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Development of Chitosan Microsphere-Based Herbal Formulations for Targeted Therapy in Obesity Management

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Abstract

The present study was aimed to prepare and evaluate curcumin-piperine-loaded microspheres, assess their sustained anti-obesity effects in obese rats, and investigate the enhancement of curcumin bioavailability through piperine. Curcumin microspheres were successfully formulated and characterized for particle size, morphology, flow properties, entrapment efficiency, and stability. FTIR analysis confirmed no significant interaction between drug and polymers, while SEM showed spherical particles with smooth surfaces and sizes ranging from 1.676 to 5.490µm. The microspheres exhibited good flow properties, high yield (44.6–97.4%), and satisfactory entrapment efficiency (46.4–73.3%), with optimal performance at a 1:4 drug-to-polymer ratio. Cross-linked formulations demonstrated reduced swelling and pH-dependent, colon-targeted sustained release, as confirmed by mucoadhesive and release kinetics studies. Pharmacokinetic and biodistribution data showed enhanced bioavailability, delayed T_{max} (8 h), and a six-fold increase in AUC compared to plain curcumin. *In-vivo* results revealed significant reductions in body weight, BMI, lipid parameters (TC, TGs, LDL), glucose levels, and organ weight, with an increase in HDL levels, particularly at the 200mg/kg dose. Stability studies over three months confirmed the robustness of the formulation, making curcumin-loaded microspheres a promising candidate for effective anti-obesity therapy with enhanced bioavailability.

Keywords: Obesity, Curcumin, Piperine, Microspheres, Poly(lactic-co-glycolic acid), SEM, *In-Vitro*, *In-Vivo*

1. INTRODUCTION

Over 650 million people worldwide suffer from obesity, which is defined as having a body mass index (BMI) of 30kg/m² or more. It is characterized by abnormal or excessive fat storage and is +strongly associated with a number of major health issues, such as type 2 diabetes, cardiovascular disorders, and various forms of cancer."

Fundamentally a result of chronic positive energy balance, obesity involves complex disruptions in lipid metabolism, adipokine signalling, insulin sensitivity, and systemic inflammation. Lipids are key contributors to the development of obesity, as their accumulation in non-adipose tissues leads to ectopic fat deposition. This process induces lipotoxicity, disrupts mitochondrial function, causes endoplasmic reticulum stress, and interferes with insulin signalling through ceramide pathways - all of which play a vital role in the emergence of metabolic syndrome and impaired glucose tolerance [1, 2]. Moreover, pro-inflammatory cytokines like IL-6 and TNF-α are released by larger (hypertropic) adipocytes, which recruit M1 macrophages into adipose tissue. This interaction fosters a persistent state of low-grade chronic inflammation, further aggravating metabolic disturbances. Conventional management approaches for obesity, including lifestyle interventions (diet, physical activity, behavioural therapy) and pharmacotherapy (orlistat, GLP-1 receptor agonists like semaglutide, and newer combinations such as cagrilintide/semaglutide), offer modest and often temporary benefits due to challenges in patient adherence, limited efficacy, and adverse effects. Bariatric surgery remains the most effective strategy for severe obesity but is invasive and costly. In response, novel drug delivery systems, particularly microsphere-based formulations, have emerged as promising alternatives for sustained, localized, and targeted therapy to modulate adipose tissue biology. Recent research highlights the potential of polymeric microspheres, such as poly(lactic-co-glycolic acid) (PLGA)-based systems, to encapsulate and release therapeutic agents over extended periods directly within white adipose tissue (WAT). A 2024 study demonstrated that PLGA microspheres loaded with the β3-adrenergic receptor agonist mirabegron, when injected locally into WAT, effectively promoted adipose browning, enhanced UCP1 expression, and increased energy expenditure without inducing the cardiovascular side effects associated with systemic dosing [3]. Similarly, DBZ (a Notch signalling inhibitor) encapsulated in PLGA microspheres facilitated beige adipocyte formation by suppressing Notch signalling pathways, improving thermogenesis and metabolic outcomes in preclinical models [4]. Furthermore, a 2025 study by Mishra et al. formulated acarbose-loaded PLGA microspheres capable of controlled gastrointestinal lipase inhibition and improved anti-obesity efficacy in vitro, offering a new strategy for oral obesity pharmacotherapy [5]. Beyond microspheres, transdermal microneedle systems have gained attention; PLGA and hyaluronic acid microneedle patches delivering browning agents such as CL316243 showed significant reductions in body weight and fat mass in high-fat diet-induced obesity mice, increasing local thermogenic protein expression and improving metabolic indices [6]. Core-shell micro-reservoir systems combining PLGA and carboxymethylcellulose have been employed to achieve spatially controlled, sustained release of adrenergic agonists and PPAR-γ activators like rosiglitazone within adipose tissue, demonstrating superior browning, inflammation reduction, and metabolic benefits in murine obesity models [7]. Collectively, these advanced drug delivery approaches - microspheres, microneedles, and composite polymer systems - address key limitations of conventional obesity therapies by enabling localized, long-acting, and precision-targeted treatment modalities, which minimize systemic exposure and adverse effects while enhancing metabolic control. Future prospects include integrating combination therapies (e.g., GLP-1 agonists and browning agents) into depot microspheres or microneedle patches, conducting large-animal and human trials, scaling up manufacturing, and navigating regulatory frameworks to ensure safety, reproducibility, and long-term efficacy. As obesity continues to rise globally, these formulation strategies offer transformative potential for developing safer,

more effective and patient-friendly interventions for sustainable weight management and metabolic disease prevention.

The main bioactive polyphenol from *Curcuma longa*, curcumin (as structure shown in Figure 1), has many therapeutic benefits, such as antioxidant, anti-inflammatory, antimicrobial, anticancer, and wound-healing effects- but is hindered by low aqueous solubility, quick metabolism, and systemic instability, which limit its clinical effectiveness [8, 9]. Piperine, an alkaloid derived from black pepper, has been extensively studied as a bioenhancer: it inhibits intestinal glucuronidation, modulates membrane fluidity, and suppresses P-glycoprotein/CYP3A4 activity, thereby enhancing absorption and reducing elimination of co-administered compounds [10]. Early trials evidenced that concurrent use of piperine with curcumin drastically increased curcumin bioavailability (~2000% increase) in humans, making this combination a cornerstone strategy for augmenting curcumin's pharmacokinetic profile [11].

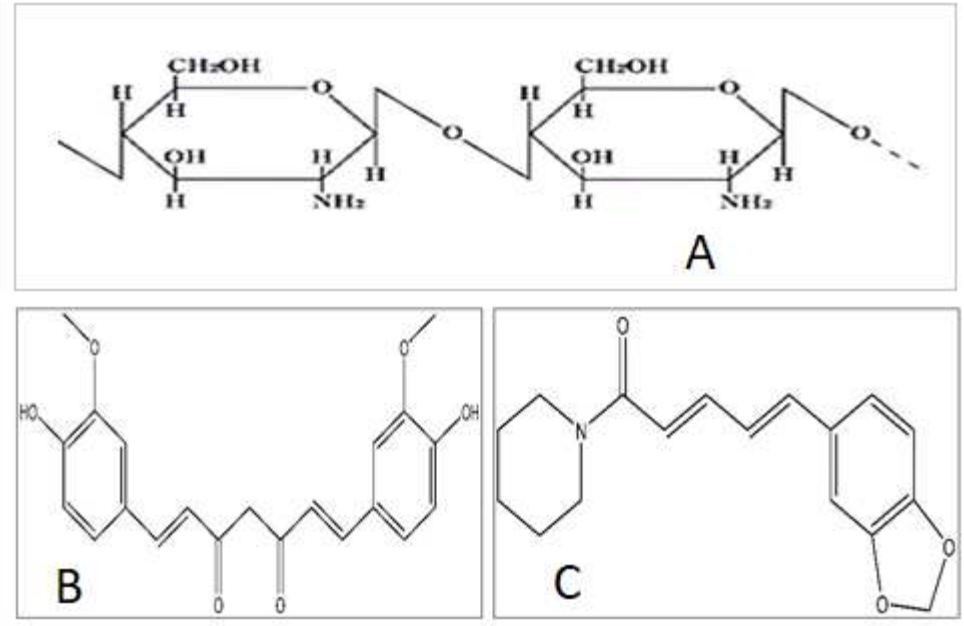


Figure 1: Structure of (A) Chitosan (B) Curcumin, (C) Piperine

Over recent years, formulation technologies including micro and nano carriers have advanced significantly to address these limitations. Nano and micro-encapsulation methods including polymeric nanoparticles, micelles, liposomes, phytosomes, nanoemulsions, and microspheres have demonstrated efficiency to improve curcumin's solubility, protect it from degradation, extend circulation time, and enhance targeted tissue delivery [12]. For example, the lipid-complex "Meriva®" (curcumin–phosphatidylcholine phytosome) has demonstrated clinical efficacy in reducing inflammation in cancer, diabetes, osteoarthritis, and neurological disorders. Likewise, curcurmin (colloidal nanoparticle dispersion) achieved ~27-fold increased bioavailability in humans and showed analgesic benefits in osteoarthritis patients. Specifically, microsphere-based systems have emerged as promising platforms for dual-curcumin and curcumin–piperine delivery. Curcumin- piperine coloaded chitosan and zein nanoparticles were developed in 2018, demonstrating synergistic anti-cancer effects in vitro [13]. More recently,

spray-dried curcumin-piperine microspheres and piperine-loaded solid lipid microparticles have been explored [14, 15]. Furthermore, an in situ-forming subcutaneous gel system embedding curcumin and piperine was introduced, designed to provide sustained release for localized delivery.

These microsphere systems provide several advantages:

- **Sustained and controlled release**: reducing dosing frequency and maintaining therapeutic levels.
- **Protection from degradation**: preserving curcumin's efficacy.
- **Targeted delivery**: particularly with injectable or implantable forms for localized action.
- **Synergistic effects**: co-delivery of piperine boosts local bioavailability of curcumin.

Moving forward, research continues to explore hybrid microsphere systems such as polymer - lipid carriers, enteric-coated microspheres, and bio responsive hydrogels. These approaches could enable oral or injectable depot formulations that deliver therapeutic doses over weeks, minimizing systemic side effects and maximizing efficacy. Continued efforts should focus on optimizing particle size, release kinetics, polymer compatibility, and in vivo biocompatibility, as well as conducting human trials to confirm preclinical promise[16, 17].

In this work we hypothesized that chitosan and gelatin based microspheres of curcumin would exhibited good flow properties, higher yield, better efficiency with stability, hence representing a promising carrier effective for the management of obesity. Aiming to minimize the curcumin degradation while still benefiting from its enhance therapeutic action, by incorporating piperine with curcumin. We have also tested particle size to estimate their effect on entrapment and flow property. This way we aimed to find the optimum therapeutic effect of drug by reducing metabolism. Some previous reports suggested the microspheres as potential carriers for drug delivery and moving forward our research focused on entering coating of chitosan-gelatin microspheres with eudragit S100 to provide therapeutic doses of drug for longer duration, minimizing systemic side effects and maximizing efficacy. Thus the main objective of the proposed work is to develop a hybrid polymer based microspheres where both polymer can contribute the synergistically biocompatibility and biodegradability, and also chitosan enhance mucoadhesion. The coating with eudragit provided protection against chitosan degradation in acidic media. Glutaraldehyde is expected to produce cross links between gelatin and chitosan molecules and thereby to slow down the rate of drug release from microspheres. The proposed system will provide the novel platform for the development of anti-obese treatment to overcome life style based weight gaining.

2. MATERIAL AND METHOD

2.1 Material

Curcumin and Piperine were obtained from Sanat Co. Pvt Ltd, New Delhi as a gift sample. Eudragit S100 polymer was provided as gift sample from Evonik India Pvt Ltd, Mumbai. Chitosan was procured from Hi-Media laboratory Pvt Ltd, Mumbai. Gelatin was obtained from CDH Laboratory Pvt Ltd, Mumbai. Chitosan were used of

microbiological grade with molecular weight of 3.8 - 20 kDa and degree of acetylation is = 75%. Rest of the materials wherever used is of analytical grade.

2.2 Purification of chitosan

Chitosan is purified as reported in our previous study[18]. Briefly 1 g chitosan powder mixed with 10 ml (1M) sodium hydroxide. After heating at 70°C with continuous stirring, filtered and the obtained solid was washed triplicate with water. The washed solid was dried at 40°C over 24 hours and dissolved in acetic acid (0.1M) with gentle stirring. After filtering of insoluble residue pH of the solution was adjusted to 8.0 in order to precipitate chitosan. The precipitate was thoroughly washed with water and dried under vaccum at 25±5 °C for 24 hours.

2.3 Preparation of Glutaraldehyde Saturated Toluene (GST) cross-linking agent

A mixture of 10 mL each glutaraldehyde and toluene was stirred for 1 hour at 1000rpm using a magnetic stirrer. The resultant solution was then allowed to stand 12 hours to stabilize it. Following this, the upper layer of toluene, saturated with glutaraldehyde, was carefully separated through decantation [19].

2.4 Preparation of microspheres

Microspheres were formulated using the chemical cross-linking method. Precisely measured amounts of gelatin and chitosan were dissolved in acetic acid (1%), with the pH adjusted to approximately 5.0. The solution was then sonicated for 10 minutes to eliminate entrapped air bubbles. Separately, 100mg of the drug was solubilize in appropriate volume of methanol and incorporated into the polymeric solution. This drug-polymer mixture was then added dropwise into an external oily phase comprising 200mL of liquid paraffin and 1 mL of Tween 80, maintained at 40 °C, with constant stirring at 1500 rpm using mechanical stirrer for 10 minutes. The obtained emulsion was cooled at 4 °C in an ice-water bath to facilitate gelatin coagulation. Subsequently, a predetermined volume of toluene saturated with glutaraldehyde was gradually added as a cross linking agent with stirring continued for 3 hours at 40 °C to complete cross-linking. The resulting curcumin-loaded microspheres were recovered by centrifugation and thoroughly washed with petroleum ether to remove residual liquid paraffin Figure 2A [20].

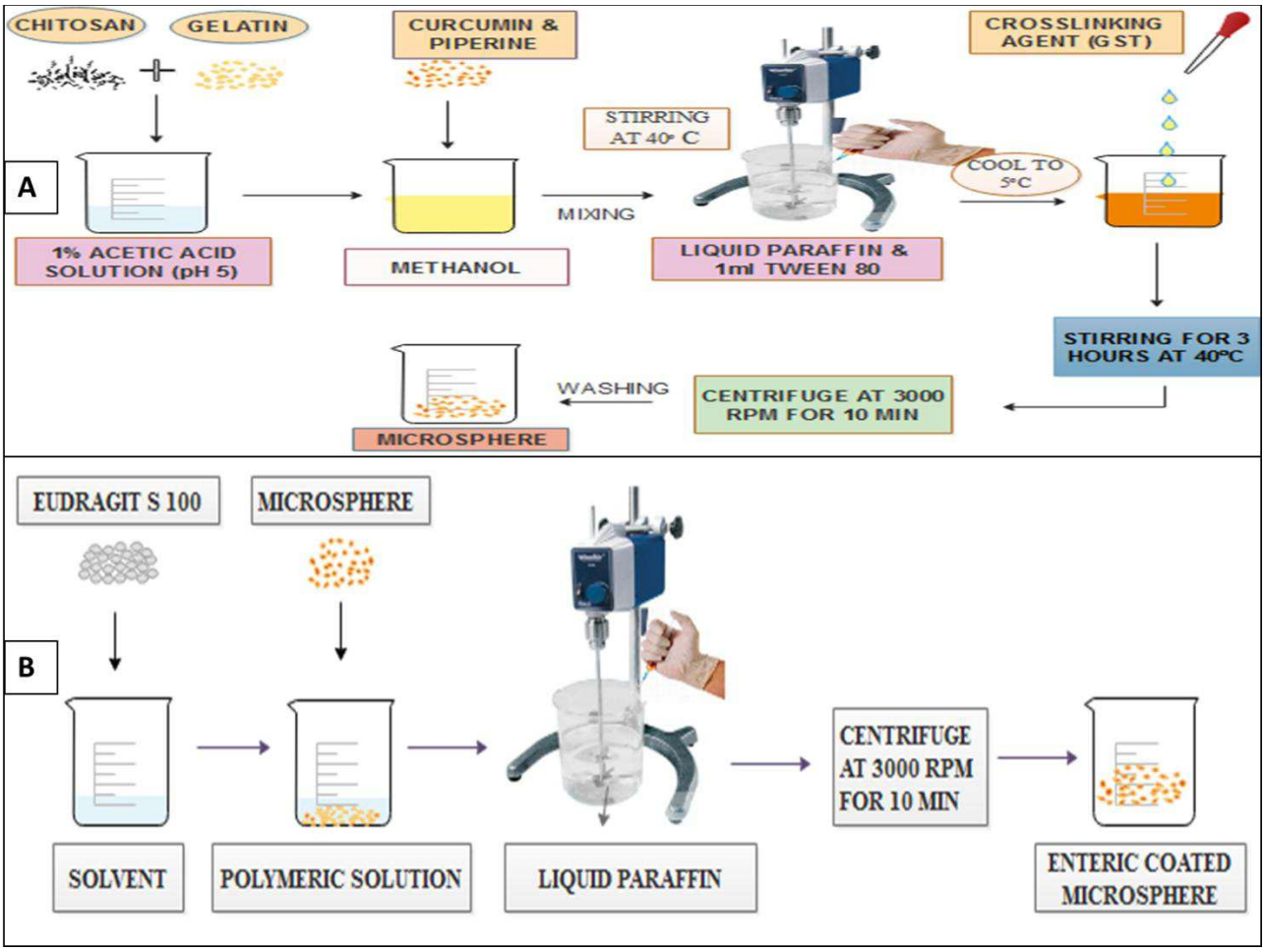


Figure 2: Preparation and coating of Herbal-Microspheres

The microspheres were coated with Eudragit S100 using solvent evaporation method according to the method previously described with slight modification [21]. Microspheres (100mg) were dispersed in coating solution prepared by dissolution of 200mg of Eudragit S100 in dichloro-methanol: isopropanol: ethanol (5:4:6) to give 2:1 coat: core ratio. . The stirring was continued until complete evaporation of organic phase. The coated microspheres were separated by centrifugation and washed with petroleum ether and n-hexane to remove liquid paraffin (Figure 1B) and stored in desiccator until analysis.

The formulations F1, F2……. F9 were prepared by full factorial design (2^3 factor) taking different ratios of cross-linking and gelatin, chitosan and constant amount of drug (curcumin+piperine) was added as bioavailability enhancer on the basis of body weight of rats. The stirring speed, stirring time were previously optimized and kept constant in preparation of chitosan gelatin microspheres and the amount of Eudragit S100 also previously optimized in coating process (Table 1) [22].

Table 1: Composition of different microspheres formulation

Formulation Code	Drug Curcumin+Piperine (in mg)	Chitosan (in mg)	Gelatin (in mg)	Cross-linking agent (ml)
F1	100:50	200	300	10
F2	100:50	100	400	10
F3	100:50	200	300	20
F4	100:50	100	400	10
F5	100:50	200	400	10
F6	100:50	100	300	20
F7	100:50	200	400	10
F8	100:50	100	400	10
F9	100:50	100	300	20
F10*	**100:50**	**100**	**400**	**10**

(*) = optimized concentration/amount

3. CHARACTERIZATION OF DEVELOPED MICROSPHERES

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy was performed on the pure drug, drug entrapped microspheres and drug-polymer mixture using a Fourier Transform Infrared Spectrophotometer (FTIR-8400S, Shimadzu, Japan) to confirm the presence of the drug within carrier. Each sample was thoroughly blended with potassium bromide (KBr) in a 95:5 ratio for making pellet, and scanned form 4000 to 400 cm^{-1} at room temperature to obtain the characteristic spectra [20].

3.2 Surface morphology

The surface morphology of the prepared microspheres was examined using Scanning Electron Microscopy (SEM) with EVO 10 instrument (Carl Zeiss). Microspheres were gently dusted over both side adhesive tape attached to an aluminium stub. Then the stub was gold-coated to a thickness of approximately 300 Å. Coated samples were scanned and photographs were captured for morphological evaluation [22].

3.3 Particle size

The particle size of the formulated microspheres was measured through a particle size analyzer (Shimadzu SALD-2201). The size analysis was performed based on laser diffraction technique, both before and after the coating process, to evaluate any changes in particle dimensions.

3.4 Micromeritic Studies

The flow properties of the prepared microspheres were assessed by micrometrics study viz. bulk density, tapped density and compressibility index, which help predict flowability. An accurately weighed sample of 5 grams was placed in a 10 mL measuring cylinder, and after recording of initial reading the cylinder then tapped 100 times on a flat wooden surface to obtain a tapped volume, which was used to calculate the relevant micromeritics indices [23]. Bulk density and tapped density were calculated by formula.

i) Bulk density (g/cm^3) = $\frac{\text{Mass of microspheres}}{\text{Bulk volume of microspheres}}$

ii) Tapped density (g/cm^3) = $\frac{\text{Mass of microspheres}}{\text{Tapped volume of microspheres}}$

iii) Compressibility Index: It was evaluated using Carr's Index, and calculated using the following formula:

$$\textbf{Carr's Index} = [1 - V/V_0] \times 100$$

Here, V represents the volume of the sample after standard tapping, while V_0 denotes the initial volume of the sample before tapping.

iv) Hausner ratio = $\frac{\text{Tapped density}}{\text{Bulk density}}$

v) Angle of repose (Ɵ): The angle of repose of the microspheres was obtained using the fixed funnel method. In this procedure, sample was flow through a funnel that was positioned at a fixed height using a support stand. Subsequently, the height and diameter of the resulting conical heap were recorded, which allowed for the calculation of the angle of repose. This measurement serves as an important indicator of the flow properties of the microspheres.

$$\textbf{Tan } \theta = h/r$$

Where h = height of the pile and r= radium of the pile formed by microspheres.

3.5 Percentage yield & % Drug entrapment

The prepared microspheres were, collected by centrifugation, dried, weighed and % yield were determined through formula.

$$\textbf{\% yield} = \frac{\text{Weight of obtained microspheres}}{\text{Total amount of polymer and drug}} \textbf{X100}$$

A sample of the drug-loaded microspheres (100 mg) was finely crushed using a glass mortar and pestle, then dispersed in methanol (100 mL). the admixture was stand for a day to ensure thorough extraction. After this period,

it was filtered through Whatman filter paper. After suitable dilution, the content of drug was quantified using UV spectrophotometry at 422nm. The efficiency of entrapment was determined using the following formula.

$$\textbf{\% Drug entrapment} = \frac{\textbf{Actual quantity of drug present in microspheres}}{\textbf{Actual initial loading dose}} \textbf{X100}$$

3.6 Swelling study

A precisely weighed sample (100mg) of microspheres was immersed in simulated intestinal fluid (pH 7.4) and set aside to swell until a constant weight was observed. The swollen microspheres were then removed, gently stained via filter paper to eliminate additional surface fluid, and change in weight was measured to evaluate their swelling capacity [24]. The degree of swelling (α) was determined using the following equation:

$$\boldsymbol{\alpha} = \frac{\mathbf{Wg - Wo}}{\mathbf{Wo}}$$

Here, W_o represents the primary weight of the dry microspheres, while Wg denotes the weight of the swollen microspheres.

3.7 Mucoadhesive study

Ten milligrams of microspheres were accurately weighed and suspended in mucin solutions at varying concentrations from 100 to 400 µg/mL. The mixtures were incubated at 37 °C for various time intervals: 30, 60, 120, and 180 minutes. After incubation, the samples were centrifuged upto 30 minutes at 10000 rpm. Free mucin concentration remaining in the supernatant was then measured through UV Spectrophotometer at a λ_{max} 257 nm [25]. The mucin binding efficiency of microspheres was determined using the following formula:

$$\textbf{\% Mucin binding efficiency} = \frac{\mathbf{Co - Cs}}{\mathbf{Co}} \textbf{x 100}$$

Here, C_o denotes the primary mucin concentration, while C_s represents the amount of unbound mucin in the supernatant.

3.8 *In-Vitro* drug release

The in vitro release performance of curcumin from different microspheres formulations were assessed using pH-dependent buffer systems: HCl buffer (pH 1.2), phosphate-buffered saline (PBS) pH 6.8, and PBS pH 7.4. These pH values mimic the physiological pH transitions within the gastrointestinal tract, ranging from the stomach (pH 1.2) to the proximal small intestine (pH 6.8) and the ileocecal region (pH 7.4). A 6 mg sample of microspheres was dispersed in pH 1.2 buffer and placed into a dialysis tube, which was sealed securely at both ends to maintain a consistent surface area. The tube was immersed in 50mL of the respective release medium and maintained under constant agitation at 100 rpm at 37 °C. At predefined time intervals, samples were collected from the external medium, after which a same amount of fresh buffer, added to sustain sink-conditions. The quantity of curcumin released was assessed determined in the samples through UV spectrophotometer at a wavelength of 429 nm.

3.9 Experimental design & high fat diet (HFD)

Male Albino Sprague Dawley rats were used in this study following all the ethical recommendations, and the protocol was approved by the Institutional Animal Ethics Committee (IAEC) of GGV. Albino rats (Sprague Dawley) male (130-140g) were used. Throughout the study, the rats were kept under conditions of 12 hour light dark cycle, relative humidity (60-70%), and constant temperature (23±2ºC). the standard pellet diet were fed to animal throughout the study with free access of drinking water. A high-fat diet (HFD), characterized as a hypercaloric diet, and was formulated by combining specific ingredients in fixed proportions. The HFD consisted of dalda (20%), coconut oil (6%), cholesterol (2%), and cholic acid (1%). The HFD was thoroughly blended, dried, and ingested daily at 8 am to experimental animals along with water for 60 days. After 60 days, obese animals were treated with pure drug and optimized formulation (F10) in different doses (100mg/kg, 150mg/kg & 200mg/kg) for 10 weeks [24, 25].

3.10 Bioavailability study

Animal for Pharmacokinetic study (Bioavailability & Biodistribution) were grouped into three groups as group I receiving curcumin, group II receiving curcumin-piperine and group III receiving microspheres. At the predetermined time intervals (1, 2, 4, 6, 8 and 24 hours) the samples of blood (0.5 mL) were collected using the cardiac puncture technique. The samples were transferred into Eppendorf microcentrifuge tubes containing EDTA as an anticoagulant. The plasma was separated by centrifuged the blood samples for 15 minutes at 2500 rpm to separate the plasma. The plasma was carefully transferred to fresh microcentrifuge tubes using a micropipette and analyzed for curcumin concentration using a UV spectrophotometer at 429 nm [26, 27].

3.11 Biodistribution study

For biodistribution study animals were grouped into 3 groups, each comprising of five male rats weighing between 150–200g. Before the study, all rats were fasted for 24 hours, although animal were received water libitum throughout the protocol. Group I given curcumin at a dose 200 mg/kg orally in water. Group II was given both curcumin and piperine (200mg/kg) in water via oral route, while Group III was given the optimized microsphere formulation of curcumin (200mg/kg) in suspension form, also administered orally. After 1, 2, 4, 6 & 24 hour, animals were killed using deep ether anaesthesia. The various organs i.e. spleen, lungs, heart, stomach, small intestine, colon, liver, fat, muscle and kidney were removed and washed with normal saline. The harvested organs were weighed, finely chopped, and homogenized in a phosphate buffered saline (PBS). The homogenates were then centrifuged, and the resulting supernatant was carefully collected. The drug present in within the tissue samples was subsequently estimated through a UV spectrophotometer at 429 nm [28].

3.12 Antiobesity study

Animals for this study were grouped into seven groups as group I health control, group II obese control, group III curcumin treated, group IV curcumin-piperine treated, group V microspheres (100mg/kg dose) treated, group VI microspheres (150mg/kg dose) treated, and group VII microspheres (200mg/kg dose) treated.

3.12.1 Body weight of experimental rats

At the starting of a study, all 7 groups of animals showed similar body weight (130-140g). The high fat diet administered to experimental rats for 60 days. The body weight (in grams) of the experimental animals was measured initially on first day of the study and subsequently recorded weekly up to 60 days using a digital weighing balance [29, 30].

3.12.2 Body Mass Index (BMI) of experimental rats

Body Mass Index (BMI) serves as a commonly accepted indicator of adiposity due to its strong connection to total body fat while minimal dependence on height. In the present study, BMI of the experimental rats was recorded both prior to and following the treatment period. The BMI values were calculated and presented in g/cm^2 [31].

3.12.3 Body Temperature of Experimental Rats

A rectal digital thermometer was used to measure the body temperature of each experimental rat on the 60^{th} day of study, both before and after the administration of the respective formulations [22].

3.12.4 Locomotor activity of experimental rats

The locomotor activity of the rats was evaluated using an actophotometer, which operates by detecting the interruption of light beams by the animal's movement. Each rat was placed separately into the device, and as the animal moved, its activity was recorded digitally based on the number of light beam interruptions [31].

3.12.5 Organ measurement of experimental rats

On the last day of study, the different organs like liver, spleen, lung, heart, muscle, fat and kidney were removed and weighed [22].

3.12.6 Lipid profile of experimental rats

Before and after drug treatment, blood was withdrawn from retro-orbital plexus and was subjected to centrifugation to collect serum for serum lipid estimation profile. Serum level of glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were determined using biochemical assay kits procured from Span Diagnostics Ltd., Mumbai, India [22].

4. RESULT AND DISCUSSION

Chitosan is purified before experiment to remove certain impurities such as heavy metals, residual proteins and endotoxin that could affect the physiological properties as well as its biological properties. After purification the average molecular weight was found to be 12.67 ± 2.54 kDa.

4.1 FTIR spectroscopy

FTIR spectroscopic analysis was conducted on pure curcumin and the optimized formulation (F10) to evaluate compatibility and assess any potential interactions between the crosslinked polymers and drug. The IR spectra of curcumin, piperine and the drug-loaded microspheres were found to be nearly equal, with no significant shifts in the

characteristic peaks of drug, demonstrating that the drug's structural integrity remained intact following successful encapsulation in the microspheres, as depicted in Figures 4 and 5, respectively. The FTIR spectrum of drug sample shows the characteristics absorption of various functional groups of curcumin and these bands are found at 3506 cm^{-1} for phenolic OH, 1730 cm^{-1} for C=O (aromatic), 1712 cm^{-1} for C=C (aromatic), 1462 cm^{-1} for olefinic C-H, 1282 cm^{-1} for C-H (aromatic) and 1028 cm^{-1} for C-O-C. The FTIR spectrum of optimized formulation revealed that Phenolic OH stretching vibration at 3506 cm^{-1} shift to 3522 cm^{-1}, aromatic ring stretching vibration at 1454 shift to 1458 cm^{-1}, C-H bending vibration at 1427 cm^{-1} shift to 1462 cm^{-1}, aromatic C-H stretching vibration at 1282 cm^{-1} shift to 1269 cm^{-1} was observed. No change was observed in C=O and C=C stretching vibration. Approximately all peaks were observed at same position with approximately same intensity, as present in individual. Therefore, we can assume that there is no chemical interaction between polymers and drugs **(Figure 3).**

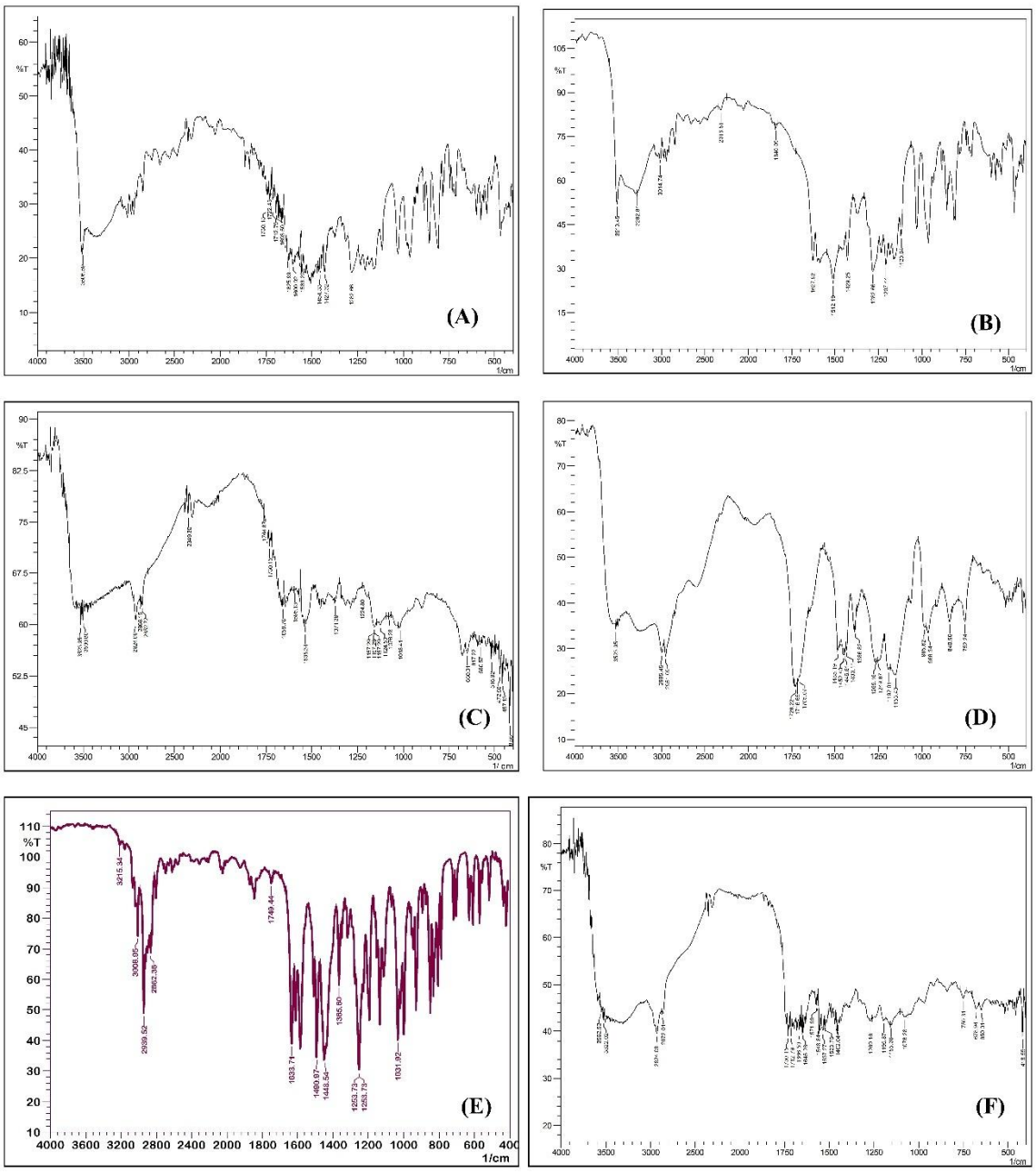


Figure 3: (A) FTIR spectrum of Curcumin (B) FTIR spectrum of Gelatin (C) FTIR spectrum of Chitosan (D) FTIR spectrum of Eudragit S100 (E) FTIR spectrum of Piperine (F) FTIR spectrum of drug loaded microspheres

4.2 Scanning Electron Microscope

The shape and surface morphology of the prepared microspheres were observed using Scanning Electron Microscopy (SEM). As illustrated in **Figure 4 (a) and (b),** the SEM images clearly reveal that the developed cross-linked microspheres possess a spherical shape with a smooth and uniform surface.

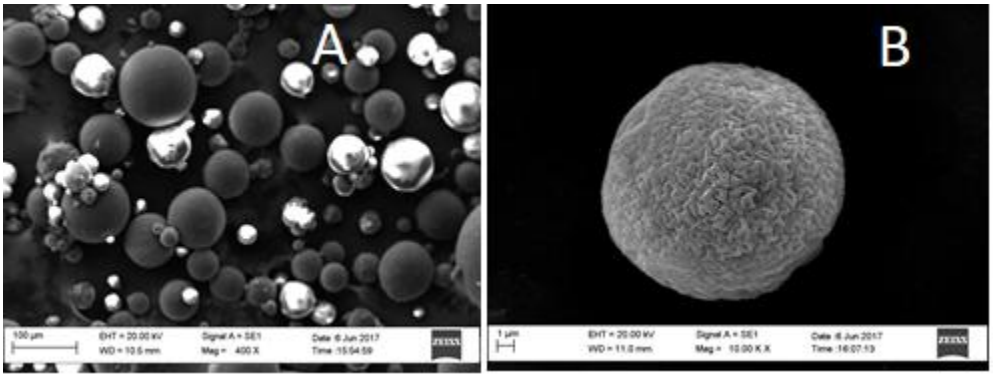


Figure 4: SEM Images of (A) F10 Microspheres (B) External surface of the prepared microspheres & enlarged view of microspheres

4.3 Morphology determine by Photo Microscopy

Shape of the microspheres was determined by photomicroscope (Zeiss) and their sizes were estimated. For each formulation, a sample was analysed under photomicroscope, in order to find out the mean diameter and standard deviation. Through photomicroscope, we can study the shape of particles (microspheres) and their surface morphology also. In the development of various formulations, an optical microscopy was initially used to measure and optimize the formulation by examining the overall physical characteristics of the product. The photomicrographs of prepared microspheres are shown in **Figure 5**.

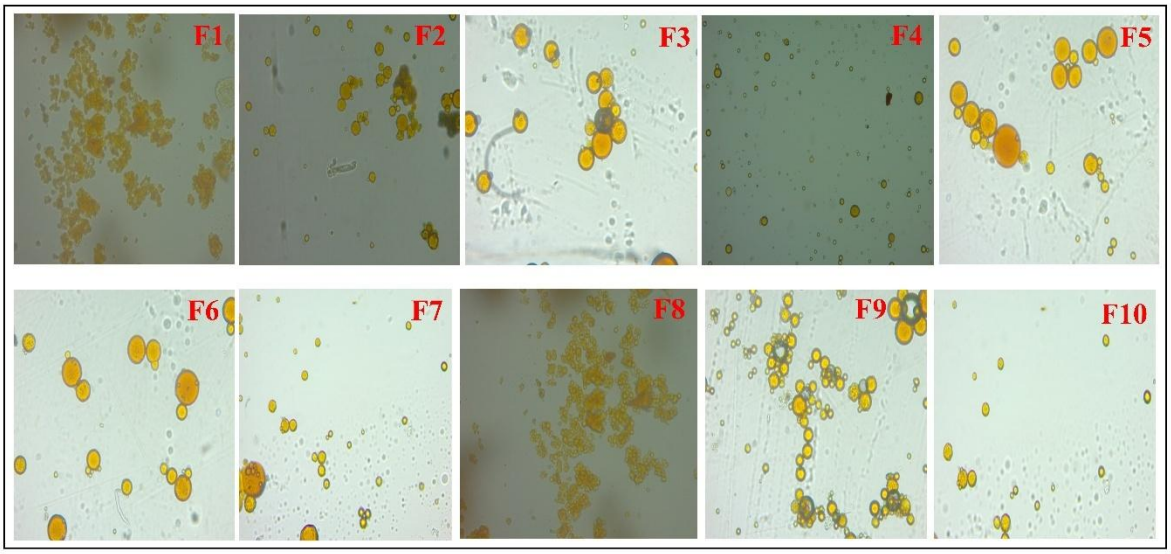
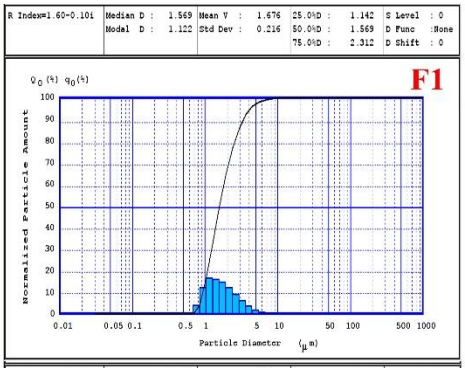


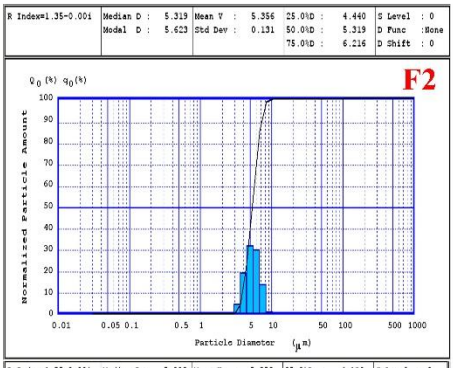
Figure 5: Photomicrographs of prepared microspheres under photomicroscope

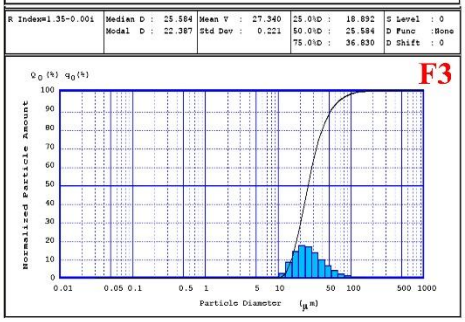
4.4 Particle size determination

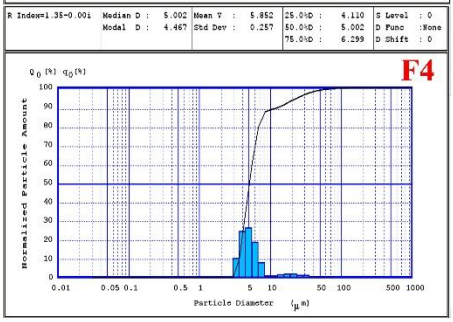
Formulation F10 showed good results with respect to particle size, as critical for microspheres, since this parameter directly impacts the stability, distribution and mucoadhesive properties of formulation. The particle size of the

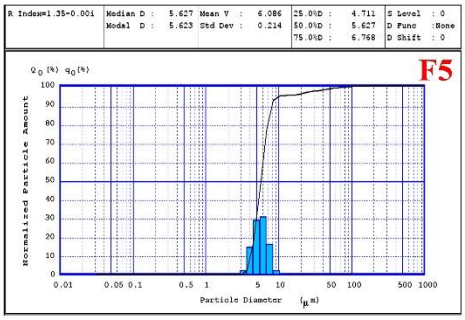
developed carriers was 1..676-5.490 µm and it was observed that as amount of drug and gelatin gradually increased, size gradually increased but presence of large amount of drug and gelatin the aggregation of microspheres occurred which may be due to high polymer gelling property and formed a more viscous dispersion which gives large droplets and subsequently increase the particle size of microspheres while too low concentration of polymer led to poor formation of microspheres. Usually, spherical microspheres can be obtained when concentration increases but when increase the concentration of gelatin increases above 400mg irregular microspheres were formed due to larger droplets of microspheres **(Figure 6 & Table 2).**

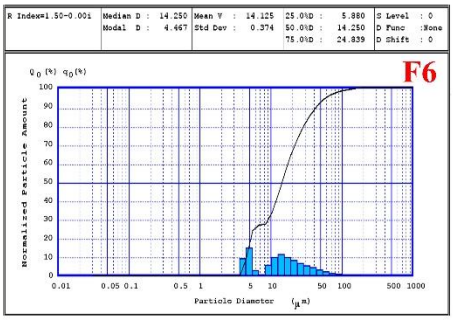

R Index=1.60-0.10i
Median D : 1.569
Modal D : 1.122
Mean V : 1.676
Std Dev : 0.216
25.0%D : 1.142
50.0%D : 1.569
75.0%D : 2.312
S Level : 0
D Func :None
D Shift : 0
F1
Normalized Particle Amount
Particle Diameter (μm)

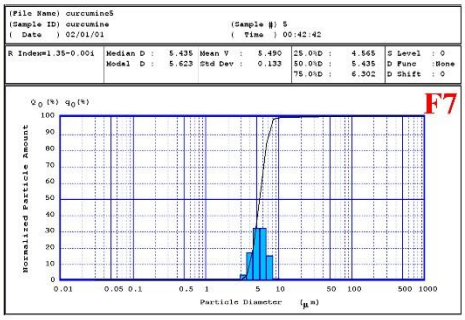

R Index=1.35-0.00i
Median D : 5.319
Modal D : 5.623
Mean V : 5.356
Std Dev : 0.131
25.0%D : 4.440
50.0%D : 5.319
75.0%D : 6.216
S Level : 0
D Func :None
D Shift : 0
F2
Normalized Particle Amount
Particle Diameter (μm)

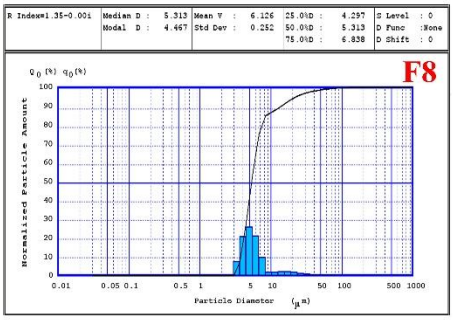

R Index=1.35-0.00i
Median D : 25.584
Modal D : 22.387
Mean V : 27.340
Std Dev : 0.221
25.0%D : 18.892
50.0%D : 25.584
75.0%D : 36.830
S Level : 0
D Func :None
D Shift : 0
F3
Normalized Particle Amount
Particle Diameter (μm)

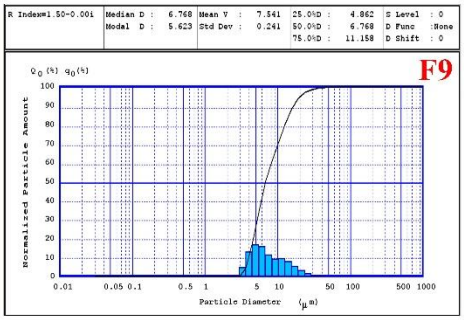

R Index=1.35-0.00i
Median D : 5.002
Modal D : 4.467
Mean V : 5.852
Std Dev : 0.257
25.0%D : 4.110
50.0%D : 5.002
75.0%D : 6.299
S Level : 0
D Func :None
D Shift : 0
F4
Normalized Particle Amount
Particle Diameter (μm)


R Index=1.35-0.00i
Median D : 5.627
Modal D : 5.623
Mean V : 6.086
Std Dev : 0.214
25.0%D : 4.711
50.0%D : 5.627
75.0%D : 6.768
S Level : 0
D Func :None
D Shift : 0
F5
Normalized Particle Amount
Particle Diameter (μm)


R Index=1.50-0.00i
Median D : 14.250
Modal D : 4.467
Mean V : 14.125
Std Dev : 0.374
25.0%D : 5.880
50.0%D : 14.250
75.0%D : 24.839
S Level : 0
D Func :None
D Shift : 0
F6
Normalized Particle Amount
Particle Diameter (μm)


(File Name) curcumin5
(Sample ID) curcumine
(Date) 02/01/01
(Sample #) 5
(Time) 00:42:42
R Index=1.35-0.00i
Median D : 5.435
Modal D : 5.623
Mean V : 5.490
Std Dev : 0.133
25.0%D : 4.565
50.0%D : 5.435
75.0%D : 6.302
S Level : 0
D Func :None
D Shift : 0
F7
Normalized Particle Amount
Particle Diameter (μm)


R Index=1.35-0.00i
Median D : 5.313
Modal D : 4.467
Mean V : 6.126
Std Dev : 0.252
25.0%D : 4.297
50.0%D : 5.313
75.0%D : 6.838
S Level : 0
D Func :None
D Shift : 0
F8
Normalized Particle Amount
Particle Diameter (μm)


R Index=1.50-0.00i
Median D : 6.768
Modal D : 5.623
Mean V : 7.541
Std Dev : 0.241
25.0%D : 4.862
50.0%D : 6.768
75.0%D : 11.158
S Level : 0
D Func :None
D Shift : 0
F9
Normalized Particle Amount
Particle Diameter (μm)

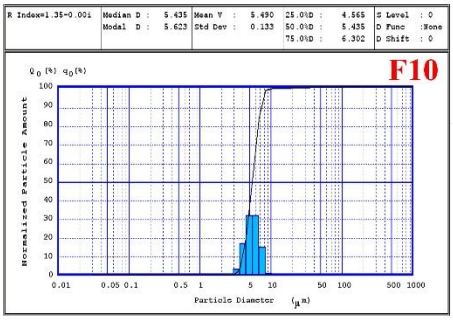

R Index=1.35-0.00i
Median D : 5.435
Modal D : 5.623
Mean V : 5.490
Std Dev : 0.133
25.0%D : 4.565
50.0%D : 5.435
75.0%D : 6.302
S Level : 0
D Func :None
D Shift : 0
F10
Normalized Particle Amount
Particle Diameter (μm)

Figure 6: Particle size of microspheres detected by particle size analyzer

4.5 Percentage yield and % Entrapment efficiency

The calculated yield and percentage entrapment efficiency in all microspheres were ranges between 44.64 to 97.36 % and 46.37 to 73.25% respectively. The results showed that, the % yield and the entrapment efficiency of the drug was increased with increase in polymer due to highly efficient drug distribution in polymer (**Table 2**). The highest drug entrapment efficiency was found with a polymer ratio of 1:4.

Table 2: Particle size, percentage yield and % drug entrapment efficiency of various formulations

S. No.	Formulation code	Particle size in µm ± S.D.	% Yield	% drug entrapment efficiency
1	F1	1.676 ±0.216	44.64 ±1.0	46.37 ±1.0
2	F2	5.356 ±0.131	98.68 ±0.1	74.97 ±0.5
3	F3	27.340±0.221	92.64 ±2.1	55.26 ±2.6
4	F4	5.852±0.257	84.25±0.3	77.23±0.3
5	F5	6.086±0.214	96.28 ±0.6	62.66 ± 2.5
6	F6	14.125±0.374	91.32±0.2	52.42 ±1.6
7	F7	5.490±0.133	94.14 ±1.1	81.23±1.3
8	F8	6.126±0.252	89.23±0.3	69.32±0.3
9	F9	7.541±0.241	77.36±0.2	65.25±2.2
10	F10	5.490±0.133	97.36±0.1	73.25±0.2

The data represent average of reading (mean ±SD; n=3)

4.6 Micromeritic studies

The prepared microspheres were assessed for bulk density, tapped density, carr's index and angle of repose. The result suggests that all the values are within the range i.e. less than 30° which indicates good flow of carriers, values shown in **Table 3.**

Table 3: Micromeritic studies of various formulations

Formulation code	Bulk density g/cm^3	Tapped density g/cm^3	Carr's index (%)	Hausner ratio	Angle of repose (Θ)
F1	0.581±0.024	0.681±0.014	14.6±1.7	1.17±0.026	23.77±0.49
F2	0.595±0.017	0.802±0.014	24.86±1.7	1.13±0.024	24.68±0.47
F3	0.531±0.017	0.707±0.014	24.8±1.7	1.32±0.026	28.75±0.64
F4	0.602±0.017	0.611±0.12	16.3±0.3	1.33±0.025	22.2±0.2
F5	0.574±0.023	0.730±0.014	21.1±1.7	1.26±0.026	25.69±0.50
F6	0.699±0.14	0.464±0.014	11.7±1.7	1.33±0.05	25.95±0.78
F7	0.545±0.12	0.652±0.02	22.2±0.8	1.12±0.03	24.36±0.2
F8	0.566±0.02	0.521±0.04	13.2±0.1	1.23±0.01	26.35±0.7
F9	0.545±0.03	0.565±0.03	11.2±0.6	1.35±0.03	29.25±0.05
F10	0.593±0.03	0.795±0.01	25.0±0.1	1.12±0.06	24.36±0.06
Values represent mean ±SD; n=3					

4.7 Swelling studies

The swelling study of various formulations was performed and significant difference was observed with cross linked microspheres and without cross linked microspheres. Without cross linking microspheres swelled 100 folds greater than cross linked microspheres. Swelling of microspheres occurred due to hydration of polymer in aqueous environment. Microspheres swelling may due to movement of polymeric chain, enhancing rapid release rates of the drug by diffusion through polymer. The cross linking of polymer alter the swelling process because cross linking agent is responsible for the formation of rigid structure that will enhance the polymer density. This would reduce mobility of polymer chain and formed a rigid as well as more stable microspheres which shows low tendncy to wsell and control release of drug through carreiers **(Figure 7**).

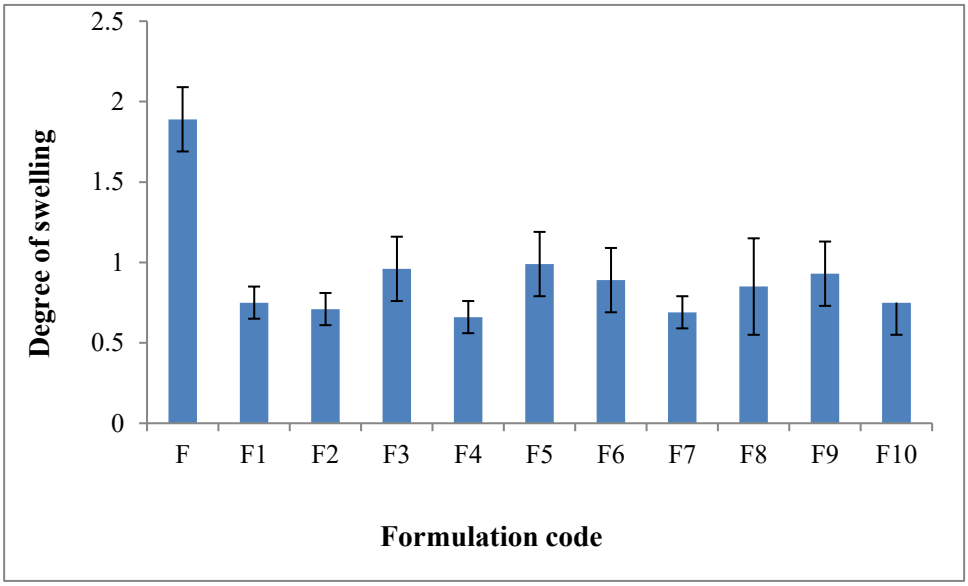


Figure 7: Degree of swelling of various formulations (Mean ± SD, n=3)

(F = Microspheres without cross linking)

4.8 *In-Vitro* Drug Release

Release of curcumin from microspheres was evaluated by in vitro release under varying pH conditions that simulate the gastrointestinal transit from the stomach to the colon. This approach was based on physiological transit times and corresponding pH environments. The cumulative drug release curve for Eudragit S 100-based microspheres indicated no release during the initial 2 hours at pH 1.2 for the first hour and pH 6.8 for the second hour. A notable increase in drug release occurred once the pH was shifted to 7.4, reflecting targeted release in the colonic environment. When compared with plain curcumin, the microspheres exhibited a slower and more controlled release pattern. The decrease in release rate with higher polymer concentrations was attributed to the strong gelling nature of gelatin and the formation of a dense cross-linked network, which acts as a barrier to drug diffusion. As shown in **Figure 8**, the results confirm that the microsphere formulation successfully delays curcumin release until it reaches the colon**.**

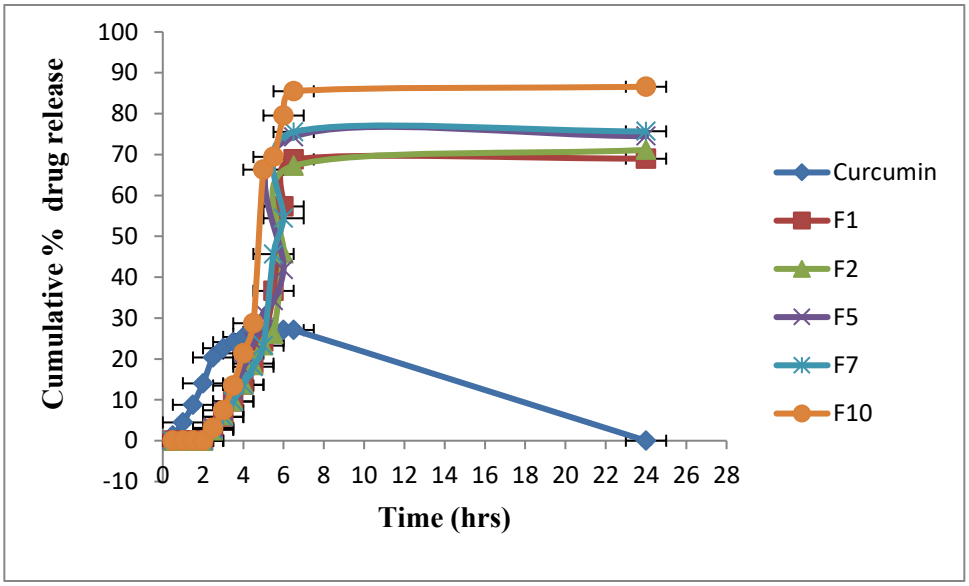


Figure 8: Cumulative (%) Drug Release of Developed Formulations (Mean ± SD, n=3)

4.9 Mucoadhesive studies

Mucoadhesive property of Cs-G microspheres was studied and strong interactions occurred between mucin and Cs-G microspheres. Chitosan exhibits electrostatic interaction with its positive amino group to negatively charged sialic acid group of mucin. Mucoadhesive microspheres could better interact with mucus layer present on the mucosal epithelium and bind to mucus molecules, thereby enhancing the retention period of the proposed carriers at the site of absorption. From the study, we concluded that Cs-G microspheres possessed mucoadhesive properties **(Figure 9).**

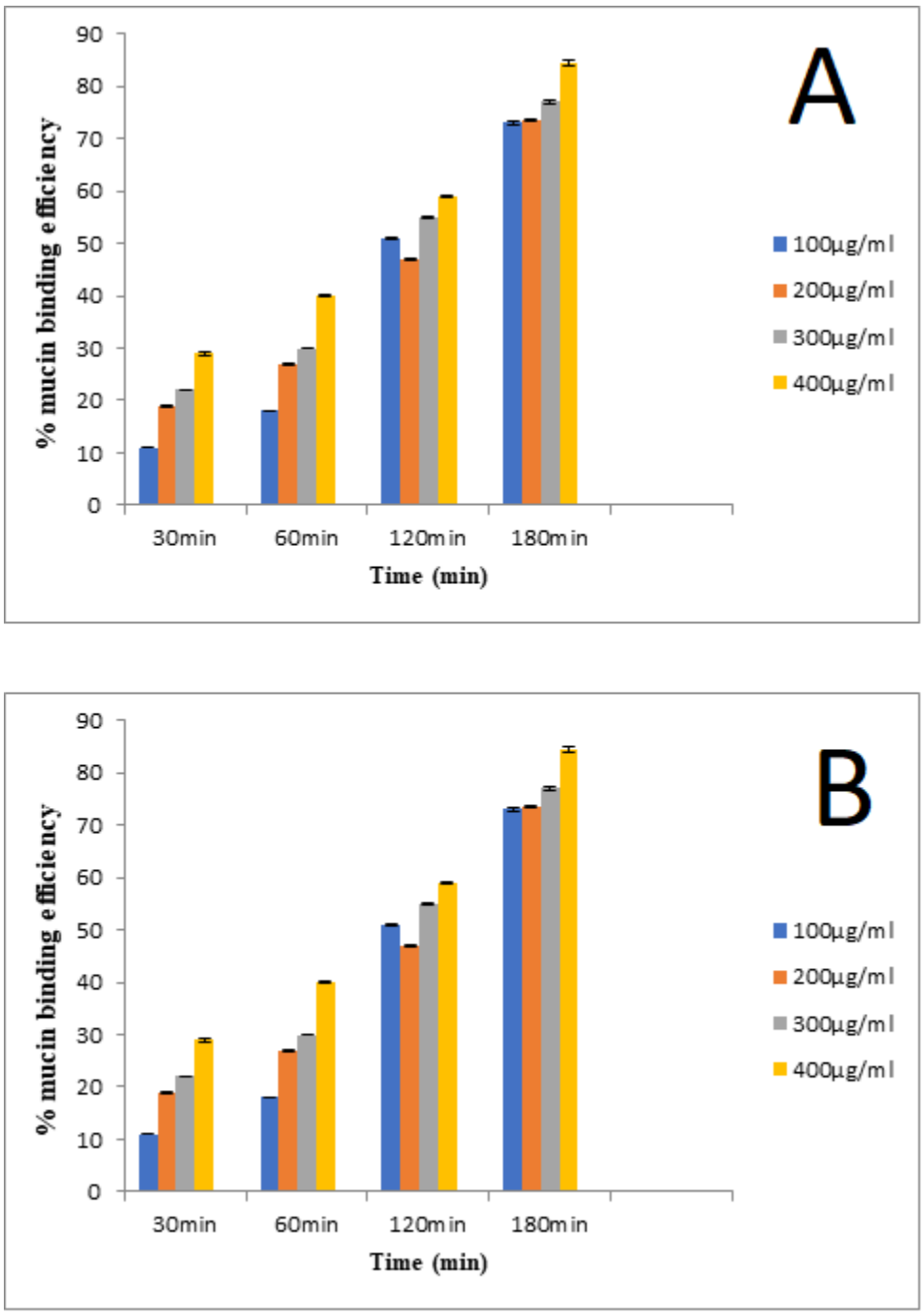


Figure 9: % Binding efficiency of mucin with microspheres (without drug) (A) and with drug (B) (Mean ± SD, n=3)

4.10 Kinetic model

Various kinetic viz. zero order, first order, Higuchi model and Hixon-crowell were applied on in vitro release of curcumin from optimized formulation. F10 follows the zero-order kinetic with R^2 value 0.854, first order kinetic with R^2 0.945, Higuchi kinetic with R^2 0.723, Korsmeyer-peppas kinetic with R^2 0.972and **(Figure 10 A,B,C,D)** respectively). The findings indicated the release pattern best fitted with Korsemeyer-Peppas model. The value of release exponent (*n*) was calculated to be 0.523. This value reflects the nature of the release behaviour which is influenced by structural characteristics of the matrix as well as release process itself. Value of $n = 0.523$ corresponds to a non-Fickian (anomalous) diffusion mechanism, suggesting that drug release is regulated by a combination of both diffusion through the polymer matrix and swelling of the polymer, as reported by the Korsmeyer-Peppas model..

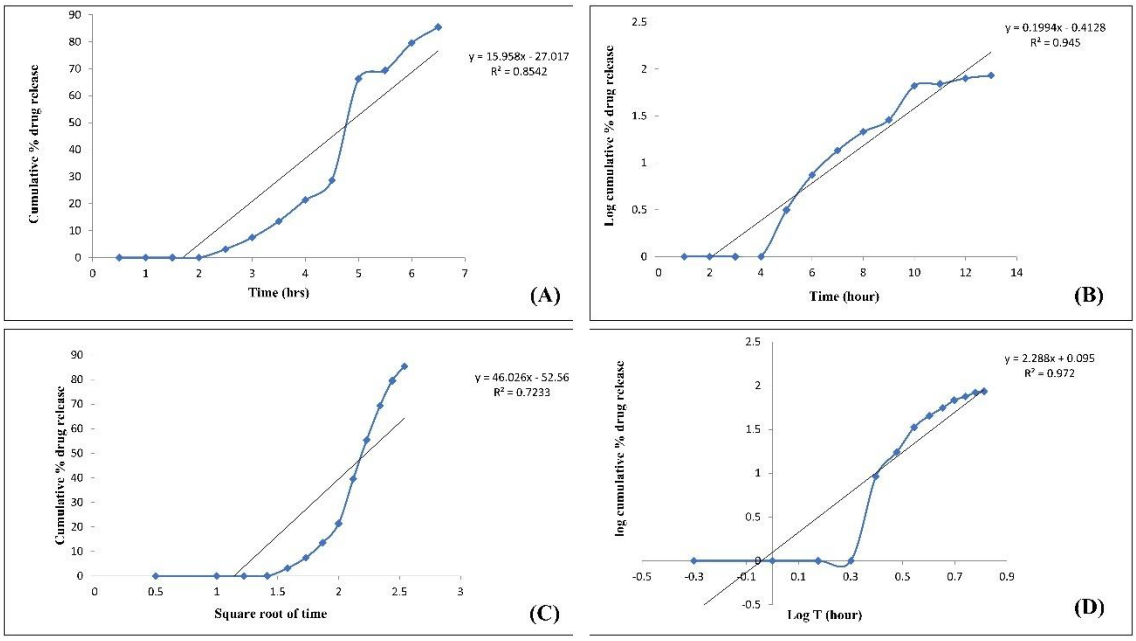


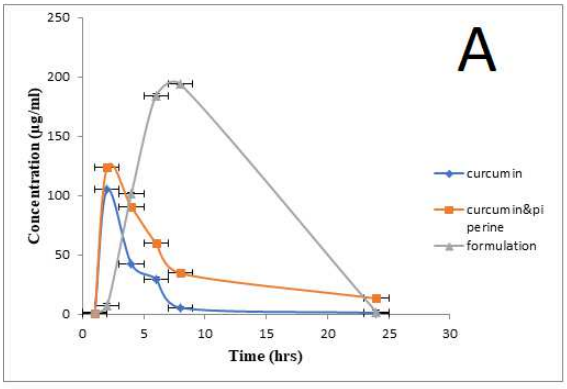
Figure 10: Kinetic model of optimized formulation F10 (A) Zero Order Kinetic (B) First Order Kinetic (C) Higuchi Order Kinetic (D) Korsmeyer Peppas Kinetic

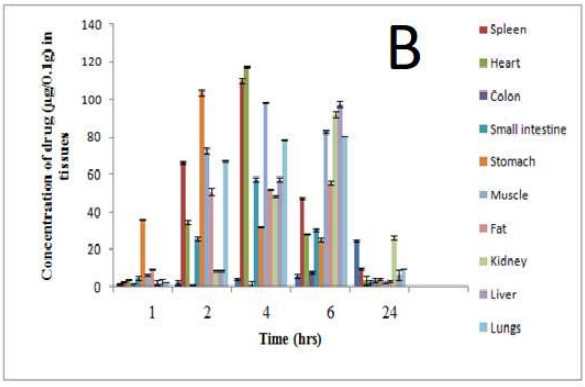
4.11 Pharmacokinetic evaluation of curcumin loaded microspheres

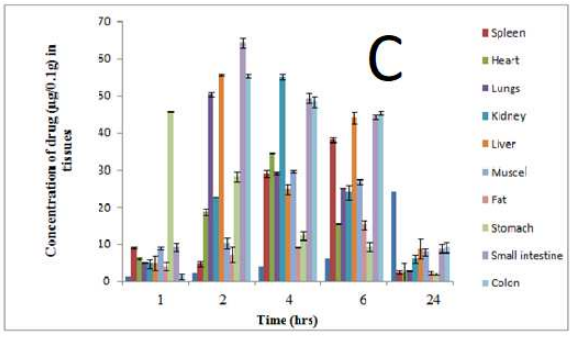
Pharmacokinetic study for curcumin was successfully performed. The plasma profile kinetics has shown that the maximum concentration (C_{max}) value for curcumin, curcumin-piperine combination and curcumin microspheres were 105 (µg/ml), 123.54 (µg/ml) & 215.76 (µg/ml) respectively and respectively and time to reach maximum concentration (T_{max}) value were 2h for both plain curcumin and curcumin-piperine combination & 8h for curcumin microspheres. The C_{max} value of curcumin microspheres was greater than plain curcumin this may be due to presence of piperine in formulation which prevents glucuronidation of curcumin. Administration of plain curcumin results in very low absorption into blood due to glucuronidation of curcumin in intestine. The value obtained from curcumin- piperine combination was little less than curcumin microspheres. The T_{max} was 8 hours for curcumin microspheres while 2 hours for plain curcumin and curcumin-piperine combination. It may due to early release of

plain curcumin and curcumin-piperine in the stomach and intestinal pH while the release from microspheres occurs after reaching colon. The area under concentration- time curve from 0 to 24 hours (AUC_{0-24}) was estimated by trapezoidal rule. AUC serves as an important pharmacokinetic parameter and represents the total amount of unchanged drug that reaches systemic circulation over the specified time period. The AUC_{0-24} value for plain curcumin, curcumin-piperine combination and curcumin microspheres were 397.82 (µg/ml/h), 925.8 (µg/ml/h) and 2024.70(µg/ml/h) respectively. The AUC_{0-24} value for curcumin microspheres was 6 folds greater than plain curcumin and 2 folds greater than curcumin-piperine combination due to sustained release of drug from microspheres.

The biodistribution study of plain curcumin, curcumin-piperine combination and optimized formulation were checked in albino male rat to found its colon targeting potential. The distribution of curcumin in rat spleen, lungs, stomach, small intestine, colon, fat, muscle, heart, liver & kidney at different time interval after administration of plain curcumin, curcumin-piperine combination and curcumin loaded microspheres is listed in **Figure 11.** The findings indicated that, after oral ingestion of plain curcumin, the highest concentration (103 µg/mL) was detected in the stomach after 2 hours, with only a minimal fraction reaching the small intestine and a negligible concentration detected in the colon. In the curcumin and piperine treated group, a C_{max} of 64.23 µg/mL was recorded in the intestine after 2 hours. In contrast, the curcumin-loaded microspheres remained intact throughout the upper GIT. The maximum curcumin concentration (177.22 µg/mL) was detected in the colon after 6 hours, while only negligible amounts were present in the stomach and small intestine. The data of in vivo results suggest that the Eudragit S100-based curcumin microspheres effectively protected the drug from premature release, enabling targeted delivery and sustained release in the colonic region.







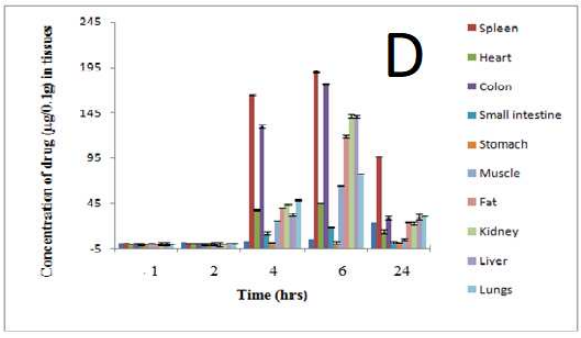


Figure 11: Plasma concentration time profile of curcumin in rat after single oral administration of curcumin (200mg/kg), curcumin-piperine combination (200mg/kg), curcumin loaded microspheres (200mg/kg) (A), Biodistribution after oral administration of curcumin (B), curcumin-piperine combination (C) and curcumin loaded microspheres (D) in rat. Values expressed in mean ± SD (n=6)

4.12 Anti-Obesity study

Curcumin significantly reduced high fat diet induced increment in body weight and relative organ weight as shown in Figure 12. The obese rats were treated with curcumin in different dose (100mg/kg, 150mg/kg & 200mg/kg) for 10 week. Here, piperine (bioavailability enhancer) is co-administered with curcumin due to less bioavailability of curcumin. Piperine has been reported to increase the bioavailability of drug by inhibition of glucurounidation in the live and small intestine **(5)**.

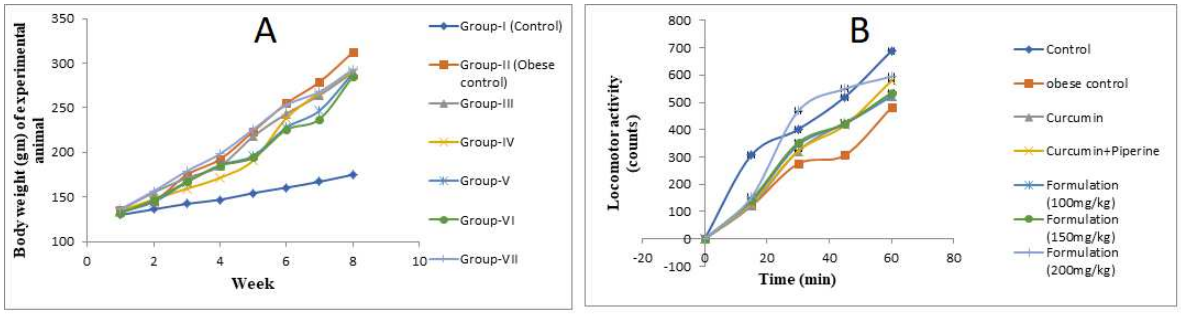


Figure 12: Body weights (gm) of experimental control & obese rats before drug treatment (A) and locomotor activity of the rats before and after drug treatment (B). Values expressed in mean ± SD (n=6)

Initial body weight of experimental rats in each group was similar. The body weight of rats fed with a HFD was greater than that of the rat fed with a normal diet, indicating that the model was successfully established. A high-fat diet (HFD) delivers significantly more calories than a standard diet, contributing to increased fat accumulation in adipose tissues. Due to its elevated fat content, HFD is recognized as a major contributing factor in the onset of obesity, promoting excessive body fat mass and overall weight gain. **Figure 12 A** show that the weight of control group significantly differed from HFD received obese rat. The obese rats were then treated with curcumin in different doses (100mg/kg, 150mg/kg & 200 mg/kg) for 10 weeks. The drug treatment significantly ($P<0.05$) reduced 16% body weight at 100mg/kg, 21% body weight at 150 mg/kg & 24 % body weight at 200 mg/kg dose

Increased in BMI was observed in obese rats as compared to normal control rats. On the final day of drug treatment period, the reduction in BMI was observed. BMI of rats treated with microspheres were revealed significant reduction from 0.997±0.008, 0.986±0.009, and 1.012±0.007 to 0.9344±0.009, 0.9346±0.01, and 0.9396±0.007 at a dose of 100mg/kg, 150mg/kg and 200mg/kg respectively.

Experimental rat receiving HFD showed a remarkable reduction in rectal body temperature and after the drug administration significant increase in body temperature was observed. Body temperature increased due to thermogenesis mechanism of body.

Locomotor activity of experimental rats was recorded before and after drug treatment. The reduction in locomotor activity was observed in HFD received rat when compared with normal control rat and significant increase in the locomotor activity was observed in drug treated group. Many peripheral and hypothalamic factors regulate the locomotor activity. Diet induced obesity has been shown to alter the dopamine system in striatum. We hypothesized that curcumin may regulate the locomotor activity by influencing the dopamine system in CNS **(Figure 12B).**

Elevated level of total cholesterol, triglyceride, HDL, LDL & glucose level were detected in HFD received rats compared to normal control rat. Oral administration of curcumin in different dose for 10 weeks to obese rats revealed significant lower level of total cholesterol, triglyceride, HDL, LDL and glucose level. Curcumin covalently binds to malonyl/acetyl transferase domain and suppress the activity of fatty acid synthase with increase in fatty acid β oxidation activity thereby decreasing triglyceride synthesis and lowered level of TG & TC. Fatty acid synthetase is key enzyme participating in energy reservation in vivo **(Figure 13).**

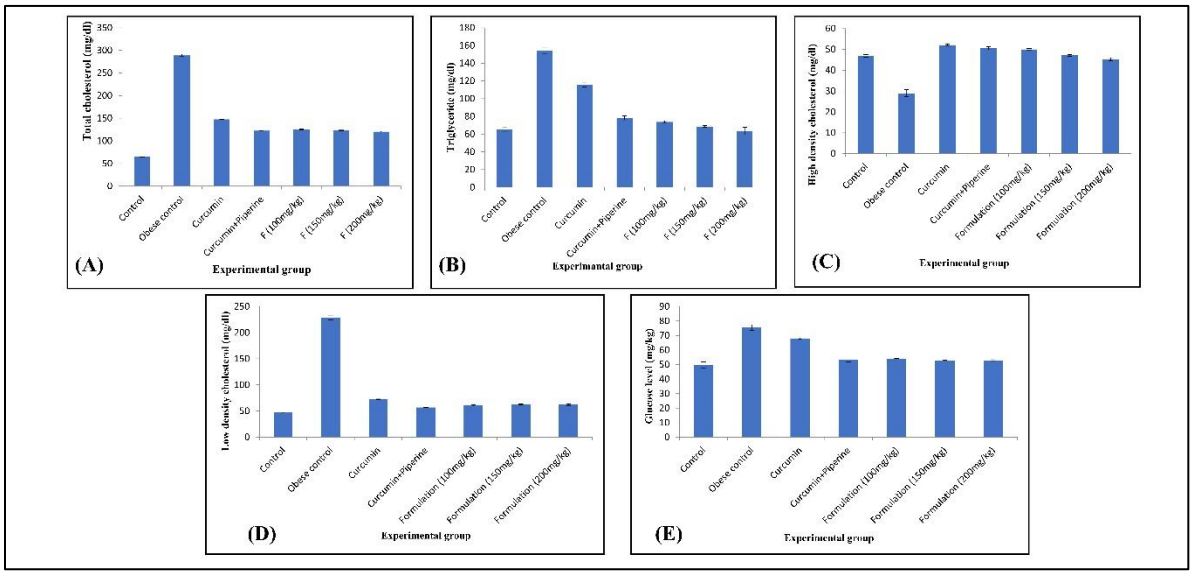


Figure 13: (A) Total cholesterol level of experimental rat before and after drug treatment, (B) Triglyceride level of experimental rat before and after drug treatment, (C) High density lipoprotein level of experimental rat before and after drug treatment, (D) Low density lipoprotein level of experimental rat before and after drug treatment, (E) Glucose level of experimental rat before and after drug treatment, Values represent as mean ± SD, (n=6)

Obesity is characterized by excessive accumulation of adipose tissue, predominantly in the abdominal region, which contributes to a rise in overall body weight as well as the weight of internal organs. At the end of study, various organs such as spleen, lung, liver, heart, muscle, fat and kidney were removed and weight was noted. It was

observed that organ weight of HFD induced rat increased when compared to normal control rat. The reduction in organ weight was observed in drug treated rats. These results showed that curcumin suppress the adipose tissues accumulation and organ weight gain.

Among the three doses (100 mg/kg, 150mg/kg and 200mg/kg), 150mg/kg & 200mg/kg doses produced a significant result as compared to 100mg/kg dose and significant reduction in body weight, BMI, organ weight and lipid profile was observed. We also found that the formulation having curcumin at dose 200mg/kg is more significant to reduce body weight compared to formulation having curcumin (150mg/kg).

All the experiments were performed in triplicate and data are expressed as mean (n=3) ± SD. Statistical analysis was performed and group differences were assessed by one way ANOVA for comparison between treated group and obese control group.

Stability studies of optimized formulations were performed at 25±2ºC/60±5%RH and 40±2ºC/75±5%RH over a period of 3 months. Mean particle size of carriers before storage was 5.490 µm which slightly increased during storage (At 25±2ºC size was 5.597 µm & at 40±2ºC size increased to 5.725µm. Slight increase in particle size may be because of absorbing atmospheric humidity and temperature effect.

Slightly rigidified surface of the microspheres was shown by SEM when microspheres stored at 40ºC, but microspheres stored at 25ºC did not differ significantly.

Drug content before storage was 73.25 ±0.5%, reduced to 73.13±0.04% at 25ºC storage condition and 73.93±0.1% at 40ºC storage condition. We concluded that there is negligible change during storage.

The drug release profile of drug from microspheres results in no significant changes before and after storage at different temperature and RH. We conclude that the curcumin release persisted without affecting by aging as no significant reduction in cumulative curcumin release was found after 24 hours of drug release study.

5. CONCLUSION

In the present work, curcumin loaded microspheres were effectively prepared by the cross-linking technique and enteric coating was done by solvent evaporation method. In vitro release test showed that curcumin loaded microspheres exhibited a sustained release property compared with the control. The microspheres were preferentially located in the colon and were retained for long time after oral administration. To conclude, the results indicated that prepared curcumin loaded microspheres are effective in lowering body weight and serum lipid levels in rat fed with high fat diet.

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Authors' contributions

Rashmi Manipuri: Research, Collect articles & Writing, Rashmi Madhariya: Writing & prepare structure, original draft, Rakesh Raj: Figure, Methodology, Alpana Ram: Investigation, Formal analysis, Conceptualization. Pooja Mongia Raj: Editing, Supervision, Resources.

Data Availability No additional data generated in this work.

Declarations Conflict of Interest Authors declare no other financial or non-financial interests related to this work.

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