Synthesis of Curcumin Loaded CMCAB Nanoparticles for Treatment of Rheumatoid Arthritis

Ashish Kumar Dewangan, Sunil Varkey, and Sonal Mazumder

***Abstract*—**The role of Curcumin for treatment of diseases like colon cancer, pancreatic cancer, arthritis and Alzheimer's disease has become the area of growing interest. It has low intrinsic toxicity and magnificent properties like anti-inflammatory, anti-mutagenic, anti-oxidant, with comparatively lesser side-effects. However, because of its poor solubility, low absorption and poor bioavailability, they are not efficient to be used for clinical purposes. Formation of nanoparticles have been proved very much promising to increase absorption and bioavailability, leading to effective drug administration. This study reports the encapsulation of Curcumin by polymeric compound like CMCAB to enhance bioavailability of the hydrophobic drug. In the present study, Curcumin – CMCAB (carboxy methyl cellulose acetate butyrate) nanoparticles are synthesized by flash nano precipitation method using Multi Inlet Vortex Mixer (MIVM). The Reynolds Number of the inlet streams was varied to optimize the particle size. The synthesized nanoparticle were separated using rotary evaporator followed by hot oven drying or freeze drying. It was observed that the particle size was dependent on the method of drying, which may be because of agglomeration occurring when it was dried in the hot air oven whereas when it was freeze dried the particles were found to be in the range of 150 – 400 nm, as measured by Dynamic Light Scattering (DLS). The Differential Scanning Calorimetry (DSC) results showed that the Curcumin – CMCAB nanoparticles were in the amorphous state. From the analysis Fourier Transform Infrared Spectroscopy (FTIR), it was observed that the nanoparticle system was chemically stable. The % drug loading was observed to be 4.5%. Further in-vitro studies and in-vivo can tell us more about the feasibility of the drug for clinical application.

***Keywords*—**Curcumin, drug loading, nano precipitation

I. Introduction

RHEUMATOID Arthritis (RA) is a devastating illness that is caused by systemic inflammatory disorder of the synovial membrane. It often leads to localized damage to articular cartilage, bone, tendon and ligament, followed by loss of function[1]. About 0.5-1 % of the worldwide population is suffering from RA[2]. This mostly occurs between 40-70 years of age, typically increases with age and 2-3 times more likely in women than men[1].

Current RA therapeutics are not efficient and RA patients suffer from systemic side effects due to frequent and long-term treatment. A number of targeted nanoparticle delivery methods for RA have shown the potential to improve the efficacy and safety profiles of RA therapeutics. There has also been development of novel biological drugs. Hence, it is required to design RA-targeted nanoparticles based on physicochemical properties of drugs and pathophysiological characteristics of the disease. The investigation of type of vehicle, size and surface chemistry is an important area of research.

Curcumin (CUR) which is used as a natural yellow pigment in the food and textile industries has shown beneficial anti-oxidant, anti-cancer, and anti-inflammatory effects[3]. It has proved to be more effective in alleviating the symptoms of rheumatoid arthritis (RA) like tenderness and swelling of joints compared to regular RA drugs. The suggested daily dose of CUR is 12 g, with no significant toxicity[4]. However, the major disadvantage of CUR is its poor aqueous solubility (at pH 5.0 is 11 ng/mL). It also shows chemical instability and metabolic susceptibility which leads to poor bio-availability in both animals and humans[5]. Also it degrades faster in neutral or alkaline buffer solution[6]. Therefore, in order to benefit from the advantages of CUR, it is essential to develop efficient drug delivery systems that would increase its solubility and chemical stability.

Many researchers have developed method to enhance CUR solubility, stability and bio-availability[6] including alternative routes of administration such as intravenous and transdermal[7]. In order to enhance oral bioavailability of CUR several strategies were developed such as chemical modification [8,9], nanoparticles[10,11] , micelles[12,13] cyclodextrin complexes[14-16], self-microemulsifying drug delivery system (SMEDDS) [17] and solid dispersions[18,19]. Another study showed an improvement in Cur bioavailability upto 20 fold by using piperine to inhibit glucuronidation[22]. There are very limited studies on nanoparticles based CUR delivery systems[11,21-24].

The current study focuses on oral administration of CMCAB-CUR nanoparticles to attain higher solubility and release of CUR. CMCAB is pH-sensitive and water-swellable when partially ionized at the neutral pH of the small intestine

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(ca. pH 6.8), but is insoluble at gastric pH (ca. pH 1.2). CMCAB has good solubility in common organic solvents. We hypothesized that the pH-dependent swelling controlled by the carboxyl groups of CMCAB might reduce CUR degradation by minimizing release in the stomach, but permitting controlled release in the small intestine. CMCAB is compatible with numerous pharmaceutical actives and has a high glass transition temperature (T_g = 137°C). It has been shown to be useful for forming amorphous solid dispersions[25]. Due to its hydrophobic character, CMCAB is a useful polymer for the slow, in some cases zero-order (with respect to time) release of hydrophobic drugs[26]. This characteristic has also been shown to lead to slow release of more hydrophilic drugs like aspirin and ibuprofen.

There have been no previous reports of preparation and testing of CMCAB/drug nanoparticles. Nanoparticles show faster and more uniform distribution in the GI tract compared to single unit dosage forms owing to their small sizes[27]. The larger surface area enhances the interaction of the nanoparticles with the epithelial lining and mucus, prolonging retention time and thereby enhancing bio-availability[28]. The benefits of using nano-formulations are enhanced dissolution, safer and more patient-compliant dosage forms, and the potential for dose escalation for improvements in efficacy. According to the Noyes-Whitney model for dissolution kinetics, the dissolution rate is directly proportional to the surface area of the drug. Also reducing particle diameter from 1 micron to 50 nanometers (nm) increases the specific area and, hence, the drug mass transfer rate by 400-fold.

Many studies of nanoparticles made with synthetic polymers have focused on controlling drug loading and characterizing drug release but, in many cases, the method of producing the particles was not rapid and scalable. We investigate the effect of processing conditions on the particle size and drug loading of nanoparticles of CUR, made with a polysaccharide – CMCAB using the flash nano-precipitation method in a multi-inlet vortex mixer (MIVM) In this method, nanoparticles form very rapidly by controlled nucleation and growth of particles, which allows for the production of nanoparticles of controlled size in a continuous manner. This is achieved by controlling the Reynolds numbers and the liquid phase composition, allowing for rapid and complete mixing.

II. Experimental Procedure

A. Materials

Curcumin and Poly Lactic Glycolic Acid (PLGA) was purchased from Sigma Aldrich, India. Carboxymethyl Cellulose Acetate Butyrate (CMCAB), was purchased from Eastman Chemical Company, USA. CMCAB was provided in its free acid form and used as received. Tetrahydrofuran (THF), purchased from Molychem, India, was used for polymer – drug nanoparticles formation.

B. Preparation of Polymer – Drug Nanoparticles

In this work, polymer – drug nanoparticles were prepared by rapid precipitation in Multi Inlet Vortex Mixer (MIVM), which can accommodate four streams [29-32]. The solution of polymer – drug in THF was injected from one of the streams whereas, the rest three streams was composed of distilled water. The four streams enters tangentially into the mixing chamber and the nanoparticle solution is collected from the exit stream situated at the center of the mixing chamber. The velocities of the inlet streams are controlled by the syringe pumps.

The set up consisted of MIVM with four inlet streams, one organic and three aqueous, the velocities of which were maintained with the help of Syringe Pumps (New Era, Unigenetics, India), NE – 1600 for aqueous streams and NE – 300 for organic stream. DISPOVAN™ syringes were used in this experiment to hold different phases in the syringe pumps.

The nanoparticle formulation was collected and after removal of THF in a rotary evaporator at a bath temperature of 50 °C and 357 mbar pressure, it was dried in the hot air oven at 50 °C for 24 hours.

C. Drug Loading

In order to determine the drug loaded in the polymer – drug nanoparticle matrix two solutions with concentrations 0.1mg/ml and 0.05mg/ml was prepared and their absorbance was measured using UV – Visible Spectrophotometer. The concentration of the drug was calculated using calibration curve prepared using pure Curcumin at a maximum wavelength of 425 nm. Finally, the drug loading was calculated from experimental concentration divided by the actual concentration.

D. Determination of Particle Size, Polydispersity Index and Zeta Potential of Polymer – Drug Nanoparticles

Particle size, polydispersity index and zeta potential were determined via Dynamic Light Scattering Technique using Zetasizer (Model – ZEN 3602, Malvern Corp, UK). The measurements were done at a fixed light scattering angle of 173° and the same cuvet cells were used for conducting zeta potential experiments.

E. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR Spectroscopy of pure Curcumin, CMCAB, PLGA, and Curcumin – CMCAB and Curcumin – PLGA nanoparticles were carried out to study the structural differences existing among the pure compounds and polymer – drug nanoparticle matrix using Perkin Elmer (Frontier-IR model) Spectrophotometer.

F. Differential Scanning Clorimetry (DSC)

Differential Scanning Calorimetry analysis was done to understand the phase transition behavior of Curcumin, CMCAB, PLGA, and Curcumin – CMCAB and Curcumin – PLGA nanoparticles using Perkin Elmer,DSC – 4000, 2013. The temperature range was set between -40 °C to 400 °C at the rate of 10 °C/min and nitrogen flow rate was maintained at 20 ml/min.

III. Results and Discussions

The polymer – drug nanoparticles were synthesized at Reynolds Number of 3275 by maintaining the organic solution and aqueous solution flow rate at 5.82 ml/min and 6.5 ml/min respectively. The nanoparticle solution collected was then

subjected to rotary evaporation for the removal of THF followed by freezing at -20 ºC for 24 hours and subsequent drying in the hot air oven at 50 ºC for 30 hours.

Two polymers were used to prepare the polymer – drug nanoparticles viz., CMCAB and PLGA. The reason behind which was that these polymers are sensitive to pH, water swellable when partially ionized at the neutral pH of small intestine (pH 6.8), but is insoluble at gastric pH of 1.2. The idea is that pH dependent swelling controlled by the carboxyl groups of CMCAB and PLGA will help in reducing Curcumin degradation in the stomach thereby increasing the bio-availability of the Curcumin. We have compared the drug loading, size and crystallinity of the nanoparticles prepared using CMCAB and PLGA. The interaction of these polymers with the Curcumin was also studied. A critical comparison of both the nanoparticles has been done.

A. Particle Size, Polydispersity Index and Zeta Potential of Polymer – Drug Nanoparticles

The nanoparticles were prepared keeping the concentration of polymers at 10 mg/ml in THF. The particle size obtained using CMCAB was 172.3 nm with PDI of 0.198 whereas PLGA gave the particle size as 201.8 nm with PDI of 0.254, when the concentration of drug was 3 mg/ml in the same solution. The zeta potential of nanoparticles with CMCAB and PLGA were respectively -47.8 mV and -34.7 mV. The effect of initial concentration of Curcumin on the particle size and PDI are shown in Figure 1 & Figure 2.

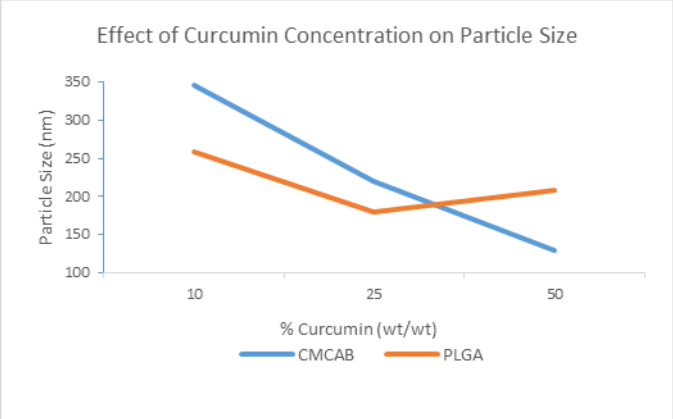

Fig 1. Effect of Curcumin Concentration on Particle Size of Nanoparticles

The particle size of CMCAB – Curcumin nanoparticles with the concentration of Curcumin follows almost a linear trend with the decrease in size as the concentration of Curcumin is increased. However, we should not increase the concentration more than 50% (wt/wt) as the objective of encapsulating Curcumin in the CMCAB matrix may not be fulfilled if we increase the drug by more than 50% (wt/wt). While in the case of PLGA – Curcumin nanoparticles the particle size was smallest when the drug concentration was 25% (wt/wt) after that the particles showed an increase in size with further increase in concentration of Curcumin.

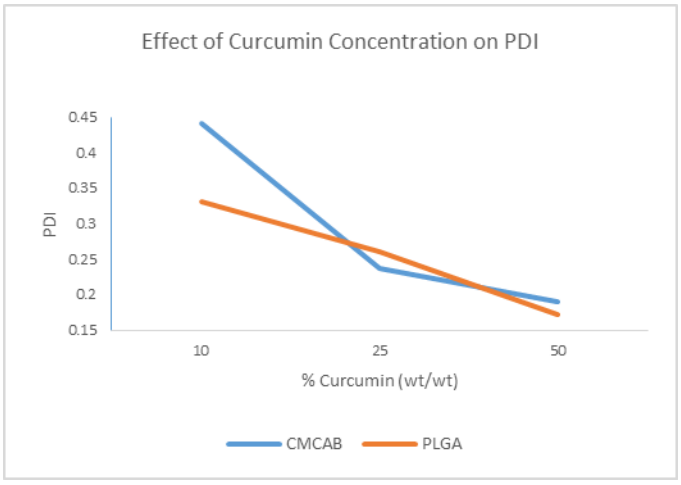

Fig 2. Effect of Curcumin Concentration on PDI of Nanoparticles

B.Fourier Transform Infrared (FTIR) Spectroscopy

FT-IR spectra of pure curcumin, CMCAB and CUR – CMCAB, PLGA and CUR-PLGA nanoparticles are shown in Figure 3-7. The nanoparticles consists of the functional groups from both the polymers and the drug. In the CUR-CMCAB nanoparticles the peaks at 3380 cm^{-1} corresponding to O–H stretch, 1625 cm^{-1} corresponding to N–H bend, 1219 cm^{-1} corresponding to C–N stretch, 810 cm^{-1} corresponding to N–H wagging from Curcumin, and the peaks at 1733 cm^{-1} corresponding to C=O, 1450 cm^{-1} corresponding to C–C stretch, 1035 cm^{-1} corresponding to C–O stretch, 598 cm^{-1} corresponding to C–Br stretch from CMCAB, were observed. Thus, FTIR results of CUR-CMCAB and CUR-PLGA proves the incorporation of both the polymers and the drug in the nanoparticles.

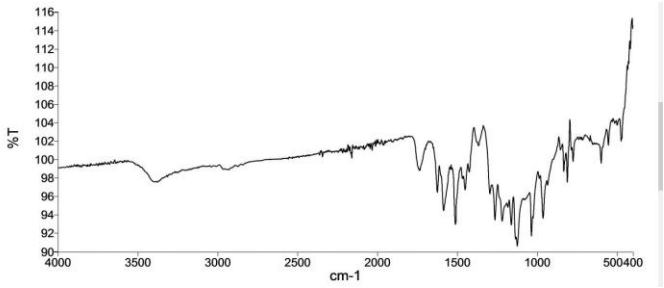

Fig 3. FTIR Result of Pure Curcumin

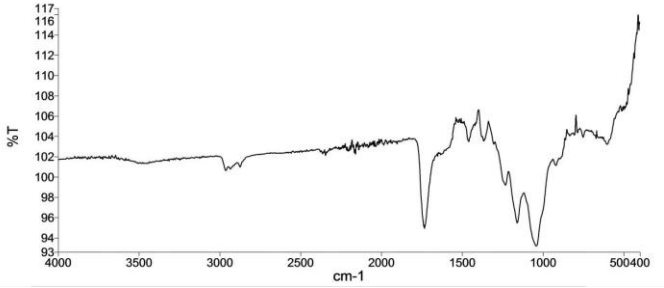

Fig 4. FTIR Result of CMCAB

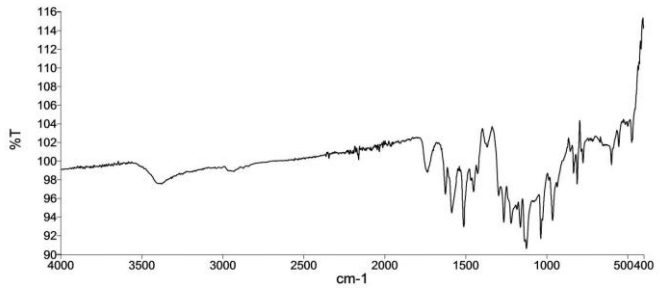

Fig 5. FTIR Result of CUR-CMCAB Nanoparticles

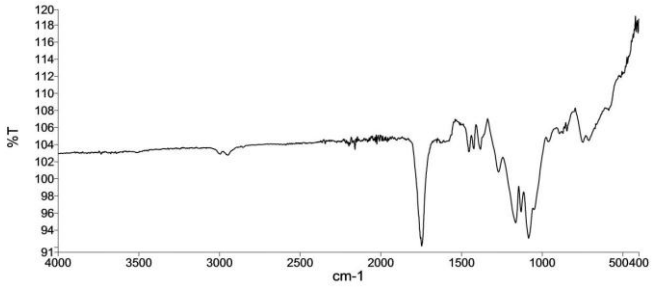

Fig 6. FTIR Result of PLGA

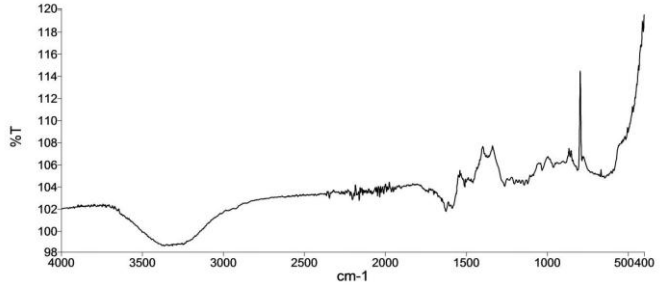

Fig 7. FTIR Result of CUR-PLGA Nanoparticles

However, in the CUR-PLGA nanoparticles peaks at 3367 cm^{-1} corresponding to O–H stretch, 1625 cm^{-1} corresponding to N–H bend, 1262 cm^{-1} corresponding to C–N stretch from Curcumin were observed and none of the peaks corresponds from PLGA, which proves that there was a strong interaction between the Curcumin and PLGA.

B. Differential Scanning Clorimetry (DSC)

DSC studies were carried out on CUR-CMCAB nanoparticles along with pure CUR and CMCAB to assess their phase transition behaviour. The glass transition temperature of pure amorphous CMCAB was observed at 158°C. The melting temperature of pure crystalline curcumin was observed as 178°C. In the nanoparticles a melting peak corresponding to the melting temperature of pure curcumin was observed at 173°C. This marked the presence of crystalline form of the drug in the nanoparticles. An attempt in future work will be to make the drug completely amorphous. DSC thermogram of CUR, CMCAB and the nanopartciles is shown in Figure 8.

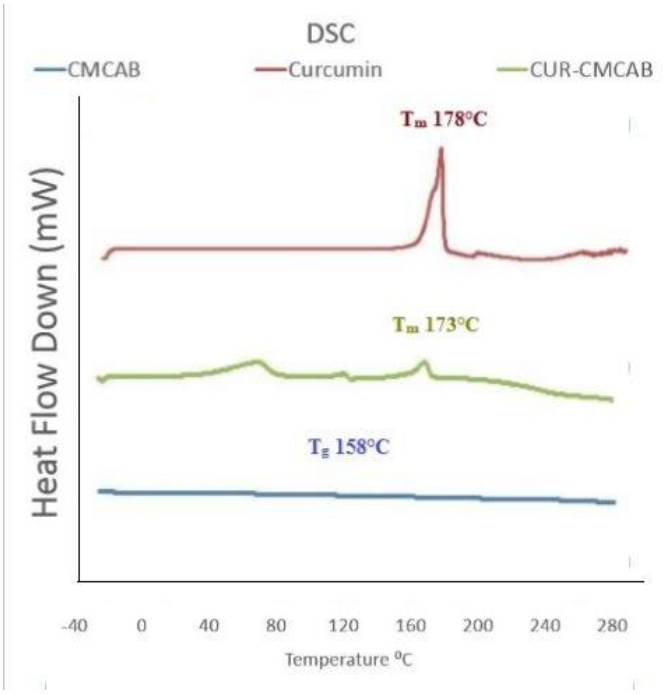

Fig 8. DSC Analysis of Curcumin, CMCAB and CUR-CMCAB

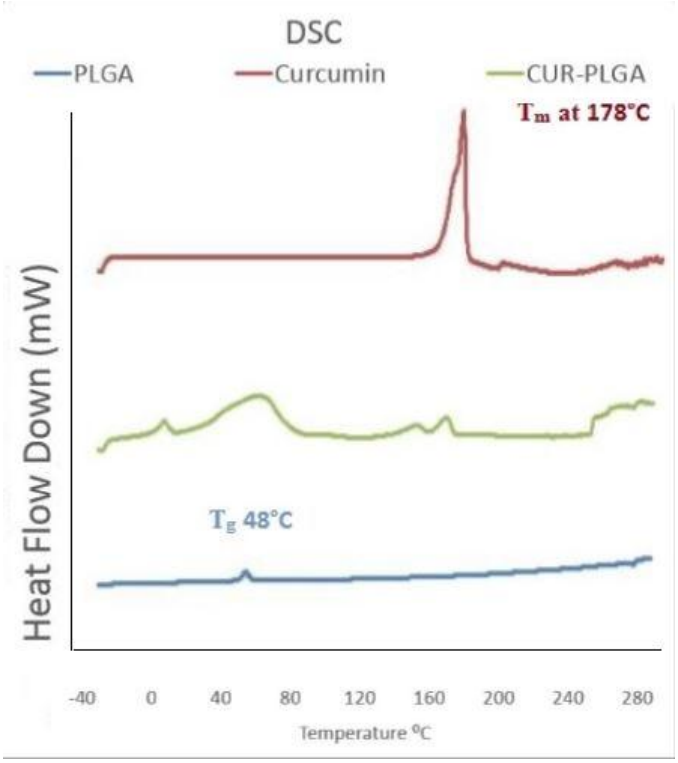

Fig 9. DSC Analysis of Curcumin, PLGA and CUR-PLGA

Similar studies were carried out for PLGA to assess its phase transition behavoiur. The PLGA was observed to be have glass transition temperature of 48°C. In the nanoparticles lots of melting peaks were observed at different temperatures. This imparted crystalline nature to the drug nanoparticles making it unsuitable to be used as a polymer for encapsulating the Curcumin. DSC thermogram of CUR, PLGA and the nanoparticles is shown in Figure 9.

C.Drug Loading

The concentration of drug present in the polymer drug nanoparticles was determined by spectrophotometric method. The characteristics curve was prepared at a fixed wavelength of 425 nm. Absorbance value of both the type of polymeric nanoparticles were determined at this fixed wavelength and it is substituted in the standard curve to get the corresponding experimental concentration. Percentage drug loaded for both the samples are tabulated in the Table 1.

TABLE I
DRUG LOADING (WT%) OF POLYMER-CUR NANOPARTICLES

S. No.	Polymer	Drug Loading %
1.	CMCAB	4.5
2.	PLGA	1.6

Drug loading for the case of PLGA is less as compared to that of CMCAB, which shows higher entrapment of drug in CMCAB matrix as compared to that of PLGA. An attempt in future work will be to further increase the drug loading percentage.

IV. CONCLUSIONS

A rapid method of synthesizing drug-polymer nanoparticles was investigated by using a multi inlet vortex mixture. The nanoparticles obtained was characterized for application in treatment of rheumatoid arthritis. For 1:1 ratio of drug:polymer the particle size 166.5 nm & drug loading of 4.5 % was observed in the case of CMCAB and 208.2 nm & 1.6 %, respectively in the case of PLGA The particles showed presence of crystalline drug. The future work will comprise of optimizing preparation methods to produce amorphous nanoparticles. These nanoparticles will also be tested in vitro and in vivo for future applications.

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