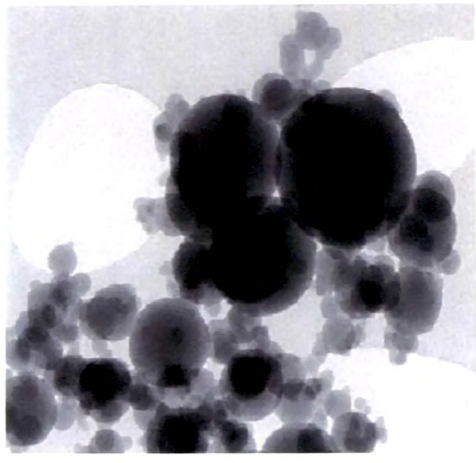
Chapter 5



PREPARATION AND CHARACTERIZATION OF NANOPARTICLES

5. PREPARATION & CHARACTERIZATION OF NANOPARTICLES

5.1 Introduction:

For the present work Nanoprecipitation (Solvent Diffusion, or Solvent Displacement) Method is adapted for prepararion of nanoparticles.

Typically, this method is used for hydrophobic drug entrapment, but it has been adapted for hydrophilic drugs as well. Polymer and drug are dissolved in a polar, water miscible solvent such as acetone, acetonitrile, ethanol, or methanol. The solution is then poured in a controlled manner (i.e. drop-wise addition) into an aqueous solution with surfactant. Nanoparticles are formed instantaneously by rapid solvent diffusion. Finally, the solvent is removed under reduced pressure (Mohanraj and Chen, 2006).

Important parameters to be considered are: polymer/surfactant ratio, polymer concentration, surfactant nature and concentration, solvent nature, viscosity, additives, active component, and phase injection (Bilati et al., 2005).

The present part of this chapter reports the preparation of Etoposide (ETN) and Quercetin dihydrate (QDN) nanoparticles using PLGA (50:50) as a nanocarrier polymer. Acetone is selected as water miscible organic solvent as it is the most common choice of solvent in nanoprecipitation methods and both the drugs exhibits good solubility properties in it. Poloxamer-407 (Lutrol F-127) is selected as a surfactant out of the three mostly used surfactants i.e. Poloxamer 407, Poloxamer 188 and PVA, as it formed the nanoparticles in the desirable size range with negative surface charge.

The characterization of nanoparticles was done by particle size analysis, zeta potential measurements and transmission electron microscopy.

The Effect of formulation variables such as amount of polymer, theoretical drug loading, surfactant concentration, aqueous and organic phase volumes was studied on nanoparticle synthesis. The conclusions were drawn on the basis of particle size measurements and entrapment efficiency determination.

5.2 Preparation of PLGA nanoparticles:

Thus Etoposide (ETN) and Quercetin dihydrate (QDN) nanoparticles were prepared by nanoprecipitation method. Initially, 100 mg of PLGA was dissolved with or without drug in 6 ml of polar, water miscible solvent acetone. This solution was then poured in a controlled manner (i.e. dropwise addition at a rate of 0.3ml/min) into 12 ml of 0.25% aqueous solution of Poloxamer-407. This addition was done with mild but constant stirring. The stirring was done by

a magnetic stirrer (Remi Equipments, India). Due to spontaneous diffusion of acetone into aqueous phase, an interfacial turbulence is created between the two phases that leads to formation of nanoparticles. Then the solvent is allowed to evaporate at room temperature with continuous stirring for 3-4 hours. The nanoparticles were then recovered by centrifugation (Sigma Centrifuge 3K30,Germany, 10,000 rpm, 20min, 2 cycles), washed twice with distilled water and lyophilized (Heto Drywinner, Denmark) with trehalose as cryoprotectant. The weight ratio used was 1:2 w/w for nanoparticles to trehalose. The dried nanoparticles were stored in the refrigerator at 4°C. Each batch was prepared in triplicate.

5.3 Optimization of Nanoparticle Formulations:

The Parameters considered for optimization were polymer concentration, surfactant concentration, organic phase volume, aqueous phase volume and theoretical drug loading. The Entrapment efficiency and the particle size were the response parameters for the optimization.

The weight of polymer PLGA $_{(50:50)}$ was varied at levels 50mg, 75mg, 100mg, and 200mg. Poloxamer 407 was used at various concentrations (0.1%, 0.25%, 0.5%, 0.75%) in the synthesis of nanoparticles and their effect on particle size and DEE was studied. The influence of aqueous to organic (W/O) phase volume ratio was studied at various levels 1.5, 2, 3, 4, and 5 to achieve optimum response parameters. Different theoretical drug loadings of ET and QD were incorporated (2.5%, 5.0%, 7.5%) in the nanoparticle formulations. The calculations of weight for QD were done by considering the dihydrate form of Quercetin. The above parameters were optimized individually for ET and QD for getting the particle size below 200nm with maximum DEE as presented in Table 5.1 and 5.2 respectively for ET and QD. Unless otherwise mentioned, all the experiments were conducted by varying one of the parameters while keeping all other parameters at a set of standard conditions which are mentioned in section 5.2 Preparation of PLGA nanoparticles. All the formulations were prepared in triplicate.

5.3.1 Particle Size and Particle Size Distribution measurement:

The particle size measurements were performed using Malvern Zetasizer Nanoseries (Malvern Instruments Ltd., Malvern, UK), which evaluates mean diameter and size distribution profiles along with Polydispersity indices (PDI) of NPs by Dynamic Light Scattering based on laser diffraction also known as Photon Correlation Spectroscopy (PCS). The instrument measures Brownian motion and relates this to size of particles. It does this by illuminating the particles with laser and analysing the intensity of fluctuations in the scattered light.

The nanoparticulate dispersions were suitably diluted in redistilled water so that the count rate of NPs lies in the range of 200 to 400 and measurements were done at 25°C. The data is presented in Table 5.1 and 5.2 and Figure 5.1 and 5.2 respectively for ET and QD.

The effect of optimization parameters which includes polymer concentration, aqueous phase volume, surfactant concentration, theoretical drug loading and organic phase volume on particle size of ETN and QDN is presented in Figures 5.3, 5.4, 5.5, 5.6 and 5.7 respectively.

5.3.2 Drug Entrapment Efficiency (%EE):

The amount of ET/QD entrapped in NPs was estimated by both direct and indirect methods.

In the direct approach, the entrapped drug was determined by suspending 10 mg of NPs in aqueous medium, and recovering the NPs pellet by centrifugation for 30 min at 20,000 rpm (Sigma 3K30, Germany). It was then dissolved in Acetonitrile and the solutions were analysed for ET and QD content in UV- Visible spectrophotometer (UV-Vis Pharmaspec, Shimadzu1700, Japan) at 283.5nm and 368.0nm respectively.

In the indirect method, the unentrapped ET/QD was analysed in the supernatant of the NP suspension collected in the centrifugation step (10,000 rpm, 20min, 2 cycles) before lyophilizaion by dissolving in Acetonitrile to assess the mass balance of the drug. The drug recoveries for ET and QD were calculated by using known amounts of ET and QD.

The DEEs for ET and QD were tabulated in the Table 5.1 and 5.2 respectively.

The effect of optimization parameters which includes polymer concentration, aqueous phase volume, surfactant concentration, theoretical drug loading and organic phase volume on DEEs of ETN and QDN is presented in Figures 5.3,5.4, 5.5, 5.6 and 5.7 respectively.

$$\text{Theoretical drug loading}(\%) = \frac{\text{Amount of drug used in the formulation}}{\text{Amount of Polymer used in the formulaion}} \times 100$$

$$\text{EE}\ (\%) = \frac{\text{Amount of drug in NPs}}{\text{Amount of drug incorporated in formulation}} \times 100$$

$$\text{DC}\ (\%\ \text{w/w}) = \frac{\text{Amount of drug in NPs}}{\text{Amount of NPs recovered}} \times 100$$

Table 5.1 Optimization of various parameters for ETN formulations

Parameters	Wt. of polymer (mg)	Aq. phase vol. (ml)	Surfactant conc. (%w/v)	Theo. drug loading (%)	Org. phase vol. (ml)	Particle size (nm)	EE (%)
Weight of polymer (mg)	**50**	12	0.25	2.5	4	147.6±4.5	33.86±2.4
	75	12	0.25	2.5	4	159.3±3.2	42.36±3.2
	100	12	0.25	2.5	4	167.6±2.8	61.45±1.3
	200	12	0.25	2.5	4	196.2±7.3	70.28±3.3
Aqueous Phase Volume (ml)	100	**10**	0.25	2.5	4	184.4±2.5	67.44±2.2
	100	**12**	0.25	2.5	4	168.7±5.4	63.39±0.7
	100	**16**	0.25	2.5	4	153.2±4.3	45.33±1.8
	100	**20**	0.25	2.5	4	148.3±4.1	32.75±4.6
Surfactant Conc. (% w/v)	100	12	**0.10**	2.5	4	178.2±1.8	65.33±1.2
	100	12	**0.25**	2.5	4	165.4±3.2	62.85±3.2
	100	12	**0.50**	2.5	4	156.5±2.0	53.88±4.1
	100	12	**0.75**	2.5	4	148.8±2.2	44.64±2.3
Theoretical Drug Loading (%)	100	12	0.25	**0**	4	125.6±1.4	-
	100	12	0.25	**2.5**	4	164.5±2.5	64.33±2.2
	100	12	0.25	**5**	4	192.4±0.8	49.64±1.5
	100	12	0.25	**7.5**	4	217.4±3.2	34.5±3.6
Organic Phase Volume (ml)	100	12	0.25	2.5	**4**	167.3±3.6	65.42±2.6
	100	**12**	**0.25**	**2.5**	**6**	**153.4±4.2**	**63.88±1.5**
	100	12	0.25	2.5	**8**	144.8±2.4	45.72±3.6
	100	12	0.25	2.5	**10**	133.4±3.2	24.66±3.4

n = 3

***The banded row indicates the optimized batch for ETN**

Table 5.2 Optimization of various parameters for QDN formulations

Parameters	Wt. of polymer (mg)	Aq. phase vol. (ml)	Surfactant conc. (%w/v)	Theo. drug loading (%)	Org. phase vol. (ml)	Particle size (nm)	EE (%)
Weight of polymer (mg)	**50**	12	0.25	2.5	4	142.4±2.3	7.65±5.4
	75	12	0.25	2.5	4	154.2±1.8	17.38±4.4
	100	12	0.25	2.5	4	164.4±1.2	24.33±3.5
	200	12	0.25	2.5	4	182.1±4.6	37.18±3.6
Aqueous Phase Volume (ml)	100	**10**	0.25	2.5	4	177.7±3.5	28.02±5.3
	100	**12**	0.25	2.5	4	160.5±3.2	31.33±3.0
	100	**16**	0.25	2.5	4	148.4±6.2	17.36±4.2
	100	**20**	0.25	2.5	4	138.5±5.3	10.31±3.4
Surfactant Conc. (% w/v)	100	12	**0.10**	2.5	4	173.0±3.2	12.73±3.2
	100	12	**0.25**	2.5	4	163.7±4.0	24.33±2.6
	100	12	**0.50**	2.5	4	153.2±2.2	37.77±4.2
	100	12	**0.75**	2.5	4	169.5±4.2	34.21±3.4
Theoretical Drug Loading (%)	100	12	0.5	**0**	4	128.0±0.4	-
	100	12	0.5	**2.5**	4	155.4±1.5	38.34±2.8
	100	12	0.5	**5**	4	178.9±2.7	29.22±1.2
	100	12	0.5	**7.5**	4	194.6±3.2	25.82±2.3
Organic Phase Volume (ml)	100	12	0.5	2.5	**4**	158.3±2.8	36.64±1.2
	100	**12**	**0.5**	**2.5**	**6**	**148.6±1.6**	**41.36±1.93**
	100	12	0.5	2.5	**8**	140.5±3.2	39.68±1.4
	100	12	0.5	2.5	**10**	130.2±2.5	20.34±2.6

n = 3

***The banded row indicates the optimized batch for QDN.**

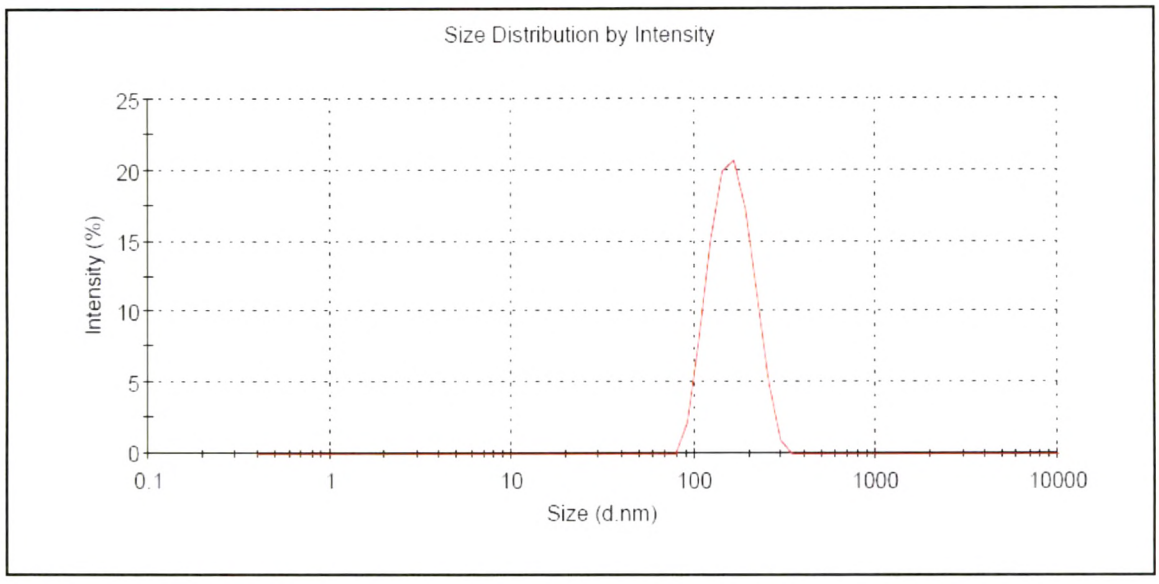


Figure 5.1 Typical particle size distribution curve of ETN formulation

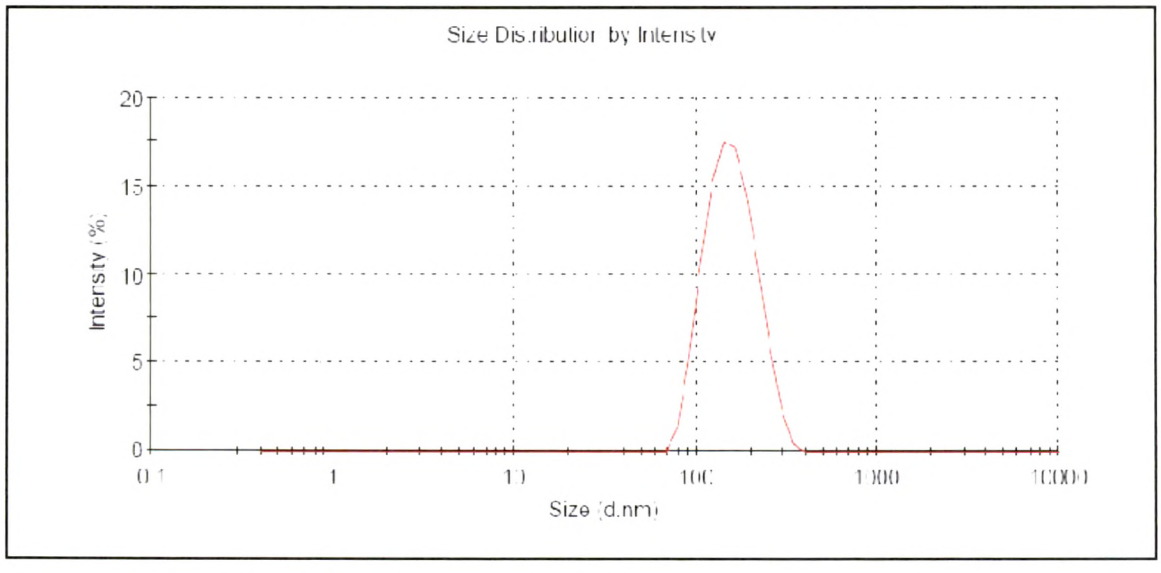


Figure 5.2 Typical particle size distribution curve of QDN formulation

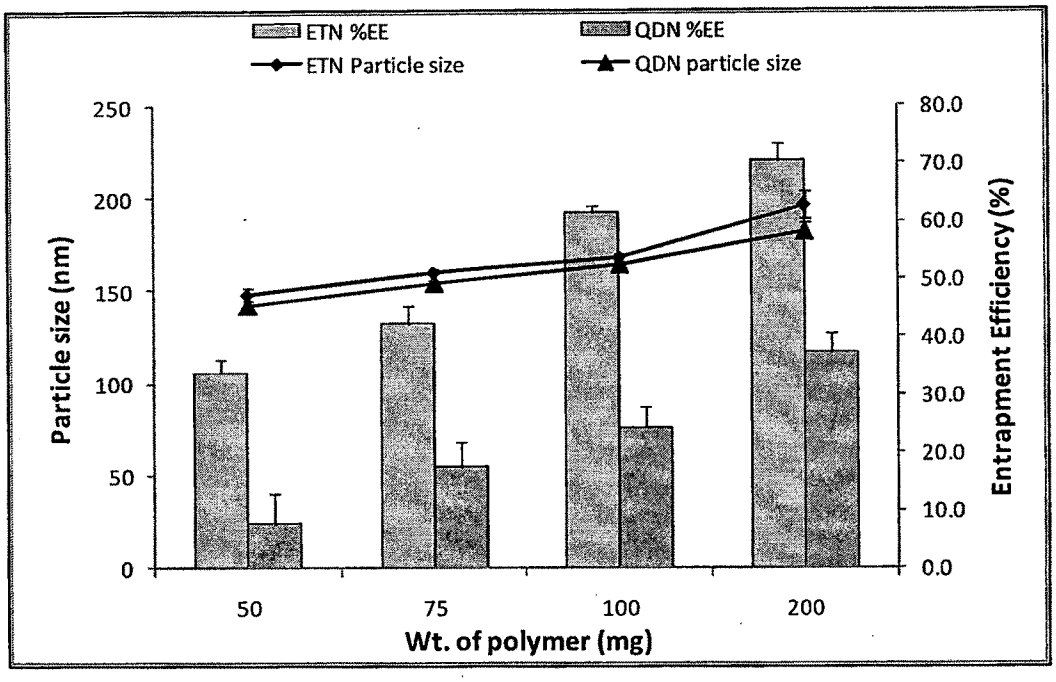


Figure 5.3 Effect of amount of polymer on particle size and DEE of ETN and QDN

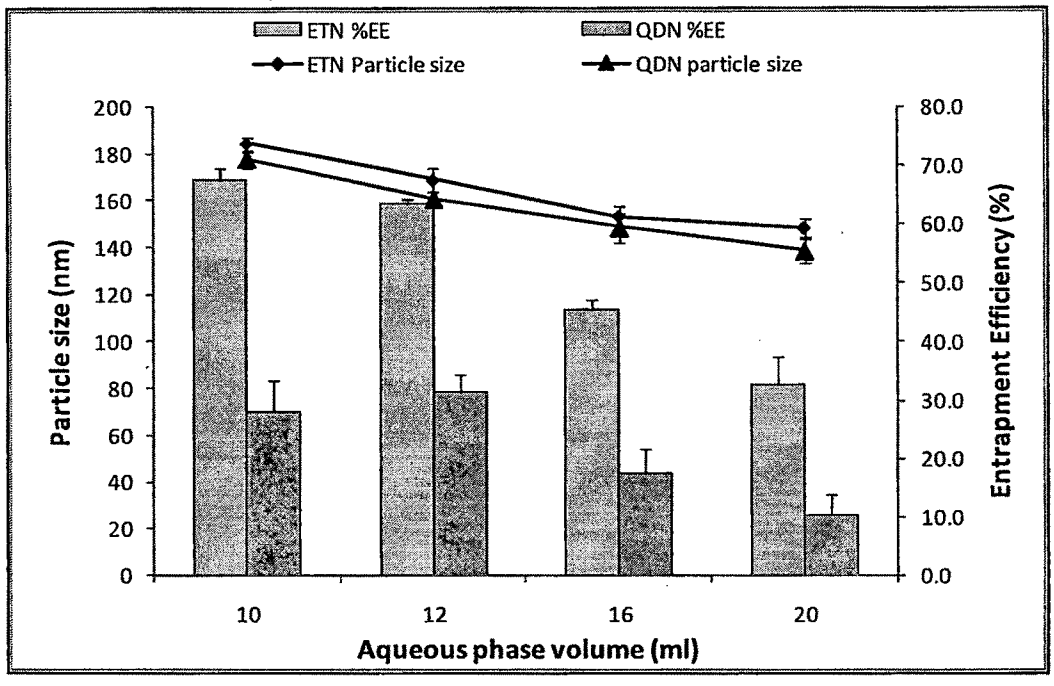


Figure 5.4 Effect of aqueous phase volume on particle size and DEE of ETN and QDN

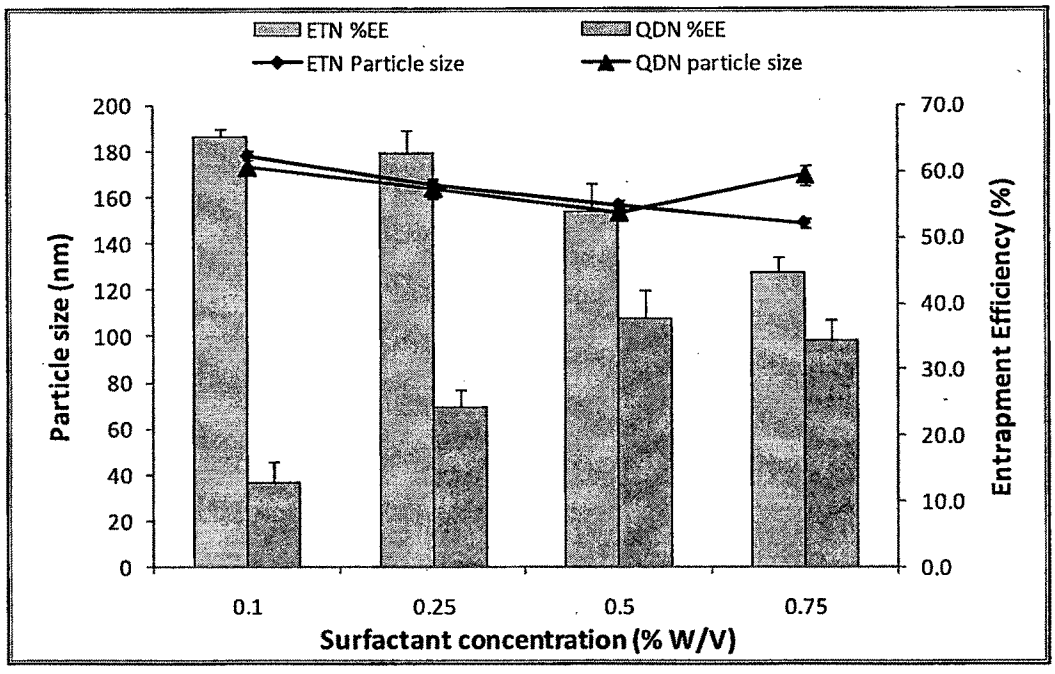


Figure 5.5 Effect of surfactant concentration on particle size and DEE of ETN and QDN

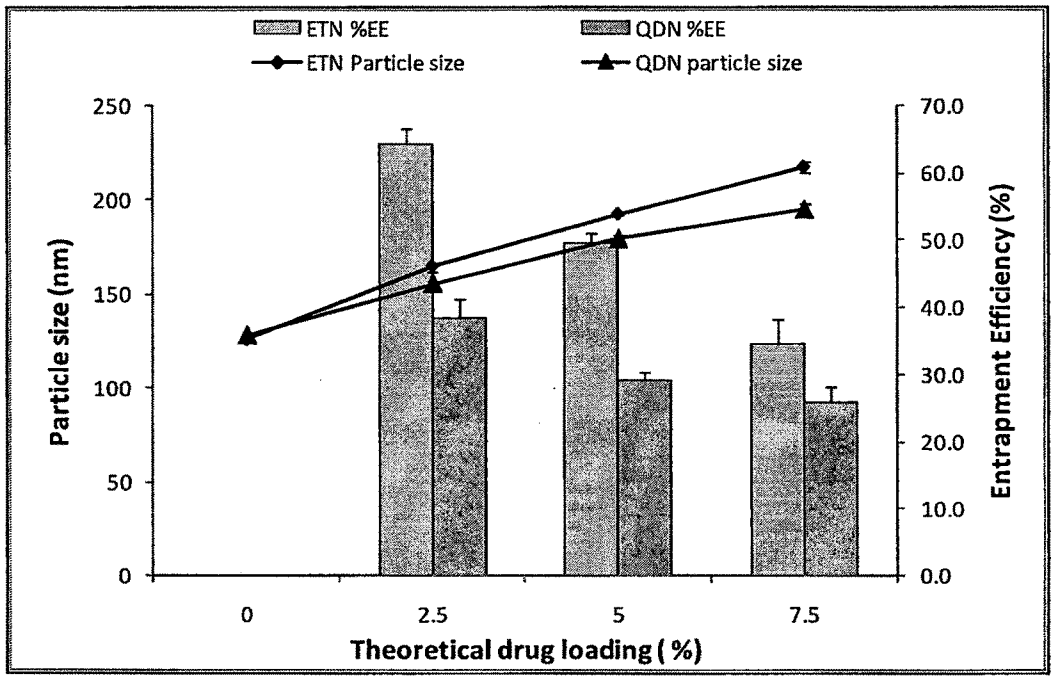


Figure 5.6 Effect of Theoretical drug loading on particle size and DEE of ETN and QDN

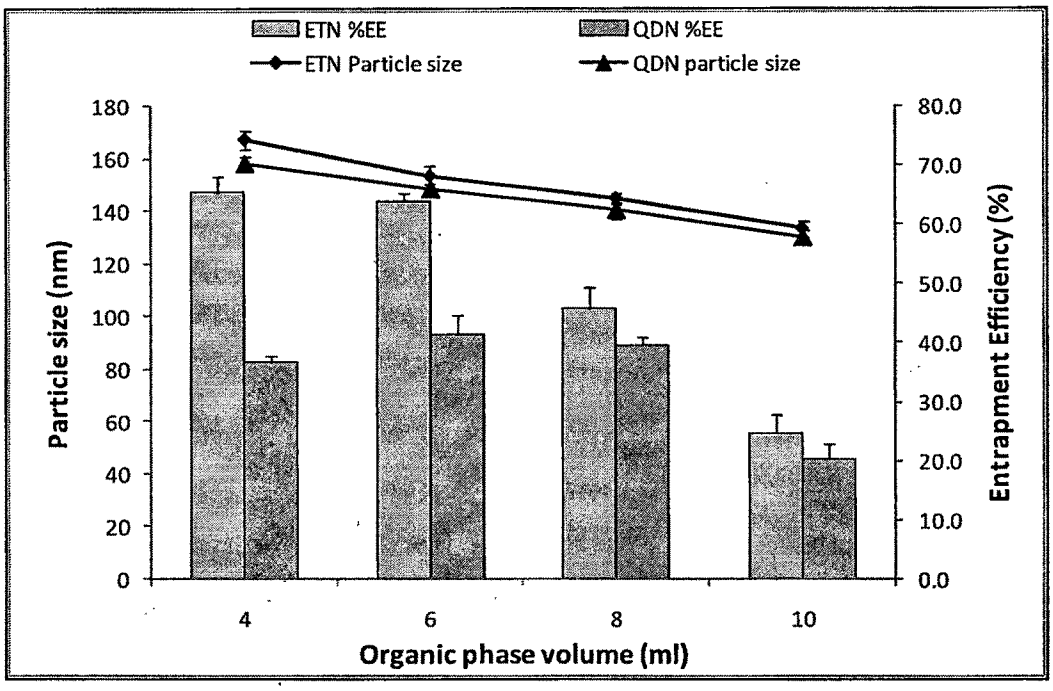


Figure 5.7 Effect of organic phase volume on particle size and DEE of ETN and QDN

5.4 Characterization of nanoparticle formulations:

5.4.1 Zeta Potential measurements:

Zeta potential of NPs was measured in redistilled water in Malvern Zetasizer Nanoseries (Malvern Instruments Ltd., Malvern, UK) at 25°C following the same dilution as for size measurement. Each measurement was done in triplicate. The Zeta potential reports were presented in Table 5.3 and Figure 5.8 and 5.9 for ETN and QDN respectively.

5.4.2 Transmission Electron Microscopy (TEM):

The morphology of the ETN and QDN was examined by Transmission Electron Microscope (Philips Tecnai-20, Holland). Before analysis, NPs were dispersed in de-ionized distilled water and one drop of the diluted dispersion was placed on 200-mesh carbon coated copper grid for observation. 2% uranyl acetate was used as staining agent for nanoparticles. The TEM images of ETN and QDN were shown in Figure 5.10 and 5.11 respectively.

5.4.3 Differential Scanning Caloriemetry (DSC):

The physical state of ET and QD loaded in NPs was investigated by DSC (Mettler Toledo, Switzerland). About 5 mg of sample of freeze-dried nanoparticles i.e. ETN and QDN was heated from 50°C to 350°C at a heating rate of 10°C/min (Figure 5.12 and 5.13). The DSC thermograms were also obtained for drugs ET and QD, $PLGA_{(50:50)}$ and plain NPs which served as controls (Figure 5.14).

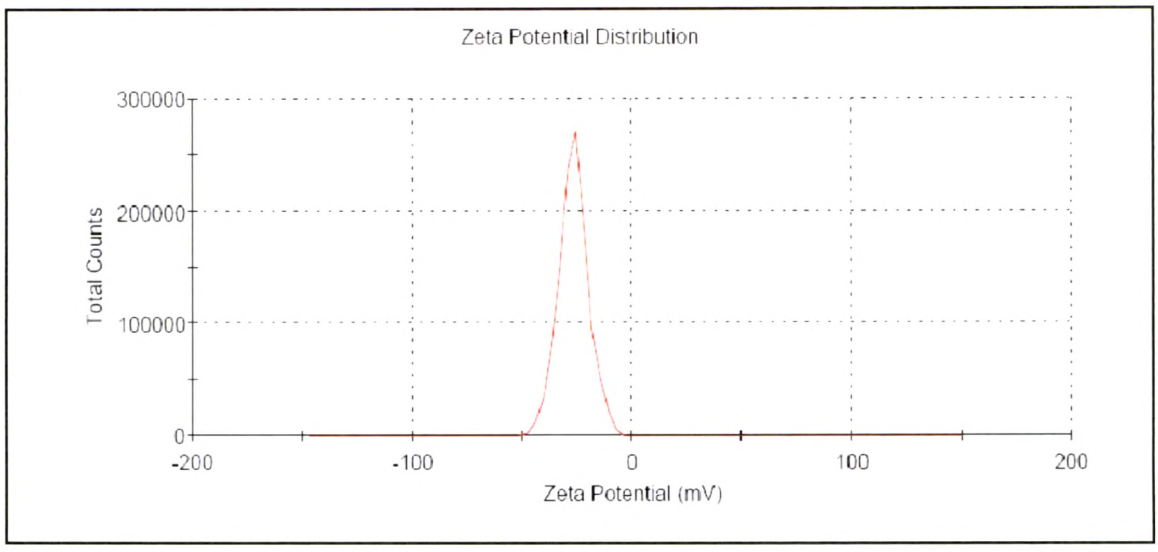


Figure 5.8 Typical zeta potential curve of ETN formulation

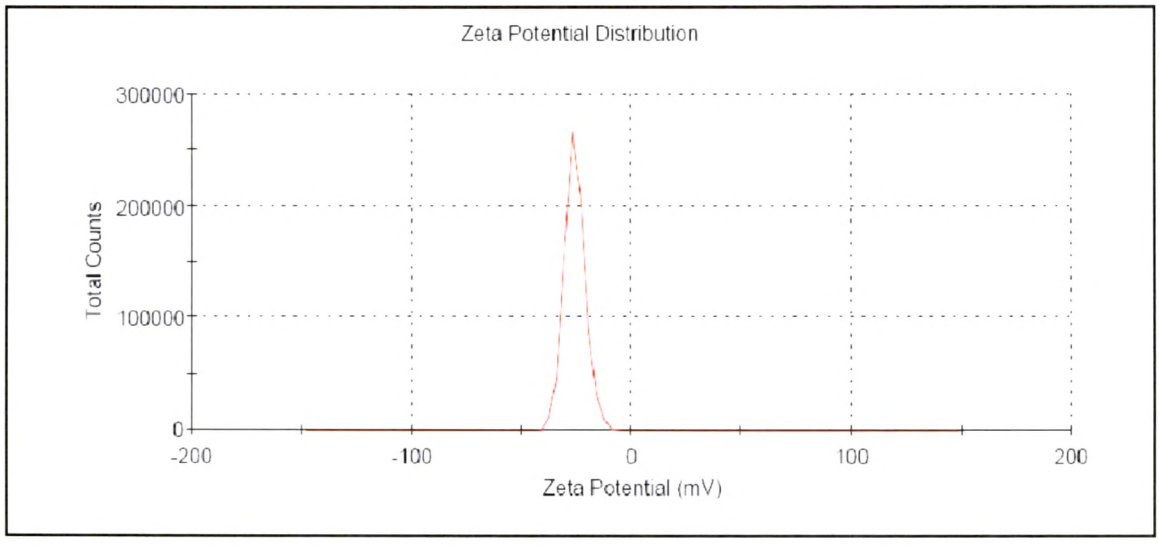


Figure 5.9 Typical zeta potential curve of QDN formulation

Table 5.3 Zeta potentials of optimized nanoparticle formulations

Nanoparticles	Zeta potential (mV)
PN	-30.2±0.6
ETN	-26.4±1.9
QDN	-27.0±0.7

n = 3

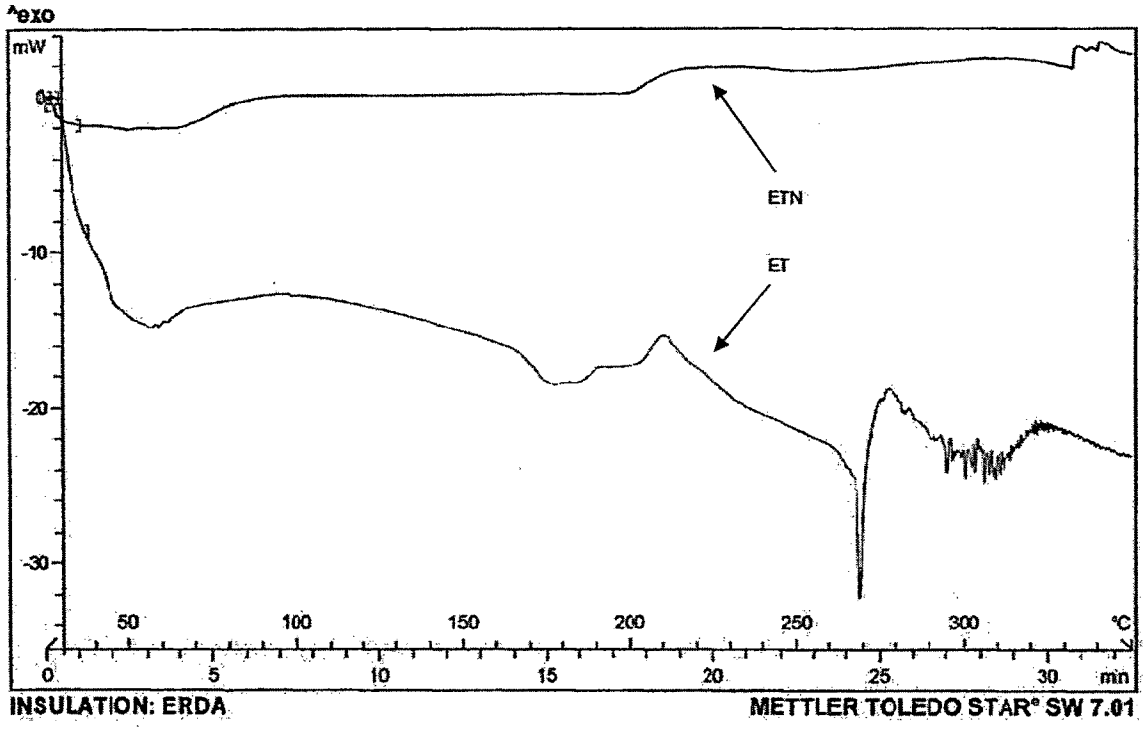


Figure 5.12 DSC curves of ET and ETN

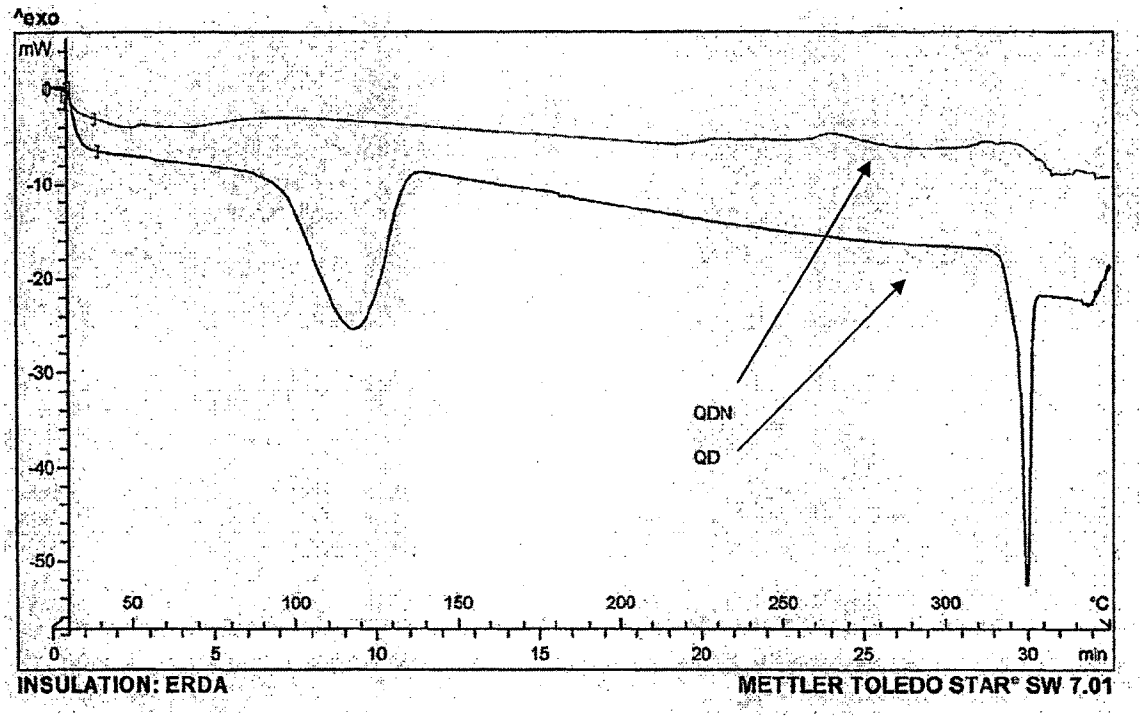


Figure 5.13 DSC curves of QD and QDN

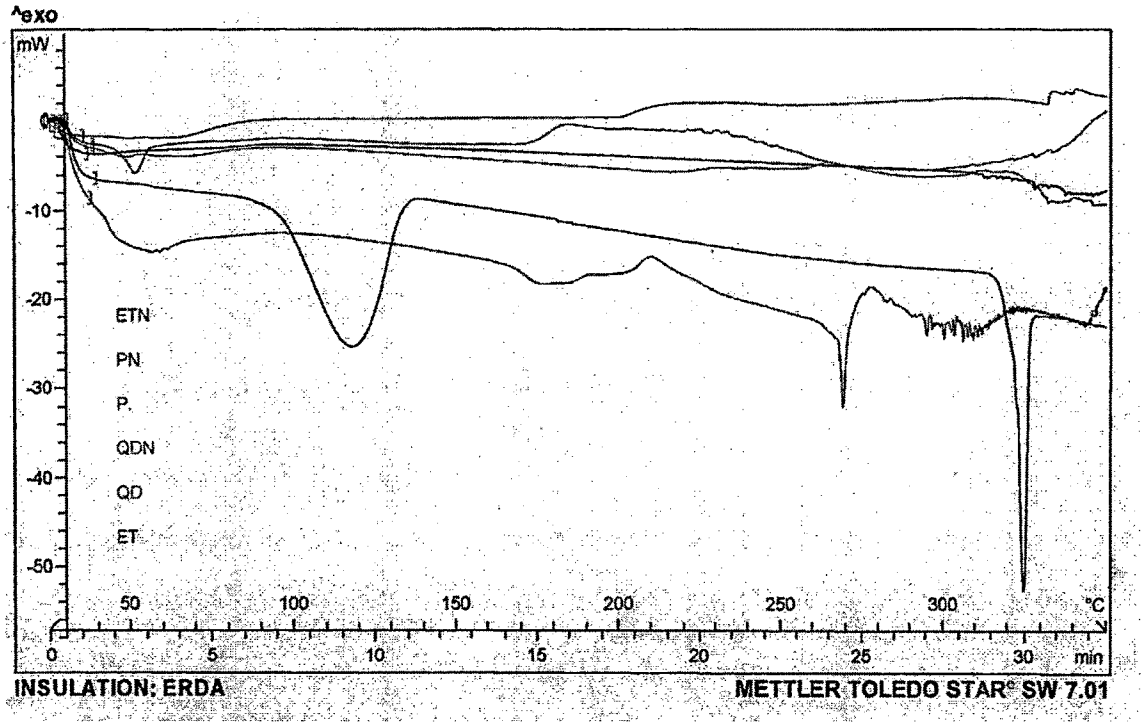


Figure 5.14 DSC curves of P, PN, ET, ETN, QD, and QDN
*** The DSC curves are shown in sequence as they are appearing in the centre of figure from top to bottom**

5.5 Stability studies:

Stability of a pharmaceutical formulation is defined as the capability of the particular product in a specific container to remain within its physical, chemical, therapeutic, microbiological, and toxicological specifications. Stability of NPs formulations is of great concern in the development of marketed preparations. The prepared formulations, ETN and QDN were tested for stability by storing them in amber coloured glass vials separately, at refrigerating temperature 2-8°C in dark and at room temperature 25±2°C at room light conditions for a period of one month and three months, followed by evaluation for particle size and drug content parameters.

5.5.1 Effect of storage conditions on particle size:

Size of NPs was determined after one month and three months by dispersing the lyophilized formulations in redistilled water using Malvern Zetasizer Nanoseries (Malvern Instruments Ltd., Malvern, UK). The results are presented in Table 5.4.

5.5.2 Effect of storage conditions on Drug content:

The samples were withdrawn at one and three month time intervals from the storage containers and analysed for the drugs ET and QD by the same procedure as explained in the section 5.3.2. The results of this experiment are presented in Table 5.5.

Table 5.4 Effect of storage conditions on the particle size of ETN and QDN

Formulations	Particle size (nm) (mean ± SD)				
	Initial Particle size	2-8°C		25±2°C	
		After 1 Month	After 3 months	After 1 Month	After 3 months
ETN	155.4±1.9	156.5±1.8	157.8±1.2	159.2±4.2	163.5±1.3
QDN	146.4±2.4	148.2±0.5	149.5±0.8	151.7±2.5	153.5±0.6

n = 3

Table 5.5 Effect of storage conditions on the drug content of ETN and QDN

Formulations	%Drug content preserved (mean ± SD)				
	*Initial Drug content (%)	2-8°C		25±2°C	
		After 1 Month	After 3 months	After 1 Month	After 3 months
ETN	100	99.13±0.82	98.01±0.24	98.32±0.2	95.52±0.6
QDN	100	98.83±0.46	97.28±0.66	97.75±0.45	94.72±0.8

n = 3

*** Initial drug content was considered 100% and compared to this the drug content for further time period is calculated.**

5.6 Results and discussion:

5.6.1Preparation of PLGA nanoparticles:

Etoposide and Quercetin dihydrate loaded PLGA nanoparticles (ETN and QDN) were prepared successfully by nanoprecipitation method as explained in section 5.2.

5.6.2 Optimization of Nanoparticle Formulations:

In this study, the effect of five preparation variables on the mean diameter and entrapment efficiencies of ETN and QDN was investigated as shown in Table 5.1 and 5.2 for ETN and QDN respectively.

The mean diameter of all formulations increased as the PLGA concentration is increased from 50mg to 200mg. Figure 5.3 shows that the mean diameter of NPs increased dramatically with the increase of PLGA concentration. Increasing PLGA concentration led to increase in the viscosity of the organic phase, hence reducing the net shear stress and promoting the formation of larger size droplets. In addition, the increasing viscosity could hinder rapid dispersion of PLGA solution into the aqueous phase, resulting in larger droplets which formed larger NPs after evaporation of the organic solvent. Moreover, with the increase of the amount of PLGA, the surfactant was probably insufficient to cover the surface of droplets completely, which might have caused the coalescence of droplets during the evaporation step and subsequent aggregation of NPs.

Figure 5.3 also shows the effect of PLGA concentration on the entrapment efficiencies of both drugs. The EE of ET increased significantly ($p < 0.05$) with the increase of PLGA concentration while the EE of QD though shows same trend but is less as compared to ET.

Increase in EE has probably resulted from the increase of viscosity. Increasing viscosity could increase the drugs resistance to diffuse into the aqueous phase and enhance the drugs incorporation into NPs. Additionally, larger NPs had higher DEEs.

From the results the optimized amount of polymer was found to be 100mg as it produced the NPs with optimum size range and with good EEs.

The aqueous phase volume (containing the 0.25% Poloxamer-407) was varied from 10 to 20ml, whereas the organic phase volume was kept constant. It was observed that the NP size of ETN as well as QDN decreased as the aqueous phase volume increased which is clearly indicated by data in Table 5.1, 5.2 and Figure 5.4. Increasing the aq. phase volume reduced the organic phase viscosity and resulted in smaller size NPs. In addition to this, the amount of surfactant increased as aq. phase volume increased, resulting in reduction of interfacial tension and thus decreasing the NP size.

The mean EEs of these two drugs decreased dramatically with the increase in aq. phase volume. This happened due to the amount of drugs partitioned into the organic phase reduced during mixing of aq. and organic phases. The drug loss still increased during solvent evaporation as the W/O volume ratio increased. This effect is more pronounced in case of QD. When W/O volume ratio increased, the amount of QD dissolved in the aqueous phase increases, resulting in less QD retention in the organic phase to interact with PLGA molecules and then lower EE of QD. This phenomenon is responsible for the lower EEs of QD as compared to ET.

From the data, the optimized **aqueous phase volume** was selected as **12ml** depending on the response variable.

The surfactant (Poloxamer-407) concentrations i. e. 0.1, 0.25, 0.5 and 0.75% W/V were tested to assess its effect on formulation characteristics. Figure 5.5 shows that the mean diameter decreased with increasing surfactant concentration. At higher concentration, more molecules of surfactant can align themselves at interface to reduce the interfacial tension efficiently, which resulted in significant increase in shear stress at constant external energy input, hence promoted the formation of smaller particles (Galindo-Rodriguez et al., 2004). However, with the increase in surfactant concentration, the viscosity of aqueous phase increases, which would result in size increase due to decrease in net shear stress. For ET, the NP size decreased with increase in concentration of surfactant due to dominance of former phenomenon. But in case of QDN, when surfactant concentration was increased above 0.5% W/V, the size increased as depicted in Figure 5.5. This may be due to dominance of the later phenomenon over the former above this concentration of surfactant.

Similarly the EE of ET decreased significantly with the increase in surfactant concentrationas shown in Figure 5.5. That was probably caused due to decrease in particle size.

The trend exhibited by QD (Figure5.5) can be explained by the behaviour of QD in different surfactant concentration. It was observed that, at lower concentration, the QD comes out of both phases and precipitate as the evaporation of organic solvent progresses, resulting in very low EE of 12.73±3.2% but the increase in surfactant concentration upto 0.5%W/V maintains the QD in a state to interact with PLGA sufficiently , thus increasing the EE to 37.77±4.2%. But if the surfactant concentration is increased beyond this level, the EE decreases because the high concentration of surfactant can increase the solubility of QD in aqueous solution.

Therefore, less QD molecules remained in the organic phase to interact with PLGA molecules when both phases were mixed.

The optimized **Poloxamer-407 concentration** was found to be **0.25%W/V for ETN** and **0.5%W/V for QDN.**

In NP formulations, the theoretical drug loading was varied from 0% to 7.5% and significant differences were observed in the size of the ETN and QDN. Increased drug loading resulted in a more viscous organic phase, thus an increase in the size of NPs was observed. In the present investigation, the low amounts of drug loading were found optimum. When theoretical drug loading was increased from 2.5% to 7.5%, the EE was greatly affected (Figure 5.6). Addition of excessive quantities of drugs during the nanoprecipitation process causes more drug loss due to limited drug entrapment capacity of the polymer, hence EES decreased accordingly.

From the obtained data, **2.5% theoretical drug loading** was considered optimum for both the drugs, ET and QD, since it produced the desired NP size.

Figure 5.7 shows the effect of organic phase volume on the particle size of ETN and QDN as well as on the EEs of ET and QD. The increase in the organic phase volume from 4ml to 10ml leads to decrease in particle size and subsequent decrease in EEs. Particle size reduction was observed due to decrease in the viscosity of system associated with more organic phase volume, leading to formation of smaller particles. Decrease in the EEs is caused due to increase in the cosolvency effect of organic solvent for the drugs, thus enhancing the aqueous phase concentrations of drugs, while lower drug concentration available for interaction with PLGA.

The optimum **organic phase volume** was noted at a level of **6ml** for both, ETN and QDN formulations.

5.6.3 Particle Size, Size Distribution and %DEE Measurement:

From the optimized conditions, the formulations, ETN and QDN, were prepared for further studies. Here onwards, ETN and QDN will refer to the optimized nanoparticle formulations of Etoposide and Quercetin dihydrate respectively.

The optimized ETN and QDN formulations exhibited mean diameters **153.4±4.2nm** and **148.6±1.6nm** respectively and corresponding drug EEs were found as **63.88±1.5%** and **41.36±3.4%.** The size distribution curves for ETN and QDN were represented in Figure 5.1 and 5.2 respectively. Both these curves reveal a narrow size distribution and monodisperse unimodal systems which is also reflected by the polydispersity indices (PDI) i.e. **0.058±0.02** for ETN and **0.088±0.03** for QDN.

5.6.4 Zeta Potential Measurement:

The zeta potential of nanoparticles is commonly used to characterise the surface charge property of the nanoparticles. The zeta potential is an important parameter when considering the stability of the nanoparticles in vitro. The more negative or positive values of zeta potential are related to more stable particles; more repulsion between particles reduce the particle aggregation.

Figure 5.8 and 5.9 depicts the zeta potential curves for ETN and QDN. The zeta potentials measured in redistilled water for optimized batches were presented in Table 5.3

The zeta potential of drug free nanoparticles (PN) was on higher negative side whereas the incorporation of ET and QD resulted in slight decrease in zeta potentials. The negative zeta potentials are beneficial in drug delivery and prolonging the circulation time property.

5.6.5 Transmission Electron Microscopy:

The morphological studies performed by TEM, indicated uniform and spherical shape, discrete particles without aggregation, and appear to be smooth in surface morphology with nano size range. The TEM micrographs (Figure 5.10 and 5.11) show the diameters **133.65nm** for **ETN** and **113.14nm** for **QDN**. These diameters were not consistent with that determined with PCS. This was mostly because of the difference in the mechanisms of the two methods. The TEM is based on diffraction technique and the PCS measures hydrodynamic radius based on scattering. The size measurement of NPs by PCS was carried out in aqueous media, thus the NPs were highly hydrated and the diameters determined by PCS were 'hydrated diameters' which are usually larger than their genuine diameters whereas sizes derived from TEM might be considerably smaller than their real diameters.(Song X et al., 2008)

5.6.6 Differential Scanning Caloriemetry:

DSC thermograms of free ET and QD as well as ETN and QDN formulation were obtained to define the physical state of the drugs in NPs.

Figure 5.12 shows thermograms for ET and ETN. DSC of ET has revealed evidence of thermal events in the range of 190-210°C, and at 268°C prior to decomposition at about 290°C. The exotherm at 206°C suggested the conversion to a different crystalline form. Further heating to 350°C revealed endotherm at 268°C which was attributed to the melting point of the newly formed etoposide (Bhaskara et al., 1995). But ETN had not shown such exothermic and endothermic peaks, indicating that ET formulated in NPs existed as an amorphous state or a solid solution.

Figure 5.13 shows thermograms for QD and QDN. DSC of QD revealed endotherms at 117.31°C and 322.32°C corresponding to a dehydration reaction and the melting process respectively. Both these endothermic peaks are completely disappeared in the thermogram for QDN. These results suggested that the drug was dispersed throughout the polymer forming a high-energy amorphous state.

Figure 5.14 shows the thermograms for P, PN, ET, ETN, QD, and QDN. These curves support the entrapment of the drugs in the polymer

5.6.7 Stability studies:

The stability conditions at which the ETN and QDN formulations were studied, storage at 2-8°C resulted in formulations which remained stable with only slight change in particle size after three months and also retained EE in limits and excellent redispersibility as compared to storage at 25±2°C (Table 5.4 and 5.5).

5.7 Conclusion:

The study demonstrates that the nanoprecipitation (solvent diffusion) technique followed by lyophilisation is a viable technique to obtain stable NPs with mean particle size of about 150nm and good entrapment efficiencies (63.88±1.5 for ETN and 41.36±1.93 for QDN). The particle size and %EE was influenced by formulation variables, which needs to be optimized. The ETN and QDN with good stability properties at refrigerated conditions (2-8°C) for a period of 3 months were obtained, thus formed a basis for further studies like *in-vitro* release and cell cytotoxicity studies and also *in-vivo* studies.

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