ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

FORMULATION TRAILS ON NANOPARTICULAR PREPARATION FOR EASY SCALE UP THROUGH DIFFERENT TECHNIQUES

INDHUMATHI D*, P.N.REMYA, S.SANGEETHA
Department of Pharmaceutics, S.R.M College of pharmacy, S.R.M University,
Kattankulathur, Chennai, India.
***Corresponding Author: Email: indhu.prathap@gmail.com**

ABSTRACT

Nanoparticles are solid colloidal particles ranging in size from 10-1000 nm(1Mu). Nanoparticles have received much attention now days due their ability to control drug release, distribution stability, higher solubility, and high degree of patient compliance. As the particles is less than 100nm, they do not produce thromboembolism problems and can easily pass through syringe needle and they are easily taken up by the reticuloendothelial system and deliver the drugs to the liver. Plumbagin is used efficiently in the treatment of rheumatism, elephantiasis activity, leucoderma and anemia. The aim is to formulate plumbagin nanoparticles using carrier polymer, polymethylcyanoacrylate, in varying proportions by the method of emulsion polymerization in continuous aqueous phase and to formulate secondary coated nanoparticles using PEG-4000, Tween80 and Span80 and also plumbagin can be coated with natural polymer like chitosan using solvent evaporation method. The surface modified nanoparticles using various polymers reveal presence of coat formulation by specific increase in particle size (115.8nm) after coating .The nanoparticles with Tween 80 produced particles of slightly bigger size (102.7nm) when compared with PEG 4000. The nanoparticles using span 80 produced irregular particles (225.3) and not uniform. Biodegradable nanoparticles prepared using chitosan were easily formed within short span (half hour) and the size was also spherical (456.3) as observed from the atomic force microscopy photographs.

KEY WORDS: Plumbagin, Polymethylcyanoacrylate, PEG-4000, Tween80, Span80, Chitosan, Emulsion polymerization.

1. INTRODUCTION

Nanotechnology is the chosen requirement of the day in all fields throughout the globe. The primary objective in the design of novel drug delivery system is controlled delivery of the pharmacological agent to its site of action at therapeutically optimal rate and dosage regimen. Nanoparticles are solid colloidal particles (Arshady, 1996) ranging in size from 10 to 1000nm. These are used as transport carrier compartments for drugs or other active molecules of non-liposome characters in the nanometer size range. Nanoparticles have received much attention over the last few years due to their ability to control drug release (Arpita Bhattacharya and Mukherjee, 2000) distribution and their quality of biodegradability. Toxicity and adverse drug reactions are reduced to a possible extent. eg. polymethylcyanoacrylic nanoparticles for targeting anticancer drug that circulate for longer period in blood and taken up least by reticuloendothelial system (RES) (Couvreur, 1992). Preparation of bio degradable nanoparticles and main steps for achieving this objective is emulsion polymerization in continuous aqueous phase using polymethylcyanoacrylate as the polymer for achieving less than 100nm sized nanoparticles; the technique of surface engineering of the nanoparticles was performed using various polymers for achieving prolonged blood circulation. The natural polymer chitosan is used in formulating biodegradable nanoparticles.

The chosen drug is plumbagin (Ravikanth Velluri and Prakash Diwan, 1999), which is an herbal drug, a small genus of herbs distributed in the tropical region, there are three species recorded from India i.e., plumbago indica, plumbago zeylinica and plumbago auriculata. Among these plumbago zeylinica is acrid and stimulates sweating, it also promotes appetite and helps in digestion; it is used for dyspepsia, piles, and skin diseases. Roots have been used as abortifacient in some indigenous practices, internally or as an irritant to the uterus. It also helps in adhesion of tissues in the body and works as antidiarrheal due to heavy properties is used as a main pharmacological esteemed remedy for leucoderma and also used efficiently in the treatment of rheumatism, elephantiasis activity, and anemia. It is isolated from dry chloroform extract of plumbago zeylanica roots containing plumbagin (98% pure).

The main objective of the study is to formulate plumbagin nanoparticles using carrier polymer, polymethylcyanoacrylate, in varying proportions, selection of the best formulation and secondary coating using PEG-4000, Tween 80, Span 80 as polymers and to formulate plumbagin with chitosan using solvent evaporation method by cross linking technique and to achieve nanoparticles of less than 100nm size.

2. MATERIALS AND METHODS

Polymethyl cyanoacrylate has been obtained as a gift sample from Anabond chemicals limited (Chennai), PEG 4000 - Rohm chemicals (Chennai), Tween 80 and Span 80 -Nice chemicals (Baroda). Reagents: methanol and acetone - Nice chemicals (Baroda).

Preparation of plumbagin nanoparticles: Preparation (5) of polymethylcyanoacrylate nanoparticles using method of emulsion polymerization in continues aqueous phase. The drug plumbagin and polymer polymethyl cyanoacrylate were dissolved in chloroform separately and then mixed. The solution is added to an aqueous solution containing polaxmer 2% and acidified with HCL to pH of 1.7. The sonication was continued for 45min at a speed of 230rpm using 1'' probe. The temperature was maintained at 20-25^{o}C through the sonication process. The nanoparticles formed are separated by fractional centrifugation using cooling centrifuge. The speed of the centrifuge was maintained at 12,000 rpm for 20 min.

Surface treating of Nanoparticles using PEG 4000, Tween 80, Span 80: In all the formulations, drug (plumbagin) and the polymer (polymethylcyanoacrylate) were dissolved in chloroform separately and then mixed. The solution is added to an aqueous solution containing polaxmer 2% and has been surface treated using polymers such as PEG 4000, Tween 80 and span 80 individually and then acidified with HCL to pH 1.7, then sonicated for 45min at a speed 230rpm using 1'' probe at a temp 20-25°C. The nanoparticles are separated by the fractional centrifugation using cooling centrifuge by maintaining speed at 12,000 rpm for 20 min. Here the polymers are expected to have cross linked with polymethylcyanoacrlyate, monomers during the polymerization process of polymethyl cyanoacrylate (Alfassi, 1989).

Preparation of chitosan Nanoparticles: Chitosan gel nanoparticles cross linked with glutaraldehyde was prepared according to a solvent evaporation technique method using ultra sonication and a cross linking technique by glutaraldehyde, plumbagin and chitosan was dissolved in 6%v/v acetic acid, stirring vigorously and then sonicated at room temperature for 15min, the preparation so obtained was poured into three necked bottom flask glutaraldehyde solution saturated with toluene containing span 80 was added slowly to the preparation by using a dropping funnel and then stirred for 8hours. The preparation obtained was centrifuged at 1x104 rpm for 15min and washed with toluene, acetone and methanol. The chitosan nanoparticle in the above procedure was prepared using three different concentration of polymer given in table.2.

Evaluation parameters

Determination of particle size: 5ml of the redispersed suspension of plumbagin nanoparticles were transferred to a cover glass, which in turn was mounted on a specimen tube. Samples (Boylon, 1983) were viewed and photographed using shimadzu scanning probe microscope model spm 9500. The Atomic Force Microscopy photographs showing the morphology of plumbagin nanoparticles with varying proportions of drug to polymer ratio were shown in figures. The particle size of plumbagin nanoparticles were tabulated in table.3 and shown in figures 2, 3 & 4.

Determination of drug content: The nanoparticles redispersed in phosphate buffer saline ph7.4 was used for the determination of drug content. To 1ml of plumbagin nanoparticular suspension, 1 ml of selective solvent was added preferentially to precipitate the polymer, to this 1ml of aqueous potassium dihydrogen phosphate solution is added and centrifuged at 16,000 rpm in cooling centrifuge at 1c for 30 minutes. The clear supernatant fluid is removed of and the nanoparticular suspension is analyzed spectrophotometrically (Jorg K Reuter, 1991) 422nm, the parameters were enlisted in table 4.

3. RESULTS AND DISCUSSION

The colloidal carriers are rapidly removed from the circulation by phagocytic cells in the liver and spleen. This limits their targeting potential. To overcome this, nanoparticles using polymethylcyanoacrylate by emulsion polymerization is tried and we had attempted to coat the formed polymethylcyanoacrylate nanoparticles with PEG 4000, Tween 80, span 80 and the natural polymer chitosan. The various polymers are only slowly biodegradable, hence to trial the characteristic of natural biodegradable nanoparticles, chitosan is being tried for formulating nanoparticles. The formulation using polymethylcyanoacrylate were tried with drug polymer ratio of 1:1, 1:2, 1:3 (B1a, B1b, B1c). From the percentage drug entrapped it is observed that the maximum entrapment (71.63%) was obtained with 1:3 ratios. Hence B1c is selected for further surface modification using PEG4000, Tween80 and span80. B1c is surface modified with PEG4000 at 1:1, 1:2,1:3 ratios (B2a, B2b, B3c), with Tween 80 at ratios of 1:1, 1:2, 1:3 (B3a, B3b, B3c) and span 80 at ratios of 1:1,1:2,1:3 (B4a, B4b, B4c). Chitosan nanoparticles were prepared at drug polymer ratios of 1:1, 1:2, 1:3 (B5a, B5b, B5c).

During the surface treatment using PEG 4000 (B2 series) the B2a with entrapment of 75.78% is selected. Tween 80 (B3 series) shows that B3b achieves an entrapment of 45.50% with B4a and for chitosan nanoparticles (B5 series); B5b is chosen as it gives an entrapment efficiency of 30.25%. The surface modified nanoparticles using PEG 4000 reveal the presence of coat formation by specific increase in particle size (115.8nm) after coating. The nanoparticles with Tween 80 produced particles of slightly bigger size (102.7nm) when compared with PEG. The nanoparticles of span 80 produced irregular particles (225.3nm) and the particles distributed were also not uniform.

The biodegradable nanoparticles prepared using chitosan were very easily formed within short span (half an hour) and the size was also spherical (456.3 nm) as observed from the Atomic Force Microscopy Photographs. From the observation of particle size (52.5nm) it is evident that nanoparticles of less than 100 nm could be easily achieved using polymethylcyanoacrylate as the carrier polymer and pluronic F68 at the concentration of 2% as the surfactant.

Table.1.Formulation of plumbagin nanoparticles by using various polymers

Formulation code	Amount of plumbagin extract (mg)	Amount of polymethyl cyanoacylate (mg)	Amount of poloxamer (%)	Amount of polymer		
				PEG 4000 (mg)	Span 80 (%)	Tween 80 (%)
B1a	100	100	2			
B1b	100	200	2			
B1c	100	300	2			
B2a	100	100	2	100		
B2b	100	200	2	100		
B2c	100	300	2	100		
B3a	100	100	2			1
B3b	100	200	2			1
B3c	100	300	2			1
B4a	100	100	2		1	
B4b	100	200	2		1	
B4c	100	300	2		1	

Table.2. Formulation of plumbagin nanoparticles by using chitosan

Formulation code	Amount of plumbagin extract(mg)	Amount of chitosan(mg)	Volume of 6%acetic acid(ml)	Concentration of glutaral dehyde (%)
B5a	100	100	10	5
B5b	100	200	10	5
B5c	100	300	10	5

Table.3 Particle size of plumbagin nanoparticles:

Formulation code	Drug:polymer	Size of particles (nm)
B1c	1:3	52.5+0.4
B2a	1:1	115.8+0.7
B3b	1:2	102.7+.1
B4a	1:1	225.3+0.5
B5b	1:2	456.3+0.7

Table.4.Drug content of plumbagin nanoparticles coated with various polymers

S.No	Polymer	Formulation code	% Drug trapped	% Drug recovery	% Drug content
1	Polymethyl cyano acrylate	B1a	29.14	22.47	53.9
2		B1b	40.56	22.86	55.03
3		B1c	71.63	26.93	60.37
4	PEG 4000	B2a	75.78	27.86	63.55
5		B2b	75.98	27.96	63.92
6		B2c	76.23	28.25	64.44
7	TWEEN 80	B3a	55.23	23.25	50.23
8		B3b	65.34	24.55	51.11
9		B3c	65.56	24.86	51.45
10	SPAN 80	B4a	45.50	20.45	46.68
11		B4b	46.52	21.22	47.56
12		B4c	46.68	21.86	47.89
13	Chitosan	B5a	25.26	20.24	40.25
14		B5b	30.25	22.86	45.23
15		B5c	31.22	23.01	45.68

4. CONCLUSION

Thus from this study it is concluded that emulsion polymerization in continuous aqueous phase is a better method of choice for the polymer namely polymethylcyanoacrylate for achieving nanoparticles of less than 100nm. The surface coating is found to be achieved better with PEG 4000 which is observed from the uniformity in size and spherical nature of the particle obtained. The biodegradable nanoparticles with chitosan exhibits better formation through the method of cross linking using glutaraldehyde and acetic acid.

Figure: 1 Aerial Parts of Plumbago Zeylanica

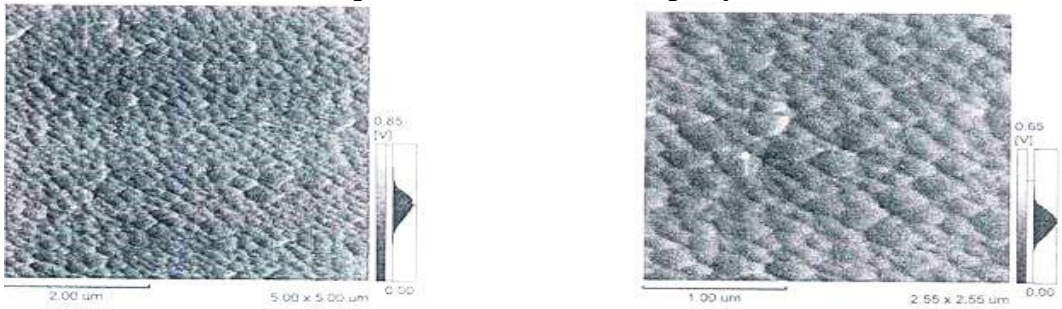

Figure: 2 AFM- Particle Size Determination of Polymethylcyanoacrylate Nanoparticle And Polymethylcyanoacrylate Nanoparticle Coated With Peg 4000

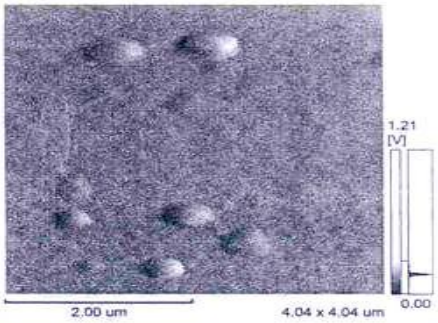

Figure: 3 AFM - Particle size determination of Polymethylcyanoacrylate nanoparticle coated with tween 80.

Figure: 4 Particle Size Determination Of Polymethylcyanoacrylate Nanoparticle Coated With Span 80 and Chitosan

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