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Preparation of PLGA nanoparticles using TPGS in the spontaneous emulsification solvent diffusion method

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D-alpha-tocopheryl poly (ethylene glycol) 1000 succinate (TPGS) is a widely used form of vitamin E that has been used as a solubilizer, an emulsifier and as a vehicle for drug delivery formulations. In this study, poly lactide-co-glycolide (PLGA) nanoparticles were prepared by spontaneous emulsification solvent diffusion (SESD) method. TPGS as an emulsifier and further as a matrix material blended with PLGA was used to enhance the encapsulation efficiency and improve the drug release profile of nanoparticles. Rifampicin and estradiol valerate were used as model drugs with different water solubility. The effect of formulation parameters such as drug/polymer ratio, oil phase combination, volume and surfactant content was evaluated. The surface morphology and size of the nanoparticles were studied by scanning electron microscopy (SEM) and laser light scattering. Drug encapsulation efficiency and *in vitro* drug release profiles of nanoparticles were determined using high performance liquid chromatography (HPLC). The nanoparticles prepared in this study were spherical with size range of 150–250 nm. It was shown that TPGS was a good emulsifier for producing nanoparticles of hydrophobic drugs and improving the encapsulation efficiency and drug loading and drug release profile of nanoparticles. However, the drug loading efficiency of rifampicin, a slightly water-soluble molecule, was significantly lower than that of estradiol valerate, a water insoluble molecule.

Keywords: TPGS; Nanoparticles; Spontaneous emulsification solvent diffusion; Rifampicin; Estradiol valerate

1. Introduction

The spontaneous emulsification solvent diffusion (SESD) has been previously used for the preparation of biodegradable nanoparticles [1]. In this method nano-sized particles of PLGA or PLA (poly lactide) can be effectively produced by pouring the polymeric

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organic solution into an aqueous phase with mechanical stirring. In this method a binary mixture of a water-miscible organic solvent such as acetone and a water-immiscible solvent such as dichloromethane as the solvent of the polymer is used for the preparation of nanoparticles. The nanoparticles are then formed via an emulsification process followed by the subsequent solvent evaporation process.

In such a process, a number of fabrication parameters can affect the nature of the nanoparticles obtained. One of the most important parameters is the type and quantity of the emulsifier. The emulsifier stabilizes the dispersed-phase droplets formed during emulsification, inhibits coalescence of droplets and determines the particle size, size distribution, the morphological and the release properties of the nanoparticles [2]. PLGA micro-/nano-particles are usually prepared by using chemical emulsifiers such as poly vinyl alcohol (PVA) [3, 4]. However, PVA has been found to have some disadvantages including low emulsification efficiency and difficulties to wash away in the formulation process [5]. Instead, TPGS has been reported to have high emulsification efficiency several times higher than PVA [6]. TPGS is a succinyl derivative of vitamin E that differs from other vitamin derivatives in that TPGS itself does not act as an antioxidant [7]. It can also greatly improve the drug encapsulation efficiency, and enhance cellular uptake of nanoparticles and thus increase the cancer cell mortality [6]. TPGS is a water-soluble derivative of natural vitamin E with its hydrophile–lipophile balance being approximately 13 [5]. The chemical structure of TPGS is similar to other amphiphile molecules comprising lipophilic alkyl tail and hydrophilic polar head portions. Its bulky structure and large surface area characteristics make it an excellent emulsifier. Moreover, it has been found that co-administration of TPGS with anti-cancer drugs could enhance cytotoxicity, inhibit P-glycoprotein mediated multi-drug resistance, and increase the oral bioavailability of anticancer drugs [6].

When choosing emulsifiers for the preparation of particulate systems, several factors should be taken into account. It is highly important to use excipients that are either approved for human use or endogenous to the human body for the development of such particulate systems. The effect of the emulsifier on different properties of particles such as morphology, surface composition, drug encapsulation capability, drug release kinetics should be also considered [8].

Feng *et al.* demonstrated that TPGS has great advantages for the manufacturing of polymeric nanoparticles for controlled release of paclitaxel as an anti-cancer drug by using the solvent evaporation/extraction technique [8]. They produced nanoparticles with a particle size range of 300–1000 nm with narrow distribution and encapsulation efficiency as high as 100%. They also reported that TPGS was an excellent emulsifier with few side effects for human health with much higher emulsification efficiency in nanoparticle formulation and *in vitro* release kinetics in comparison with PVA [8].

The present work investigated the use of TPGS as emulsifier and further the possibility of applying TPGS as a matrix material blended with PLGA for the preparation of nanoparticles containing estradiol valerate or rifampicin as model drugs with different water solubility. The size of the colloidal carriers is a key factor for the biological fate of the nanoparticles. Nanoparticles of smaller than 100 nm can bypass the mononuclear phagocyte system macrophages uptake. Therefore the effect of TPGS on the size of nanoparticles was also investigated.

2. Materials and methods

2.1. *Materials*

Rifampicin (USP 27) and estradiol valerate (USP 27) were donated from Alhavi Pharma Co, Iran and Abureihan Pharma Co, Iran respectively. PLGA (50 : 50, Resomer® RG 504 H, MW 48000) was purchased from Bohringer Ingelheim, Germany. TPGS NF grade (MW 1513) was purchased from Curtec, Holland. PVA (MW 22000) was obtained from Sigma-Aldrich, USA. Dichloromethane (DCM) and acetone (analytical grade) were purchased from Merck, Germany. Acetonitrile and methanol used as mobile phase in HPLC was purchased from Merck, Germany. Deionized water was used throughout the experiment. The *in vitro* release measurement was carried out at pH 7.4 at 37°C in phosphate buffer medium. All other chemicals used were of reagent grade.

2.2. *Methods*

2.2.1. Preparation of nanoparticles. Nanoparticles were prepared using spontaneous emulsification solvent diffusion method [1]. Briefly, known amounts of polymer and drug were added into the mixture of DCM/acetone (in the case of rifampicin), and DCM/acetone/ethanol (in the case of estradiol valerate) and stirred for 15 minutes to ensure that all material was dissolved. This solution of organic phase was slowly poured into an aqueous solution containing emulsifier using a high speed homogenizer (T 18 basic ULTRA-TURRAX, IKA, USA) at 14000 rpm for 5 min. Stirring continued for 12 h to allow for the evaporation of the internal phase. The polymer was then precipitated and the nanoparticles were isolated by using a centrifuge (Sigma 3K30, Sigma, Germany) at 21,000 g for 15 min and washed trice with deionized water. The suspension was then freeze-dried for 48 h (Lyotrap Plus, LTE Scientific Limited, UK) to obtain a fine powder of nanoparticles, which was then kept in a desiccator.

2.2.2. Encapsulation efficiency. The drug content of nanoparticles was determined by HPLC method. A reversed phase C_{18} Column (25×0.46 cm, pore size 5 μm) (Teknokroma, Spain) was used. The mobile phase for rifampicin consisted of a mixture of methanol, phosphate buffer (pH 5.2) and acetonitrile (50 : 33 : 17 by vol.) delivered at a flow rate of 1.00 ml/min with a pump (WellChrom K-1001, Knauer, Germany). The mobile phase for the measurement of estradiol valerate was a mixture of acetonitrile and ammonium nitrate buffer (70 : 30 by vol.) delivered at a flow rate of 2 ml/min. Analysis for each drug was done separately. A 20 mg sample of nanoparticles was dissolved in 1 ml of acetonitrile followed by the addition of 2 ml of methanol to precipitate the polymer. The sample was then centrifuged for 5 min at 21,000 g and a 20 μl aliquot taken from the supernatant was analyzed by the HPLC system. The column effluent was detected at 337 nm and 280 nm for rifampicin and estradiol valerate respectively with a UV detector (WellChrom K-2600, Knauer, Germany). The calibration curves for the quantification of both drugs were linear over the range of standard concentrations of each other at 0–50,000 ng/ml with a correlation coefficient of $r^2 > 0.999$.

The encapsulation efficacy was obtained as the mass ratio between the amount of each drug incorporated in nanoparticles and that used in the nanoparticles preparation.

2.2.3. Nanoparticle characterization. *Size and size distribution*. The particle size and size distribution of the nanoparticles were measured by laser light scattering (Zetasizer ZS, Malvern, UK). The samples were prepared by suspending the freeze dried nanoparticles in 10 ml deionized water (10 μg/ml).

Morphology. Scanning electron microscopy (XL 30 scanning microscope, Philips, the Netherlands) was employed to determine the shape and surface morphology of the produced nanoparticles. To examine the morphology of nanoparticles, a small amount of nanoparticles was stuck on a double-sided tape attached on a metallic sample stand, then coated under vacuum with a thin layer of gold before SEM.

2.2.4. *In vitro* drug release. Drug release from the nanoparticles was studied using a modified dissolution method [9]. The media was a 0.05 M phosphate buffer solution. A known mass of nanoparticles was suspended in tubes of buffer solution at pH 7.4. Three replicates were used for each sample. The tubes were placed in a shaker bath (WB14, Memmert, Germany) at 37°C and shaken horizontally at 30 cycles/min.

At selected time intervals the tubes were centrifuged at 21,000 g for 10 minutes and an aliquot of 900 μl was taken from the supernatant. A volume of 100 μl of ethanol was added and analyzed by HPLC. A calibration curve was prepared prior to the start of dissolution using a phosphate buffer-ethanol (9 : 1 by volume) media. The HPLC method used was the same as described above. After the aliquots were removed the entire supernatant was replenished in order to maintain sink conditions. Drug release data was normalized by converting drug concentration in solution to a percentage of the cumulative drug release.

2.2.5. Statistical analysis. One-way analyses of variance (ANOVA) test was performed on the data to assess the impact of the TPGS on drug releases.

3. Results and discussion

3.1. *Nanoparticle characteristics*

Different formulations used for the preparation of rifampicin and estradiol valerate nanoparticles are presented in tables 1 and 2 respectively. Figure 1 shows the SEM photograph of R9 and E9 samples. All nanoparticles had a fine spherical shape. However in some cases small bridges between the particles may be seen. This may be due to the remaining PVA on the surface of the nanoparticles. However with through washing of the nanoparticles with deionized water, there may be other reasons for these bridges such as the attachment of nanoparticles during the freeze drying process or during gold coating of particles for SEM observation. However light scattering measurement showed that these bridges are broken easily and there is not a significant difference between the size of the nanoparticles before or after drying ($p > 0.05$).

Table 1. The rifampicin nanoparticles and their properties.

Sample	% PVA in external phase	% TPGS in external phase	PLGA: TPGS ratio	Drug content (%) ± SD	Encapsulation efficiency (%) ± SD	Mean diameter (nm) ± SD
R1	1	0	1 : 1	0.70 ± 0.05	7.0 ± 0.5	222 ± 20
R2	1	0	1 : 2	0.70 ± 0.03	7.0 ± 0.2	230 ± 12
R3	1	0	2 : 1	0.50 ± 0.02	5.0 ± 0.4	212 ± 23
R4	1	0	1 : 4	2.0 ± 0.20	19.0 ± 0.6	245 ± 24
R5	0	0.25	1 : 0	*	*	*
R6	0	0.50	1 : 0	–	–	560 ± 18
R7	0	1	1 : 0	–	–	470 ± 23
R8	1	1	1 : 0	–	–	378 ± 21
R9	1	0	1 : 0	0. 09 ± 0.01	0.98 ± 0.05	210 ± 23
R10	0	0	1 : 0	*	*	*

*Nanoparticles not formed. – Drug loading: 10%.

Table 2. The estradiol valerate nanoparticles and their properties.

Sample	% PVA in external phase	% TPGS in external phase	PLGA: TPGS ratio	Drug content (%) ± SD	Encapsulation efficiency (%) ± SD	Mean diameter (nm) ± SD
E1	1	0	1 : 1	8.7 ± 0.2	92 ± 2	200 ± 20
E2	1	0	1 : 2	9.1 ± 0.3	96 ± 3	224 ± 12
E3	1	0	2 : 1	6.3 ± 0.3	75 ± 2	215 ± 23
E4	1	0	1 : 4	9.5 ± 0.7	100 ± 7	231 ± 24
E5	0	0.25	1 : 0	*	*	*
E6	0	0.50	1 : 0	–	–	560 ± 18
E7	0	1	1 : 0	–	–	470 ± 23
E8	1	1	1 : 0	–	–	378 ± 21
E9	1	0	1 : 0	4.8 ± 0.3	55 ± 1	186 ± 10
E10	0	0	1 : 0	*	*	*

*Nanoparticles not formed. – Drug loading: 10%.

The resulting nanoparticle characteristics are summarized in tables 1 and 2. It is shown that the surfactant used in the fabrication process is an important factor to influence the particle size and the drug loading efficiency of nanoparticles [5]. This is highly important as the particle size of nanoparticles influence the drug release kinetics, cellular uptake and thus the therapeutic effects of the drug-loaded nanoparticles [10].

The emulsification-diffusion method has previously been successfully used to prepare biodegradable nanoparticles in an efficient and reproducible manner [11]. In this study nanoparticles were prepared by a modified emulsification-diffusion method or SESD. The technique is based on the rapid diffusion of the solvent from the internal phase into the external phase, which thereby provokes polymer aggregation in the form of solid colloidal particles. Structurally, TPGS has a dual nature, similar to an amphiphile molecule, with one part of the molecule exhibiting lipophilicity and another part exhibiting hydrophilicity, which is necessary for use as a surface-active agent [8]. Zhang and Feng in 2006 reported that using 0.03% of

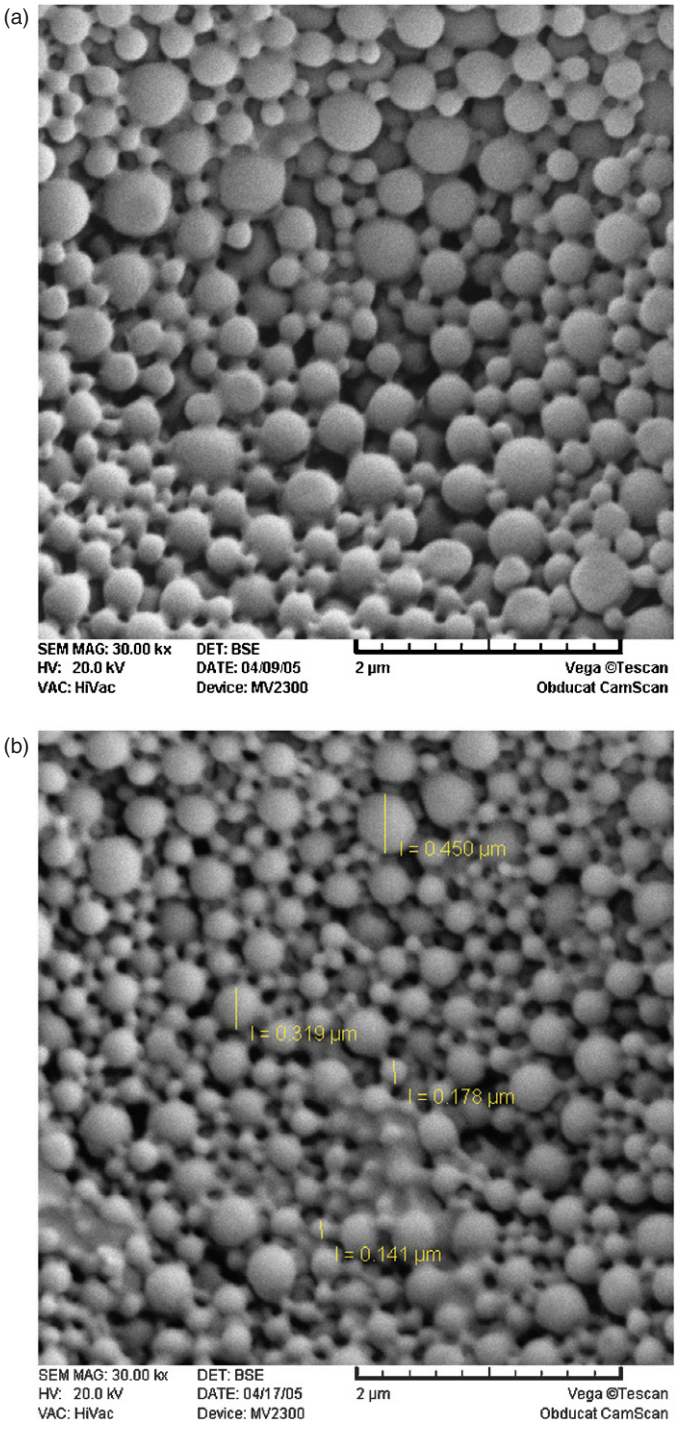

Figure 1. SEM micrographs of; (a) rifampicin (R9) and (b) estradiol valerate (E9) nanoparticles showing the shape and the surface characteristics.

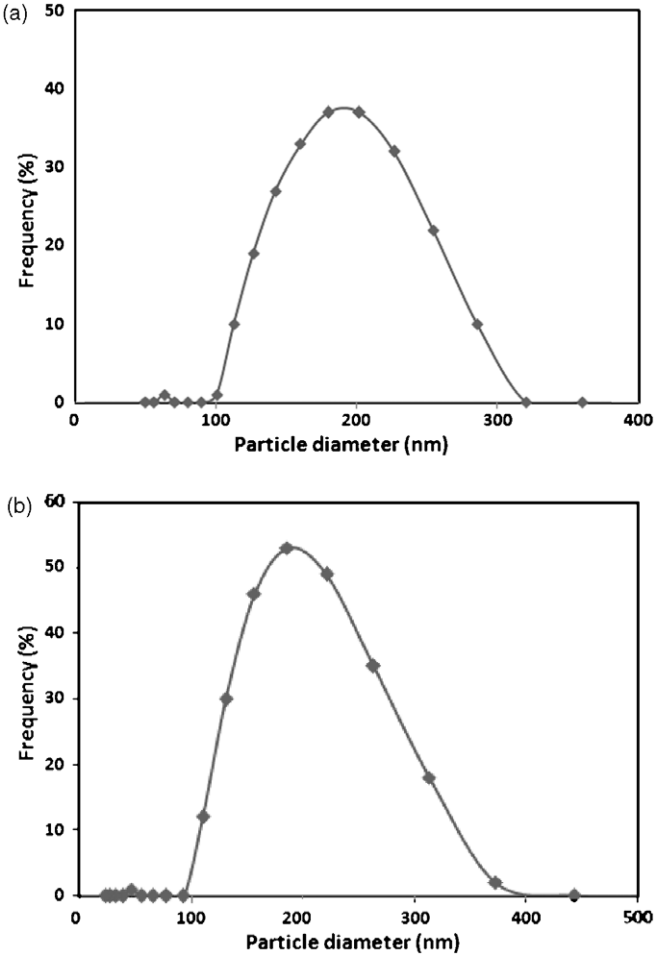

Figure 2. Size distribution curves of; (a) rifampicin (R9) and (b) estradiol valerate (E9) nanoparticles determined by light scattering technique.

TPGS as an emulsifier was enough to produce nanoparticles of the size of about 300 nm [12]. However in our study, it was found that higher concentrations of TPGS ($>0.25\%$) was required to obtain nanoparticles with the same size range. At lower concentrations of TPGS, the nanoparticles were not formed and two phases of the emulsion were separated. This may be due to the difference in the nanoparticle preparation methods used in this study. In this study acetone, a water-soluble solvent used as the main solvent of the internal phase, tends to make hydrogen bonds with water of the external phase. This may be a reason for extra TPGS needed as emulsifier to break these bonds.

Figure 2 shows a typical laser graph for the size distribution of nanoparticles. As can be seen the size distribution of nanoparticles is normal. The impact of the amount of TPGS on the size of nanoparticles is shown in tables 1 and 2. As can be seen when TPGS was blended with PLGA as a matrix material of nanoparticles, the size of the

nanoparticles was larger than 400 nm at any concentration of the TPGS. The mixture of PLGA and TPGS has a self-emulsifying effect, which can form nanoparticles with no need to add another surfactant stabilizer. In this case, the particles size was high. To achieve the smaller particles, different ratios of TPGS/PLGA in the internal phase and different concentration of PVA in the aqueous phase, were used. Lowering PVA amount from 4% to 1% in the aqueous medium led to an improvement in drug lading efficiency. This may be attributed to less drug penetration to the continuous phase from the dispersed phase as a result of lower concentration of emulsifier [5, 13]. The optimum results regarding smaller size and higher drug loading efficiency was achieved when 1% PVA was used. It can also be seen that using TPGS in preparation of nanoparticles can result in a satisfactory drug loading efficiency which was found to increase from 1% for PLGA nanoparticles without TPGS to 7% for PLGA : TPGS (1 : 4 by volume) for rifampicin nanoparticles and from 55% for PLGA nanoparticles without TPGS to 100% for PLGA : TPGS (1 : 4 by volume) for estradiol valerate nanoparticles. The amphiphile surfactants align themselves at the oil-water interface to promote the stability of the particles by lowering the surface energy and thus resist coalescence and flocculation of the particles. Too little emulsifier would not be enough to cover the interface and too much emulsifier would cause particle aggregation [5]. As can be seen in tables 1 and 2, altering the PLGA : TPGS ratio did not affect the particle size significantly. It can be therefore concluded that an appropriate amount of TPGS for the preparation of PLGA nanoparticles of either rifampicin or estradiol valerate was when the ratio of TPGS to PLGA used was four to one. At this level nanoparticles with an optimum size and drug loading efficiency were produced.

As it is shown in the tables, drug loading efficiency of rifampicin nanoparticles was lower than that of estradiol valerate nanoparticles. As rifampicin is a slightly water-soluble drug, rapid diffusion of acetone into the aqueous phase in SESD method may be the reason for such a low rifampicin loading efficiency.

TPGS had an excellent effect on drug loading of the nanoparticles, especially for estradiol valerate with a loading efficiency of up to 100%. Also using TPGS could improve the drug loading efficiency of rifampicin as a model drug of slightly soluble agents.

3.2. In vitro *drug release*

A typical *in vitro* drug release from PLGA nanoparticles prepared in this study is shown in figure 3. Figure 3(a) and (b) show the release profiles for nanoparticles containing rifampicin and estradiol valerate respectively fabricated using 1% w/v PVA solution and various PLGA/TPGS ratios in phosphate buffer (pH = 7.4).

The burst release was prominent for all formulations during the first hours of release. However, the release gradually decreased and remained constant. The initial burst could be due to the diffusion release of rifampicin distributed near or at the surface of the nanoparticles. Due to the fast release rate of drug molecules from nanoparticles the mechanism of drug release is rather diffusion controlled than biodegradation of PLGA.

For both drugs, the drug release from the PLGA/TPGS nanoparticles was much faster than the PLGA nanoparticles without TPGS. With no TPGS in the formulations,

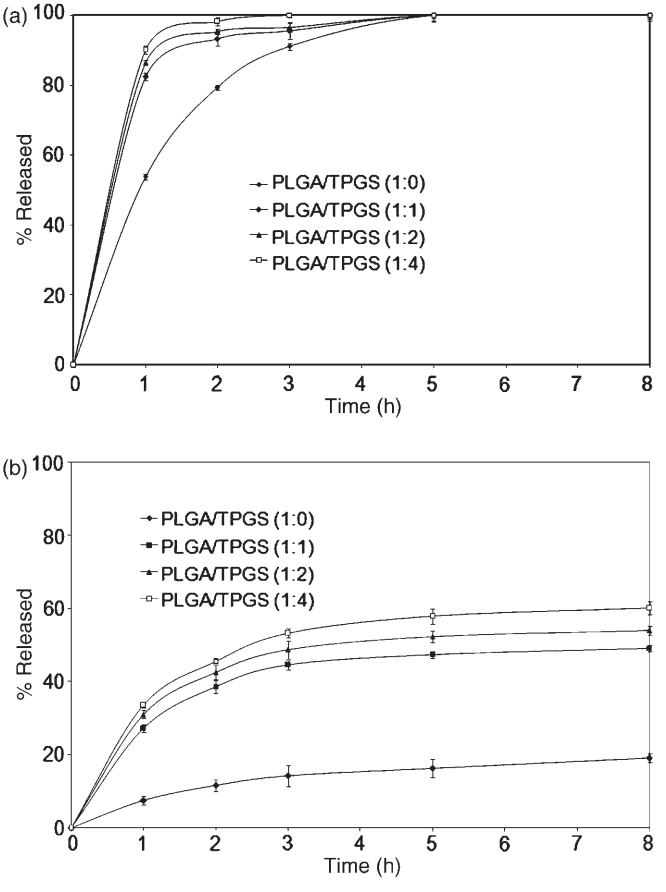

Figure 3. *In vitro* release curves of; (a) rifampicin and (b) estradiol valerate nanoparticles in phosphate buffer (pH 7.4).

estradiol valerate was released very slowly which may not result in a therapeutic level of drug concentration in the blood. The faster drug release from the PLGA/TPGS nanoparticles may be attributed to the amphiphilic nature of the TPGS, which causes rapid absorption of water molecules into the polymeric matrix, hence promoting the drug diffusion through polymeric matrix of the nanoparticles. This is another advantage of the PLGA/TPGS nanoparticles over the traditional PLGA nanoparticles, which were found to release the drug too slowly to meet the therapeutic needs.

In the case of rifampicin, although the release was faster when PLGA/TPGS was used for the preparation of nanoparticles, the difference was not significant ($P > 0.05$), because the drug molecule itself is hydrophilic, hence diffuses into the buffer medium easier. The difference is only significant when estradiol valerate nanoparticles are prepared. As is shown in figure 3, by using TPGS estradiol valerate the release was found to increase from 19% to 60% during the first 8 hours. As can be seen the higher the amount of TPGS, the faster the rate of drug release was achieved.

4. Conclusion

The present research proposed a novel formulation of rifampicin and estradiol valerate nanoparticles by applying the SESD method using TPGS either as emulsifier or as a component of the matrix material to fabricate nanoparticles. Different formulations with various drug : polymer ratios, oil phase combination and volume and surfactant were evaluated. Although the amount of the TPGS used had no significant effect on the nanoparticle size and morphology, the drug loading and release profile of nanoparticles were highly influenced by the use of TPGS. Our results demonstrated that TPGS is a good emulsifier for producing nanoparticles of hydrophobic drugs with desired particle size, size distribution and morphological properties.

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