Formulation and Evaluation of Curcumin Loaded Gel Embedded Beads for Solubility Enhancement

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Abstract

Curcumin is a tautomeric complex that exists in the form of enolic in natural diluters & in water as a keto form. Natural pigment present in the Curcumin which containing anti-fungal, anti-bacterial, anti-viral, anti-inflammatory, anti-malarial, anti-oxidant, anti-mutagenic agent, abilities of wound healing & enhances anti-tumor property in contradiction of various types of tumor cells but curcumin is very poorly soluble in water which leads to an very low oral bioavailability. The aim of this research was to prepare a novel microbead formulation, and to solubilize and increasing its solubility. Initially, curcumin was solubilized with DMSO and then microencapsulated by cross linking polymer SA with $CaCl_2$. A three-factorial design was employed to obtain the optimum curcumin loaded micro size bead formulation, specifically the best entrapment efficiency and *in vitro* curcumin release. The independent variables were SA, Gelatin and $CaCl_2$ concentration, and the weight of curcumin solution, while the dependent variables were entrapment efficiency and in vitro curcumin release. The optimized microbeads formulation was CB8 of gel embedded beads. Results showed that high concentrations of SA, Gelatin & $CaCl_2$ could increase the entrapment efficiency. In vitro curcumin release of gel embedded microbeads (wet beads) is very much efficient from the dry beads due to its ready to release phenomenon. In conclusion, the optimum microbeads formulation increased the solubility of curcumin, and achieved a high entrapment efficiency and in vitro curcumin release.

Keywords: Sodium Alginate (SA), Calcium Chloride ($CaCl_2$), Curcumin, Microbeads, CB (Curcumin beads).

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1. Introduction:

The most preferred form of delivery are oral dosage forms. However, one of the key tasks in delivery is to remove gastrointestinal obstruction and prevent the drug from being released into the stomach. Over the past decade, an enormous increased has been shown in the growth of orally administered anticancer agents, as one-fourth of all presented anti-cancer medications are nowadays orally administered. Beads shall be produced in size range of mm (millimeter), µm (micrometer) or nm (nanometer).[1-3] The size shall be identified by consuming the approximate space technique, with 3 dimensions respectively: first is the main diameter (the max diameter of the max expected area), second is the intermediary diameter (the min diameter of the max expected area, or the max diameter of the min estimated area), and the nominal diameter (i.e., the smallest diameter of the region). Beads shall be used for continuous drug release, hormones, antibiotics and vaccines. Eg: By using prime advantage of the properties of the beads, away from the basic purpose, the beads can deliver a large SA (surface area) and mass transfer behavior and easy assessment of diffusion. The technique defined in this, is similar to that obtained through natural polymers. The key rewards are that the natural polymers do not cause any systemic toxins on biochemical, biodegradation and administration. [4-6]

Micro-Encapsulation is a recognized technique of delaying & modifying release of drug features. In lieu of oral usage, it has been used toward promote release of drugs and can decrease or exclude GI (gastrointestinal) irritation. It is the procedure of shutting off µ (micron) solid

particles or liquids or gases in an inactive covering that results in the construction of micro particles. Since multi-component delivery of drug leads to a widespread and undeviating spread across gastrointestinal tract, high confined localization may be avoided. Additionally, multi-component delivery systems are evenly distributed throughout the GIT.[6-8] This outcomes in more frequent absorption of drug and decreases resident irritation equated to the single unit dosage form like polymeric matrix tablets that do not separate. Abdominal abortion is a difficult method that creates the *vivo* function of delivery of drug methods uncertain; however, floating or hydro dynamically CDDS (controlled drug delivery systems), floating micro size spheres, tiny beads, and microcapsules are helpful in overcoming such uncertainties. Highly connected microbeads, depending on biodegradability and biocompatibility, can be given as long lasting drugs.[8-11]

Microbeads are defined as spherical polymicrobial diameters ranging from 0.5 to 500 µm, which are small, hard, thin and freely flowing cell carriers or crystal-like form, forming continuous or numerous releases of treatment. Micro size beads have been used in variety of different products & one of the utmost important applications of these polymeric micro size beads is the ability to identify the presence and presence of numerous biological species in the presence of body fluids to determine the occurrence of a disease.[9, 10] Liable on the definite application, various polymers are used to produce micro size beads. The supreme polymers are polystyrene. Numerous lively agents without main side effects. Originated microbeads from many polymers like Cationic polymers (Chitosan), Ionic polymers (Sodium alginate (SA)) and Binding constituents (Gelatin, Chondroitin sulfate) predetermined proportions. [12-15] Due to the uniform distribution of drug residues in the gastrointestinal tract, many single dosage forms, like microphages or microbeads, have become popular as oral drug delivery systems due to greater uniform drug absorption, local irritation and polymeric content.[16-18]

When non-newtonian fluids like alginate or other polymer are normally used for cell stabilization and encapsulation. The most standard & modest technique of finding the beads is the crosslink of the alginate solution. Therefore it is not shocking that this is the classic method of select for most encapsulation & bead manufacturers. Necessary to differentiate among the size of the alginate droplet & the size of the bead of alginate formed by the encapsulation mechanism, that is, the size of the drop once exposed to the hardening / crosslinking solution.[19-22] A two percent solution of sodium alginate was extracted at 25 ◦ C using an encapsulation device equipped with a nozzle (50-500 micro meter in diameter) of 110 mM $CaCl_2$, sampled from a hardening solution on bead. The minimum duration, and the size distribution of the beads, were examined within 60 minutes.[23-25]

1.1 Solubility

Solubility is the property of a liquid, solid or gaseous substance that is soluble in a solvent, solid or liquid to form homogeneous of solute in solvent. The solubility of a material depends on the solvent even at temp. (Temperature) & pressure. Amount of solubility of an ingredient in a particular solvent is defined as saturation density, where more solute residues are added to the solution to increase its concentration. The solvent is usually liquid, which may be a natural substance or a mixture of both drinks. You may additionally talk about a strong solution, however very rarely dissolve in fuel.[26, 27] The volume of solubility ranges broadly, from infinitely soluble (fully miscible) which includes ethanol in water, to poorly soluble, which include silver chloride in water. The time period insoluble is regularly applied to poorly or very poorly soluble compounds. The melting factor occurs whilst the two procedures continue at a consistent charge. In a few instances, a supersaturated solvent can be referred to as a metastable, which can also exceed the solubility equilibrium. Solubility ought to no longer be confused with its potential to dissolve or immerse an object, as those approaches can arise now not most effective by means of dissolving, but additionally by chemical reactions. Solubility is also not dependent on size of particle or other kinetic features; given enough time, even larger cells will eventually melt. [28, 29] If the concentration of the solute is equivalent to its solubility, the solution is said to be filled with solute. If the concentration of the solute is less than its solubility, the solution is said to be incomplete. A solution that contains a

low solute concentration is called a dilute, and the one with the highest concentration is called concentrated. Solutions can be prepared when the concentration of the solute surpasses its solubility so these such solutions are said to be supersaturated, and are interesting examples of unequal regions. [30, 31]

International Union of Pure and Applied Chemistry (IUPAC) defines solubility is expressed as the methodical composition of the saturated solute in proportion of solutes employed in a particular solvent. Concentration, molarity, mole fraction, mole ratio are the units stated of solubility. It is usually expressed in terms of mass (grams of solute per kilogram, g per 100 mL), molarity, molarity, mole fraction or other similar details of concentration. The maximum equilibrium amount of solute to the volume of a solvent is the solubility under certain conditions. The advantage of manufacturing melting in this manner is its simplicity, but the disadvantage is that it depends very a great deal on the existence of other solvents inside the solvent (e.g., ordinary ion and many others.). Dispersed solutions of low ionic soluble compounds are every so often described as solubility constants. It's far a balanced process. This refers back to the ratio among dissolved ions in salt and dissolved salt. Like other equilibrium constants, temperature is likewise a number of solitude. The amount of this steady is typically liberated of the existence of other species within the solvent. The drug is taken into consideration very soluble while its most quantity dissolves in 250mL or underwater media at a pH range of 1.0-7.5. An extent of 250mL is received from positive bioequivalence research agreements that determine the remedy of a drug with one glass of water. All of those elements are critical due to the fact eighty five% of the great-promoting tablets in the USA and Europe are given orally. [31- 33]

When the solubility of ingredients in aqueous media is restricted, formulation approaches are required in drug product finding and they endure of crucial importance for the development of key materials choice and commercial product growth. Numerous methods are used in an effort to enhance the solubility & solubility of water soluble components which contain as following:[34-36]

a) Reduction in particle Size

b) Nanonization

c) Cosolvency

d) Hydrotrophy

e) Alteration in pH

f) Sono-crystallization

g) Supercritical Fluid (SCF) Method

h) Solid Dispersion

i) Inclusion Complexation

j) Self Emulsifying or Self Micro Emulsifying Methods

k) Liquisolid Techniques.

1.2 CURCUMIN:

Natural pigment present in the Curcumin which containing anti-fungal, anti-bacterial, anti-viral, anti-inflammatory, anti-malarial, anti-oxidant, anti-mutagenic agent, abilities of wound healing & enhances anti-tumor property in contradiction of various types of tumor cells. Curcumin is a tautomeric complex that exists in the form of enolic in natural diluters & in water as a keto form. Turmeric is the natural plant recognized for its remedial uses, in history 4,000 years to the Vedic arts of India, turmeric have been added as a spice in cooking & has religious meaning. Turmeric is a dried, boiled, refined & shiny curcumalanga. After reaping the all rhizomes are collected. The rhizomes are generally about 2 cm - 8 cm in length & 1 cm - 2 cm wide with splits & bulbs. Dehydrated rhizomes are re-processed to get turmeric powder. Turmeric has different names for different countries and different cultures. Turmeric has at least 53 names only in Sanskrit. [37] Turmeric is the outmost active nutritional supplement existing. In India, It has been used for thousands of years as a healing remedy and a as a spice. Nowadays, science has started to support the traditional claim that turmeric holds compounds with therapeutic properties [38]. The main active ingredient in the turmeric is Curcumin. Curcumin has strong anti-inflammatory properties & it is a very powerful anti-oxidant. However, curcumin does not enter your bloodstream properly. To get the complete effect of curcumin the bioavailability (the level at which your body absorbs the drug) of curcumin essentials to increase. Oxidative impairment is supposed to be one of the reasons of aging & disease, which include loose radicals,

notably active molecules with unpaired electrons. Loose radicals often react with important organisms, including fatty acids, proteins, or DNA. The primary cause that anti-oxidants are very beneficial, is that they guard your frame from unfastened radicals. This is an effective anti-oxidant that can lessen free radicals due to its chemical shape [39, 40]. Further, animal and mobile studies show that curcumin can also inhibit the action of loose radicals and can promote the action of different antioxidants. Additional clinical studies are needed for humans to verify these benefits. It raises the brain hormones up to the level, which promotes the development of new neurons & may help fight the numerous processes of dementia. Curcumin has beneficial effects in a number of areas that are known to contribute to heart disease. In addition, it is an anti-oxidant and anti-inflammatory agent. Curcumin tops to numerous variations in cellular levels that can help prevent and possibly even cure cancer. It can cross the barrier in brain (blood brain barrier) & has been exposed to top to numerous developmental pathological processes of Alzheimer's disease. The anti-inflammatory effect has been shown before now for a multiplicity of syndromes like Parkinson's disease, Alzheimer's disease, multiple sclerosis, epilepsy, and brain damage. Arthritis is a common disease characterized by inflammation of the joints. Numerous studies show that curcumin may be helpful in treating arthritis symptoms and, in some cases, even more effective than anti-inflammatory drugs. Because of its numerous good health properties, like its ability to avoid from heart disease & cancer and can help with longevity. [41, 42].

Curcumin is understood and used worldwide in lots of exclusive ways for its many ability fitness benefits. for example, turmeric is utilized in India - which includes curcumin - used in curries, turmeric is utilized in Japan, offered in tea, turmeric is utilized in Thailand- used for cosmetics, turmeric is utilized in China- used as a color, turmeric is utilized in Korea- provided drinks, turmeric is utilized in Malaysia- used as a disinfectant; in Pakistan- used as an anti-inflammatory agent & turmeric is utilized within the U.S., it's miles applied in sauces, cheese, butter, and chips, as a preservative and in coloration, further to drugs and powders. This is presented in an expansion of forms which consist of capsules, pills, ointments, energy beverages, soaps & cosmetics. Curcuminoids are permitted via the United States Food, Drug and Administration (FDA) as "commonly identified as safe" (GRAS), and good tolerance and protection profiles have been verified in clinical trials, even at doses of among 4000 and 8000 mg / day and doses of as much as 12,000 mg / day, 95% attention of three curcuminoids: curcumin, bisdemethoxycurcumin, and demethoxycurcumin.[43, 44]

Curcumin, a polyphenol, has been validated to direct many signaling molecules even as additionally showing hobby on the mobile degree, which has helped resource its many health advantages [45]. It has been proven to be beneficial for anti-inflammatory conditions, metabolic syndrome, pain, and to assist manage anti-inflammatory and degenerative eye conditions. Similarly, it is been tested to gain the kidneys. Despite the fact that there appear to be infinite restoration benefits in curcumin supplementation, maximum of those advantages are because of its anti-oxidant & anti-inflammatory effects. No matter its described blessings of anti-inflammatory and anti-oxidant procedures, one of the key trouble with the advent of curcumin itself is its negative bioavailability, which is clear in most cases because of negative absorption, speedy metabolism & elimination. A few sellers had been tested to increase its bioavailability via masking those numerous equipment. A lot of them are designed to block the metabolic pathway of curcumin if you want to beautify its bioavailability. E.g., piperine, a well-known enhancer, is a main constituent of black pepper and is associated with a 2000% upward thrust in the bioavailability of curcumin. Therefore, the problem of awful bioavailability seems to be solved through adding piperine-like dealers that enhance bioavailability, accordingly developing a curcumin complex. [46, 47]

Numerous high-quality studies display that turmeric has main profits for your body & brain. Many of these profits come from its main active ingredient i.e., curcumin. It's far a vivid yellow chemical produced with the aid of vegetation of the Curcuma longa species. [48].

Curcumin was termed in 1815 when Vogel and Pierre Joseph Pelletier described the first separation of a "yellow coloring-matter" from the rhizomes of turmeric. At later stage, it was originate to be a mixture of resin and turmeric

oil. In 1910, Milobedzka and Lampe reported the chemical structure of curcumin to be as diferuloylmethane. Later in 1913, the same group accomplished the synthesis of the compound. Although curcumin has been used historically in Ayurvedic medicine, its potential for medicinal properties remains unproven as a therapy when used orally. [49, 50]

1.2.1 CURCUMIN SIDE EFFECTS:

It has a long history of safety established. E.g., according to reports of "JOINT UNITED NATIONS & THE WORLD HEALTH ORGANIZATION EXPERT COMMITTEE ON FOOD ADDITIVES" (JECFA) and "EUROPEAN FOOD SAFETY AUTHORITY" (EFSA), Daily intake acceptable value of curcumin is 0 mg -3 milligram/kilogram weight of body. [51]. A limited trials on healthy objects have supported the safety & efficacy of curcumin. Despite this well-established security, adverse effects are addressed. 7 objects receiving 500-12,000 milligram in dose reaction study & followed 72 hours experienced headache, diarrhea, rash, & yellow stools [52]. In another study, some subjects receiving 0.45 grams - 3.6 grams/day curcumin for 1 to 4 months reported diarrhea & nausea as well as a growth in serum alkaline phosphatase and lactate dehydrogenase content. [53].

1.3 POLYMERS:

In the development of cutting edge medicine, polymer-based totally hydrogel performs a crucial function in lots of biomedical packages. HG (hydrogels) have many blessings over exclusive drug providers due to their H2O (water) absorption volume, biocompatibility & biodegradability. But HG (hydrogels) have some drawbacks along with unrestricted release rate and uncontrolled swelling, main to many issue results. to manipulate the rate of launch and inflammatory residences of hydrogels, positive materials are protected into hydrogels along with clay minerals, floor coatings and distinctive polymers which include chitosan, poly-L-Lysine. Controlled doses have many benefits in commonplace paperwork which encompass decreasing the fee of launch, reducing component outcomes and preserving drug overdose at active degrees in plasma. Water-soluble polymers have a wide range of commercial applications which include meals, remedy, paint, textiles, paper, homes, adhesives, adhesives, water purifiers, and many others. [54] On this paper, water-soluble polymers are divided into categories:

1.3.1 Synthetic and natural.

Polymers formed in soluble water are solvents, dissolving or soluble in water and, thus, altering the physical properties of aqueous systems in the form of gelation, solidification or emulsification / stabilization. These polymers usually have duplicate units or unit blocks; polymer chains contain hydrophilic groups that are partially or embedded in the spine. Hydrophilic groups may be nonionic, anionic, cationic or amphoteric. [55]

Chemicals include technology, which includes new chemicals that are water soluble despite their high therapeutic effect. These have limits in their potential use in the preparation of bioavailable medicinal products. Rate limiting factors in all the cases for absorption of drug is the dissolution rate of the API in the gastrointestinal fluids. Therefore, the availability of oral bioavailability and solid oral dosage forms of water soluble drugs is currently part of single of the main goals and major trials in the field of developing new drugs. "Solid dispersion" is still more favorable than the previous one, a policy that overcomes this limitation Due to its simplicity from the point of view of process scalability and manufacturing, solid dispersion has converted one of the supreme dynamic and auspicious research fields for cement companies with great potential. In addition, such formulations have significant advantages over commonly used methods, particularly for micro needling. Therefore, the popularity of concrete dispersal is expected to increase speedily. The word stable dispersion refers to strong nation combinations, which might be made by using dispersion, generally via solvent vaporization or by dissolving one or extra energetic substances in an inert carrier matrix. In those extensions, the motor particle may be dispensed in a totally crystalline nation (inside the form of strong drug debris), in a semicircular state, and in a completely amorphous kingdom (nice particle dispersion) within the service. Such structures look like very powerful in enhancing the fee of elimination of slow-melting pills. [56] Poly (ethylene glycol) (PEG) and poly vinyl Pyrrolidone (PVP) are the main drug providers to put together for solid dispersion preparations because of their strong hydrophilic houses and

their potential to shape molecular addicts with many compounds. The presence of hydroxyl or carbonyl organizations within the regenerative gadgets of those polymers increases the solubility of the drug and improves its bioavailability. The fee of elimination of the stable dispersion element is generally as a result of one of the following strategies; Eutectic formation, improved surface area because of rain inside the carrier, formation of stable answer, restore because of near contact with hydrophilic service, rain in metastable crystalline form or lower in fabric luster. Both drug and drug aggregate residences and production methods could have an effect at the type of stable dispersion that is shaped, as well as the following stable dispersion behavior. Solid dispersion is usually done in one of two ways: dissolving the company-company mixture or dissolving the solvent and carrier in the regenerative solvent after solvent removal. For example, direct soluble extraction in solid gelatin capsules or compression formation of transdermal patches may occur in large preparations. Hydrophilic polymers have been extensively investigated as solvents. [57] This method requires at least a solvent to dissolve. We used different polymer carriers in this study. Poly vinyl Pyrrolidone (PVP) has been selected as a water-soluble polymer. Hydroxy propyl methyl cellulose (HPMC) was selected as a water soluble and polymer dispersion. The bodily & chemical properties of fluconazole in strong dispersion are characterized by way of calciferous calorimetry & powder X-ray diffraction, as well as the outcomes of various hydrophilic solid dispersion providers on its solubility have been investigated. The extra impact of assembling a number of these polymers need to be taken into consideration. Ultimately, with the aid of evaluating the stable dispersion traits produced via this technique the usage of the same old solvent evaporation approach. [58]

2. Material and Method

2.1 Materials: Curcumin gift sample was received by Sigma-Aldrich.com, Dimethyl Sulfoxide, Calcium chloride and Ethanol was received by Qualikems FineChem Pvt. Ltd., Mumbai, India, Sodium Alginate and Gelatin was received by Central Drug house Ltd. New Delhi, India.

2.2 Method

2.2.1 Preformulation Studies

2.2.1.1 Melting Point

Melting point of curcumin was determined by capillary fusion method. Small quantity of curcumin filled in capillary tube sealed at one end. Capillary filled by pressing the open end into small heap of powder curcumin then turning open end up and vibrating it, so that curcumin get tightly packed to a depth of 2-3 mm. Further, melting point was measured with help of thermometer using auto melting point apparatus and compared with value given in literature. [59, 60]

2.2.1.2 UV Spectrophotometry

10 mg of Curcumin weighed and dissolved in 10 ml of phosphate buffer 7.4 with dimethyl sulfoxide as a cosolvent, from this 0.01 ml of solution withdrawn and volume made up to 10 ml with phosphate buffer 7.4 to obtain concentration 1 µg/ml. This solution then scanned under UV spectrophotometer in the range of 400-800 nm in basic spectrum mode λ_{max} and compared with value given in literature.

2.2.1.3 FT- IR Spectroscopy

FTIR spectrum of Curcumin was determined by KBr pellet method using Fourier transform infra-red spectrophotometer (Shimadzu- 8400S, Kyoto, Japan) with DRS attachment and spectrum of KBr as background. The range of scanning was 400-4000 cm^{-1} at which sample is overlapped. The FTIR spectrum was compared with the reference spectrum.

2.2.2 Preparation of Calibration Curve

2.2.2.1 Preparation of Calibration Curve of Curcumin in Phosphate Buffer pH 7.4

(a) Composition of Phosphate Buffer

Phosphate buffer (pH 7.4) are prepared by dissolving 2.38 gm of disodium hydrogen phosphate, 0.19 gm of potassium dihydrogen phosphate and 8.0 gm of Sodium chloride 1000 ml distilled water and pH is adjusted to 7.4.

(b) Preparation of Stock Solution

10 mg of Curcumin weighed on digital balance and transferred into 10 ml of volumetric flask containing 10 ml of phosphate buffer pH 7.4 with dimethyl sulfoxide as cosolvent. Further 0.1 ml of sample was withdrawn from this solution

and diluted up to 10 ml using phosphate buffer pH 7.4 (10µg/ml).

(c) Procedure for Estimation

Aliquots of 0.04, 0.08, 0.12, 0.16, 0.20 and 0.24 ml of solution withdrawn from stock solution using calibrated pipette and volume in tune to 10 ml with phosphate buffer (pH 7.4) to obtain aliquots of 0.04, 0.08, 0.12, 0.16, 0.20 and 0.24 µg/ml. Calibration curve was prepared by measuring absorbance of these solution against blank under UV spectrophotometer at 424 nm.

2.2.3 Validation of Analytical Method

The analytical method was validated as per USP guideline for assay in category I and as per ICH Q2A guideline. The linearity of the calibration curve was determined the curve of absorbance (y) versus concentration (x) of curcumin in the concentration range of (0.04-0.24 µg/ml). The accuracy of the method was performed by adding known amounts of curcumin to placebo solution and then comparing the averaged measured concentration to the nominal concentration, and was expressed in percentage. To determine the intra-day precision of the method, samples were analyzed within the day three times. To determine the inter-day, the inter-day accuracy and precision assay was repeated on three consecutive days. Precision was evaluated by calculating the relative standard deviation (R.S.D) of measured concentration at each sample.

2.2.4 Preparation of Microbeads:

In this technique (Ionotropic Gelation Technique), Curcumin, Gelatin and Sodium alginate were taken in various amounts according to factorial design in a 100 ml distilled water to make a solution of these two polymers. Dimethyl sulfoxide was used as cosolvent for drug. After that, the solution was added dropwise into the calcium chloride solution with 21 gauze needle size from syringe. Allow beads for 1 hour to rigidize and dry at 50^{o}C in oven. Formulation design for the curcumin microbeads is shown below in table 1.

Formulation Code	Amount of Curcumin Drug	Sodium Alginate	Gelatin	Calcium Chloride
CB1	350 mg	1.50 gm	0.250 gm	0.500 gm
CB2	350 mg	1.50 gm	0.250 gm	2.0 gm
CB3	350 mg	1.50 gm	0.500 gm	0.500 gm
CB4	350 mg	1.50 gm	0.500 gm	2.0 gm
CB5	350 mg	1.75gm	0.250 gm	0.500 gm
CB6	350 mg	1.75gm	0.250 gm	2.0 gm
CB7	350 mg	1.75gm	0.500 gm	0.500 gm
CB8	350 mg	1.75gm	0.500 gm	2.0 gm

Table 1: Formulation Design for the Curcumin Microbeads

2.2.5 Evaluation of Curcumin Microbeads

2.2.6.1 Entrapment Efficiency

Weighed accurately 10 milligram quantity of prepared curcumin microbeads were grounded and dispersed in phosphate buffer pH 7.4 and kept the solution for 24 hours at room temperature. The prepared microbeads was then sieved and present curcumin was investigated using U.V at 424 nm.

% EE = Drug amount present in microbeads/Total drug amount incorporated × 100

2.2.5.2 Particle Size

The randomly sampling of prepared microbeads has been done and their size was examined using a calibrated optical microscope using stage micro meter and eye piece under regular polarized light. 50 random beads were inspected and each bead was carried out in triplicate. Optical microscope was used for determining the particle size of microbeads under regular polarized light, and the mean particle size was calculated.

2.2.5.3 Differential Scanning Calorimetry (DSC)

The arcs of Curcumin, placebo microbeads, and curcumin loaded microbeads have been noted using thermogravimetric analyzer and Rheometric scientific, model DSC-SP. The evaluation turned into carried out by means of heating every sample from 40-600°C at the heating price of 10 °C in step with minute under N (nitrogen) surroundings.

2.2.5.4. Scanning Electron Microscopy (SEM)

It is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The shape of the microbeads letters was detected using the SEM with a speeded up voltage of 20 kV.

2.2.5.5 Morphology

Surface morphology of microbeads became investigated by way of Scanning Electron Microscopy (SEM). The microbeads, lined with Platinum by way of ion auto high-quality coater, for twenty second at 1.1 Volts underneath Ar ecosystem were established onto metal stubs the usage of twice micrographs were taken.

2.2.5.6 Fourier Transform Infrared Spectroscopy

FTIR spectrum of Curcumin was determined by KBr pellet method using Fourier transform infra-red spectrophotometer (Shimadzu- 8400S, Kyoto, Japan) with DRS attachment and spectrum of KBr as background. The range of scanning was 400-4000 cm^{-1} at which sample is overlapped. The FTIR spectrum was compared with the reference spectrum.

2.2.5.7 *In-vitro* Release

The *in-vitro* release of curcumin from the microbeads was considered in phosphate buffer at pH 7.4. The curcumin loaded micro beads were engaged in basket apparatus in 900 mL of phosphate buffer pH 7.4 incubated at 37 ± 0.5°C at 100 rpm. At appropriate time intervals the solution was taken out (5, 10, 15, 30, 60, 120, 180, 240, 300, 360, 420, and 480) and swapped with the freshly prepared standard. Models were examined at Lambda max (λ_{max}) of 424 nanometer. Release of curcumin were calculated from the curve of calibration and signified as cumulative percentage of curcumin release vs. time.

2.2.5.8 Selection of Optimized Formulation

In the name of high entrapment performance, the percentage of accumulated drug releases with a record of very low Polydispersity, full particle size, and Scanning Electron Microscopy formations are selected.

2.2.6 Preparation of Gelatin Gel

Gelatin gel was prepared as, gelatin was taken (20% w/v) in distilled water and heated at 55-75°C. Then the warm solution was kept at room temperature for 2-3 days for air drying to make gel.

2.2.7 Addition of Beads into Gelatin Gel

When the gelatin gel is completely cooled then the prepared beads were added into it which make the beads wet with stirring with glass rod.

2.2.8 Filling the Capsule with Gelatin Gel Containing Beads

The capsules were taken of hard gelatin shell, capsules were filled according to the dose of the drug.

2.2.9 Characterization of Capsules Containing Gel Embedded Beads

5.2.9.1 Drug Content

The 1 capsule were taken randomly and mixed in the determined quantity of phosphate buffer pH 7.4, then the absorbance checked on UV spectrophotometer at 424 nm and drug content was measured by:

Drug content = Amount of drug present in capsule/ Actual drug in capsule * 100

2.2.9.2 Weight Variation of Capsules

Weigh 20 capsules individually and determine the average weight. The individual weight should be with in limit of 90-110% of average weight, if not all of capsules fall within the limits, Weigh 20 capsules individually. Remove the net content of each capsule with the aid of a small brush. Weigh the empty shells individually.

2.2.9.3 Stability

For the stability study of the microbeads and capsules, both visual control and analysis rate of drug content was determined. For this study microbeads was placed in a climatic chamber for 3 months at 25 ± 2°C temperature and 60% relative humidity. This microbeads was kept in amber colored bottle with a lid. The drug content was measured from the beginning and also at the end of the 3 months spectrophotometrically at λmax 424 nm.

3. Result and Discussion

3.1 Pre-formulation Study

3.1.1 Melting Point

Melting point of Curcumin was found to be 181°C which complies with the literature value of 179-183°C indicating the identity and purity of drug sample.

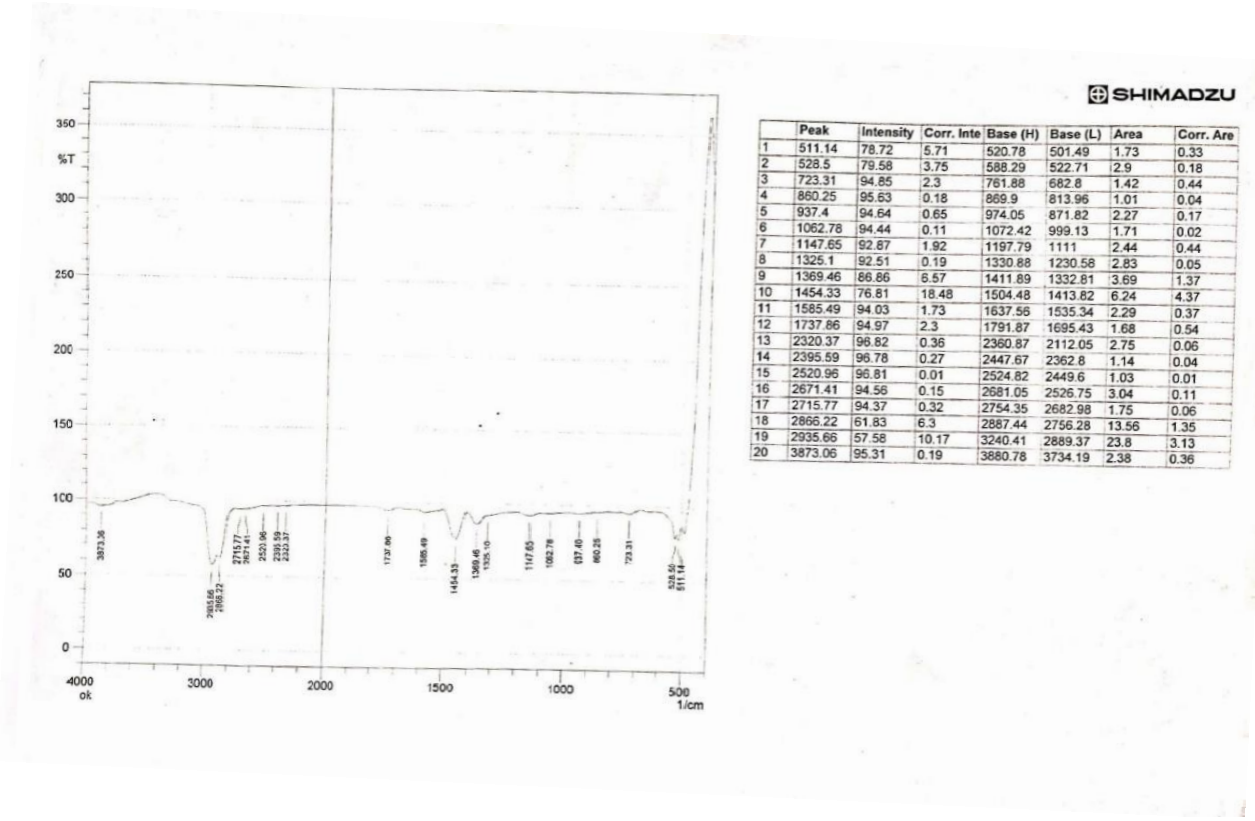
3.1.2 UV Spectrophotometry

Spectrophotometric studies were carried out in order to determine the λmax of Curcumin at different physiological pH i.e. phosphate buffer (pH 7.4), In Table 2 it is shown that experimental absorption maxima at 424 nm which complies with the literature value of 424 nm.

S. No.	Media	Literature(λ_{max})	Experimental(λ_{max})
1	0.00001% (w/v) solution of Curcumin in Phosphate buffer 7.4	424 nm, 428 nm, 418 nm	424 nm

Table: 2 - Spectrophotometric determination of Curcumin.

3.1.3 FT-IR Spectroscopy: Infra-red spectrum of Curcumin. The major peaks observed and corresponding functional groups. Infra-red spectrum shows peak characteristic of structure of Curcumin. FTIR interpretation of Curcumin is shown in the Fig 1.



	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	511.14	78.72	5.71	520.78	501.49	1.73	0.33
2	528.5	79.58	3.75	588.29	522.71	2.9	0.18
3	723.31	94.85	2.3	761.88	682.8	1.42	0.44
4	860.25	95.63	0.18	869.9	813.96	1.01	0.04
5	937.4	94.64	0.65	974.05	871.82	2.27	0.17
6	1062.78	94.44	0.11	1072.42	999.13	1.71	0.02
7	1147.65	92.67	1.92	1197.79	1111	2.44	0.44
8	1325.1	92.51	0.19	1330.88	1230.58	2.83	0.05
9	1369.46	86.86	6.57	1411.89	1332.81	3.69	1.37
10	1454.33	76.81	18.48	1504.48	1413.82	6.24	4.37
11	1585.49	94.03	1.73	1637.56	1535.34	2.29	0.37
12	1737.86	94.97	2.3	1791.87	1695.43	1.68	0.54
13	2320.37	96.82	0.36	2360.87	2112.05	2.75	0.06
14	2395.59	96.78	0.27	2447.67	2362.8	1.14	0.04
15	2520.96	96.81	0.01	2524.82	2449.6	1.03	0.01
16	2671.41	94.56	0.15	2681.05	2526.75	3.04	0.11
17	2715.77	94.37	0.32	2754.35	2682.98	1.75	0.06
18	2866.22	61.83	6.3	2887.44	2756.28	13.56	1.35
19	2935.66	57.58	10.17	3240.41	2889.37	23.8	3.13
20	3873.06	95.31	0.19	3880.78	3734.19	2.38	0.36

Fig 1: FTIR interpretation of Curcumin

FTIR study of drug shows that the drug is pure in nature and not contain any impurities or foreign particles because of the peaks in graph which are come after IR study shows only drug's peaks which compared to literature.

3.1.4 Preparation of Calibration Curve

In order to obtain standard calibration curves the mean absorbance values (n=6) of different concentrations in range of 0.04-0.24 µg/ml (Table: 3) of stock solution in phosphate buffer (pH 6.4) was prepared and the absorbance were plotted against concentration and shown in (fig 2)

Sr. no.	Concentration	Absorbance*± S.D
1	0.04	0.097 ± 0.003
2	0.08	0.179 ± 0.005
3	0.12	0.279 ± 0.008
4	0.16	0.330 ± 0.006
5	0.20	0.421 ± 0.002
6	0.24	0.507 ± 0.004

Table: 3 Calibration curve of Curcumin in phosphate buffer (pH 7.4)

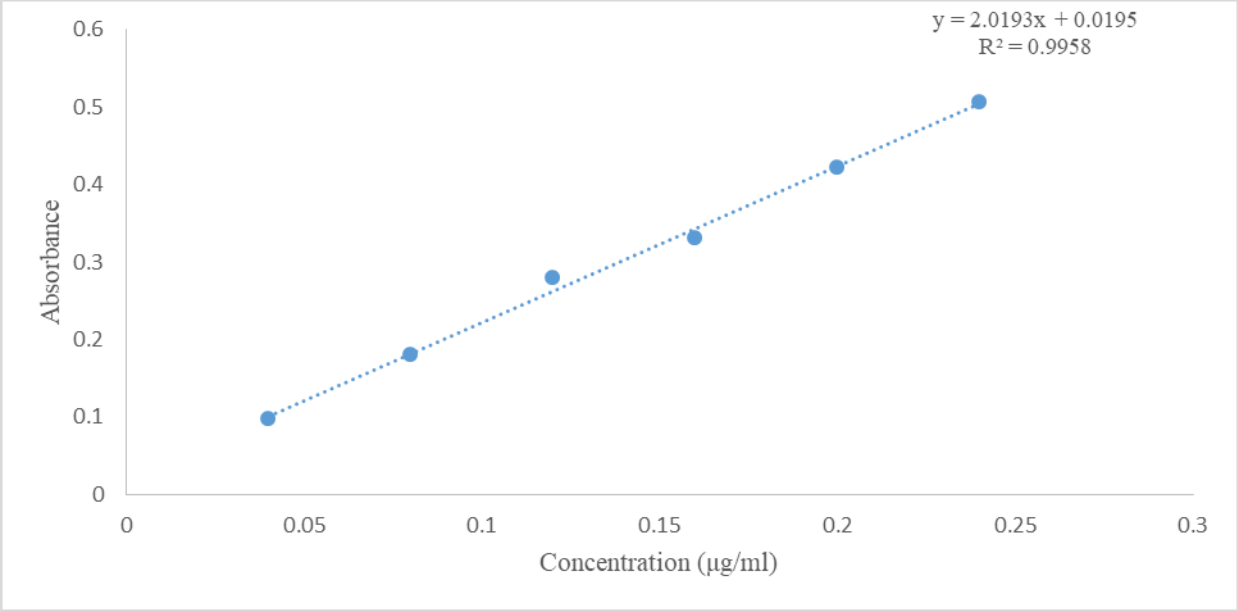


Fig 2: Calibration Curve of Curcumin in Phosphate Buffer (pH 7.4)

3.1.5 Validation of Analytical Method

Validation must demonstrate that the analytical procedure is able to accurately and precisely predict the concentration of unknown samples. The closeness of the test result to the exact value is known as accuracy which must be need to meet the requirement for planned analytical application. Precision is known as the repeated measurement of the single test result when the method is applied multiple times repeatedly of homogenous sample and it is expressed as standard deviation or relatively standard deviation. Validation of analytical method is shown in the table 4. The values of % RSD between calibrations curves of three consecutive days and at three consecutive time points were found to be less than 2 when analyzed for inter and intraday precision as shown in table 5 and 6. The calibration showed the linearity in the range of 0.04-0.24 µg/ml with an accuracy of 97.64-101.51 % range. This reflects that the inter-day and intraday precision were between calibration curves. The lowest concentration of the sample which can be detected is known as the limit of detection. The lowest concentration of analyte which can be detect with acceptable accuracy and precision under the experimental condition. The data which is determined under stated experimental conditions indicated that the drug was stable during complete study period. The data is showed in (table 6).

Parameter	Observation
Accuracy(% Recovery)	97.64-101.51%
Range (µg/ml)	0.04-0.24
Regression line equation	y = 2.0193x + 0.0195
(R^2)	R^2 = 0.9958
Interday precision (%RSD)	±1.4971
Intraday precision (%RSD)	±1.2629
LOD (µg/ml)	0.413
LOQ (µg/ml)	1.4324

Table: 4 Validation of Analytical Method

Concentration	Day 1	Day 2	Day 3	Mean	SD	%RSD	Average
0.04	0.11	0.114	0.116	0.1133	0.0030	2.6956	1.4971
0.12	0.289	0.298	0.295	0.2940	0.0045	1.5586	
0.20	0.421	0.422	0.423	0.4220	0.0010	0.2369	

Table no. 5: Interday Precision in Phosphate Buffer 7.4

Concentration	Morning	Afternoon	Evening	Mean	SD	%RSD	Average
0.04	0.115	0.118	0.114	0.11567	0.0020	1.7997	1.2629
0.12	0.288	0.294	0.298	0.29333	0.0050	1.7158	
0.20	0.422	0.424	0.422	0.42266	0.0011	0.2731	

Table no. 6: Intraday Precision in Phosphate Buffer 7.4

In the Interday precision, three absorbance readings were taken in the three consecutive days at the same time interval and in Intraday precision absorbance readings were taken at the same day with the three hour of time interval like morning (09:00), afternoon(12:00) and evening (16:00).

3.2 Characterization of beads containing Curcumin (CB1-CB8)

3.2.1 Entrapment Efficiency

The drug entrapment efficiency was found to be in the range of (78.82 ± 1.56 to 93.34 ± 0.74) as shown in (Table 7). As the Quantity of polymer and calcium chloride is increasing, the percentage of entrapped drug is increasing like at low polymer concentration the entrapped drug is 78.82% and at high polymer concentration, the entrapped drug is 93.34%.

3.2.2 Particle size:

The optical microscope is firstly calibrated with the help of stage micrometer and eye piece under regular polarized light by this method:

100 divisions of an eye piece corresponds to 14 divisions of stage micrometer.

And 1 division of eye piece = 0.01 mm (millimeter)

Therefore, 14 division of stage micrometer = 14 x 0.01 = 0.14 mm

100 division of eye piece = 0.14 mm

Therefore, 1 division of eye piece = 0.14/100 mm

= (0.14/100) x 1000 µm (micrometer)

So, the calibration factor is = 1.4 µm

To calculate the size of beads, 1 division of eye piece = 1.4 µm of stage micrometer

Therefore, size will be calculated just by multiplying the size with 1.4 µm.

The formulation 1 shows minimum particle size i.e. 67.99 µm which results that the quantity of polymer is responsible for the less size of the beads. The quantity of polymer is low in the CB1 and high in the CB8 and CB7 which result 76 and 78 µm respectively that directly reveals that the polymer concentration is responsible for particle size of the beads because in the ionotropic gelation method, the amount of polymer is increased than the size will increase due to the bulk of the drop dropped from the syringes. Sodium alginate polymer have the good literature in preparation of microbeads and in this research it plays a vital role in preparing these beads with lesser bead size. Gelatin is also helpful for sodium alginate to maintain the size of beads.

Formulation	% E E * SD	Particle Size* SD (µm)
CB1	78.822 ± 0.546	68.453 ± 1.234
CB2	79.765 ± 0.834	67.993 ± 0.596
CB3	89.057 ± 1.228	70.874 ± 0.978
CB4	87.794 ± 1.034	71.249 ± 0.789
CB5	91.293 ± 0.986	74.948 ± 0.109
CB6	84.435 ± 1.100	73.938 ± 0.098
CB7	90.043 ± 1.198	78.837 ± 0.123
CB8	93.349 ± 0.745	76.987 ± 0.679

Table 7: Entrapment efficiency and particle size of formulations.

3.2.3 Scanning Electron Microscopy (SEM)

Examination of the shape of optimized micro beads developed (sodium alginate and gelatin curcumin-containing micro beads were performed by scanning electron microscopy (Fig 3) SEM studies revealed that microbeads are almost round in shape and have a rough surface from the outer side (Fig 3). SEM study revealed that, microbeads are almost circular in shape with irregular outer surface. This shows that, beads have well mechanically strength. Rough surface from the outer side with wrinkles appeared for Curcumin-loaded sodium alginate and gelatin beads due to the entrapment into the matrix of curcumin.

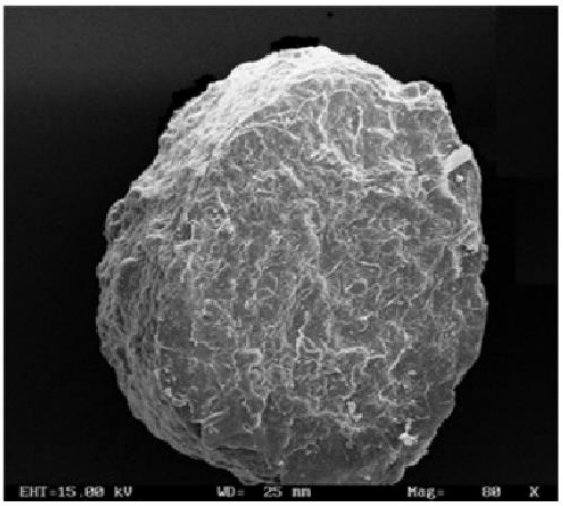


Fig 3: SEM Image of the Curcumin Alginate Microbeads.

3.2.4. DSC Analysis

The DSC thermograms for Curcumin indicated as "a", placebo microbeads indicated as "b", curcumin filled microbeads indicated as "c", are proven in fig 4. The curve of placebo microbeads indicates endothermic peaks at 87 & 205°C. DSC

curve of Curcumin indicates endothermic top at 181°C because of its melting, at the same time as no such height has been found in curcumin filled microbeads indicates the guarantees that the curcumin is molecularly distributed microbeads.

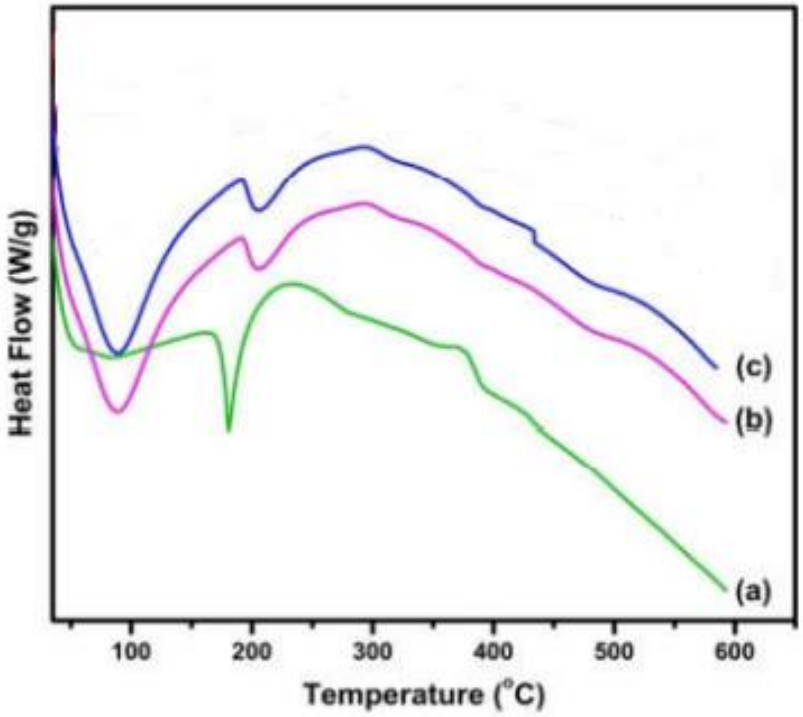
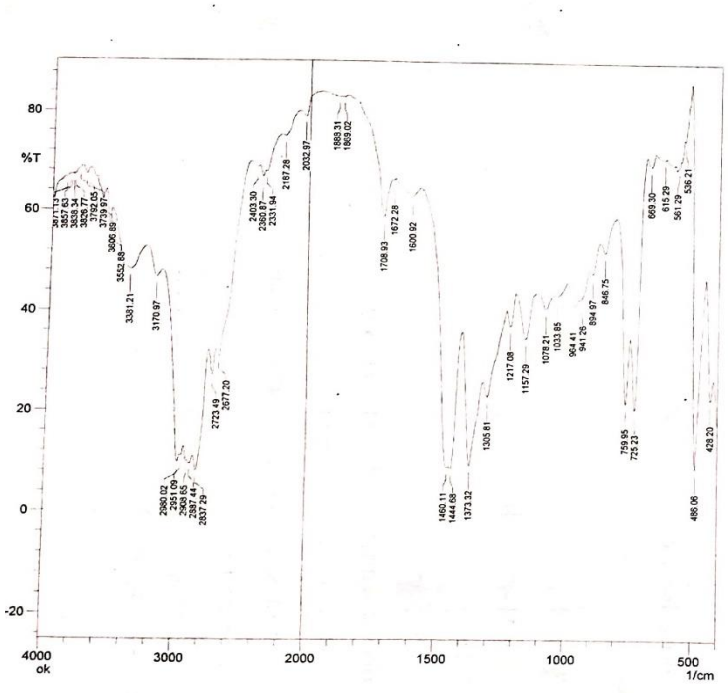


Fig 4: DSC image of the Curcumin Alginate Microbeads.

3.2.5 FTIR

Infra-red spectrum of Curcumin alginate beads. The major peaks observed and corresponding functional groups. Infra- red spectrum shows peak characteristic of structure of Curcumin alginate beads. FTIR of optimized formulation is shown in the fig 5.



SHIMADZU

	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	428.2	23.112	11.583	447.49	416.62	16.26	2.277
2	486.06	9.216	60.128	514.99	449.41	32.369	19.462
3	536.21	75.014	2.104	540.07	516.92	2.296	0.169
4	561.29	69.685	0.513	563.21	540.07	3.316	0.114
5	615.29	71.367	0.121	624.94	611.43	1.975	0.007
6	669.3	69.973	2.1	684.73	651.94	4.903	0.249
7	725.23	21.227	25.504	742.59	686.66	22.579	6.535
8	759.95	22.414	19.348	802.39	744.52	23.907	4.638
9	846.75	52.706	3.372	864.11	804.32	15.132	0.609
10	894.97	48.456	2.155	904.61	866.04	11.239	0.323
11	941.26	43.269	0.933	947.05	906.54	13.832	0.304
12	964.41	42.056	2.539	991.41	948.98	15.267	0.535
13	1033.85	43.962	0.798	1045.42	993.34	18.11	0.304
14	1078.21	41.386	3.013	1109.07	1047.35	22.606	0.837
15	1157.29	35.365	9.202	1195.87	1111	33.262	3.477
16	1217.08	37.856	4.596	1230.58	1197.79	13.062	0.981
17	1305.81	23.706	5.449	1321.24	1232.51	45.393	2.928
18	1373.32	9.619	23.394	1402.25	1323.17	57.843	17.807
19	1444.68	9.019	4.736	1452.4	1404.18	34.754	1.698
20	1460.11	9.243	2.946	1570.06	1454.33	49.737	0.727
21	1600.92	63.27	1.926	1633.71	1571.99	11.927	0.46
22	1672.28	66.554	0.194	1676.14	1666.5	1.697	0.006
23	1708.93	59.288	10.387	1851.66	1678.07	23.188	2.117
24	1869.02	83.185	0.189	1882.52	1853.59	2.297	0.014
25	1888.31	83.21	0.109	1905.67	1884.45	1.685	0.009
26	2032.97	79.098	2.636	2077.33	1957.75	10.613	0.545
27	2187.28	75.206	1.191	2214.28	2079.26	14.974	0.392
28	2331.94	67.978	0.454	2337.72	2216.21	17.054	0.067
29	2360.87	66.942	1.616	2385.95	2339.65	7.805	0.222
30	2403.3	68.743	0.567	2457.31	2387.87	11.066	0.113
31	2677.2	31.111	4.738	2698.41	2459.24	76.716	3.608
32	2723.49	27.392	5.019	2750.49	2700.34	26.277	1.736
33	2837.29	8.215	7.461	2860.43	2752.42	88.192	11.214
34	2887.44	9.578	0.959	2902.87	2862.36	40.421	1.042
35	2908.65	10.087	0.502	2931.8	2904.8	25.86	0.424
36	2951.09	11.115	0.742	2958.8	2933.73	23.296	0.404
37	2980.02	10.008	5.674	3124.68	2960.73	93.857	3.261
38	3170.97	46.855	2.966	3253.91	3126.61	39.208	1.445
39	3381.21	48.259	8.056	3529.73	3255.84	78.152	10.201
40	3552.88	56.38	5.662	3579.88	3531.66	10.877	0.922

Fig 5: FTIR of the optimized formulation.

The compatibility of Curcumin with sodium alginate with gelatin polymers has been investigated by IR-spectroscopy. The Infra-Red spectra of a polymer & drug compound was linked with the pure drug spectra. Where there was no substantial change in peaks, indicating drug stability during the encapsulation process that suggested Curcumin into beads was well combined & there might be a weak inter-molecular collaboration between Curcumin, sodium alginate & gelatin. In Curcumin loaded SA and gelatin, maximum of the curcumin peaks (2935, 2715, 2671, 2395, and 2320 cm^{-1}) are combined with the active SA and gelatin group peaks.

3.2.6 *In-vitro* release of drug:

In-vitro release of drug of all formulation are shown in the table 8 and 9. In this we include dissolution of capsule containing gel embedded beads and dried beads, this is done for the comparison of these two. The % release shows that there is a difference between the release profile of dried beads and wet beads inside the capsule as shown in the fig 6 and 7.

In wet beads inside the capsule shows a rapid and better drug release as compare to dried beads, the CB5-CB8 shows highest drug release due to the higher amount of polymers i.e., sodium alginate, gelatin and calcium chloride for ion exchange. The highest drug release shown by CB8 due to highest amount of the polymers. This shows that the polymers shows impact on drug release as shown in table 9. And in the dried beads they did not show the rapid release due to dryness of beads. In this CB8 shows highest drug release due to polymers as shown in table 8.

Time	CB1	CB2	CB3	CB4	CB5	CB6	CB7	CB8
0	0	0	0	0	0	0	0	0
10	1.138	1.242	1.245	1.345	1.353	1.343	1.452	1.354
30	3.831	2.353	3.877	3.436	3.875	2.456	3.656	4.355
60	6.889	5.35	5.341	6.312	5.245	5.234	6.322	6.953
90	10.981	9.533	8.242	10.242	9.344	8.3423	10.243	10.453
120	14.419	13.324	12.383	14.374	13.335	12.347	13.484	15.483
180	18.965	17.383	16.982	17.483	17.394	16.484	16.936	18.936
240	20.846	20.935	19.303	19.484	21.496	19.393	20.383	22.835
300	23.956	23.863	22.945	22.389	24.384	22.393	23.498	25.968
360	25.906	26.454	25.484	25.433	27.594	25.595	25.484	28.395
420	28.074	28.857	27.738	29.854	29.964	29.854	28.853	31.854
480	31.955	30.837	31.836	32.484	32.484	33.487	30.947	34.494

Table 8: % Cumulative Drug Release of Dried Beads in Phosphate Buffer 7.4

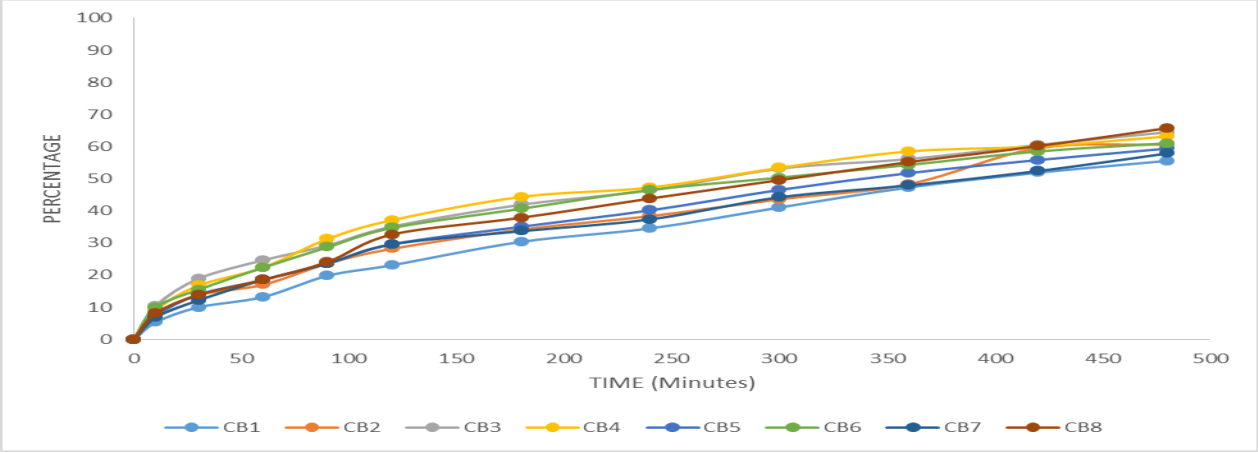


Fig 6: % Cumulative release of dried beads in phosphate buffer 7.4

Time	CB1	CB2	CB3	CB4	CB5	CB6	CB7	CB8
0	0	0	0	0	0	0	0	0
10	5.284	7.831	10.378	9.104	7.322	9.869	6.812	8.086
30	9.996	13.816	18.910	16.745	13.944	15.472	12.161	13.816
60	13.179	17.000	24.640	22.348	18.528	22.348	18.528	18.528
90	19.801	23.622	29.266	31.266	23.622	28.715	23.622	24.131
120	23.112	28.206	35.120	37.120	29.607	34.700	29.607	32.663
180	30.372	34.191	41.924	44.378	35.082	40.685	33.682	37.884
240	34.573	38.393	46.393	47.307	40.176	46.543	37.375	43.869
300	41.067	43.614	53.161	53.420	46.543	50.363	44.251	49.599
360	47.307	48.326	56.146	58.513	51.764	54.311	47.944	55.203
420	51.892	59.532	60.551	60.042	55.839	58.513	52.401	60.169
480	55.585	60.678	64.499	63.225	59.405	61.060	57.877	65.772

Table 9: % Cumulative drug release of capsule containing gel embedded beads in phosphate buffer 7.4.

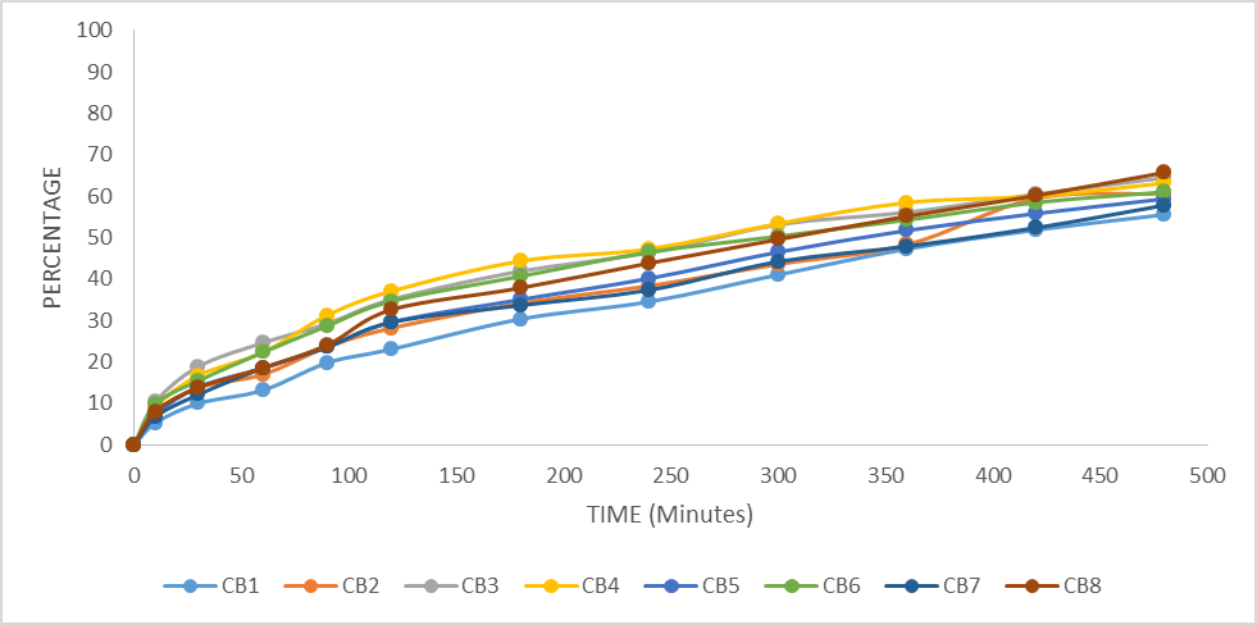


Fig 7: % Cumulative Release of Capsule Contains Gel Embedded Beads in Phosphate Buffer 7.4.

Rate of dissolution is the amount of drug substance that goes in solution per unit time under the standard conditions of temperature and solvent composition. The needs of dissolution model is necessary for developing *in-vitro in-vivo* correlation, to reduce the cost and speed up the development. Capsules containing gel embedded beads follow the Higuchi model which directly show that the drug release depend upon the diffusion process based on the Fick's law which is square root time dependent. Stagnant layer is the formation of a thin film at the interface which helps to release the drug more efficiently. In capsule containing gel embedded beads the release is solubility independent and it is barrier dependent which means need to change the diffusion of the solid mass but the dried beads follows the first order model in which the rate directly depends upon the concentration of the drug which means if we

change the concentration than solubility will change. First order is concentration dependent process which leads to solubility dependent not barrier dependent if we increase the concentration of the drug than it may be harmful for the body because the side effects of drug will also increase which are severe. So the capsule containing gel embedded beads are more effective at same concentration than the dried beads which is the rational of this study.

3.2.7 Selection of Optimized Formulation

CB8 was selected as optimized formulation with highest drug release and entrapment efficiency.

3.3 Characterization of Capsules

3.3.1 Drug Content

This was measured for surety of drug quantity inside the capsules for its performance. The dose was calculated according to the adult person's weight for the optimum results of the drug. The measured drug content is shown in the table 10 and the drug content is in the range.

3.3.2 Weight Variation of Capsules

The weight variation test is necessary for the verification of the formulation inside the capsule. The weight variation test shows that, all the capsules are under the limits of tests. As shown in the table 10.

Formulation no.	Weight variation* SD	Drug Content * SD
CB1	376.123 ± 0.373	65.434 ± 0.023
CB2	375.239 ± 0.652	71.242 ± 0.532
CB3	369.453 ± 0.733	73.333 ± 0.434
CB4	377.453 ± 0.563	74.863 ± 0.985
CB5	375.574 ± 0.473	80.947 ± 0.173
CB6	381.344 ± 0.354	79.563 ± 0.836
CB7	385.344 ± 0.687	75.384 ± 0.153
CB8	382.345 ± 0.683	84.463 ± 0.936

Table 10: Weight variation and drug content of capsules containing beads.

3.3.3 Stability Studies:

For optimized formulation (CB8) stability test has been performed. No significance difference in drug content was found at zero i.e. (84.463 ± 0.936) and 3rd month (79.674 ± 0.163), that indicating no chemical degradation, phase separation and sedimentation at the time of stability study. So the result was found to be the formed formulation maintained their integrity.

4. Conclusion

Alginate beads are considered as effective and superior for increasing solubility and stability of drugs from other polymers, alginate beads containing curcumin which overcome the drawback of solubility and permeability and also minimize side effects of that particular drug. The developed formulation shows rapid and sustain release of the drug which helps to increase patient compliances related to Curcumin. The formulation proved better permeability of Curcumin from other formulations. The release from wet beads i.e. capsule containing gel embedded beads shows better results as compared to dried beads which gives the straight point towards the increased solubility and permeability of Curcumin. The capsules containing gel embedded beads follow the Higuchi model which results that permeation is depend upon diffusion process. This formulation have ease of manufacture and required less polymers.

Furthermore, this formulation is ready to release formulation which have no lag time for releasing the drug because the microbeads are already swelled and wet in a gelatin gel and it also sustain the release of Curcumin.

5. References:

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