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Formulation and evaluation of Isoniazid loaded -å-polycaprolactone nanoparticles

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ABSTRACT

The present study was aimed at developing a sustained release formulation of Isoniazid (INH) nanoparticles using the biodegradable polymers, poly-e-caprolactone (PCL) as carrier. The PCL nanoparticles (NP) of hydrophilic dug isoniazid (INH), a first line antitubercular drug are developed and the entrapment efficiency of drug in the NPs has been improved. The PCL NPs were prepared by an oil-in-water emulsion solvent evaporation with ultrasonication technique. 3^2 factorial designs employed to optimized formula. Two independent variables like ultrasonication time and PCL Concentration were selected for factorial design for its significant effect on particle size, entrapment efficiency of NPs. The prepared PCL NPs were characterized by SEM analysis, entrapment efficiency, zeta potential, FTIR, DSC, in-vitro diffusion studies. These results indicate that INH loaded PCL nanoparticles could be effective in sustaining its release for a prolonged period. The PCL NPs could be alternate method for delivery for INH with prolonged drug release profiles and better therapeutic effect can be achieved for the treatment of tuberculosis. However, further studies are needed to confirm its performance in vivo.

Key words: Polycaprolactone, Nanoparticles, hydrophilic drugs, isoniazid

INTRODUCTION

The potential of site specific drug delivery in optimizing drug therapy [1] has given impetus to significant advancement in the pharmaceutical engineering of novel dosage forms such as nanoparticles, which are solid colloidal polymeric carriers less than 1µm in size [2]. Several review articles have highlighted the ability of such NP to reduce associated adverse effects of various drugs [1,3,4]. Some of the commonly reported methods of preparing NP from biodegradable polymers includes solvent evaporation [5], monomer polymerization [6], Nanoprecipitation [7], and the salting out procedure [8]. The Nanoprecipitation method developed by Fessi et al. [9] represents an easy and reproducible technique and has been widely used by several research groups to prepare NP [7, 10, 11]. This method is based on the interfacial deposition of polymer following displacement of a semi polar solvent miscible with water from a lipophilic solution [9]. The nanoprecipitation method [12] was also widely used by several research groups to prepare nanoparticles [13, 14]. However, this technique suffers the drawback of a poor incorporation efficiency of water soluble drugs owing to the rapid migration and therefore loss of drug into the aqueous phase. Govender et al. [15] have recently improved the incorporation of a water-soluble drug into poly(DL lactide- co-glycolide) nanoparticles prepared by this technique by either increasing the aqueous phase pH and thereby decreasing the solubility of the drug, or including various excipients into the formulations. A NP system with maximal drug loading and high entrapment efficiency will reduced the quality of carrier required for the administration of sufficient amount of active compound to the target side as well as drug wastage during manufacturing. Mainly water insoluble drugs have been incorporated into NP using the Nanopreciptation technique with typical drug content values being: Indomethacin, 2.0% w/w [9] or 5.8%w/w [12] ; dexomethasone, 0.9% w/w [9] and Itraconazole, 4.1% w/w [13]. The difference between emulsion solvent evaporation and nanoprecipitation is that the main phase in the solvent evaporation method stay immiscible at all times, only to be removed later by evaporation. Emulsion solvent evaporation involves two steps. The first step requires emulsification of the polymer solution into an aqueous phase. During the second step, the solvent used in polymer solvent is evaporated, inducing polymer precipitation as nano spheres [16]. Isoniazid is a class III drug (high solubility and low permeability) having an aqueous solubility of approximately 140 mgl per ml. The drug is characterized by a short half-life ranging from 1 h to 4 h, depending on the rate of metabolism.

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MATERIALS AND METHODS

Materials

Isoniazid was a gift sample from Macleod Pharmaceuticals, Mumbai; Poly-e-caprolactone (PCL M_w 65000 g/mol) was purchased from Sigma Aldrich. Polyvinyl alcohol (PVA M_w 88000 g/mol), DCM, Phosphate buffer, all are of analytical grade was purchased from Qualichens, New Delhi.

Method of preparation of PCL Nanoparticles

In this procedure, specific amount of PCL was dissolved in 10mL of solvent i.e. Methylene chloride containing 20 mg of Isoniazid. A PVA (2.5g /100 ml) solution was prepared in PBS solution. The PCL solution was added to 40 ml of the PVA solution. The total mixture in 250 ml centrifuge bottle was then placed in an ice bath and emulsified using a Vibra-cell Probe Sonicator (VC 5040, Sonics and Maternals, USA) a specific time to obtain an oil-in-water emulsion. Another 40mLof the PVA solution was then added to the emulsion. The final emulsion was stirred for 12 hour at 300 rpm on a magnetic stir plate to allow the evaporation of methylene chloride to allow the formation of the nanoparticles. The suspension was then centrifuged at 11,000 g for 20 min. The pellet was resuspended in distilled water and centrifuged three more times at 1200 g for 20 min each. Then washing steps were followed to remove un encapsulated PVA and Isoniazid. The nanoparticles were collected and

frozen at -80°C for at least 1.5 hour and subsequently freeze dried. The freeze dried nanoparticles were stored at 4°C.

Physical Characterization

Measurement of particle size, polydispersity index and zeta potential

Particle size distribution of INH loaded PCL NP was determined by laser scanning technique using Malvern instrument after appropriate dilution with distilled water. The mean particle size, polydispersity index and zeta potential were calculated for each formulation maintained at 25^0 C and polydispersity index will measure the size distribution of nanoparticles population.

Scanning Electron Microscopy (SEM)

The SEM analysis of prepared PCL NP was performed for morphological studies. The formulations are poured in to circular aluminum stubs using double adhesive tape, and coated with gold in HUS -5GB vaccum evaporator , and observed in Hitachi S-3000N SEM at an acceleration voltage of 10 Kv and a magnification of 5000X.

Differential Scanning Calorimetry (DSC)

DSC analysis was performed in order to investigate the melting and recrystallization behavior of crystalline materials like PCL NP. The samples were sealed in aluminium pans and measurements were recorded using DSC instrument. The samples were heated from 25 to 2000 C at a heating rate of 100 C /min under nitrogen atmosphere.

Total drug content

From the prepared PCL NP formulation 1ml of suspension is dissolved in the 10 ml of 7.4 PBS buffer and ethanol mixture. The amount of isoniazid was determined using UV spectrophotometer at 266nm.The placebo formulation prepared similarly to drug loaded PCL NP is used as blank. The total drug content was calculated.

Encapsulation efficiency (EE)

The prepared PCL NP dispersion was centrifuged at 15000 rpm for 30min at 0^0 C using REMI cooling centrifuge. Then the supernatant is analysed for the free drug content.

EE= {total drug content - free drug content/ total drug content}*100

Drug Loading = (Mass of INH determined / Mass of NP produced)*100

In vitro diffusion studies

This is performed by using a modified franz diffusion cell at 37^0 C which is fitted with a dialysis membrane having a molecular weight cut off 3500 Da. The membrane was soaked in boiling distilled water for 12 hours before mounting in a franz diffusion cell. PCL NPS dispersion 2 ml is placed in to the donor compartment and the 20ml of PBS (7.4) is used to fill receptor compartment. With 24 hours interval 1ml of sample is withdrawn and analyzed using UV Visible spectrophotometer at 266 nm.

Statistical analysis

Statistical analysis was performed using EASE Design -Expert-8 for its factorial design. 3^2 factorial designs were selected for optimization study of above mention formulation. 3-D (response surface) and 2-D (contour plot) shows the effect of polymer and ultrasonication time in response variable.

RESULT AND DISCUSSION

Effect of Polymer concentration

It was observed that there were no significant differences in particle mean size between two PCL concentrations (Table -1). So In this study, two PCL concentrations, 3 and 5 g/100 mL, were selected. Those concentrations showed the best result in preliminary test. But the encapsulation efficiency was increased by increasing PCL concentration from 3 to 5 g/100 mL (Table -5).

Table no.1: Influence of concentration and molar mass of polymer (PCL) in the organic phase (PCL + Methylene chloride) on nanoparticles mean size[a]

Mass of PCL (mg) in 10 mL of organic phase	Molar mass of PCL in g/mole	Mean size of NP (nm)
100	65000	453±7
200	65000	471±9
300	65000	413±6
500	65000	458±4
800	65000	475±9

a- Experimental conditions: 1 g of PVA (80000 g/mol) in 40 mL aqueous phase (2.5% w/w) and varying amounts of PCL in organic phase (10 ml); sonicated for 5 minutes.

By increasing the polymer concentration in the organic phase, the viscosity of the solution was increased. Increasing viscosity can decrease the INH diffusion into the aqueous phase and thus increase the entrapment into the nanoparticles. Consequently, the encapsulation efficiency of nanoparticles was increased by increasing the polymer concentration. Conversely, INH loading (%) was decreased by increasing PCL concentration (Table -5). Increasing viscosity increased the total mass of PCL in the nanoparticles. This decreased the ratio of INH to the total mass of nanoparticles, thus decreasing % loading.

Effect of ultrasonification time on nanoparticles mean diameter

The particle mean size was decreased by increasing ultrasonification time from 1 to 5 min (Table -2). The increased time of ultrasonification led to the formation of smaller nanoparticles. But the encapsulation efficiency was decreased by increasing ultrasonification time from 1 to 5 min (Table-5). Increasing the ultrasonification time resulted in a reduction in the encapsulation efficiency due to the decreasing particle mean size.

Table no.2: Effect of ultrasonification time on nanoparticles mean diameter

Sonication time (min)	NP Mean Diameter
1	568±23
2	408±25
3	354±38
4	327±12
5	235±29

(n=3)

Effect of concentration of PVA properties of nanoparticles

Different concentration of PVA were taken for the preparation of NP taking only polymer in organic phase which shows that one gram of PVA having molecular weight 88000 in 40 ml showing the best optimized result of size of nanoparticles with less standard deviation (Table -3)

Table no.3: Effect of concentration of PVA properties of nanoparticles

Mass of PVA (g) in 40 ml of aqueous phase	Molar mass of PVA in g/mole	Mean size of NP (nm)
0.2	88000	385±15
0.5	88000	393±27
1	88000	335±4
1.5	88000	1139±63
1	31000	438±12
1	88000	469±4

(n=3) Experimental conditions: 0.5 g of PCL (65000 g/mol) in organic phase (10 mL); sonicated for 5 minutes.

Table no.4: Coding factor of drug, polymer and sonication time

Formula	Amount of Drug (in mg)	Amount of PCL (in mg)	Sonication time (min)
NPI 1	20	300	1
NPI 2	20	300	3
NPI 3	20	300	5
NPI 4	20	400	1
NPI 5	20	400	3
NPI 6	20	400	5
NPI 7	20	500	1
NPI 8	20	500	3
NPI 9	20	500	5

(n=3)

Table no. 5: Optimization of Nanoparticle formulation by solvent evaporation technique

Formula	Encapsulation	Loading	NP Mean size	Polydispersity	Zeta potential
NPI 1	41.57±1.36	1.03±0.25	570±65	0.8±0.06	-14.53±0.52
NPI 2	39.22±1.52	1.15±0.21	408±14	0.8±0.03	-12.63±0.19
NPI 3	28.53±1.29	2.48±0.36	354±34	0.38±0.06	-10.37±0.49
NPI 4	48.84±1.45	0.83±0.11	527±78	0.95±0.42	-9.28±0.43
NPI 5	41.21±1.87	0.56±0.19	335±31	0.47±34	-11.21±.25
NPI 6	34.42±1.65	1.26±0.24	219±56	0.69±0.04	-11.79±0.82
NPI 7	69.72±6.25	1.29±0.15	548±5	0.50±0.04	-11.57±0.21
NPI 8	54.95±2.39	2.16±0.09	370±8	0.37±0.09	-12.02±0.12
NPI 9	46.57±1.52	1.94±0.13	248±13	0.79±09	-10.08±0.32

(n=3)

SEM analysis

The SEM analysis shows that the NPs are of having the mean particle size (volume) and the polydispersity of all samples were determined (Table 5). It can be observed that the size of particles is the smallest when the sonication time is more and polymer amount is less but at the same time entrapment efficiency is less with % drug loading is more, However, the entrapment efficiency of the NPs was more when the polymer concentration is more and also the size with increase in entrapment efficiency and decrease in % drug loading.(Table 5).

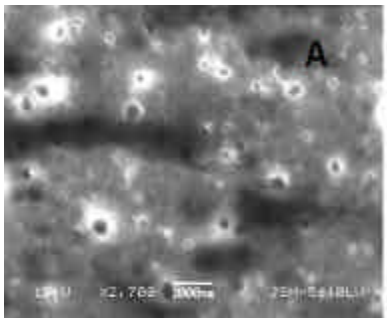


Fig-1

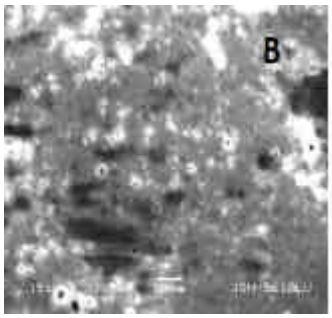


Fig-2

A, NPI 8 sample (INH:PCL, 20:500, PVA 2.5 %, Methylene Chloride 10 ml, sonication time 3 minutes).
B, NPI 3 sample (INH:PCL, 20:300, PVA 2.5 %, Methylene Chloride 10 ml, sonication time 3 minutes).

Fig.1 & 2 SEM micrographs of nanoparticles showing the shape and the surface characteristics.

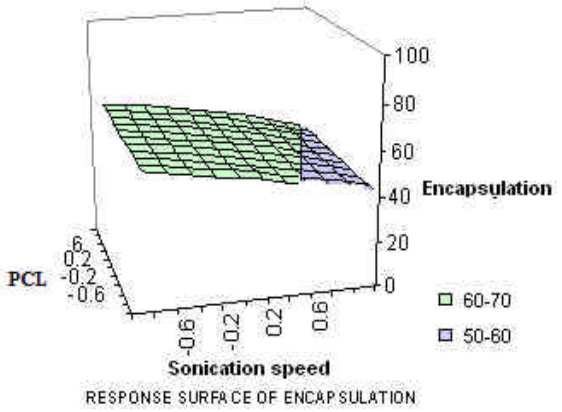
Table no.6: Intensive Search of Optimized Formulation

Formulation	PCL	Sonication Time	Encapsulation	NP size
NPI 1	0.4	2	56.12	389
NPI 2	0.2	0.2	56.96	365
NPI 3	0.4	0.2	60.14	374
NPI 4	0.6	0.2	63.52	398
NPI 5	0.2	0	69.15	395
NPI 6	0.4	0	70.14	349
NPI 7	0.6	0	70.95	400
NPI 8	0.2	2	49.87	370
NPI 9	0.6	2	52.33	398

Table no.7: 3^2 Factorial Design of Encapsulation

PCL/ S.T -0.6	Encapsulation -0.6	-0.4	-0.2	0	0.2	0.4	0.6
-0.4	32.11	39.68	41.12	40.19	56.12	41.22	40.32
-0.2	33.95	41.21	44.41	40.32	51.21	42.15	39.12
0	44.53	48.77	52.25	41.57	50.32	42.25	38.45
0.2	49.51	50.11	56.96	69.15	49.87	41.28	37.19
0.4	50.12	50.47	60.14	54.95	50.21	41.32	36.11
0.6	50.14	51.21	63.52	69.72	52.33	40.87	35.21

3^2 Factorial Design of Encapsulation shows that as increase the concentration of PCL the encapsulation is increase with optimum sonication time, which shown in response surface and contour plot. Further increase the concentration of PCM does not change any considerable encapsulation.



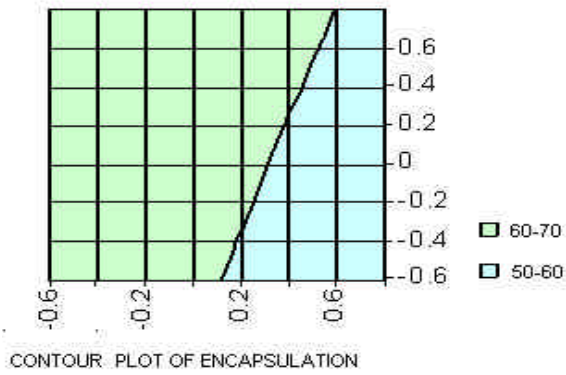
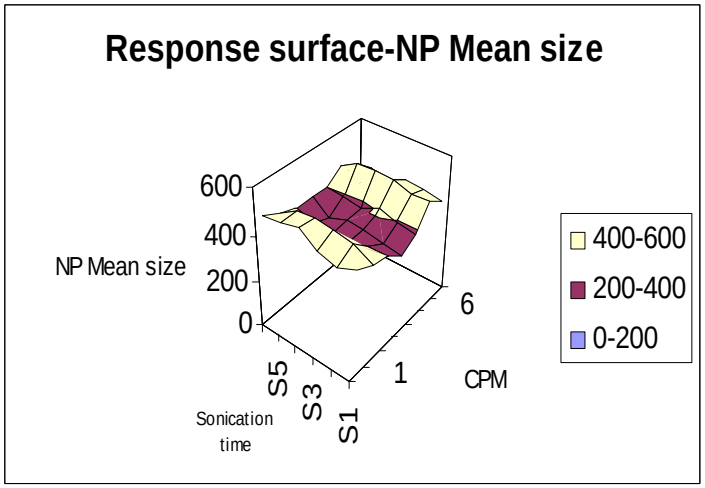


Figure no.3: Response surface and Contour plot of Encapsulation

Table no.8: 3^2 Factorial design of NP mean size

PCL/ S.T	NP Mean Size						
-0.6	**-0.6**	**-0.4**	**-0.2**	**0**	**0.2**	**0.4**	**0.6**
-0.4	454	420	400	350	248	478	431
-0.2	421	461	395	358	397	470	439
0	438	471	377	371	412	491	447
0.2	495	485	365	370	375	502	412
0.4	470	477	374	349	394	497	433
0.6	465	420	398	354	398	455	368

3^2 Factorial Design of NP size shows that as increase the sonication time the nano particle size of PCL is decrease, but it is up to beyond certain limit, further increase in time change the behavior of nano particles and also size is increase, besides moderated sonication time shown remarkable result which shown in response surface and contour plot. The marked area shows the optimized formulations.



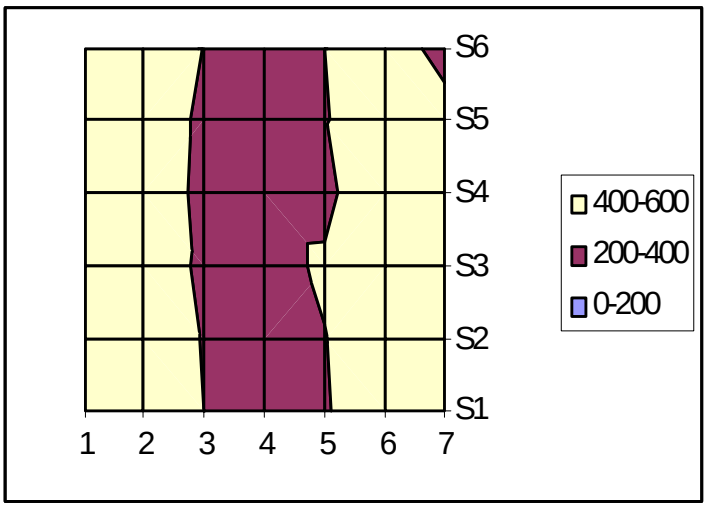


Figure no.4: Response surface and Contour plot of NP Mean size

From the intensive search and optimization two formulations (NPI-3 and NPI-4) were selected for the dissolution studies.

Mathematical Model.

Simply referred to as the model, it is an expression defining the dependence of a response variable on the independent variables. Mathematical models can either be empirical or theoretical. An empirical model provides a way to describe this factor-response relationship. It is most frequently, but not invariably, a set of polynomials of a given order. Most commonly used linear models are shown in Eqns 1-3

$E(y) = b_o + b_1 X_1 + b_2 X$ …(1)

$E(y) = b_o + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2$ …(2)

$E(y) = b_o + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2$ …(3)

Where E(y) represents the measured response and X_i , the value of the factors. b_o, b_i, b_{ii} and b_{ij} are the constants representing the intercept, coefficients of first-order terms, coefficients of second-order quadratic terms and coefficients of second-order interaction terms, respectively. The coefficients are calculated either by multiple linear regression (MLRA) or by the method of contrasts. Eqns. 1 and 2 are linear in variables, representing a flat surface and a twisted plane in 3-D space, respectively. Eqn. 3 represents a linear second-order model that describes a twisted plane with curvature, arising from the quadratic terms.

Table no.9: Drug Release profile of Nanoparticles in pH 7.4

Time (day)	Drug Release NPI8	Drug Release NPI3
1	5.27	9.53
2	23.75	26.39
3	35.63	37.28
4	45.25	48.39
5	58.53	59.35
6	63.76	66.53
7	74.49	75.95
8	85.56	85.53
9	92.67	86.62
10	96.05	91.21

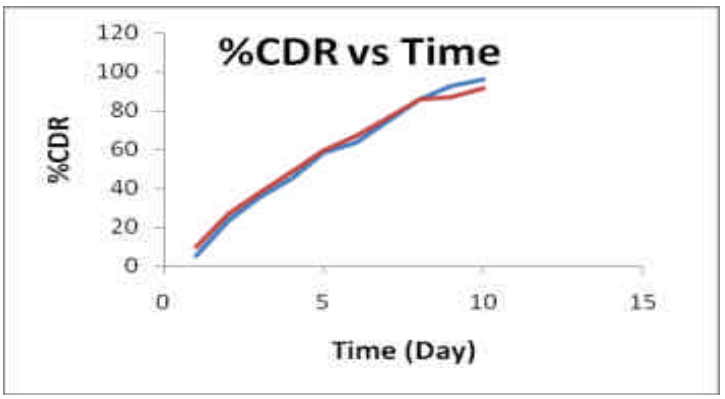
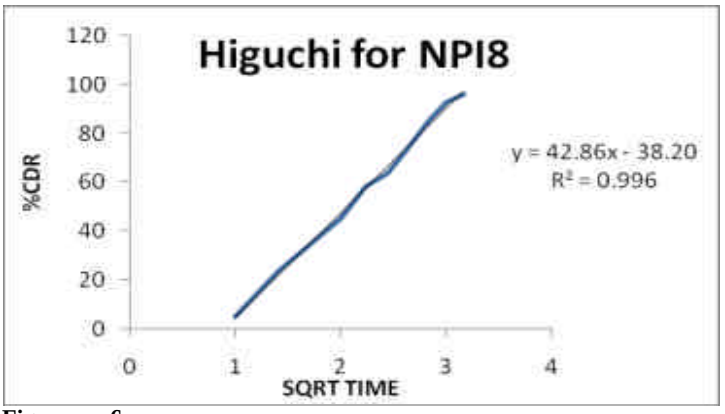


Figure no.5:



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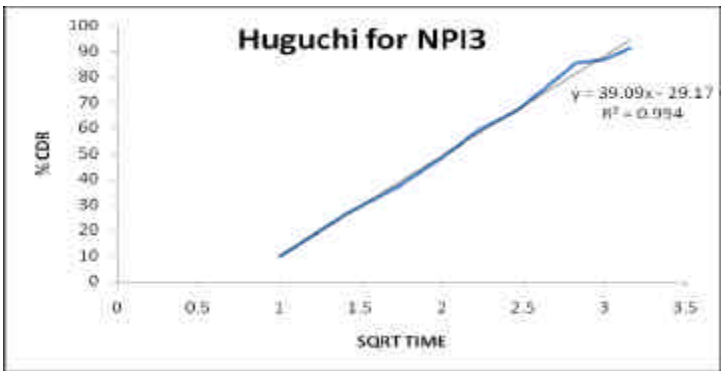

Figure no.7:

The drug release profile (Table no 9) follows the Huguchi equation which concludes that the dissolution is diffusion controlled.(Fig no 6,7).

CONCLUSION.

A novel approach for the preparation of INH-loaded NPs is followed by applying oil-in-water emulsion solvent evaporation with ultrasonication technique. Investigation of the preparation, Optimization of formulation, characterization, in vitro release of NPs was carried out. The different formulations with various polymer concentration and sonication time were evaluated. Our results demonstrated that this method is simple and easy, and has the efficacy to produce NPs with desired size and size distribution and morphological properties with high entrapment efficiency with moderate drug loading.

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