Optimization of Product Parameters of Drug Loaded Polymeric Nanoparticles Prepared by Rapid Precipitation

Sunil Varkey, Ashish Kumar Dewangan , 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 has been proved very much promising to increase absorption and bioavailability, leading to effective drug administration. This study is the extension of our work in the preparation of Curcumin-CMCAB nanoparticles by flash nano precipitation using Multi Inlet Vortex Mixer where Reynolds number is optimized based on the polydispersity index and particle mean size. In order to further analyse the variation in the properties of nano particles with varying conditions, we alter the polymer drug ratio (90:10, 75:25, 50:50). For each set of ratio, the preparation and characterization is carried out. Selective polymers and separation techniques will be used to get the most well defined nanoparticles. The optimization of process parameters will be carried out for rapid preparation of polymer drug nanoparticles.

Keywords---- Curcumin, drug loading, nano precipitation

I. Introduction

THE importance and effectiveness of Curcumin for the treatment of rheumatoid arthritis is clearly established. But its poor bio availability, absorption, photo stability etc led to the development for drug loaded nano particles. These nano particles were found to have improved physicochemical and pharmacokinetic properties, with better active targeting of inflamed region. They also provide a sound basis for reduction in the dosage frequency and drug dosage due to faster and uniform distribution in gastro intestinal (GI) tract and larger surface area. Since the focus is on obtaining the best particle size and poly dispersity index (PDI), the optimization of product parameters is inevitable. The optimization can be achieved with respect to the Reynolds number, ratio of polymer to drug and varying different polymers. The experiment at different Reynolds number is obtained by varying the inlet flow rates of streams in the Multi Inlet Vortex Mixer (MIVM). Also different polymers such as Carboxy Methyl Cellulose Acetate Butyrate (CMCAB), Cellulose Acetate Propionate (CAP), Cellulose Acetate Butyrate (CAB), Poly lactide-co-glycolides (PLGA), and Hydroxy Propyl Methyl Cellulose Acetate Succinate (HPMCAS) are used for the purpose.

The optimization process was basically carried out with the help of parameters such as poly dispersity index, average size and zeta potential which were obtained using Dynamic Light Scattering (DLS) technique. The ratio of weight average molecular mass to the number average molecular mass is called polydispersity index. The value ranges from 0 to 1. The smaller the value, more the system is mono disperse. The zeta potential is one of the primary parameter known to affect stability. In this case, higher the magnitude of its value, better the stability of the colloidal solution.

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II. Materials And Methods

A. Preparation of Drug Loaded Nanoparticles

The preparation of the polymeric nano particle is done by the rapid precipitation method with the help of an MIVM. Initially, a solution comprising of 30 mg of Curcumin drug, 100 mg of polymer such as CMCAB and 10 ml of Tetra hydro furan (THF) is prepared and sonicated in order to obtain a uniform mixture of the sample. The prepared sample is taken in a syringe and attached to one of the inlet of the MIVM. The other three inlets are attached to syringes filled with distilled water. The flow rates of the inlet streams can be set in accordance to the Reynolds number to be achieved. The pump is started and the required amount of the sample is collected in a flask. In order to remove the THF from the solution, a rotary evaporator is used. After the process, the sample is collected and stored in vials. To determine the various parameters of the prepared nano particles, Dynamic Light Scattering technique is required.

B. Multi Inlet Vortex Mixer

The MIVM works on the principle of flash nano precipitation, which is used for preparing functional nanoparticles having improved optical, mechanical and chemical properties. The MIVMs are of special consideration due to its flexibility in adjusting the flow rates of the inlet streams. Basically it consists of a small cylinder with four tangential inlet ports. The rapid mixing of the solvent and the non solvent causes high super saturation which initiates the flash nano precipitation. The rapid mixing helps from agglomeration of the particles and produces nanoparticles of

narrow size range. Inside the MIVM, turbulence is caused by the strong collisions. The injected stream also forms a vortex pattern inside the chamber. The main advantage of this mixer with other mechanisms is its flexibility with choice of chemicals, the attainment of different intensities of supersaturation by manipulating the amount of solvent and anti solvents etc.

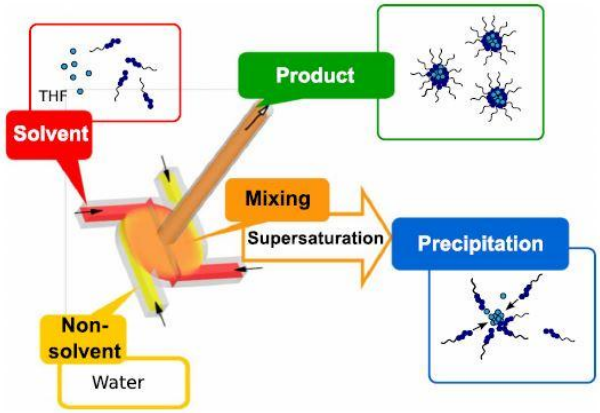

Fig 1 Illustration of MIVM applied to flash nano precipitation

C. Optimizing Reynolds Number

The equation proposed by Liu et al correlating the flow rates and Reynolds number is given as:

$$Re = \sum_{i=1}^{N} \frac{u_i D_{mix}}{v_i} = D_{mix}\left(\frac{u_1}{v_1} + \frac{u_2}{v_2} + \frac{u_3}{v_3} + \frac{u_4}{v_4}\right) \quad (1)$$

Where D_{mix} is the diameter of the mixture chamber, $u_{1\text{-}4}$ are the velocities of four individual inlet streams, $v_{1\text{-}4}$ are kinematic viscosities of each solvent stream. In order to obtain different Reynolds number, the flow rate of the syringe consisting of solvent is kept constant and the flow rate of the other pump is varied. The samples are collected for different Reynolds number and are characterized using DLS technique.

D. Optimizing Polymers

After obtaining the optimized Reynolds number, further experimentation is carried out with different polymers. The different polymers used for the purpose includes CMCAB, CAP, CAB, HPMCAS and PLGA. Curcumin drug loaded nanoparticles are prepared using all these polymers at the optimized Reynolds number. The prepared samples are then characterized using DLS. From the results from DLS technique, the best one or two polymer is selected for further optimization.

E. Optimizing Polymer-Drug Concentration

The best two polymers are used for further optimization by varying its concentration with drug. Three different polymers to drug ratio is chosen for further preparation and characterization. The ratios used are 90:10, 75:25 and 50:50. All these samples again undergo DLS and the best concentration ratio is obtained. After the complete optimization, the sample is prepared in bulk and is used for performing further characterization techniques such as FTIR, UV-VIS, DSC, DLS, XRD, SEM etc and various release studies.

III. Results

A. Optimized Reynolds Number

The different runs were carried out keeping the syringe containing the solvent at a fixed value, say 5.82 ml/min. The flow rate of the three syringes containing distilled water is varied sequentially for each run. The different flow rates used were at 5,10,15,20 and 24 ml/min. By performing DLS on each of these samples, it was found to show that best particle size and polydispersity index were in the range between 2776 and 4439 Reynolds number. Further experiments were focused on the flow rates whose Reynolds number was in between these two values. Finally it was found out that the best parameters for the nano particles were achieved at a Reynolds number of 3275. The corresponding flow rates were 5.82 and 6.5 ml/min.

TABLE I
VALUES OF PARTICLE SIZE, PDI AND ZETA POTENTIAL WITH VARYING REYNOLDS NUMBER

Qsolvent (ml/min)	Qanti-solvent (ml/min)	Reynolds Number	Particle Size(nm)	PDI	Zeta Pot.(mV)
5.82	5	2776	186.1	0.208	-47.8
5.82	6.5	3275	172.3	0.198	-48.3
5.82	10	4439	184.6	0.244	-55
5.82	15	6102	204.8	0.295	-44
5.82	20	7765	205.7	0.278	-44.9
5.82	24	9095	237.4	0.232	-48.2

B. Optimized Polymers

An optimized Reynolds number of 3275 was obtained by the initial set of runs. In order to optimize using different polymers, another set of experiments were carried out using polymers such as CMCAB, CAP, CAB, HPMCAS and PLGA for the same Reynolds number. Polymers like CAB, CAP, and HPMCAS gave very poor result with respect to average particle size and polydispersity index. Hence these were discarded from further studies. While the polymers such as CMCAB and PLGA gave very positive findings and opened the scope for more works with these polymers. The average paricle size of CMCAB loaded nano particles was 200.8 nm with PDI of 0.191; whereas the particle size for PLGA loaded nano particles were found to be 201.8 nm with PDI of 0.254. Since the values of zeta potential are greater than 30, it reflects that the solution is stable.

TABLE II
VALUES OF PARTICLE SIZE, PDI AND ZETA POTENTIAL WITH DIFFERENT POLYMERS

Drug	Particle Size(nm)	PDI	Zeta Pot.(mV)
CAB	370.0	0.447	-35.0
CAP	322.1	0.367	-30.1
CMCAB	200.8	0.191	-40.6
HPMCAS	611.3	0.782	-25.4
PLGA	201.8	0.254	-34.7

C. Optimized Polymer- Drug Concentration

The results till now proved that the polymers CMCAB and PLGA are the best ones to be compatible with Curcumin drug and its preparation. In order to optimize with respect to different drug loadings, we chose the drug weight percentage as 10%, 25% and 50%. The same procedure was repeated with these drug loadings and characterization was done using DLS. For CMCAB loaded nano particles, the best value for particle size and PDI was found at 50% drug weight. The average particle size was found to be at 166.5 nm with PDI of 0.190. Whereas for PLGA loaded nano particles, the drug loading at both 25% and 50% weight gave good results. But the values at 50% weight were considered due to better PDI values. The particle size was found to be 208.2 nm and PDI as 0.172 for this polymer.

TABLE III
VALUES OF PARTICLE SIZE, PDI AND ZETA POTENTIAL WITH VARYING DRUG-POLYMER CONCENTRATION

Drug	Drug Weight %	Particle Size(nm)	PDI	Zeta Pot.(mV)
CMCAB	10	345.3	0.487	-31.6
CMCAB	25	219.7	0.238	-51.2
CMCAB	50	166.5	0.190	-44.5
PLGA	10	258.0	0.331	-30.6
PLGA	25	179.3	0.260	-34.7
PLGA	50	208.2	0.172	-35.2

IV. DISCUSSION

Rapid precipitation is being seen as one of the most advanced technologies to produce nano particles. The drug and the stabilizing polymer is being rapidly mixed with a non solvent in order to obtain high supersaturation over a time scale which is shorter than its usual characteristic nucleation and growth time interval for the nano particles. The polymer gets uniformly adsorbed on to the drug which helps in obtaining uniform size distribution and ceases the formation of particles in narrow size range.

The Multi Inlet Vortex Mixer (MIVM) produced rapid mixing and precipitation for obtaining nano particles with adequate ease of operation. Its principle is based on the fact that the momentum from each of the stream adds independently to cause micro mixing inside the mixer. Good mixing and precipitation is obtained even when the streams flow at different rates.

It's been found that the best optimized values of average particle size and polydispersity index for the Curcumin-CMCAB nano particles are produced when the Reynolds number lie between 2500 and 4000. The experiment results show that nano particle size decreases with decrease in Reynolds number. But it finally confines within a particular range due to the requirement of adequate turbulence for mixing. The use of other polymers such as CAB, CAP, and HPMCAS with Curcumin gave poor results and found as less compatible. However, other than CMCAB, experiments with PLGA showed promise with Curcumin drug and produced nano particles of acceptable size and polydispersity index. Further, varying the concentration ratio of polymer to drug gave more insight to the effect on the particle size. The best particle parameters resulted at a polymer to drug ratio of 1:1. This might be due to the better control of precipitation and stabilization of particles which prevented further growth. Also, there seemed a trend that the particle size increased with the decrease in amount of drug concentration.

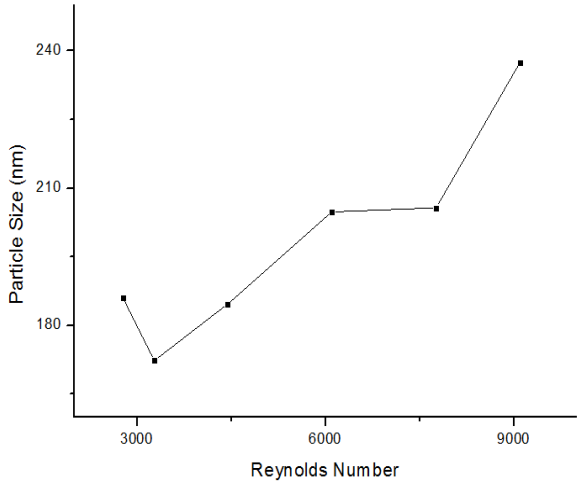

Fig 2 Variation of particle size with reynolds number

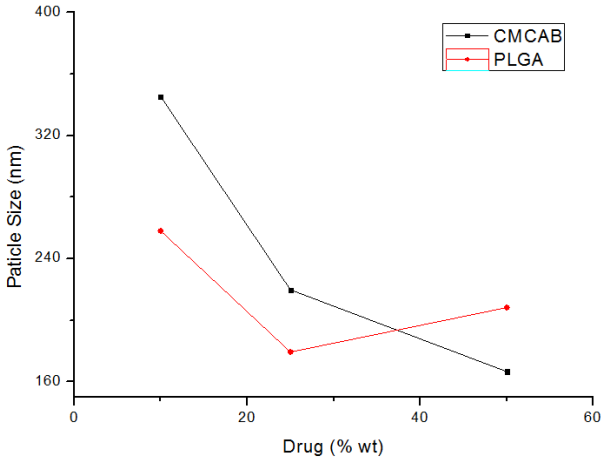

Fig 3 Comparison of the effect of particle size with drug weight percentage

V. CONCLUSION

The work was done to optimize the product parameters of drug loaded nano particles by rapid mixing and precipitation. Optimization is considered as an art, science and mathematics which select the best from finite and infinite alternatives. The optimization was based on the values of Reynolds number, different polymers and the concentration of polymer and drug. The initial experiment started with obtaining the best result on basis of Reynolds number. The optimized Reynolds number was then used on different polymer- drug combination to obtain the compatibility of these polymers with Curcumin drug. The final optimization completed with having a study on variation of the product parameters with polymer-drug concentration.

ACKNOWLEDGMENT

This work is supported by University Grants Commission, Government of India. The authors would like to thank Mr. Maulick Chopra, BITS PILANI for providing help and guidance throughout the course of work and Mr. Emil Joseph, BITS PILANI for making arrangements in doing DLS.

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