve with a regression value of 0.999, slope of 0.022 and intercept of 0.007 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25µg/ml.

Table 3: Calibration curve data for Cytarabine in simulated gastric fluid pH 1.2

CONCENTRATION (µg /ml)	ABSORBANCE
0	0
5	0.122
10	0.242
15	0.346
20	0.454
25	0.569

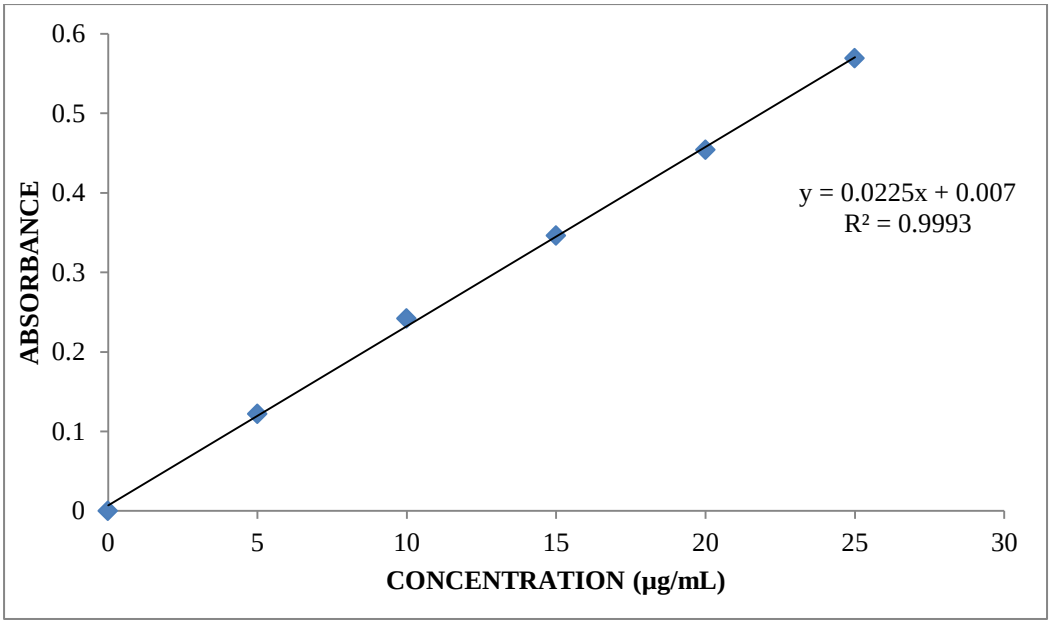

Figure 5: Standard graph of Cytarabine in simulated gastric fluid pH 1.2

Calibration curve of Cytarabinein pH 7.4 phosphate buffer

Table 8.2 shows the calibration curve data of Cytarabine in pH 7.4 phosphate buffer at 275nm. Fig. 8.2 shows the standard calibration curve with a regression value of 0.999, slope of 0.027 and intercept of 0.007 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 5-25µg/ml.

Table 4: Calibration curve data for Cytarabine in pH 7.4 phosphate buffer

CONCENTRATION (µg /ml)	ABSORBANCE
0	0
5	0.148
10	0.286
15	0.432
20	0.557
25	0.691

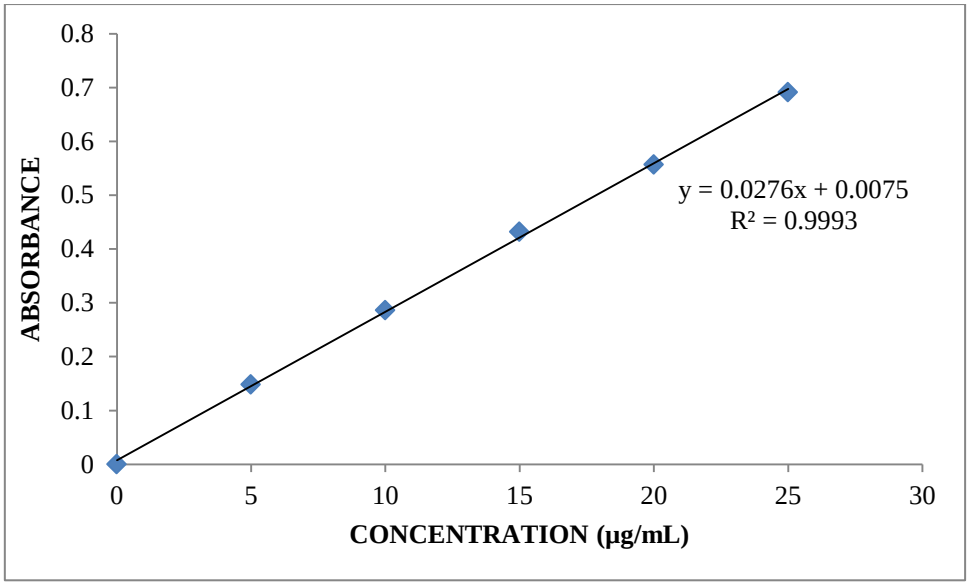

Figure 6: Standard graph of Cytarabine in pH 7.4 phosphate buffer

EVALUATION AND CHARACTERISATION OF MICROSPHERES

Micrometric Properties

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing HPMC15cps +Carbopol 934p had a size range of 320.25 µm to 457.31µm. Microspheres containing HPMC15000cps + Carbopol 934p as copolymer exhibited a size range between 305.45 µm to 385.19µm.

The particle size data is presented in Tables 8.3. The effect of drug to polymer ratio on particle size is displayed in Figure. The particle size as well as % drug entrapment efficiency of the microspheres increased with increase in the polymer concentration.

The bulk density of formulation C1 to C6 containing HPMC15cps +Carbopol 934p and HPMC15000cps + Carbopol 934p formulation was in the range of 0.44 ± 0.03 to 0.55 ± 0.011 gm./cm^3 (as shown in table 8.3), Tapped density 0.50± 0.061 to 0.62 ± 0.011and hausners ratio 1.12 ± 0.015 to 1.16 ± 0.032.

The Carr's index of formulation C1 to C6 containing different grades of HPMC15cps +Carbopol 934p and HPMC15000cps + Carbopol 934p 11.29 ± 0.35 to 14.28 ± 0.47 respectively. The angle of repose of formulation C1 to C6 containing HPMC15cps +Carbopol 934p and HPMC15000cps + Carbopol 934p formulation was in the range <28.53 respectively (as shown in table 8.3) The values of Carr's index and angle of repose indicate good flow properties.

Table 5: Micromeritic property of floating microspheres of Cytarabine

Formulation code	Mean particle size	Bulk density ((gm./cm^3))	Tapped density (gm./cm^3)	Hauseners ratio	Carr's index	Angle of repose
C1	320.25	0.44 ± 0.03	0.50± 0.061	1.13 ± 0.012	12 ± 0.58	26.12 ± 0.1
C2	338.15	0.48 ± 0.06	0.56 ± 0.08	1.16 ± 0.032	14.28 ± 0.47	28.53 ± 0.57
C3	457.31	0.55 ± 0.08	0.62 ± 0.011	1.12 ± 0.015	11.29 ± 0.57	25.46 ± 0.57
C4	305.45	0.53 ± 0.09	0.61 ± 0.071	1.15 ± 0.021	13.1 ± 0.15	27.61 ± 0.63
C5	351.12	0.49 ± 0.01	0.56 ± 0.08	1.14 ± 0.012	12.5 ± 0.21	25.15 ± 0.58
C6	385.19	0.55 ± 0.011	0.62 ± 0.06	1.12 ± 0.023	11.29 ± 0.35	26.08 ± 0.51

PERCENTAGE YIELD

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug- polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range of 86.15 to 98.17 % for microspheres containing HPMC15cps +Carbopol 934p, 82.24 to 95.12 % for microspheres containing HPMC15000cps + Carbopol 934p.

DRUG ENTRAPMENT EFFICIENCY

Percentage Drug entrapment efficiency of Cytarabine ranged from 76.29 to 98.38 % for microspheres containing HPMC15cps +Carbopol 934p, 80.77 to 93.54% for microspheres containing HPMC15000cps + Carbopol 934p. The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The % drug entrapment efficiency of the prepared microspheres is displayed in Table 8.4, and displayed in Figures.

Table 6: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

S.No.	Formulation code	% yield	Drug Content (mg)	% Drug entrapment efficiency
1	C1	86.15	90.40	76.29
2	C2	90.60	92.83	85.14
3	C3	98.17	97.10	98.38
4	C4	82.24	83.91	80.77
5	C5	86.46	89.73	90.16
6	C6	95.12	92.50	93.54

Swelling studies

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swellability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Swellability data revealed that amount of polymer plays an important role in solvent transfer. It can be concluded from the data shown in Table 8.5 that with an increase in polymer concentration, the percentage of swelling also increases. Thus we can say that amount of polymer directly affects the swelling ratio. As the polymer to drug ratio increased, the percentage of swelling increased from 80.42 to 93.69% for microspheres containing HPMC15cps +Carbopol 934p as polymer, 76.10 to 93.22% for microspheres containing HPMC15000cps+Carbopol 934p as polymer. The percentage of swelling of the prepared microspheres is displayed in Figures. The percentage of swelling of the prepared microspheres is displayed in Figures. The effect of drug to polymer ratio on percentage swelling is displayed in Figure Table 8.5: Percentage swelling of the prepared microspheres.

Table 7: Swelling studies

S.NO.	FORMULATION CODE	INITIAL (Wt)	FINAL (Wt)	PERCENTAGE SWELLING
1	C1	15	18.65	80.42
2	C2	15	17.15	87.46
3	C3	15	16.01	93.69

4	C4	15	19.71	76.10
5	C5	15	17.36	86.40
6	C6	15	16.09	93.22

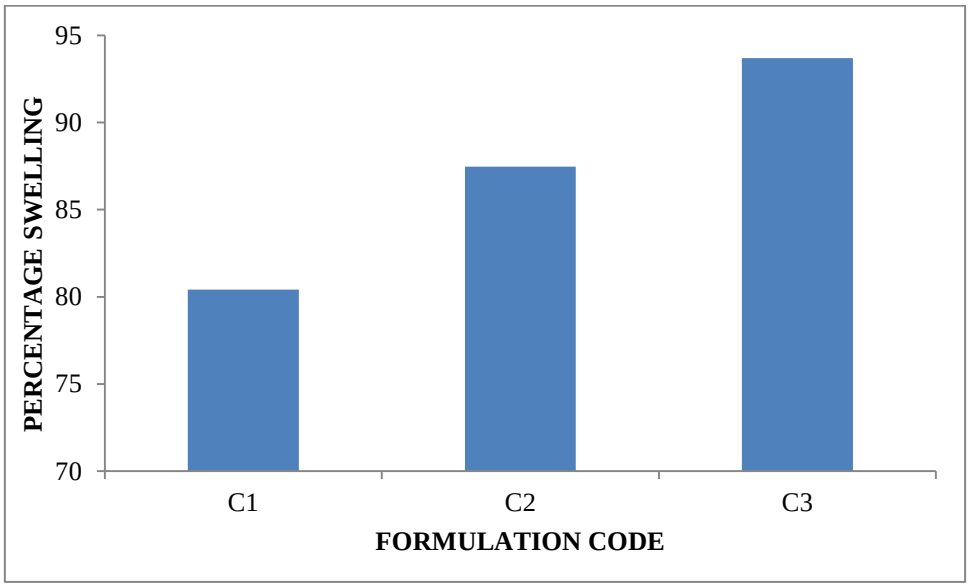

Figure 7 : Percentage swelling of microspheres containing HPMC15cps +Carbopol 934p

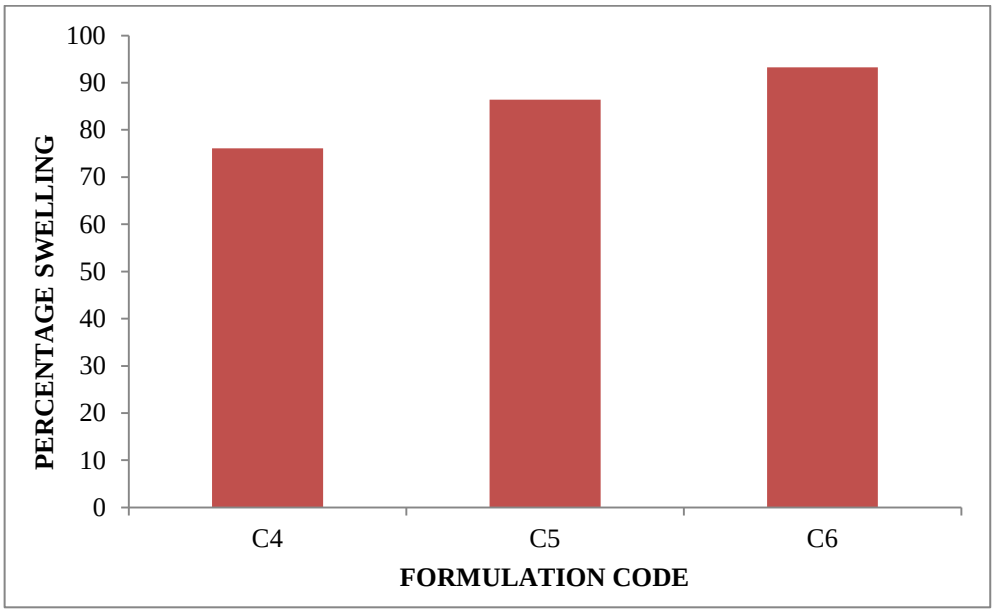

Figure 8: Percentage swelling of microspheres containing HPMC15000cps + Carbopol 934p

***In-vitro* mucoadhesion test**

As the polymer to drug ratio increased, microspheres containing HPMC15cps +Carbopol 934p exhibited % mucoadhesion ranging from 53.41 to 90.14 %, microspheres containing

HPMC15000cps+Carbopol 934p exhibited % mucoadhesion ranging from 62.78 to 87.63%. The results of *in-vitro* mucoadhesion test are compiled in Table 8.6. Comparative depiction of % mucoadhesion is depicted in Fig.8.5 and 8.6.

Table 8 : Percentage mucoadhesion of the prepared microspheres

S.NO.	FORMULATION CODE	No. OF MICROSPHERES		PERCENTAGE MUCOADHESION
		INITIAL	FINAL	
1	C1	15	10.31	53.41
2	C2	15	13.47	71.51
3	C3	15	14.96	90.14
4	C4	15	11.85	62.78
5	C5	15	13.41	70.14
6	C6	15	14.28	87.63

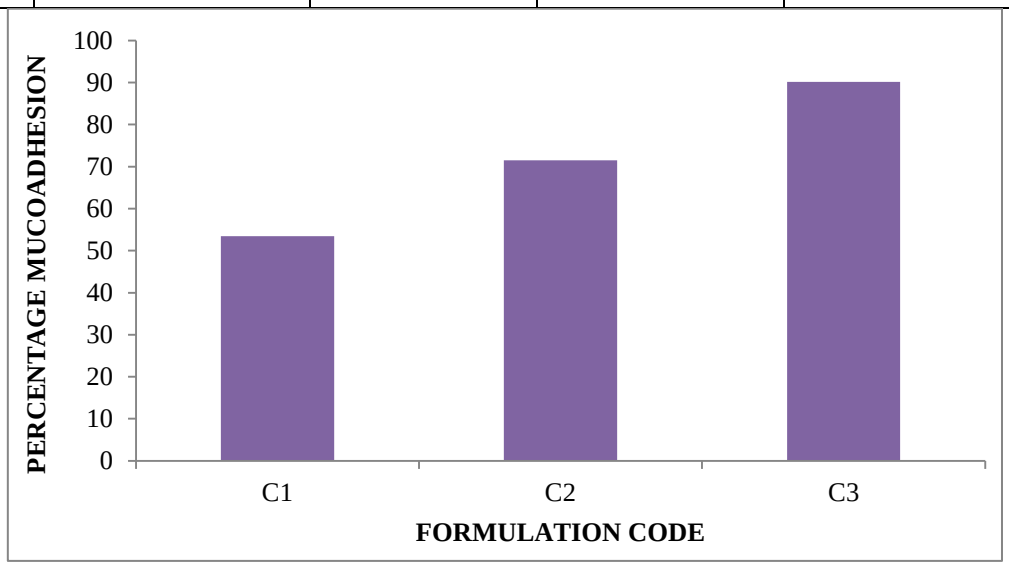

Figure 9: Percentage mucoadhesion of microspheres containing HPMC15cps +Carbopol 934p

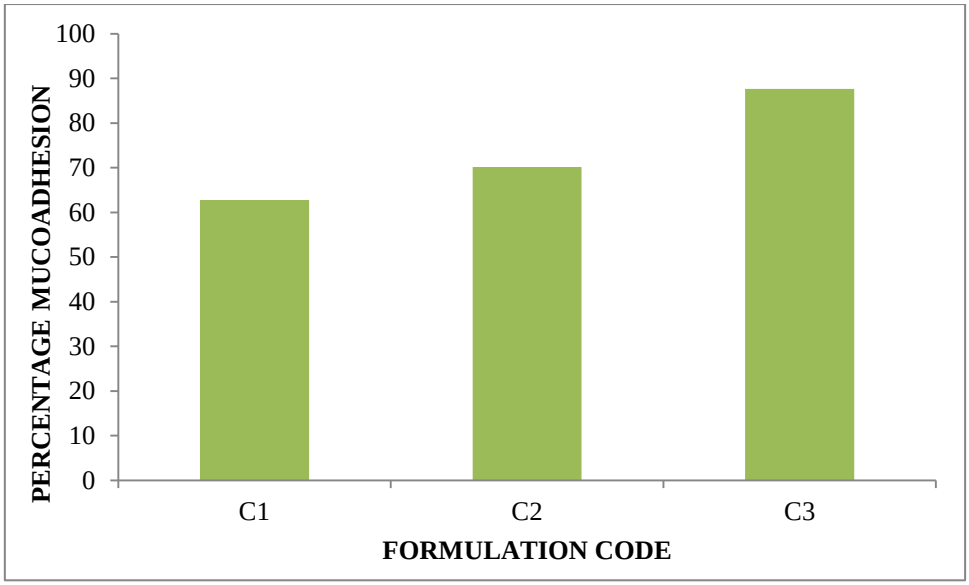

Figure 10 : Percentage mucoadhesion of microspheres containing HPMC15000cps + Carbopol 934p

***IN-VITRO* DRUG RELEASE STUDIES**

Dissolution studies of all the formulations were carried out using dissolution apparatus USP type I. The dissolution studies were conducted by using dissolution media, pH 1.2. The results of the *in-vitro* dissolution studies of formulations C1 to C6 are shown in table. The plots of Cumulative percentage drug release Vs Time. Figure shows the comparison of % CDR for formulations C1 to C3, figure for formulations C4 to C6.

The formulations C1, C2 and C3 containing HPMC15cps+Carbopol 934p showed a maximum release of 96.14 % at 9 hours, 97.82 % after 10 hours and 99.13 % after 12 hours respectively.

The formulations C4, C5 and C6 containing HPMC15000cps+Carbopol 934p showed a maximum release of 95.93% after 9 hours, 96.85% after 11 hours and 97.12% after 12 hours respectively.

This shows that more sustained release was observed with the increase in percentage of polymers. As the polymer to drug ratio was increased the extent of drug release increased.

Table 9: *In-vitro* drug release data of Cytarabine microspheres

TIME (H)	CUMULATIVE PRECENT OF DRUG RELEASED					
	C1	**C2**	**C3**	**C4**	**C5**	**C6**

0	0	0	0	0	0	0
1	19.72	17.53	13.98	25.19	11.20	08.91
2	30.90	27.19	23.51	32.25	18.86	13.58
3	35.45	32.26	30.60	39.13	23.32	18.16
4	43.83	38.37	35.19	46.56	31.12	25.93
5	55.42	45.20	41.99	50.83	37.28	31.75
6	60.01	50.12	48.45	66.93	43.67	39.54
7	79.95	68.86	53.72	78.54	50.49	43.83
8	87.50	77.10	60.02	90.17	56.53	58.76
9	96.14	85.23	65.14	95.93	62.68	65.12
10		97.82	71.23		88.94	79.43
11			89.27		96.85	91.86
12			99.13			97.12

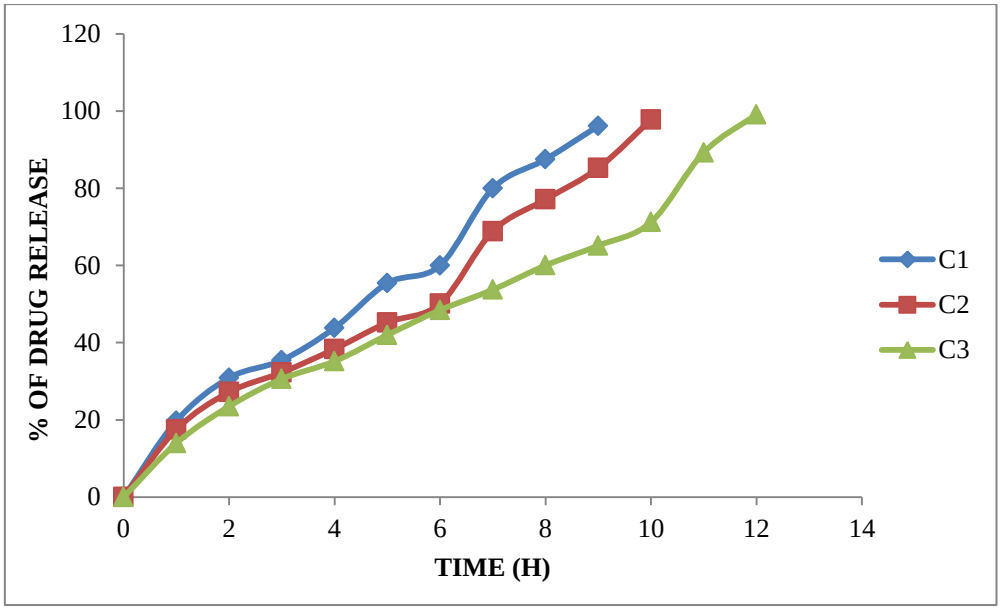

Figure 11 : *In-Vitro* drug release profile of Cytarabine microspheres containing HPMC15cps +Carbopol 934p

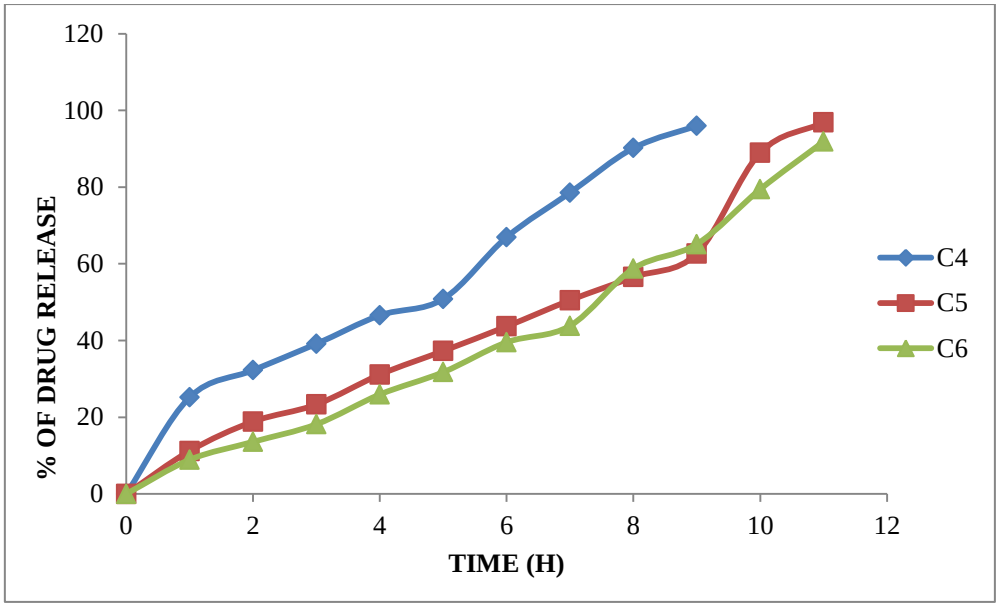

Figure 12: *In-Vitro* drug release profile of Cytarabine microspheres containing HPMC15000cps + Carbopol 934p

IN-VITRO DRUG RELEASE KINETICS

For understanding the mechanism of drug release and release rate kinetics of the drug from dosage form, the *in-vitro* drug dissolution data obtained was fitted to various mathematical models such as zero order, First order, Higuchi matrix, and Krosmeyer-Peppas model. The values are compiled in Table 8.9. The coefficient of determination (R^2) was used as an indicator of the best fitting for each of the models considered. The kinetic data analysis of all the formulations reached higher coefficient of determination with the peppas drug release model whereas release exponent value (n) ranged from 0.983. From the coefficient of determination and release exponent values, it can be suggested that the mechanism of drug release follows Korsmeyer-Peppas model along with non-Fickian diffusion mechanism which leading to the conclusion that a release mechanism of drug followed combination of diffusion and spheres erosion.

Table 10: Release kinetics studies of the optimized formulation (C3)

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
13.98	1	1.000	1.146	0.000	1.935	13.980	0.0715	-0.854	86.02	4.642	4.414	0.227
23.51	2	1.414	1.371	0.301	1.884	11.755	0.0425	-0.629	76.49	4.642	4.245	0.397
30.6	3	1.732	1.486	0.477	1.841	10.200	0.0327	-0.514	69.4	4.642	4.109	0.532
35.19	4	2.000	1.546	0.602	1.812	8.798	0.0284	-0.454	64.81	4.642	4.017	0.625

41.99	5	2.236	1.623	0.699	1.764	8.398	0.0238	-0.377	58.01	4.642	3.871	0.770
48.45	6	2.449	1.685	0.778	1.712	8.075	0.0206	-0.315	51.55	4.642	3.722	0.920
53.72	7	2.646	1.730	0.845	1.665	7.674	0.0186	-0.270	46.28	4.642	3.590	1.051
60.02	8	2.828	1.778	0.903	1.602	7.503	0.0167	-0.222	39.98	4.642	3.419	1.222
65.14	9	3.000	1.814	0.954	1.542	7.238	0.0154	-0.186	34.86	4.642	3.267	1.375
71.23	10	3.162	1.853	1.000	1.459	7.123	0.0140	-0.147	28.77	4.642	3.064	1.577
89.27	11	3.317	1.951	1.041	1.031	8.115	0.0112	-0.049	10.73	4.642	2.206	2.436
99.13	12	3.464	1.996	1.079	-0.060	8.261	0.0101	-0.004	0.87	4.642	0.955	3.687

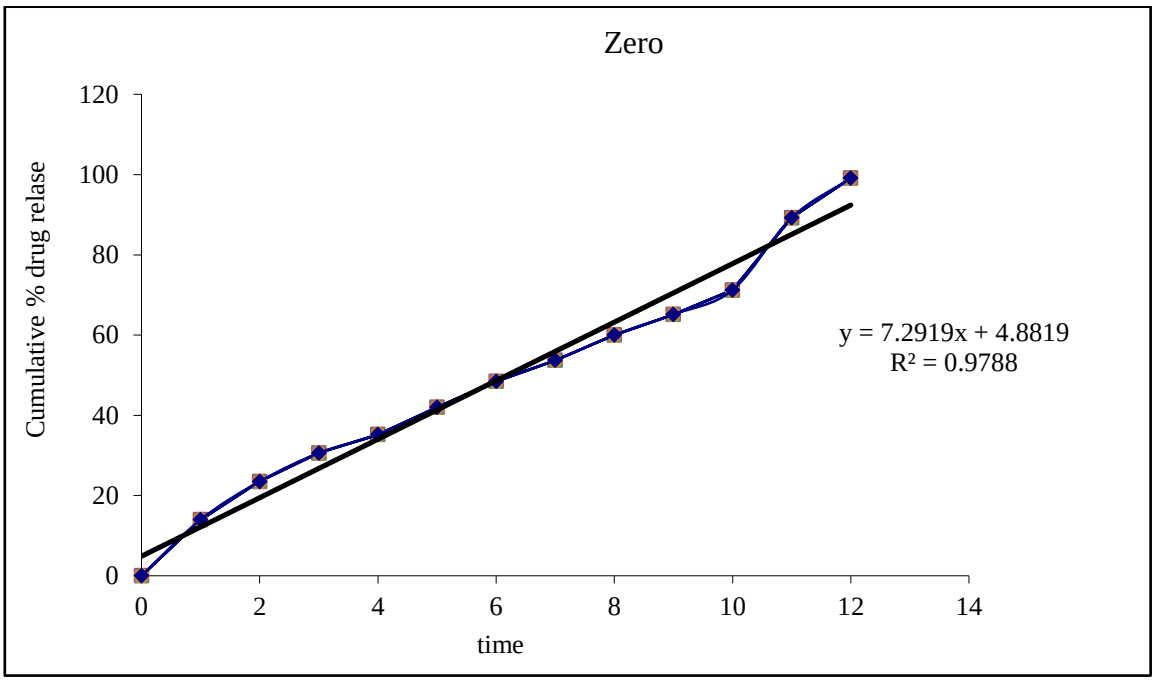

Figure:13: Graph of zero order release kinetics of optimized formula

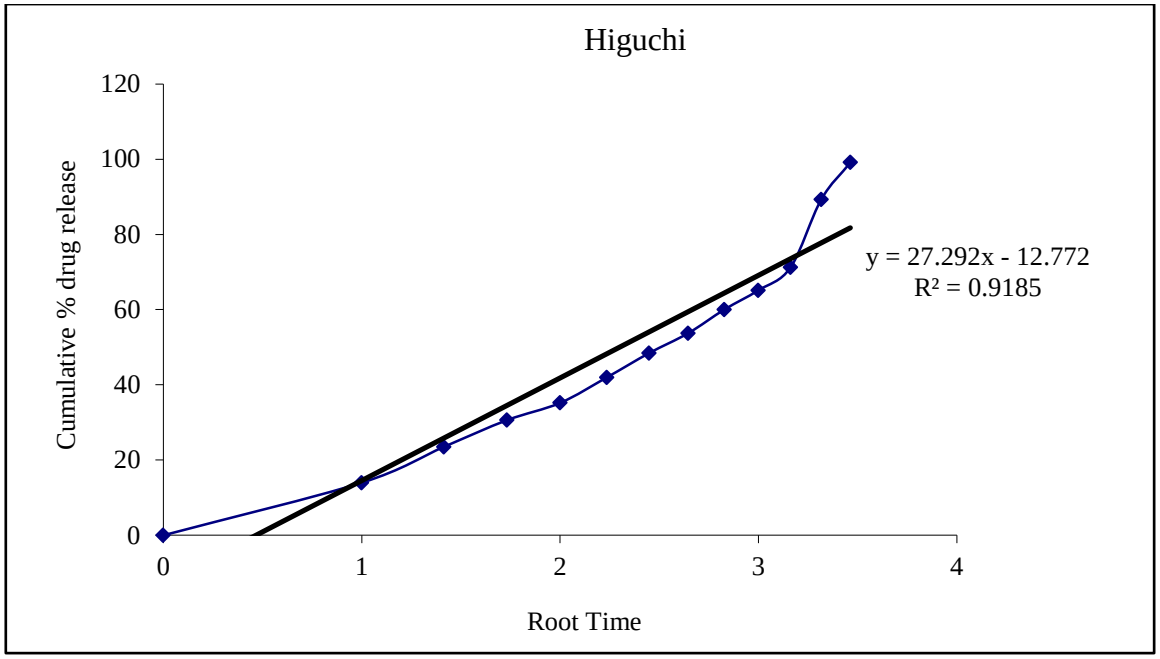

Figure:14 : Graph of higuchi release kinetics of optimized formula

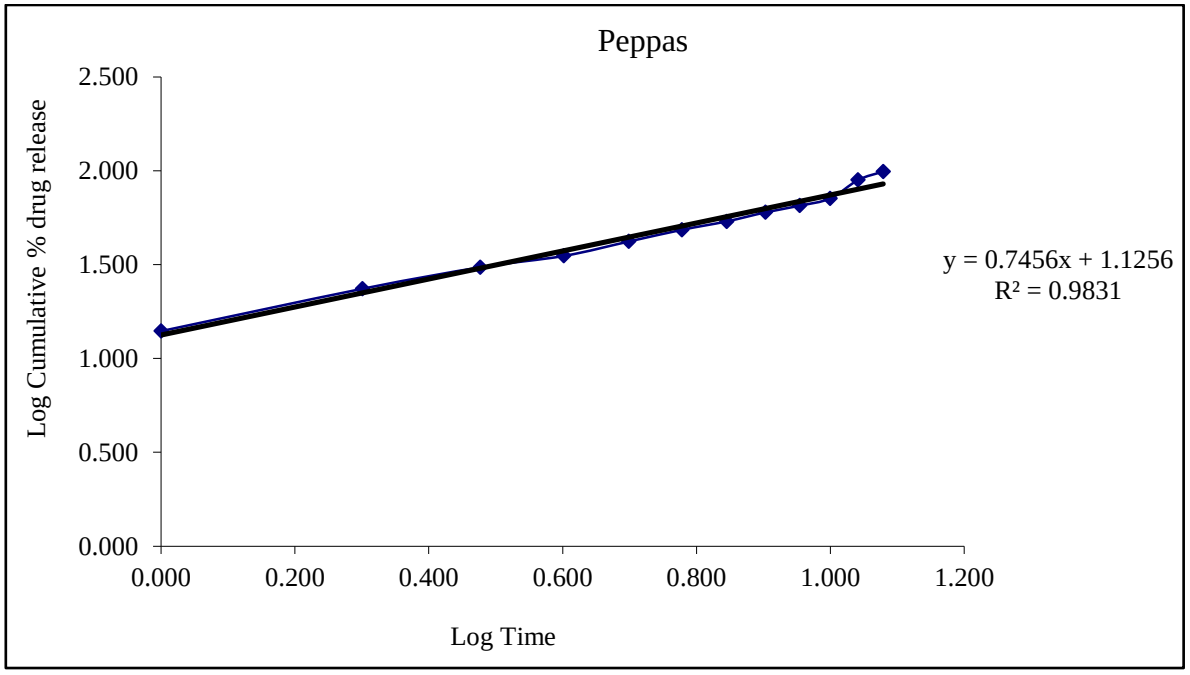

Figure 15: Graph of peppas drug release kinetics of optimized formula

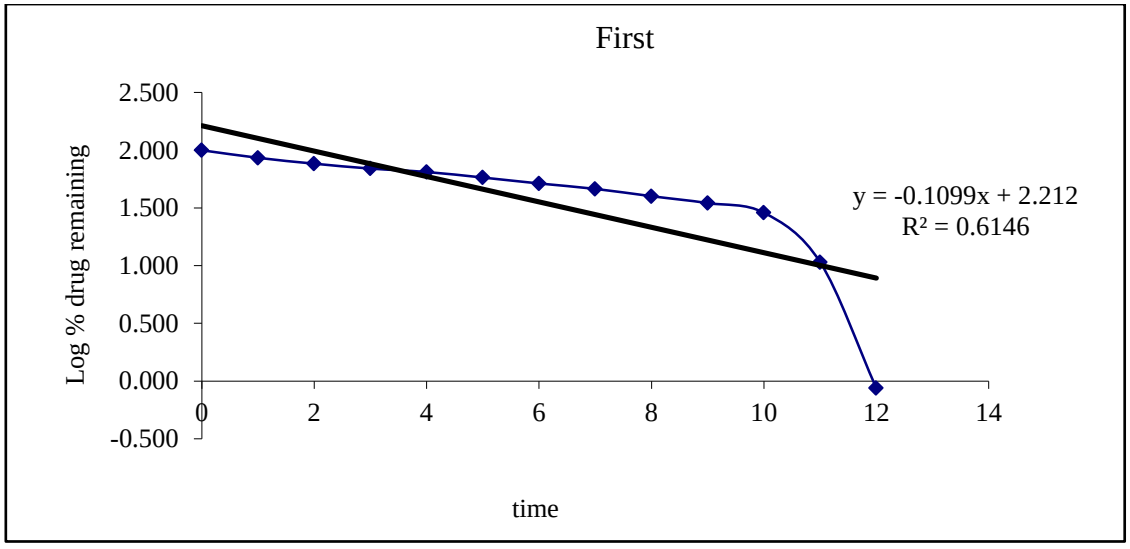

Figure:16: Graph of first order release kinetics of optimized formula

Optimised formulation C3 was kept for release kinetic studies. From the above graphs it was evident that the formulation C3 was followed peppas drug release kinetics.

Compatibility studies

Drug polymer compatibility studies were carried out using Fourier Transform Infra Red spectroscopy to establish any possible interaction of Drug with the polymers used in the formulation. The FT-IR spectra of the formulations were compared with the FTIR spectra of the pure drug.

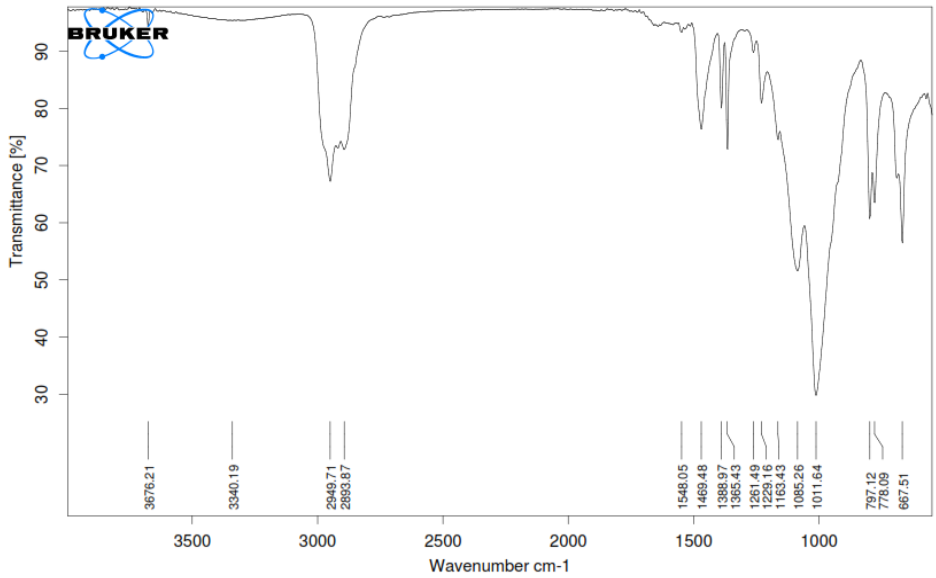

Figure 17: FT-IR spectra of Pure drug

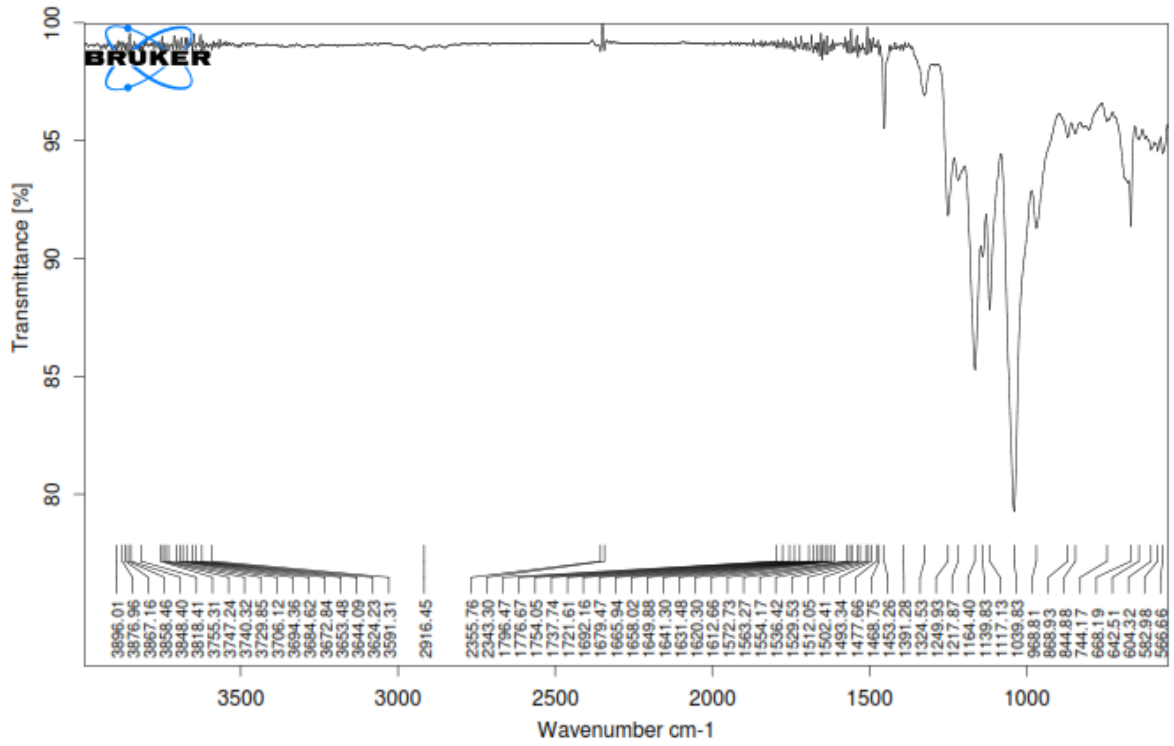

Figure 18: FT-IR spectra of Optimised formulation

9. CONCLUSION

Cytarabine loaded microspheres were prepared solvent evaporation technique. Six batches of formulations, of drug: polymer ratio i.e. 1:1,1:2 and 1:3, were prepared and evaluated for particle size, % yield, drug content, drug entrapment efficiency, Swelling studies, *In-vitro* mucoadhesion test, *in vitro* drug release and Flow property had shown satisfactory results. The IR Spectra's revealed that, there is no interaction between polymer and Cytarabine. The polymer used is compatible with the Cytarabine. The basis of release data and graphical analysis formulation C3 showed a good sustained release profile with maximum entrapment efficiency because of high polymer concentration. Hence, from all the above obtained data it can be summarized that it is possible to formulate a promising sustained release mucoadhecive microspheres of Cytarabine by solvent evaporation technique using an ideal polymer like HPMC15cps +Carbopol 934p.

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