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CURCUMIN LOADED CASEIN MICROCARRIER SYSTEMS TO MODIFY WATER DISPERSIBILITY AND DELIVERY CHARACTERISTICS

Roopa G.[1,*], Jayanthi C.[1], Srinidhi[1], Roopa Karki[2], Hanumanthachar Joshi[1] and Goli Divakar[2]

[1]Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysore-560004, Karnataka.

[2]Acharya and B M Reddy College of Pharmacy, Bangalore-560107, Karnataka, India.

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***Corresponding Author**
Roopa G.
Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysore-560004, Karnataka.

ABSTRACT

The aim of the present study is to prepare curcumin loaded casein microcarrier Systems to modify water dispersibility and delivery characteristics. Casein based curcumin microparticles were prepared by simple coacervation phase separation using pH change method. Morphology and surface characteristics of the formulations were assessed by scanning electron microscopy. The Mean particle size of casein curcumin loaded microparticles, were found to be in the range of 32 ± 0.22 to 63 ± 0.45 µ. The yield of the formulations varied from 85 ± 0.79 to 95 ± 0.92%. The percent of drug loading was in the range of 23 ± 0.21 % to 45 ± 0.45 % and the percentage encapsulation efficiency was in the range of 84% to 94%. The FT-IR spectra ascertained the compatibility of curcumin with the polymer. Microparticulate formulations containing 1:1 ratio of curcumin and casein exhibited comparatively faster and complete release (CCMP1, 4 and 7) than 1:2 and 1:3. Results obtained revealed that cross linking time of 2-6 h with glutaraldehyde 25% v/v did not influence the dissolution profiles of microparticulate formulations. SEM images exhibited irregular shaped discrete particles. CCMP3 was identified as the optimum formulation owing to sustenance of drug release.

KEYWORDS: Curcumin, casein, microparticles, glutaraldehyde, pH coacervation phase separation, controlled release.

INTRODUCTION

Curcumin (CCM) (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadien-3,5-dione) is the principle yellow pigment, constitutes 80% of curcuminoids of the rhizomes of *Curcuma longa L.* and saffron. The other curcuminoids are demethoxycurcumin (15%) and bis-demethoxycurcumin (5%).[1] This polyphenol modulates various targets through direct interaction or modulation of gene expression. Curcumin physically binds to as many as 33 different proteins, including thioredoxin reductase, cyclooxygenase-2 (COX-2), protein kinase C (PKC), 5-lipoxygenase (5-LOX), and tubulin. CCM possess diverse pharmacological actions including antitumor[2], antiamyloid and anti-inflammatory activities. Preclinical studies demonstrated that it suppresses carcinogenesis in a number of cell lines including breast, cervical, colon, gastric, hepatic, leukemia, oral epithelial, ovarian, pancreatic and prostate cancer cell lines.[3-4] Thus CCM has a challenging role in research and development. Oral delivery, the most natural, safest, convenient, economical and popular route of drug administration. CCM has shown poor bioavailability less than 1 % hence limited clinical benefits owing to its poor water solubility, degradation in the GIT, rapid first pass metabolism and low permeability, thus categorized in the fourth position according to biopharmaceutical classification of drugs.[5-8] Casein, the major milk protein, has been the integral component of the daily diet all over the world. Casein exhibits several interesting characteristics making it a potential candidate for conventional and novel drug delivery systems.[9] Strategies are aimed to associate bioactive molecules to casein to investigate their efficacy in modulating the release and/or enhancing the bioavailability. Casein's ability to modify drug dissolution from compacts have been reported. Casein microparticles entrapping bioactive molecules have been fabricated via emulsification-chemical crosslinking with Glutaraldehyde, enzymatic crosslinking by transglutaminase, simple coacervation and electrostatic complexation. Casein nano-formulations are fabricated to deliver nutraceuticals and synthetic drugs via enzymatic crosslinking, graft copolymerization, heat-gelation and polyelectrolyte ionic complexation. Casein-based formulations are concluded as promising bio materials for controlled drug delivery.[10-14] Coacervation commonly classified as simple and complex coacervation, are the techniques employed for microencapsulation. Simple coacervation involving addition of a strong hydrophilic substance to a solution of a polymer colloid, which results in two phases, one rich in colloidal droplets and the other poor in such droplets. Microencapsulation by simple coacervation consists of a core material, a polymer colloid, and a dispersion medium. A coacervate rich in polymer is then formed and deposited on the core material, where, due to the decrease in free interfacial energy, a layer of wall

material is formed. After solidification with a cross linking agent, microcapsules are formed and can be isolated from the solution phase. A coacervate can also be formed by adding either a second polymer or a nonsolvent, or by simply changing the temperature or pH of the dispersion system. Protein-based polymers especially casein's are advantageous as coating materials as they are biodegradable, eco friendly, economical, and readily available. Protein coatings prevent the UV exposure of prepared biological antibiotics that are very sensitive to UV light. Crosslinked casein microparticles have gained popularity as a carrier for several drugs. It is also demonstrated that reaction of the free amino groups in the protein with the cross linker decreases its proteolytic degradation in the GIT. The aim of the present study is to prepare curcumin loaded casein microcarrier Systems to modify water dispersibility and delivery characteristics.[15]

MATERIALS AND METHODS

Curcumin and Tween 80 were purchased from Himedia laboratories Pvt. Ltd. Mumbai. Casein was purchased from Spectrochem Pvt. Ltd. Mumbai, Glacial Acetic acid and Potassium dihydrogen phosphate anhydrous and Glutaraldehyde were purchased from Merk specialities Pvt.Ltd. Mumbai. Sodium Hydroxide and Hydrochloric acid were purchased from Loba chemicals, Pvt. Ltd. Mumbai.

Formulation of casein based curcumin microparticles by pH Coacervation phase separation method.

Weighed quantity of Casein was dispersed in 10ml of 1 N NaOH using glass rod, kept agitated on the magnetic stirrer for one hour. Specified quantity of Tween 80 was dropped into the solution. One hundred mg of curcumin was added to the above solution and the mixture was allowed for 10 m stirring, which resulted in the formation of cherry red solution. To this sufficient amount of 1 N HCl was added to adjust the pH to 4.7, during which period coacervates were formed. Yellowish orange microparticles obtained were hardened by adding 1ml of Glutaraldehyde solution (25 % v/v), stirred for predetermined h. Slurry was centrifuged, supernatant removed, 10ml of distilled water was added, redispersed, centrifuged again and supernatant removed to wash the microparticulate sediment. This sediment was suspended in 2 ml distilled water, placed in a petry dish and dried in an hot air oven at 60°c for 45 m. Dried free flowing microparticles were obtained by smoothly scraping the thin film, weighed and stored in dessicator for further investigations. Totally 9 formulations were prepared as mentioned in Table 1.[16 -17]

Evaluation of microparticles

Particle size analysis

Particle size of microparticles was measured using optical microscopy. A drop of the suspension was placed on a slide with the coverslip and mounted on a mechanical stage. The microscope eyepiece was fitted with a micrometer by which the size of the particles was determined using 10x. Size of 100 particles was measured. Mean particle size was calculated using the equation[18-19]

$$MEAN\ PARTICLE\ SIZE = \frac{\sum nd}{\sum n}$$

Yield

The yield was calculated by dividing the weight of microparticles by the total weight of the input materials, i.e., weight of CCM and casein.[20]

$$\% \text{ yield} = \frac{\text{Wt of microparticles}}{\text{Wt of Curcumin + Wt of Casein}} \times 100$$

Drug loading and encapsulation efficiency

Ten mg of microparticulate formulation was triturated in a glass mortar, using aliquots of methanol, quantitatively transferred to 10 ml volumetric flask and shaken overnight on a mechanical shaker. The mixture was filtered through whatman filter paper, 0.05 ml of the filtrate diluted to 10 ml with methanol. Absorbance was measured at 425 nm. Percentage drug loading was calculated using the formula[21],

$$\text{Percent drug loading} = \frac{\text{Conc. from standard graph(µg/ml) x dilution factor x 100}}{\text{Weight of Microparticles (mg) x1000}}$$

Percentage encapsulation efficiency is calculated using formula

$$\% \text{ Encapsulation efficiency} = \frac{\text{Percent drug loading}}{\text{Percentage of drug added in the formulation}} \times 100$$

Drug – Excipient compatibility

Preformulation studies data of drug – polymer interactions are very critical in selecting appropriate polymers. FT-IR spectroscopy was employed to ascertain the compatibility of CCM and casein. Samples were finely ground with IR grade Potassium bromide and then pressed into pellet, and IR spectra were taken in transmission over the range of 4000-500 cm^{-1} at ambient temperature using Shimadzu FTIR – 8400S.

Dissolution profiles of pure Curcumin and Microparticles in 2% aqueous solution of Tween 80.

In vitro release of CCM from the microparticles was performed on a magnetic stirrer (Tarsons: Spinit model MC- 01) at 37±0.5°c, for assessing relative dissolution profiles of various microparticulate formulations. Ten mg of the formulation was dispersed in 10ml of 2% tween 80, as the dissolution medium, stirred at 100rpm. At designated time intervals, 0.1 ml of the release medium was sampled through thin layer of cotton plugged 1ml pipette, which was diluted to 10ml, absorbance was measured at 425nm. All experiments were run in triplicates.[22]

SEM analysis

Surface and shape characteristics of pure curcumin and microparticle formulation CCMP3 were evaluated by means of scanning electron microscopy (Model Hitachi S3400N, Japan). The scanning electron microscopy samples were prepared by lightly sprinkling the microsphere powder on a double adhesive tape, which was stuck to an aluminum stub. The stubs were then coated with gold to a thickness of ~300 Å using a sputter coater, and the photographs of samples were taken (Figure 2).

RESULTS AND DISCUSSION

Casein curcumin microparticles prepared by simple coacervation phase separation by pH change method were found to be fine, free flowing, humidity resistant. Initial batches prepared without tween 80 exhibited more of aggregated particles, which drived us to incorporate tween 80 in the formulation. This surfactant might act as de aggregating agent. Totally 9 formulations were prepared with varying the concentrations of casein from 0.1g to 0.3g and the rigidisation time as 2, 4, 6 h. As casein was insoluble in water, it was dissolved in 1N NaOH for one h, during which time, formed sodium caseinate might undergo solvation in aqueous environment. Addition of CCM to alkaline (1N NaOH) casein solution, resulted in the formation of cherry red colour solution. CCM is insoluble in water, but soluble in alkaline casein solution. In this solution CCM might exist as molecular and colloidal dispersion. Adjustment of pH to 4.7 resulted in the formation of coacervates / microparticles of casein with entrapped or embedded CCM. Slurry was centrifuged, supernatant removed, 10 ml of distilled water was added, redispersed, centrifuged again and supernatant removed to wash the microparticulate sediment and was dried in the hot air oven at 60°c for 45 m. Prepared formulations were packed in air tight glass vials, labeled and stored in dessicator.

The Mean particle size of microparticles, CCMP1 to CCMP9 were found to be in the range of 32 ± 0.22 to 63 ± 0.45µ. Studies showed decrease in mean particle size with increase in casein concentration. The yield of the formulations of CCMP1 to CCMP9 varied from 85 ± 0.79 to 95 ± 0.92%. The prepared nine microparticle formulations gave satisfactory yield, as recorded in (Table 2). As observed there has been a marginal increase in the yield with increase in the concentration of casein from 0.1g through 0.2g to 0.3g. Rigidisation time of 2 - 6 h has not affected the yield of microparticles. The percent of drug loading was found to be in the range of 23 ± 0.21 % to 45 ± 0.45 % and the percentage encapsulation efficiency was in the range of 84% to 94% (Table 2).

Drug loading refers to percentage of drug cargo contained in the delivery system. In the microparticulate system CCM, have got encapsulated by the casein coacervates formed during pH coacervation and phase separation process. Casein is practically insoluble in water but was solubilized by the addition of 10ml of predetermined strength of Sodium hydroxide (1N), which produced pH above 12 and was in the colloidal state (micelles). Solution was translucent. It was observed that casein-sodium hydroxide solvation time of 1h was satisfactory to yield particulate free solution. Addition of 1drop (40mg) of tween 80 and 0.1g of CCM yielded cherry red colour with very few and sedimentable CCM particles. Change of pH during addition of 1N Hcl reduced solubility of CCM resulting in microprecipitation and simultaneous coacervation and phase separation of casein. During this period there could be formation of co precipitates of casein and CCM as well as encapsulation of insoluble CCM at acidic pH by the coacervated casein molecules. Drug loading and Encapsulation efficiency are the important quality control tests to assess the efficiency of drug entrapped. Percentage drug loading found to be dependent on the proportion of casein employed in the formulation. Greater percentage of CCM loaded is associated with lower proportion of casein as observed in Table 1. Formulations 1, 4 and 7 exhibited percentage loading in the range of 42-44%. Percentage entrapment efficiency is found to be independent of casein concentration, all formulations exhibited appreciable % EE endorsing the suitability of chosen formulation and procedure. Percentage Drug loading might influence the drug release or dissolution profile of the formulation, as it might provide driving force for the diffusion of CCM within the microparticles or in the dissolution medium.

Drug-polymer compatibility

The compatibility of CCM and casein was assessed by FT-IR spectra. Comparison of peak position of drug in the spectra of physical mixture and microparticulate formulation with that of pure drug ascertained the compatibility of drug and the polymer. Pure drug CCM, casein, physical mixture and CCMP3 formulation were subjected to FT-IR analysis. The recorded spectra are given in Figure 1. Characteristic peaks of CCM were compared with the corresponding peaks of the formulation. These characteristic bands for the drug were identifiable and there was no major shift in them when combined with polymers in the microparticles. This indicates that the drug is intact and has not reacted with the excipients used in the formulation and hence they are compatible. Pure CCM spectrum (Figure 1a) exhibited the characteristic peaks in the intensities of O-H stretch at 3510 cm^{-1}, the C=O stretching frequencies of ester linkage at 1627 cm^{-1}, the C--C stretch (aromatic ring) at 1508 cm^{-1}, (CCC) stretch, (CCH) in plane bending, (C-O-C) of aromatic and inter ring chain of pure curcumin at 1427 cm^{-1}, (CCC) stretch and (CCH) in plane bending of the aromatic "keto" part at 1280 cm^{-1} which are also observed in the physical mixture (Figure 3c) and CCMP3 microparticle formulation (Figure 3d) with minor shifts in the peaks implying the successful entrapment of curcumin in the casein microparticle formulation. The major region of spectra of casein (Figure 3b) showed absorption peaks at 3396 cm^{-1} (O-H). The peak at 2920 cm^{-1} (C-H) also appeared in the physical mixture (2939 cm^{-1}) and CCMP3 microparticle formulation (2926 cm^{-1}) with minor shifts in the peaks, which confirmed the incorporation of casein in the microparticulate system.[23]

Dissolution profiles have been depicted in the Figure 2 and charted in the Table 2. We have chosen 2% tween 80 as the dissolution medium, as it provides sink conditions for performing *in vitro* drug release studies for relative assessment of various prepared microparticulate formulations. Dissolution of drug from its formulation into the biological environment is a step prior to *in vivo* absorption across biomembrane. *In vitro-in vivo* correlation is of paramount importance to ascertain the therapeutic performance of a developed formulation. Microparticulate formulations containing 1:1 ratio of CCM and casein exhibited comparatively faster and complete release (CCMP1, 4 and 7) than 1:2 and 1:3 proportions. With lower casein concentrations the percentage drug loading is higher, which might be the driving force for providing concentration gradient for faster release and also due to lesser viscosity ie..diffusional path length of hydrogel particles. Results obtained reveal that cross

linking time of 2-6 h with glutaraldehyde 25% v/v did not influence the dissolution profile of microparticulate formulations.

The Scanning Electron Micrographs (SEM) showing the shape and surface characteristics of the particles of the pure curcumin and the optimized microparticulate formulation CCMP3 are seen in Figure 3a and 3b respectively which showed non-uniform surfaces with dents. The CCMP3 formulation exhibited discrete particles with irregular surface features and a glassy surface could also be seen.

Table: 1. Formulations chart of Curcumin Casein microparticles using 3^2 Factorial Design

Ingredients/ Process	Formulations								
	CCMP1	CCMP2	CCMP3	CCMP4	CCMP5	CCMP6	CCMP7	CCMP8	CCMP9
Curcumin(g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Casein(g)	0.1	0.2	0.3	0.1	0.2	0.3	0.1	0.2	0.3
Concentration of NaOH.	1N	1N	1N	1N	1N	1N	1N	1N	1N
Tween 80(g)	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Glutaraldehyde(ml)	1	1	1	1	1	1	1	1	1
Rigidization Time(h)	2	2	2	4	4	4	6	6	6

Table: 2 Evaluation data of microparticle formulations.

Evaluation Parameters	Formulations									Curcumin (Pure drug)
	CCMP1	CCMP2	CCMP3	CCMP4	CCMP5	CCMP6	CCMP7	CCMP8	CCMP9	
Average Particle Size in µ (± S.D)	63±0.45	52±0.32	35±0.29	65±0.42	54±0.52	32±0.22	61±0.52	58±0.68	34±.35	
%Yield (± S.D)	87±0.83	92±0.94	94±0.89	89±0.76	91±0.90	92±0.84	85±0.79	94±0.68	95±0.92	
%Drug loading (± S.D)	44.5±0.53	30±0.42	23±0.21	45±0.45	29±0.30	24±0.22	42±0.39	29±0.28	23±0.21	
%Encapsulation Efficiency (± S.D)	89±0.75	92±0.94	91±0.90	86±0.75	89±0.76	94±0.65	84±0.69	88±0.78	93±0.81	
% Dissolved at 0.5h(± S.D)	20±0.11	22±0.12	19±0.91	24±0.32	21±0.11	20±0.12	18±0.12	24±0.31	26±0.31	15±0.02
% Dissolved at 3h(± S.D)	48±0.72	44±0.61	35±0.52	64±0.22	46±0.21	41±0.12	66±0.23	47±0.22	37±0.62	80±0.23
% Dissolved at 6 h (± S.D)	75±0.12	67±0.13	39±0.21	84±0.28	68±0.01	42±0.25	86±0.41	65±0.22	39±0.21	98±0.75
% Dissolved at 24 h (± S.D)	92±0.31	70±.01	40±0.32	94±0.31	72±0.12	45±0.42	96±0.52	73±0.43	44±0.32	98±0.75

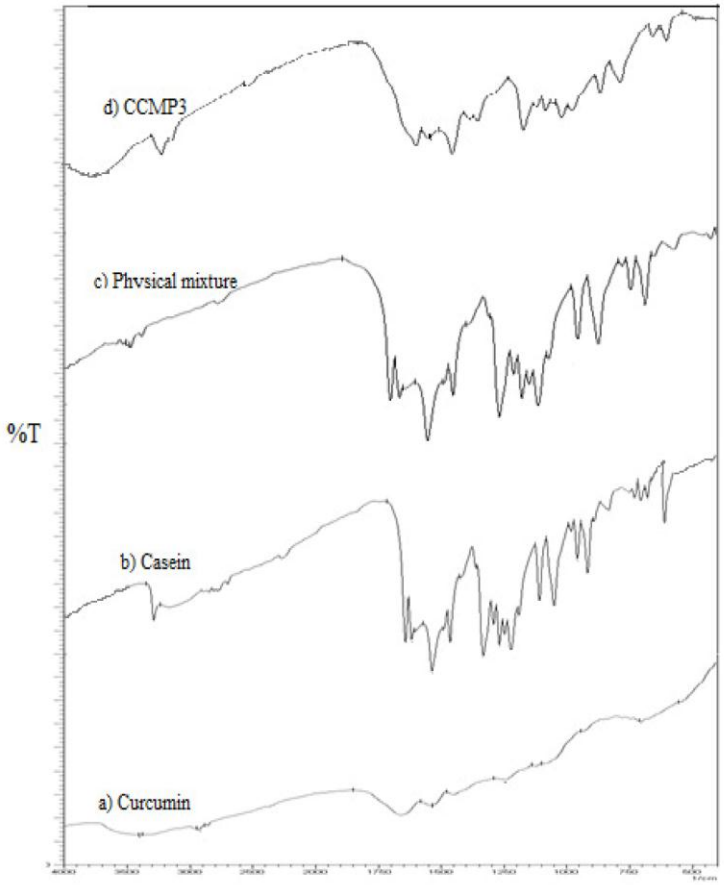

Figure 1: a) FT-IR spectra of Curcumin b) Casein c) Curcumin Casein Physical Mixture d) CCMP3 formulation.

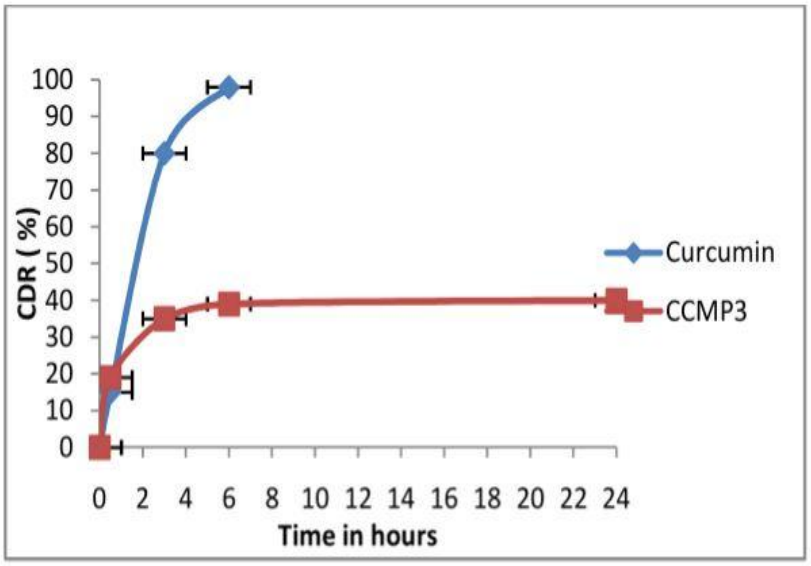

Figure 2 In-vitro release studies

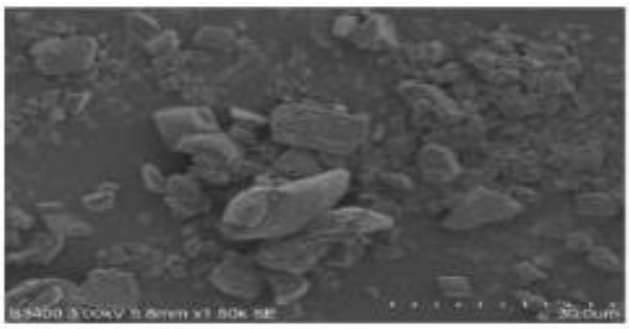

Figure 3a: SEM image of pure curcumin.

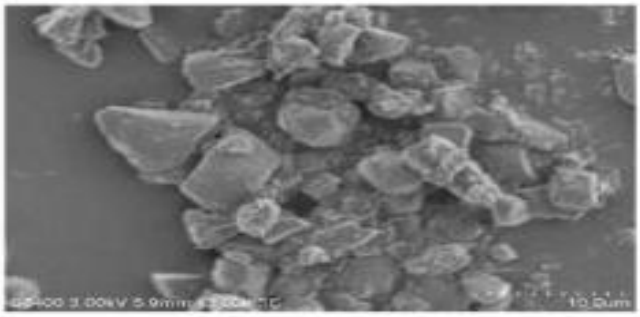

Figure 3b: SEM image of CCMP3 formulation.

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CONCLUSIONS

Casein curcumin microparticles prepared by simple coacervation phase separation by pH change method were found to be fine, free flowing, humidity resistant. CCMP1 exhibited better drug loading, entrapment efficiency and dissolution profile. CCMP3 showed sustained release effect. FT-IR spectra confirms the compatibility of CCM with casein which holds promise for further studies. SEM images exhibited irregular shaped discrete particles. Formulation CCMP3 was identified as the optimum formulation owing to sustenance of drug release.

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