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Effect of solvents quality on determination of particle size and polydispersity of nanoparticles

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Nanotechnology is of great interest to researchers and industrialists and nanocolloidal carrier systems for drug delivery have been studied in great detail, but while much research has been carried out on the preparation of nanoparticles using a variety of techniques employing various solvents, no attention has been given to the quality of solvents used in the process of nanoparticles characterization. The present investigation aimed to study the effect of solvents quality on the chitosan nanoparticles characterization for average particle size (Z) and polydispersity index (P.I.). Particle size distribution study showed that the number of particles contributed by solvents significantly affects both the Z and P.I. of the nanoparticulate suspensions leading to ambiguous results. While the Z decreases upon dilution with organic solvents, the phosphate buffer and water causes a net increase in Z because of the introduction of larger extraneous nanoparticles. The P.I. was found to increase with dilution because of the differences in the size of the polymeric nanoparticles and the nanoparticles introduced by the solvent upon dilution. The study thus recommends use of the highest quality of solvents in nanoparticles manufacturing and characterization process to avoid the generation of erroneous results.

Keywords: Quality of solvents; Chitosan; Nanoparticles; Particle size; Polydispersity; Formulation

1. Introduction

Nanoscience can be defined as the study of phenomenon and the manipulation of materials at atomic, molecular, and macromolecular scales where properties differ significantly from those at large scale, and *nanotechnology* as the design, characterization, production, and application of structures, devices and systems by controlling shape and size at the nanometer scale. Thus nanotechnology is the study and design of the systems at the nanometer scale (10^{-9} m) — the scale of atoms and molecules [1]. Nanotechnology application in medical or pharmaceutical sector represents the maximum interest (about 30%) of all nanomaterial companies operational in the

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field [2] and this has lead to the large number of special reports, companies and products with the nano-prefix [3]. Demand for nanotechnology based health care products in the US is projected to increase nearly 50% per year to $6.5 billion by 2009 from the current figures of $906 million in 2004.

Nanoparticles used in drug delivery can be either the polymeric nanoparticles (where polymer acts as a carrier for the therapeutic agents) or the nanosuspensions of the active therapeutic agent itself produced by various milling or homogenization procedures to increase the surface area and hence to increase the saturation solubility, dissolution rate and/or bioavailability. The production techniques for these nanoparticles involve the use of various solvents, aqueous (water) [4, 5] or non-aqueous (e.g. acetone [6–8], ethanol [4, 6], methanol [9], methylene chloride [6, 10–12], etc.) at one stage or the other, along with various other excipients. In fact, solvents constitute a major proportion of the formulation and their use in formulation and preparation of nanocolloidal carrier systems remains almost indispensable. Residual solvents in pharmaceutical products not only possess a potential toxic risk to human health; they may also affect the physicochemical properties of the pharmaceutical product and excipients, which in turn could govern the manufacturing process or the preparation conditions [13]. Residual solvent analysis in pharmaceutical products have attracted considerable interest during last ten years with demands for international harmonization of limits and International Conference on Harmonization (ICH) has developed guidelines for residual solvents as impurities in new drug substances and new drug products. Additionally, these solvents are reported to be used for dilution purposes to minimize the inter-particle interactions and aggregation in particle size and size distribution (polydispersity) studies of nanoparticles by Dynamic Light Scattering (DLS) or Photon Correlation Spectroscopy (PCS) [14–16].

Based on the hypothesis that the solvents used for the preparation or dilution of nanoparticles suspension contribute significant foreign nanoparticles and may affect the actual particle size and size distribution of nanoparticles by introduction of extraneous nanoparticles, the major goal of the present investigation was to quantify the effect of quality of solvents on the particle size and polydispersity of chitosan nanoparticles. Chitosan was selected as the polymer of choice for present study because of its insolubility in almost all organic solvents and also in water at neutral pH, which ensures the absence of any nanoparticles surface-solvent interactions.

2. Materials and methods

2.1. *Materials*

The polymer chitosan (specifications: low molecular weight, viscosity of 1% w/v solution in 1% v/v acetic acid – 130 cps, deacetylation degree >80%) was received as a gift sample from India Sea Foods. Sodium alginate was purchased from CDH Labs Ltd. Pluronic F-68 was a generous gift from BASF Corp. HPLC grade solvents were purchased from E. Merck (India) Ltd. All other solvents and materials used were of analytical grade.

2.2. *Methods*

2.2.1. Preparation of chitosan nanoparticles. The nanoparticles were prepared by modified coacervation method as reported by Calvo *et al.* [17]. Briefly, the aqueous solution of sodium alginate (0.1% w/v) was sprayed into the chitosan solution (0.1% w/v) containing Pluronic F-68 (0.5% w/v) under continuous magnetic stirring at 1000 rpm for 30 min. Nanoparticles were formed as a result of the interaction between the negative groups of sodium alginate and the positively charged amino groups of chitosan (ionic gelation). Nanoparticles were collected by centrifugation (REMI high speed, cooling centrifuge, REMI Corp., India) at 18,000 rpm for 30 min at 4°C. For particle size and size distribution study these nanoparticles were redispersed in 5 ml of HPLC grade water.

2.2.2. Morphological analysis of chitosan nanoparticles. Morphological analysis of the chitosan nanoparticles was performed using Transmission Electron Microscopy (TEM) (Philips CM 10). Samples of the nanoparticles suspension (5–10 µl) were dropped onto Formvar-coated copper grids. After complete drying, the samples were stained using 2% w/v phosphotungstic acid. DigitalMicrograph and Soft Imaging Viewer software were used to perform the image capture and analysis using particle sizing.

Particle size and polydispersity index study was carried out at 25°C by photon correlation spectroscopy (PCS) using disposable cells at detection angle of 90°. Sample volume used for the analysis was kept constant i.e. 5 ml to nullify the effect of stray radiations from sample to sample. Studies were carried out in triplicate ($n = 3$) and the standard deviation (S.D.) was recorded.

2.2.3. Study of the effect of solvents on nanoparticles characterization. To study the effect of solvents quality on nanoparticles characterization process, re-dispersed chitosan nanoparticles were diluted with various HPLC grade solvents viz. acetone, acetonitrile, methanol, isopropyl alcohol (IPA), phosphate buffered saline (PBS, pH 7.4), water (HPLC grade) and double distilled water (DDW). The particle size and size distribution (polydispersity) studies were carried out for various increasing dilutions (1:1, 1:2, 1:5, and 1:10) with different solvents. All solvents used for reconstitution and/or dilutions were previously filtered through 0.45μ filter (Millipore, USA).

In particle size and size distribution study by PCS, measurements were made in triplicate for each sample at 25°C using disposable cells at detection angle of 90°. The counts per second (cps) of the nanoparticulate dispersions were always maintained above the required minimum of 5000 cps and for various dilutions of chitosan nanoparticles dispersion prepared, the relationship between cps and dilution was studied. Data were then analyzed by *PhotoCor* software to calculate average particle size (Z) and polydispersity index (P.I.) to relate with the dilution of nanoparticulate dispersions with different solvents and compared with the water (HPLC grade) (ANOVA, 95% confidence interval, $p < 0.05$). Blank solvents were also observed for the cps and used as the control.

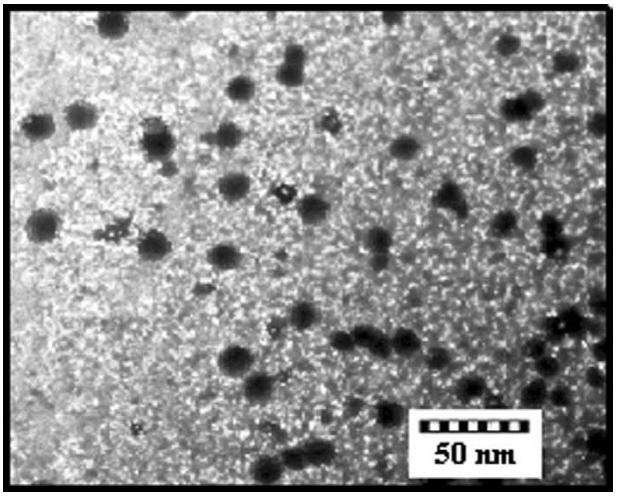

Figure 1. TEM image of chitosan nanoparticles.

3. Results and discussion

Chitosan is a natural cationic polysaccharide obtained by the N-deacetylation of chitin, a product found in the shells of crustaceans. Sodium alginate is an anionic polysaccharide consisting of linear copolymers of α-L-gluronate and β-D-mannuronate residues. Alginates are hemocompatible, have not been found to accumulate in any major organs and show evidence of *in vivo* degradation [18]. Chitosan nanoparticles were prepared by an ionic gelation method where alginate-chitosan polyionic complexes are formed via interaction between the carboxyl group of alginate and the amine group of chitosan [19].

Chitosan nanoparticles were chosen as the model nanoparticles to study the effect of solvents quality on nanoparticles characterization process because chitosan is soluble in water only at acidic pH and is almost insoluble in all organic solvents. Thus any change in the average particle size or polydispersity of chitosan nanoparticles after dilution with solvents can be ascribed to the changes in particle distribution intensities brought-out by particles contributed by solvents rather than the nanoparticles surface–solvent interactions.

Average particle size (Z) and polydispersity index (P.I.) for the originally reconstituted chitosan nanoparticles suspension were found to be 285 ± 14 nm and 0.219 ± 0.011, respectively. Transmission Electron Microscopy (TEM) study of the chitosan nanoparticles showed quite uniform and almost spherical nanoparticles (figure 1). Table 1 shows the effect of solvent quality on Z and P.I. upon increasing the dilution with various aqueous as well as non-aqueous solvents.

Blank solvents when studied for particle size and size distribution studies, the cps observed was in the range of 1100–1350 cps for organic solvents and 1700–1975 cps for DDW and PBS. The average particle size and polydispersity could not be recorded by instrument because of the required minimum of 5000 cps.

Table 1. Effect of solvent quality on average particle size (Z) and polydispersity index (P.I.) of chitosan nanoparticles.

Sr. No.	Solvents used for reconstitution/dilution	Particle size (Z)* and Polydispersity Index (P.I.)* for various dilutions of nanoparticulate dispersions							
		1 : 1		1 : 2		1 : 5		1 : 10	
		Z (nm)	P.I.	Z (nm)	P.I.	Z (nm)	P.I.	Z (nm)	P.I.
1	Acetone, HPLC grade	275 ± 11	0.259 ± 0.018	268 ± 15	0.276 ± 0.016	261 ± 9	0.339 ± 0.028	243 ± 19	0.352 ± 0.024
2	Acetonitrile, HPLC grade	264 ± 8	0.283 ± 0.027	271 ± 11	0.321 ± 0.028	253 ± 17	0.346 ± 0.032	236 ± 8	0.382 ± 0.017
3	Methanol, HPLC grade	249 ± 13	0.249 ± 0.015	236 ± 8	0.273 ± 0.011	232 ± 19	0.296 ± 0.019	215 ± 13	0.341 ± 0.034
4	Isopropyl alcohol, HPLC grade	257 ± 5	0.267 ± 0.009	263 ± 17	0.289 ± 0.021	245 ± 12	0.309 ± 0.025	223 ± 11	0.337 ± 0.029
5	Phosphate buffered saline, pH 7.4	236 ± 17	0.321 ± 0.031	247 ± 13	0.362 ± 0.033	263 ± 7	0.348 ± 0.018	297 ± 21	0.311 ± 0.015
6	Water, HPLC grade	251 ± 12	0.273 ± 0.016	243 ± 6	0.255 ± 0.014	237 ± 11	0.291 ± 0.009	228 ± 15	0.319 ± 0.021
7	Double distilled water	223 ± 9	0.336 ± 0.030	256 ± 11	0.312 ± 0.017	268 ± 14	0.351 ± 0.017	279 ± 8	0.389 ± 0.027

*Study carried out in triplicate and values of Z and P.I. shown here are average ± S.D.

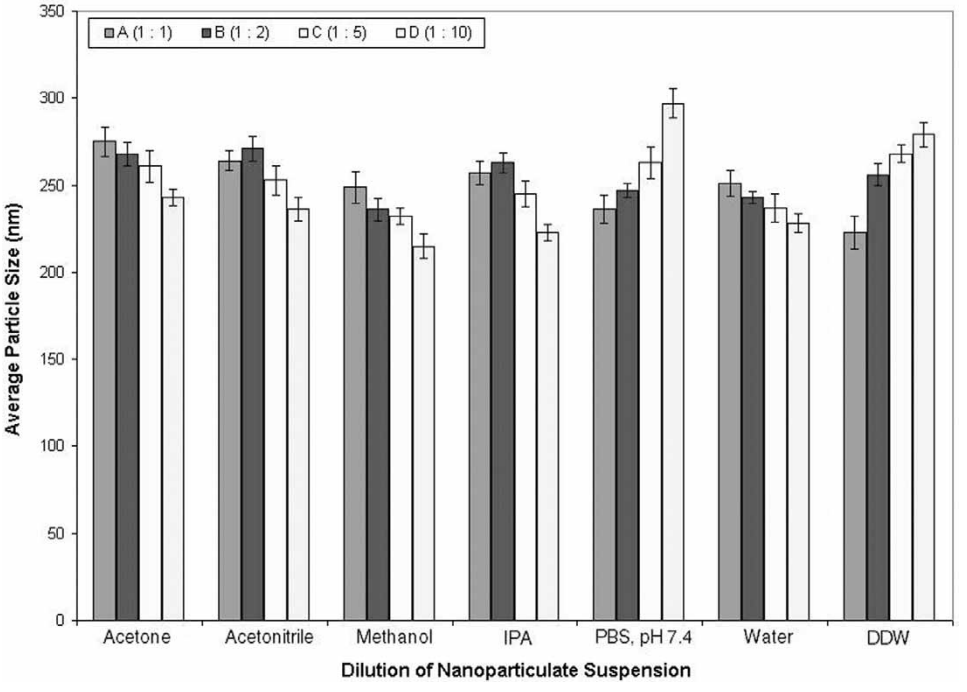

Figure 2. Effect of increasing dilution of nanoparticulate dispersions on Z.

It was observed that on diluting the samples and increasing the dilution from 1:1 to 1:10, the average particle size of the chitosan nanoparticles decreases (except in case of PBS and DDW) because of the large number of very small foreign nanoparticles contributed by the solvent (figure 2). But in case of PBS and DDW, the Z increases with dilution and approaches to close of the actual size of nanoparticles because of the relatively larger nanoparticles contributed by these solvents. It can be ascribed further to the impurities or nanoparticles generated by the solute particles dissolved in PBS. These results were in agreement with the relationship between cps and dilution (figure 3), which showed the number of extraneous nanoparticles introduced, increases with dilution from 1:1 to 1:10. The maximum number of nanoparticles was introduced by PBS, pH 7.4 followed by DDW and acetonitrile.

Figure 4 shows the effect of increasing dilutions of nanoparticulate dispersions as % deviation from average particle size, and the negative values obtained upon dilution showed that the observed particle size is lower than the actual Z. The % deviation varied from –24.56% to 4.21%. Only in case of PBS the positive deviation could be observed, as the observed particle size was higher than Z because of larger size particles contributed by PBS.

Polydispersity index was found to increase on increasing the dilution of nanoparticulate dispersions from 1:1 to 1:10 (figure 5). Percent (%) deviation from average P.I. was observed to vary from 13.70% (1:1 dilution with methanol) to 77.63% (1:10 dilution with DDW) (figure 6). It can be attributed to the differences in particle

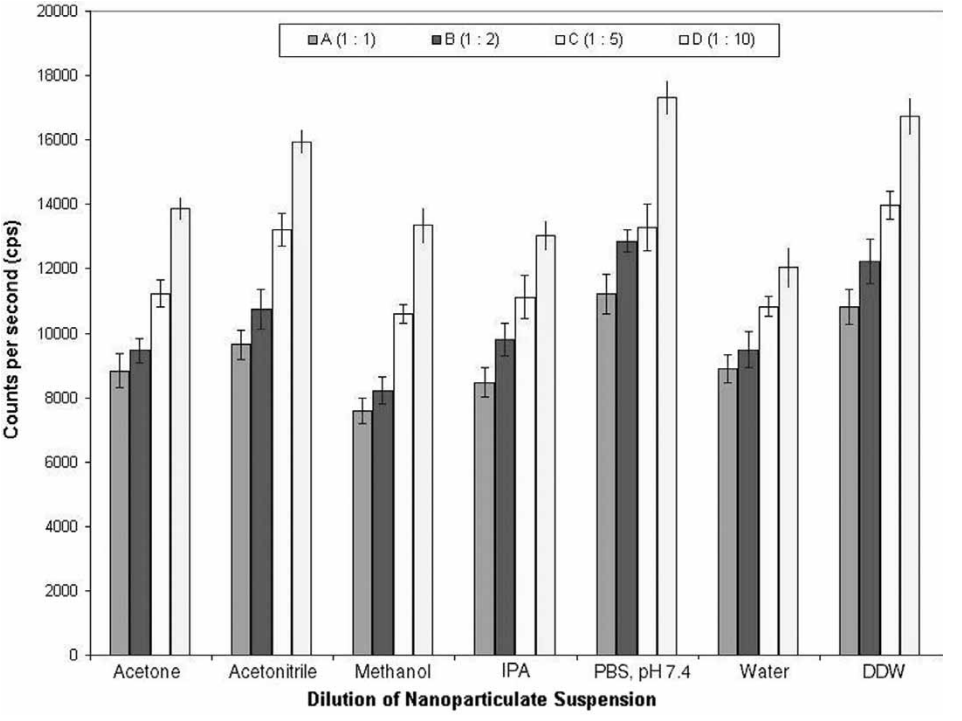

Figure 3. Effect of increasing dilution of nanoparticulate dispersions on counts per second.

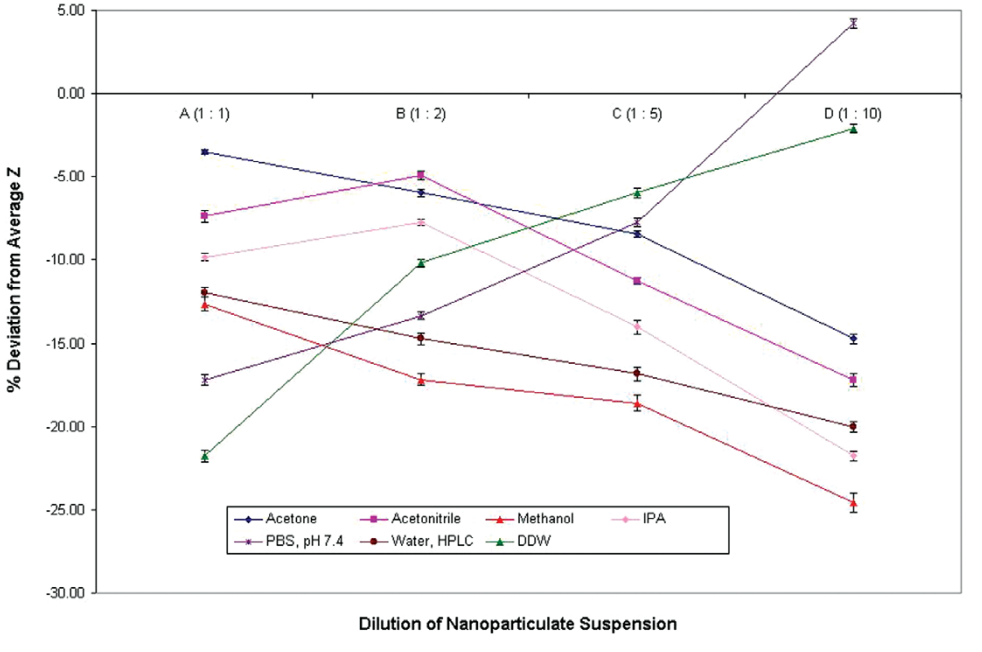

Figure 4. Effect of increasing dilution of nanoparticulate dispersions on % deviation from Z.

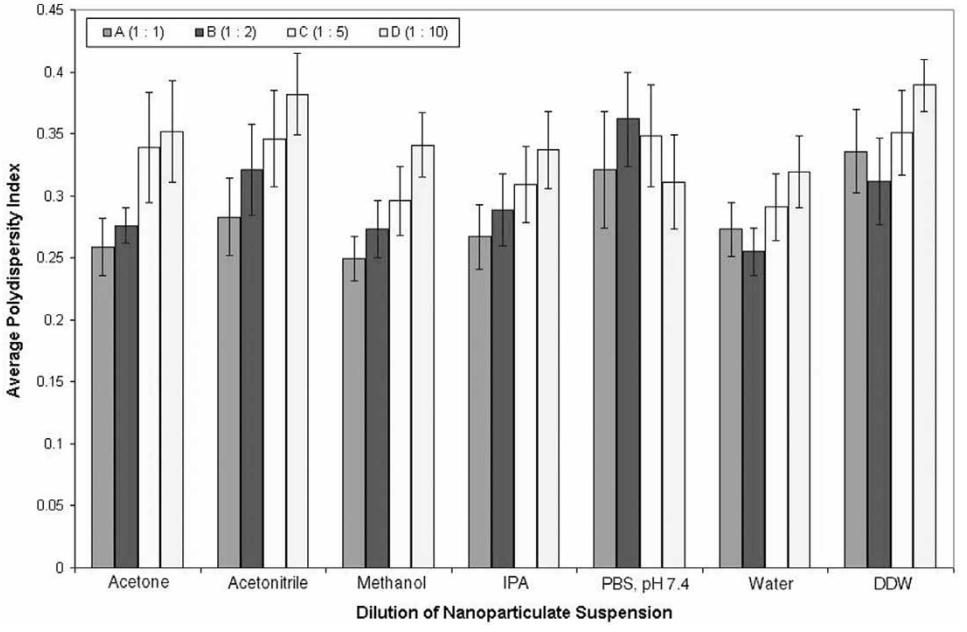

Figure 5. Effect of increasing dilution of nanoparticulate dispersions on P.I.

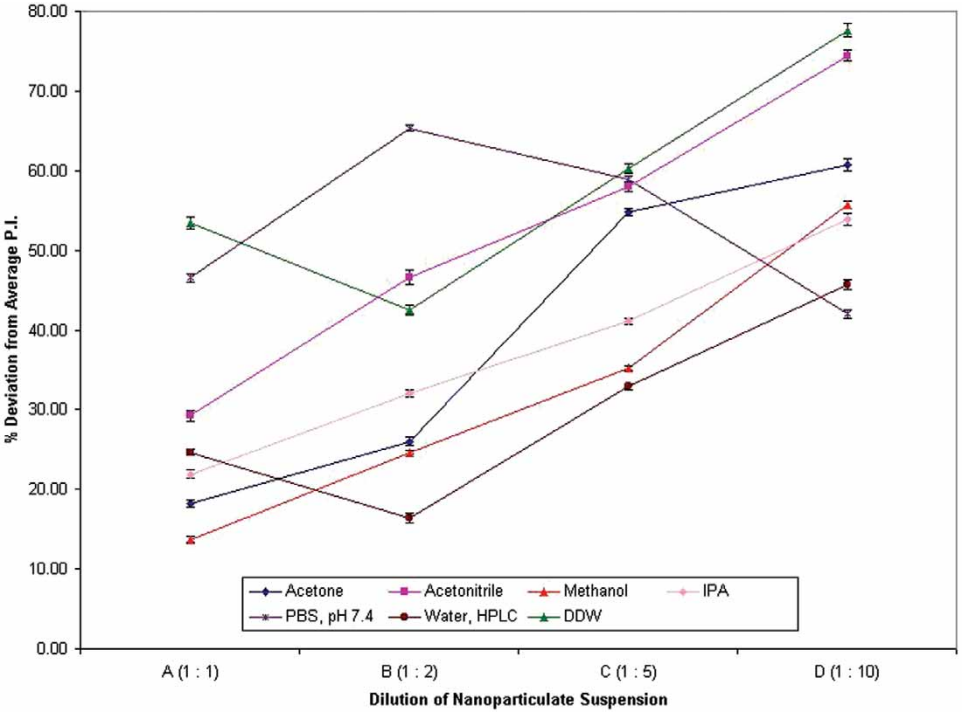

Figure 6. Effect of increasing dilution of nanoparticulate dispersions on P.I.

size of the polymeric nanoparticles and those of extraneous particles contributed by the solvent. However, the difference in size of the particles contributed from PBS and polymeric nanoparticles was small, which leads to the actual decrease in P.I. with increasing the dilution of nanoparticulate dispersions from 1:1 to 1:10 for PBS. Thus, more than 75% deviation from average P.I. suggests that the dilution of nanoparticulate dispersions should be given proper consideration.

It was also observed that when the particles counts per second (cps) are low (5000–7500 cps) at lower dilutions, the average particle size of the original suspension is shifted to lower values with increasing dilutions but at higher dilutions of the nanoparticles, the polydispersity was found to be higher because of significant contribution of very small nanoparticles by the solvent. At higher cps (25,000–35,000 cps) i.e. in concentrated nanoparticle dispersions, the contribution or interference by solvent generated nanoparticles is insignificant and the actual polymeric nanoparticles are primarily counted. However, the possibility of having false higher average particle size increase because of the aggregation or association of nanoparticles in concentrated suspension. So an optimum dilution may help in solving the problem of false higher or false lower particle size.

4. Conclusions

The solvent selection in nanoparticle preparation or characterization processes is most important and has a significant effect on the average particle size and polydispersity index of nanoparticles, depending upon the number of particles contributed by solvent (quality) and particles' counts per second (dilution). While the simple solvents contribute a smaller number of very small nanoparticles, the use of buffers (e.g. PBS, pH 7.4, etc.) as solvents for dilution may contribute significant number of larger nanoparticles and might shift the average nanoparticles size to higher values. Although the total cps of the blank solvents was low to determine the average particle size and polydispersity of these extraneous nanoparticles, they affect the outcome significantly when gets counted with polymeric nanoparticles in the characterization process. Based on the results, it can be concluded that highest purity grade (HPLC Grade) solvents, filtered through 0.45 μm membrane filters should be used in nanoparticles preparation and/or characterization process. This will avoid the introduction of extraneous nanoparticles from solvents and will have little or no effect on the actual particle size and P.I. of nanoparticles.

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