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Full Length Research Paper

Evaluation and optimization of factors affecting novel diclofenac sodium-eudragit RS100 nanoparticles

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The major concern of the present study was to evaluate the processing conditions and formulation factors affecting diclofenac sodium –eudragit RS100 nanoformulation size and their optimization to reach optimized nanoparticle (size below 200 nm). Diclofenac sodium –eudragit RS100 nanoparticles were formulated using nanoprecipitation – solvent deposition technique (the single emulsion technique). The effect of several process parameters, that is, homogenization type (homogenizer or sonicator), speed of homogenization, dispersing agent characteristics, the quantity and ratio of phases, drug-polymer content and ratio and also temperature of quasi-emulsion in the time of preparation were considered on the size of the nanoformulations. Particle size and size distribution of nanoparticles were studied by applying laser diffraction particle size analyzer, and morphology of the nanoparticles was also inspected by transmission electron microscopy (TEM). All the prepared formulations using eudragit RS100 resulted in nano-range size particles with relative spherical smooth morphology and drug loading efficiency of nearly 100%. According to these findings, nanoprecipitation – solvent deposition technique was able to engineer diclofenac sodium –eudragit RS100 nanoparticles to reach target size that could undergo more studies for evaluation and comparison of the anti-inflammatory effect of drug in nanoparticles with classical dosage forms following its ocular administration.

Key words: Nanoparticles, diclofenac sodium, eudragit RS100, particle size.

INTRODUCTION

Alternatively or along with corticosteroids, non steroidal anti-inflammatory drugs (NSAIDs) are used for the management of inflammatory diseases. Clinical specialists prescribe diclofenac, as salts, for management of pain, inflammation and miosis of eye and symptoms of seasonal allergic conjunctivitis (Ahuja et al., 2008), but delivery of diclofenac sodium (DS) using classical dosage forms throw eye tissues, due to low capacity of the eye, fast pre-corneal loss caused by tear drainage and less permeability of ionized form of potassium and sodium salts in physiologic pH, has been faced with the problem. These delivery deficiencies of classical dosage forms could lead delivery systems to low efficacy and require high dose dosage forms to be effective and consequently increase the incidence of site toxicities (Ahuja et al., 2008; Agnihotri and Vavia, 2009). So in the last 15 years, outstanding efforts have been made to improve drug delivery systems' (DDS) efficacy, applying novel carrier based drug delivery systems (Ahuja et al., 2008; Zimmer and kreuter, 1995) as colloidal systems, especially nanoparticles for ophthalmic delivery because of their high stability, higher bioavailability and long acting property (Zimmer and

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Kreuter, 1995; Kreuter, 1994) using biocompatible polymers like eudragits (Agnihotri and Vavia, 2009; Kreuter, 1994; Pignatello et al., 2002; Adibkia et al., 2011). Also as other nanoparticles, eudragit RS100 nanoparicles are biocompatible without any irritant effect on eye tissues until 24 h after application (Agnihotri and Vavia, 2009). Polyacrylic (and other polymers) nanoparticles preparation by desolvation from an water-miscible organic polymer solution (Kreuter, 1994), which was defined as nanoprecipitation - solvent deposition (Anilkumar and Harinath, 2011) and quasi-emulsion solvent diffusion technique (Pignatello et al., 2002) in literatures, is one of the widely used nanoparticles preparation technique employed for lots of drugs as ibuprofen, indomethacin, propranolol HCl and simvastatin. In this method, relatively hydrophilic copolymers (Eudragit RS or Eudragit RL) and drug, after dissolution in water-miscible solvents (as dispersed phase), gently are added to the water (as continues phase) then depositing water-miscible dispersed phase in aqueous phase causes organic solution to be supersaturated, and this phenomenon leads to nanoprecipitation (Kreuter, 1994). One of the requirement of this method is that, both polymer and drug have to be insoluble in continues phase (Gao et al., 2006; Dhoka et al., 2011). The processing conditions and formulation factors that control the particle size in solvent deposition method, as like solvent evaporation technique, are homogenization type (homogenizer or sonicator), homogenization rate and duration, stir rate over stabilization period, dispersing agent characteristics (type and content), viscosity of both phases, the configuration of the vessel and stirrer, quantity and ratio of phases, polymer and drug content and ratio, size and direction of dripping needle and temperature of quasi-emulsion in the time of preparation and nanoprecipitation by deposition of solvent (Kreuter, 1994; Pignatello et al., 2002; Li et al., 2008). Processing conditions and formulation factors had shown different effect on the size characteristics of nanoparticles depending on the materials and methods employed for the preparation of the nanoparticles. What is more, in literatures, the effect of these factors had been mentioned in separate studies having diverse condition, so it is not definite to compare them (Kreuter, 1994; Li et al., 2008; Dehghan et al., 2010; Joseph and Sharma, 2007). In this study, more efforts are made to consider the effect of all of the factors in the size of diclofenac sodium-eudragit RS100 nanoparticles (DSENs) in similar condition.

Eudragit RS100 is a copolymer of poly (ethylacrylate, methyl-methacrylate and chlorotrimethylammonioethyl methacrylate), with content of quaternary ammonium groups between 4.5 to 6.8% responsible for the bioadhesive characteristics of this polymer. This property increase nanoparticles interaction time with tissue, and as a result, tear drainage effect decrease, and consequently efficacy of delivery system increase. What is more, hydrophilic property of making polymer able to swell up is consequence of quaternary ammonium groups. Eudragit RS100 is insoluble at the physiological pH (Pignatello et al., 2002; Adibkia et al., 2011).

Also literature defines nanoparticles as colloidal drug delivery system having particle size under 1000 nm (Mudshinge et al., 2011), but size of ophthalmic nanoparicles is below 300 nm (Agnihotri and Vavia, 2009; Zimmer and kreuter, 1995; Pignatello et al., 2002; Adibkia et al., 2007; Cetin et al., 2010; Gupta et al., 2010). This article is presenting the details of factors that affect particle size and optimization of them to achieve suitable particle size of DSENs for ocular delivery prepared from nanoprecipitation-solvent deposition method.

MATERIALS AND METHODS

Diclofenac sodium from Amoli (Mumbai, India) was a kind gift from Zahravi Pharmaceutical Co. (Tabriz, Iran). Eudragit RS100 from Degussa (Darmstadt, Germany) was a kind gift from Akbarieh Co. (Tehran, Iran). Poly vinyl alcohol (PVA, MW 95000, degree of hydrolysis 95%), Plouronic F-68 and Tween 80 were obtained from Sigma (Deisenhofen, Germany). All the other chemicals were of the highest available grade from Merk (Darmstadt, Germany).

Preparation of nanoparticles

The DSENs with the different ratios of DS/eudragit RS100 (that is, 1:1, 1:3 and 1:5) were prepared using nanoprecipitation - solvent deposition technique (the single emulsion technique) which diffusing of phases to each other led to decrease solubility of internal phase content and precipitation of them as drug loaded particles (Pignatello et al., 2002). Normally, drug and polymer were co-dissolved in ethanol at room temperature by using sonicator (Starsonic 35 LIARRE frequency us: 28-34 KHz). The resulted solution was slowly poured with a constant speed (0.5 ml/min) into acid buffer (pH 3.2) saturated with DS and containing surfactant as external phase. During this process, the mixture was agitated using a high speed homogenizer (Heidolph, Germany) or sonicator. The formed oil-in-water (O/W) quasi-emulsion was gently stirred at room temperature for 24 h to stabilize resulted particles. Then for obtaining all the dispersed nanoparticles, the resulted nanosuspensions were centrifuged several times at 14,000 rpm, 20°C for 20 min (Hettich, Germany). The collected nanoparticles were washed (3x) with DS saturated acid buffer (pH 3.2) using previously described centrifugation approach and then lyophilized using lyophilizator (Christ Alpha 1-4, Germany).

The aim of using acidic buffer as external phase was making an insoluble medium for drug. DS (pKa=4.0) is practically insoluble in acidic pH. The data for this claim had not presented.

Optimization of nanoparticles

For the optimization process, in order to obtain a suitable size of nanoparticles, the processing conditions and formulation factors' values were changed and their effect on the size of nanoparticles was considered by the following parameters: ratios of drug to polymer, total amount of drug-polymer, volume of ethanol and volume of acid buffer (pH 3.2). Types and amounts of surfactant in external phase, speed of homogenizer as agitating parameter and bath temperature of sonicator in the time of agitation for the first-end of the sonication are 20 to 32; 32 to 44 and 44 to 51, and 51 to 54ºC, respectively. Evidently, only one parameter was adjusted in each series of experiments. Stirring rate over stabilization period is considered as a variable parameter too. In order to decrease

Table 1. Effect of formulative variables on the mean size of DSENs.

Formulation	Total amount (mg)	Drug-polymer ratio	Continuous phase volume (ml)			Homogenization rate (rpm)	Particle size, (nm)±SD	PDIa±SD
			PVA 0.5%	PVA 1%	PVA 2%			
PV1	100	1-3	-	10	-	13000	866±5.7b	0.221±0.065
PV2	100	1-3	-	20	-	13000	780±82	0.268±0.050
PV3	100	1-3	-	30	-	13000	658±24	0.366±0.080
PV4	100	1-3	-	50	-	13000	605±24	0.338±0.013
PV5	100	1-3	-	100	-	13000	533±27	0.381±0.071
PV6	100	1-3	100	-	-	13000	462±24	0.388±0.041
PV7	100	1-3	-	100	-	13000	533±27	0.381±0.071
PV8	100	1-3	-	-	100	13000	616±44	0.335±0.032
PV9	100	1-3	-	100	-	7000	733±79	0.303±0.042
PV10	100	1-3	-	100	-	13000	533±27	0.380±0.071
PV11	100	1-3	-	100	-	19000	448±79	0.531±0.141
PV12	100	1-1	-	-	100	13000	546±7	0.435±0.212
PV13	100	1-3	-	-	100	13000	616±44	0.335±0.032
PV14	100	1-5	-	-	100	13000	765±57	0.227±0.048
PV15	150	1-1	-	-	100	13000	567±55	0.325±0.020
PV16	150	1-3	-	-	100	13000	689±32	0.340±0.031
PV17	150	1-5	-	-	100	13000	828±40	0.277±0.066
PV18	300	1-1	-	-	100	13000	695±16	0.328±0.042
PV19	300	1-3	-	-	100	13000	831±71	0.200±0.034
PV20	300	1-5	-	-	100	13000	946±25	0.144±0.016

[a]PDI, stands for polydispersity index. [b], mean value of 3 replications ± standard deviation. Mean size and polydispersity index with standard deviation for the formulations with PVA. Dispersed phase volume (ethanol) was 2 ml.

the complexity, the formulations mentioned in the tables were used.

With the aim of simplifying the evaluation of parameters, the subsequent formulations were classified into two main classes that differ in agitating method: (a) Homogenizer class and (b) Sonicator class. Homogenizer class (Class a) subdivided into three different subclasses, that differ in the type of surfactant in continuous phase, which includes subclass aI) Tween (%.02 w/v) + Plouronic f68 (0.5% w/v), subclass aII) PVA (0.5% w/v) and subclass aIII) Plouronic f68 (0.5% w/v). Details of class (a) formulations and class (b) formulations are mentioned in Tables 1, 2 and 3 and 4, respectively. The nanoparticles obtained from class b method, prepared with the sonicator (S4, S5 and S6), were preferred for further assessments due to it having smaller particle size with monomodal distribution in comparison to other formulations. Formulation conditions for S4, S5 and S6 were as follows: Bath temperature from 51 to 54°C directing 30 ml of external phase containing Plouronic f68 (0.5% w/v), 2 ml dispersed phase, and a total amount of 100 mg drug – polymer.

Nanoparticles size and morphology

The particle size and size distribution of the prepared nanoparticles were determined via laser diffraction particle size analyzer (Shimadzu, Japan) equipped with the Wing software (version 1201). So as to prevent clumping, the dried powder samples were suspended in DS saturated acid buffer (pH 3.2) and slightly sonicated before measurement. The mean diameter and size distribution of the resulted homogeneous suspension were assessed, subsequently. Each value resulted from triplicate determinations. The morphology of the nanoparticles was also inspected by Transmission electron microscopy (TEM) model 906 (LEO, Germany). Prior to assessment, samples were suspended in DS saturated acid buffer (pH 3.2).

Loading efficiency of nanoparticles

Nanoparticles, equivalent to 100 mg of diclofenac sodium, was accurately weighted and dissolved in ethanol. Spectrophotometery was applied (n=6) at 278 nm to determined Drug loading efficiency.

Statistical analysis

The Mann-Whitney U test was conducted for statistical analysis. Data was represented as mean values ± SD (standard deviation). A p value less than 0.05 were assumed for the statistically significant differences.

RESULTS AND DISCUSSION

Size and morphological properties of the nanoparticles

A solubility characteristic of the drug is the main factor to decide about the specific method of encapsulation (Ubrich et al., 2004). In the current study, the single emulsion nanoprecipitation-solvent deposition process

Table 2. Effect of formulative variables on the mean size of DSENs.

Formulation	Total amount (mg)	Dispersed phase volume (ml)	Continuous phase volume (ml)	Homogenization rate (rpm)	Particle size (nm)±SD	PDI±SD
TP1	100	3	25	7000	663±68	0.337±0.021
TP2	100	3	25	13000	568±42	0.321±0.015
TP3	100	3	25	19000	462±69	0.458±0.038
TP4	100	1.5	25	13000	810±63	0.323±0.132
TP5	100	3	25	13000	568±42	0.321±0.015
TP6	100	6	25	13000	497±54	0.381±0.078
TP7	100	3	10	13000	781±62	0.319±0.012
TP8	100	3	25	13000	568±42	0.321±0.015
TP9	100	3	50	13000	401±28	0.472±0.040
TP10	50	3	50	13000	211±104	1.275±.464
TP11	100	3	50	13000	401±28	0.472±0.040
TP12	200	3	50	13000	592±26	0.511±0.299
TP13	300	3	50	13000	704±27	0.291±0.020

Mean size and polydispersity index with standard deviation for the formulations with Tween 0.02% + Plorounic f68); Drug-polymer ratio was 1 to 3.

Table 3. Effect of formulative variables on the mean size of DSENs.

Formulation	Dispersed phase volume (ml)	Continuous phase volume (ml)		Homogenization rate (rpm)	Particle size (nm)±SD	PDI±SD
		Plouronic (f68 0.5%)	Plouronic (f68 1%)			
P1	2	-	10	13000	606±42	0.334±0.034
P2	2	-	20	13000	533±35	0.290±0.010
P3	2	-	30	13000	497±22	338±0.077
P4	2	-	50	13000	530±82	352±0.050
P5	2	-	30	7000	558±22	0.307±0.007
P6	2	-	30	13000	497±22	0.338±0.077
P7	2	-	30	19000	487±21	0.251±0.073
P8	2	30	-	13000	200±100	0.486±0.315
P9	2	-	30	13000	497±22	0.338±0.077
P10	5	-	30	13000	553±13	0.360±0.055

Mean size and polydispersity index with standard deviation for the formulations with Plorounic f68. Drug-polymer ratio was 1 to 3 and total weight was 100 mg.

was employed for the encapsulation of DS, water-insoluble in acidic pH, into eudragit RS100. Obviously, no more than one parameter was changed in each series of experiments. All last prepared formulations resulted in a nano-range size and the size distributions were relatively monodisperse in all of the formulations with the polydispersity index (PDI) values between 1.279 and 1.515.

Formulations prepared using homogenizer (a)

(al) PVA as surfactant

In terms of the total amount of drug-polymer, various formulations were studied in three drug-polymer ratio (1:1, 1:3 and 1:5), which no significant differences observed ($p>0.05$) between 100 and 150 mg, but 150 mg showed significant differences ($p<0.05$) compared with 300 mg, which increasing the total amount resulted in bigger particle sizes. In the entire three total amount of drug-polymer, we observed that increasing the polymer portion (from 1:1 to 1:5) significantly ($p<0.05$) led to bigger particle size. In the preliminary studies, Anilkumar and Harinath (2011) and Li et al. (2008) believed that increasing viscosity of the external phase and consequently, increasing droplet size causes this phenomenon. Then 100 mg was selected to the rest of the formulations. Increasing external phase volume, having 1% PVA, resulted in significant decrease ($p<0.05$) in particle size, in the way among 10, 20, 30, 50 and 100 ml, the smaller size belongs to the 100 ml. The same results have been reported by Patel et al. (2010). By the

Table 4. Effect of formulative variables on the mean size of DSENs**.**

Formulation	Sonicator bath temperature during formulation (°C)		Drug-polymer ratio	Particle size (nm)±SD	PDI±SD
	At the beginning	At the end			
S1	20	32	1-3	407±51	0.427±0.064
S2	32	44	1-3	290±36	0.637±0.145
S3	44	51	1-3	266±87	1.085±0.401
S4	51	54	1-3	107±12	1.515±0.059
S5	51	54	1-1	103±6	1.492±0.043
S6	51	54	1-5	170±36	1.279±0.386

Mean size and polydispersity index with standard deviation for the formulations with Plorounic f68); details of formulation was as like as formulation P8.

attitude that emulsion droplets move freely in medium and have less chance to collide with each other, then 100ml kept on to the rest of the formulations. The next evaluated parameter was agitating speed, in which three homogenization speeds, 7000, 13000 and 19000 rpm, were applied. The one with 13000 rpm significantly resulted in more ($p<0.05$) smaller particle size than 7000 rpm, but the difference between 13000 and 19000 rpm was not significant ($p>0.05$). So it was suitable to use 13000 rpm for the evaluation of other parameters. It is noticeable that contrary reports acquired from literature about the effect of agitating speed on the particle size (Adibkia et al., 2010; Patel et al 2010). These contrary results about agitation speed could be vindicated as follows: Increasing speed of agitation result smaller droplet size and consequently particle size decrease, at the other hand, we hypothesize that, over agitation in solvent diffusion methods could cause to faster deposition of solvent to each other that result in faster precipitation with bigger particle size. This furthermore causes the accumulation of resulted particle which are not stabilized. We believe that this phenomenon is more common when emulsifier concentration is low to support particles smaller than expected size. The last parameter that was studied about PVA is its content in external phase applying 2, 1 and 0.5% of surfactant. The results showed significant differences ($p<0.05$) in particle size that decreasing surfactant content led to smaller particle size; this outcome was in contrary to Anilkumar and Harinath's (2011) claim. This contrary result could be vindicated by relation of concentration of surfactants with their structure in solvents, which over micelle forming concentrations result bigger particle size. None of formulations above could lead to our expected nano-sized particles (below 200 nm) (Table 1).

(aII) Tween (%.02 w/v) + Plouronic f68 (0.5% w/v) as surfactant

In this sub group, the first parameter that varied was agitating speed as PVA formulations, but none of them show any significant differences ($p>0.05$) in particle size. 13000 rpm, by the aim of similarity to the PVA formulations, kept on to evaluation of other parameters. In the evaluation of dispersed phase volume, three amount of ethanol were used (1.5,, 3 and 6 ml) which by increasing phase volume, particle size decreased, but the difference between 1.5 and 3 ml was observed significant ($p<0.05$) and the difference between 3 and 6 ml was not significant ($p>0.05$). Moreover, increasing continuous phase volume, worked on 10, 25 and 50 ml, caused to decrease significantly ($p<0.05$) in particle size. These two recent phenomena could be vindicated by the viscosity of the dispersed and continuous phases that is explained by Li et al. (2008). Then 50 ml kept on to evaluation of total drug-polymer amount variation on the size characteristics. For this purpose 50, 100, 200 and 300 mg were analyzed. As like PVA, Results showed a significant ($p<0.05$) decrease in particle size by decreasing total amount of drug-polymer (Table 2).

(aIII) Plouronic f68 (0.5% w/v) as surfactant

Evaluation of continuous phase volume by this surfactant showed different results in regard to aforesaid surfactant contents. In this study, no regular relationship observed between volume of external phase and particle size. Indeed, an optimum volume (30 ml) has obtained for the smallest particle size. Since the differences in particle size between 10 and 20 ml and between 30 and 50 ml was not significant ($p>0.05$), but on the other hand thedifferences between 10 and 30 ml became significant ($p<0.05$), and by increasing external phase volume, particle size decreased. We hypothesize that mechanism of this phenomenon is as below: further increasing in continuous phase volume decrease dispersing effect of surfactant, by fast nanoprecipitation which is consequence of fast deposition of phases to each other,(that at first size decreased and then increased by further increase in continuous phase volume). Then 30 ml of continuous phase used for other evaluations as agitating speed, dispersed phase volume and surfactant

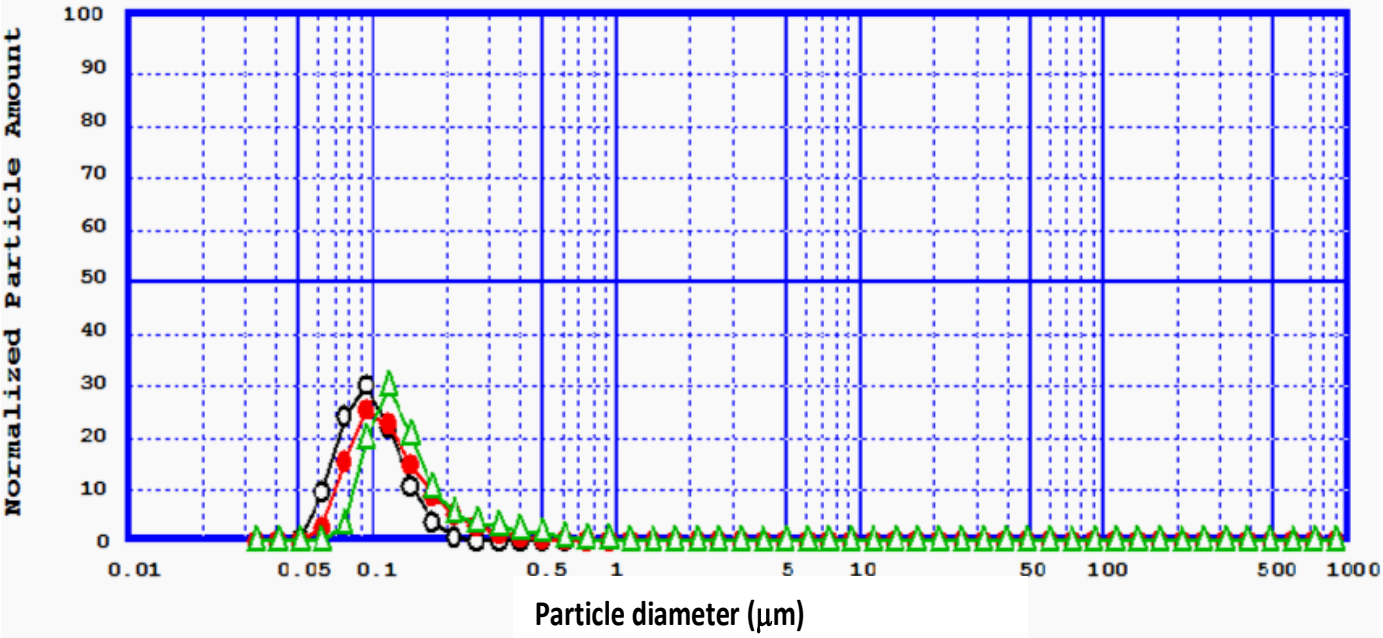

Figure 1. DSENs size distribution curves (Drug-polymer ratios from left to right are ○, 1 to 1, ●, 1 to 3 and △, 1 to 5, respectively).

content results in agitating speed in the same way as PVA and about the next two parameters, less volume (2 ml in regard to 5 ml) and less surfactant content (0.5% in regard to 1%) showed significantly ($p<0.05$) smaller particle size, the mechanism behind this phenomenon is explained in Table 3.

Formulations prepared using sonicator (b)

P8 formulation characteristics were applied to the formulation of a nano-range size, but agitated by sonicator instead of homogenizer. Ding et al., (2011) applied Sonication in the preparation of nanoparticles. Only sonicator bath temperature varied to receive the final formulation. Bath temperature at the beginning and at the end of the formulations were as followed; 20 to 32, 32 to 44, 44 to 51 and 51 and 54°C. By increasing environmental temperature, particle size significantly ($p<0.05$) decreased. Probably, increasing solubility of drug and polymer in phases by increasing temperature that preventing sudden precipitation of particles give more time to quasiemulsion to agitate by sonication and precipitate gradually is the reason of this phenomenon. Finally, two other ratios of drug-polymer (1-1 and 1-5) were prepared, which by increasing polymer portion particle size significantly ($p<0.05$) increased, but all of the three ratios were in the expected nano-range size (Table 4).

Laser diffraction particle size analyzer profiles (Figure 1) and TEM photograph (Figure 2) that are confirmatory to each other illustrates intended particle size. TEM experiments revealed a spherical shape with a relative smooth surface for the resultant nanoparticles (Figure 2).

Drug loading efficiency

As external phase was saturated with drug, nearly 100% loading resulted for nanopartices, in all three ratio of drug-polymer.

Conclusion

As indicated in this study, nanoprecipitation – solvent deposition technique was able to engineer DSENs to reach target size. The smallest size was obtained by applying sonication method, in which higher temperature of the sonicator bath resulted in smaller particle size. On the other hand, agitation speeds of homogenizer depend on dispersing agent; correctly interfering materials in the formulation, can have or may not have significant effect on the size of the nanoparticles and effect of increasing dispersing agent content, in contrast to literatures, caused bigger particle size. In terms of phase's volume, increasing continuous phase volume caused smaller particle size, albeit the optimum volume of continuous phase of Plouronic f68 was obtained. Meanwhile, dispersed phase showed contrary results between formulations having different dispersing agents. Increasing polymer portion of formulation in both groups of aI and b resulted in bigger particle sizes. Finally,

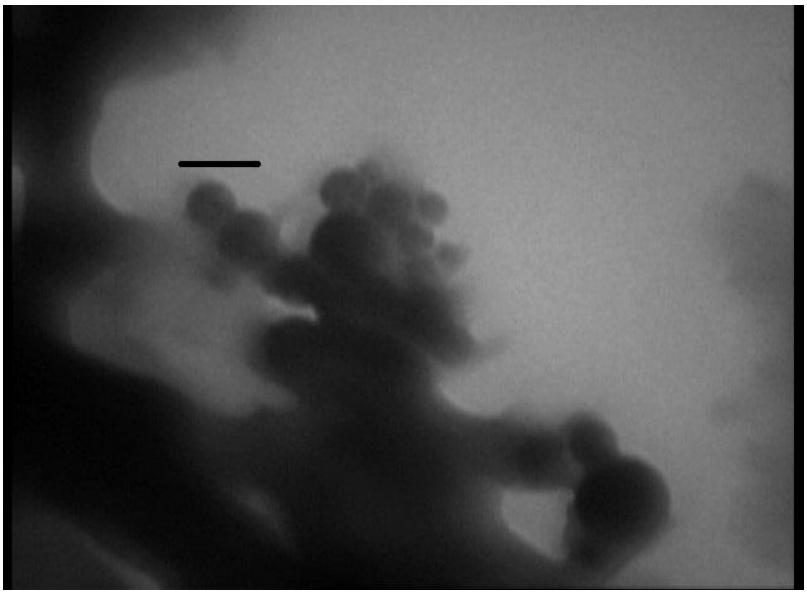

Figure 2. Transmission electron micrograph of DSENs at the ratio of 1:3 (Showed line is equal with 278 nm).

increasing total amount of drug – polymer led to bigger particle size in the studied groups aI and aII.

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