INTRODUCTION

Glycosylation is a post-transitional modification of the protein, where sugar molecules covalently bound to free amino groups of protein residue. Glucose react, nonenzymatically with human hemoglobin to form amino-deoxy fructose adducts and initiate advanced glycation [1,2]. It was reported that such reactions take place both in vivo and in vitro. Non-enzymatic glycosylation of human hemoglobin leading to advanced glycation end products (AGEs) formation [3]. Maillard reaction indicates that reducing sugars react with amino acids, peptides, and proteins in solution producing dark-colored products. Thus, Maillard reaction results in some important, highly active intermediates such as Di-carbonyl products, glyoxal, and methylglyoxal [4]. The aldehyde functional group of sugars, non-enzymatically react with a thiol or amino groups of a protein (or another biomolecule) forming a Schiff base. Then, the Schiff bases rearrange to produce ketoamine or amadori products [5]. These amadori products undergo rearrangements and other reactions such as cyclization, oxidation, and dehydration to form more stable AGEs by both oxidative and non-oxidative pathways [5]. Impaired glucose metabolism is associated with oxidative stress. Under normal physiologic conditions also the formation of AGE takes place but is accelerated in hyperglycemia [3,5].

Recent studies indicated that curcumin decreases blood glucose levels and glycated hemoglobin levels [6,7]. Curcumin is a yellow colored, hydrophobic polyphenolic pigment derived from rhizomes of Curcuma longa L, belonging to the family, Zingiberaceae. C. longa L contains curcuminoids, the three major components of curcuminoids are curcumin (77%), demethoxycurcumin (17%), and bisdemethoxycurcumin (3%). Curcumin is insoluble in water and ether but is soluble in ethanol, dimethylsulfoxide, and other organic solvents. Curcumin has been reported to play a beneficial role in various activities such as antioxidant, anti-inflammatory, antimicrobial, anticancer, antioxidant, antidiabetic, antihyperlipidemic, and hepatoprotective activity [8,9]. However, low-water solubility, poor bioavailability, and rapid metabolism are major factors that limit the utility of curcumin [10]. Various formulations have been designed to improve the oral bioavailability of curcumin [11]. Sodium alginate has been widely used polymer for oral delivery because it is a biocompatible, biodegradable polymer, and minimal processing required for formulation development [12].

The purpose of the present study was to formulate curcumin microspheres and investigate its glycosylation inhibitory potential.

MATERIALS AND METHODS

The standard (quercetin) was purchased from Sigma-Aldrich, USA; curcumin purchased from Changsha Staherb Natural Ingredients Co., Ltd., China, other chemicals such as sodium alginate, calcium chloride, and polyethylene glycol 400 (PEG 400) were purchased from Loba Chemie Pvt. Ltd. Mumbai.

Compatibility study

Fourier-transform infrared (FTIR) analyses of curcumin, sodium alginate, and their physical mixture were obtained using an FTIR spectrophotometer (Shimadzu R prestige-21 FTIR spectrophotometer). The pellets were prepared under hydraulic pressure of 150 kg/cm² on the KBr-press (Technosearch Instruments, Maharashtra, India); the
spectra were scanned over 4000-400 cm⁻¹ spectral range at ambient temperature.

**Preparation of curcumin-microspheres**
The curcumin microspheres were prepared by an ionic gelation technique which involves crosslinking in the presence of multivalent counterions. Curcumin was first triturated with PEG 400 and then entrapped in the polymer matrix of sodium alginate.

**Determination of microsphere yield**
It is expressed as a ratio of the total weight of microspheres to the total weight of all non-volatile ingredients including excipients used for the preparation of formulation. The microspheres in with particle size 371–559 µm were used to determine percentage yield.

**Percentage entrapment efficiency (%EE)**
Dried microspheres (100 mg) were ground in a mortar and then dispersed in ethanol. It was then sonicated for several hours to ensure exhaustive extraction of curcumin from microspheres. The resulting solution then filtered through 0.45 µm membrane. Then, the absorbance of the resulting solution was taken at 424.5 nm with ultraviolet (UV)-spectrophotometer (Shimadzu–UV-1800), and pure ethanol was used as blank.

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\text{%EE} = \frac{\text{Actual amount curcumin in microspheres}}{\text{Theoretical amount of curcumin}} \times 100
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**Particle size and polydispersity index (PDI)**
The particle size and PDI of formulation batches (F1–F6) were examined by a Zetasizer NanoZS90 (Malvern Instruments, Malvern, UK) at least three repetitions for each sample.

**Scanning electron microscopy (SEM)**
The microspheres were evaluated for surface and shape characteristics using SEM. The dry microspheres were randomly scanned and photomicrographs were taken on carbon stub coated with gold in an ion sputter Carl Zeiss Supra 5 model (Germany). The voltage provided was between 5 and 10 kV.

**In vitro dissolution studies**
To study in vitro drug release from microspheres, USP II dissolution apparatus was used. The test carried at 50 rpm in the 900 ml of 1M HCl (pH 1.2) for 2 h followed by 900 ml phosphate buffer (pH 7.4) for 10 h. The dissolution media were maintained at 37°C ± 0.5°C. Samples 5 ml each were withdrawn at intervals and analyzed spectrophotometrically at 424.5 nm. To maintain the sink condition, the release medium was replenished with the same amount of fresh medium.

**Statistical analysis**
To determine the kinetics of drug release from delivery systems, mathematical models were used. The obtained data were fitted to models such as zero-order, first-order, Higuchi, Korsmeyer–Peppas model, and Hixson’s Crowell model [12,13].

**In vitro human hemoglobin glycosylation assay**
Blood was collected from healthy donors after obtaining their consent. The samples were collected in ethylenediaminetetraacetic acid containing bottles. Preparation of hemolysate was based on the principle of hypotonic lysis [14]. As per the procedure [5], the blood was collected and then was washed with 0.14M NaCl solution, thrice. Then, 1 volume of the suspension of red blood cells was lysed with 2 volumes of 0.01M phosphate buffer, pH 7.4 and 0.5 volumes of carbon tetrachloride. The hemolysate was then centrifuged at 2300 rpm for 15 min at room temperature so as to free it from the debris. The upper layer (hemoglobin rich fraction) was separated and stored into the sample bottle in the refrigerator at −10°C until required for use.

**Estimation of hemoglobin glycosylation**
The hemoglobin concentrations were estimated by a reported method at 540 nm [15]. Non-enzymatic glycosylation inhibition was estimated according to the assay [16]. To 1 ml of a solution of glucose (in 0.01 M phosphate buffer pH7.4), 1 ml hemoglobin fraction was added. The contents were kept incubated in the dark for 72 h at room temperature. The final concentrations of glucose were 2, 10, and 20 mg/ml. This release hydroxyl-methyl-furfural and its estimation correspond to the degree of glycosylation. Blank solution where glucose was omitted, used as control.

**Assay: Effect of curcumin and its formulation on hemoglobin glycosylation**
In this experiment, the effect of quercetin (used as standard), suspension of curcumin and curcumin microparticles on hemoglobin glycosylation was tested. To 1 ml of hemoglobin solution, 5 µl of gentamycin, quercetin, suspension of curcumin, and its formulation in concentration (50–1000 µg/ml) was added. To this, 0.01 M phosphate buffer pH 7.4 and incubated in the dark at room temperature. The concentrations of glycated hemoglobin at the incubation period of 0, 24, and 72 h were estimated colorimetrically at 443 nm [5]. The test was conducted in triplicate.

**RESULTS AND DISCUSSION**
**FTIR spectroscopy**
FTIR spectrum of curcumin shows (Fig. 1) characteristic peaks at 803.42/cm, 1111.32/cm, 1275/cm, 1286.68/cm, and 1687.68/cm.
The peak at 1687.68/cm is attributed to the enol group in curcumin. The strong and broad absorption peak at 3675.04/cm and 3550.34/cm due to stretching of –OH group in sodium alginate while stretching vibrations at 1287.24/cm, 1231.31/cm, and 1081.23/cm due to -C-O and –C-O-C- groups of the polymer. However, the physical mixture of curcumin and sodium alginate retain their respective peaks indicating no physical interaction.

**SEM**

Scanning electron micrograph of batch F4 (Fig. 2) indicated that alginate microspheres are discrete and spherical in shape with rough surface morphology.

%EE, percentage yield, and particle size

The percentage yield of microspheres was in the range of 43.05%–81.4%. The percentage entrapment efficiency was found to be in the range of 57.18%–84.24% (Table 1). The concentration of sodium alginate and calcium chloride has an effect on percentage yield, percentage entrapment efficiency, and particle size. Increase in the concentration of sodium alginate increases percentage yield and percentage entrapment efficiency. However, manufacturing of microspheres became difficult as the concentration of alginate increases to 3% due to increase in viscosity [17]. At 2%, the concentration of alginate fabrication of microspheres was smooth. Impact of concentration of crosslinker on particle size, entrapment efficiency, and percentage yield was observed. In the case of F3 and F4 formulations as a percentage of crosslinker increases yield and entrapment efficiency increases. This may be due to the availability of more Ca$^{2+}$ ions with the carboxyl group of sodium alginate. In both F3 and F4 formulation, the increase in crosslinker concentration decreased in particle size was noted. Similar observations were made by Hariyadi *et al.* [17].

**In vitro release study**

All the batches were subjected to in vitro release study. Cumulative drug release after 12 h from different batches was shown in Fig. 3. The F3 and F4 showed sustained release pattern when compared with other batches. It can be seen from Fig. 3 that about 15% drug release in acid pH 1.2 and about 85% drug release at pH 7.4. Fig. 3 also displayed the effect of polymer concentration and crosslinking on in vitro drug release, as polymer concentration increases drug release from polymer matrix found to be decreased. The formulation batches F5 and F6 with high polymer concentration show drug release up to 55%–60%. The increased concentration of polymer may show increased swelling and decreases drug diffusion from the polymer matrix. Literature reported similar discussion [18]. Extend of crosslinking also affect drug release from formulation. As the concentration of crosslinker increases, retarded drug release was observed. This may be due to limitations in easy transport of drug molecule from polymer matrix [18,19]. Maximum release from microspheres was observed with phosphate buffer (pH7) as dissolution media. The release involves ion exchange between Na$^+$ present in phosphate buffer and Ca$^{2+}$ bound to carboxylic acid group of alginate. A higher percentage of crosslinker prolong the duration of ion exchange. Similarly, dense gel structure due to the high concentration of

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**Fig. 2:** Scanning electron micrograph of batch F4 microspheres and surface microstructures of prepared

**Fig. 3:** Comparative percentage release of curcumin from formulation batches (F1–F6)
sodium alginate may restrict diffusion and delay ion exchange process. Similar observations have been made by Voo et al. where the effect of polymer and crosslinker on dissolution was studied [19].

On the basis of percentage yield, entrapment efficiency, and particle size F4 batch was found to be optimum and was further characterized for the study of release kinetics. In all the cases, the best-fit model was found to be Korsmeyer–Peppas with “n” value between 0.85 (Table 2) suggesting super Case II transport mechanism, i.e., erosion of the polymeric chain stresses hydrophilic polymers which swell in water, followed by diffusion controlled release zero-order mechanism. The results were in agreement with a study conducted by Mazumder et al. [20].

**CONCLUSION**

In this study, curcumin microspheres were successfully developed using the ionic gelation technique. The entrapment efficiency, yield, and drug release were influenced by sodium alginate and calcium chloride concentrations. Administration of curcumin inhibits glycosylation of hemoglobin. Together our, data suggest that curcumin microspheres may be more effectively utilize curcumin in therapeutic applications.

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**AUTHORS’ CONTRIBUTIONS**

Aditi S. Kulkarni as author carried out the experimental work, data collection, data analysis, and manuscript drafting. Dr. S. S. Bhujbal is principal advisor involved in designing of work, data interpretation, and provided resources for research work.
CONFLICTS OF INTEREST
No conflicts of interest associated with this work.

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