SPIONs and curcumin co-encapsulated mixed micelles based nanoformulation for biomedical applications

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Abstract

In this work, we have synthesized oleylamine (OM)-coated hydrophobic monodispersed SPIONs with an average particle size of ~9 nm via thermal decomposition method. The as-prepared hydrophobic SPIONs are co-encapsulated along with a drug (curcumin, Cur) within the mixed micelles based nanoformulations which is made of d-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) and Pluronic F127 while keeping the TPGS:F127 ratios at 100:0, 75:25, 50:50, 25:75 and 0:100. Then, the nanoformulations are characterized for hydrodynamic size via dynamic light scattering (DLS) technique, and drug/SPIONs encapsulation efficiencies are determined via UV-vis spectroscopy. Among all the nanoformulation, the mixed micelle with 50:50 TPGS:F127 has exhibited relatively lower hydrodynamic diameter (Dh) (~ 84 nm), better encapsulation efficiencies of Cur and SPIONs (~95% / 56%), and high yield (above 90%). Moreover, morphology and encapsulation of SPIONs/Cur inside the optimized 50:50 TPGS:F127 nanoformulation is confirmed by TEM. In addition, only 10% of Cur is released during 12h time period from optimized nanoformulation indicating the sustained-release property, whereas ~68% of Cur is quickly released in free Cur experiments for the same time period. Hence, the SPIONs/Cur are efficiently co-encapsulated inside the TPGS:F127 mixed micelle based nanoformulation which could be used for further biomedical applications.

Keywords: Mixed micelles, SPIONs, curcumin, drug delivery, nanoprecipitation.

Introduction

Polymeric micelles are extensively studied as the drug delivery systems for various cancer treatments because of their ability to (i) solubilize poor water-soluble chemotherapeutic drugs (CHDs), and (ii) consequently improve their bioavailability in tumor regions. Generally, the formation of the polymeric micelles is a thermodynamically driven process, where the amphiphilic polymers (i.e., polymers having a hydrophobic tail and a hydrophilic head) spontaneously self-assemble to form a core-shell based aggregated structure, where the core is hydrophobic, and the shell is hydrophilic in nature. The hydrophobic core can be effectively used for encapsulation of the CHDs based on the hydrophobic-hydrophobic interactions.

Usually, the polymeric micelles are made of single di-/tri-block amphiphilic copolymers. Nowadays, mixed polymeric micelles (i.e., mixture of two or more amphiphilic copolymers) have gained more care due to their increased stability as compared to their single polymeric counterparts. In addition, the mixed micelles based nanoformulations have huge potential to overcome the existing problems such as unfavorable bio-distribution of CHDs and subsequent toxicity to the normal tissues, and inability of drugs to cross the physiological barriers in brain tumors [**1-5**]. Thus, the mixed micelles based nanoformulations can be effectively used for cancer treatments.

Moreover, in recent times, superparamagnetic iron oxide nanoparticles (SPIONs) have gained great deal of attention for their usage in several biomedical applications such as drug delivery, magnetic fluid hyperthermia (MFH) therapy, magnetic resonance imaging (MRI), and magnetic targeting in cancer treatment due to their excellent chemical stability, biocompatibility and superparamagnetic behavior [**6-10**]. High quality (highly crystalline and monodispersed) SPIONs can be synthesized by thermal decomposition of an iron precursor in presence of organic solvents/surfactants at higher temperature [**11-13**]. However, the obtained SPIONs are hydrophobic in nature and thereby cannot be directly used in their biomedical applications. Therefore, the surfaces of these SPIONs should be modified further to convert them into hydrophilic**.** In addition, the hydrophobic SPIONs can be co-encapsulated along with CHDs inside a delivery system for effective combined therapies (MFH and chemotherapy) in cancer treatments**.** However, the issues that exist for the co-encapsulation of SPIONs/drugs inside the current delivery system are as follows: (i) low encapsulation efficiency of SPIONs/drugs, (ii) low yield of nanoparticles and (iii) larger hydrodynamic sizes [**14 -**

16]. So, there is a need for developing a delivery system by co-encapsulation of the hydrophobic SPIONs and CHDs for their effective usage in the cancer treatments. Hence, in this work, we report co-encapsulation of the hydrophilic SPIONs and a model drug (curcumin) inside a novel mixed micelle based delivery system, which is made of d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and Pluronic F127 (F127).

Experimental

Materials

Iron (III) acetylacetonate ($Fe(acac)_3$), oleylamine (OM), Pluronic® F-127, D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS,$C_{33}O_5H_{54}(CH_2CH_2O)_{23}$), and tetrahydrofuran (THF) are purchased from Sigma Aldrich. All the chemicals are reagent grade and used without any further purification.

Methods

(i) Synthesis of hydrophobic SPIONs

Initially, the hydrophobic OM-coated SPIONs are synthesized by thermal decomposition method as reported elsewhere [**17**]. Typically, 2 mmol of $Fe(acac)_3$ and 20 ml of oleylamine are added to a round bottom flask (RBF) to form a mixture solution, which is magnetically stirred under a continuous flow of nitrogen (N_2) gas. Afterward, the mixture solution is dehydrated by heating at 120 °C for 1h, and then quickly heated to 300 °C and maintained at this temperature for another 1h. The resultant black color solution is cooled down to room temperature by removing the heat source. Then, the as-synthesized nanoparticles are precipitated and collected by centrifugation at 10,000 rpm, followed by three times washing with ethanol/hexane mixture. Finally, the hydrophobic SPIONs are dispersed in ethanol and dried in oven at 40 °C for overnight to obtain the powder of OM-coated SPIONs. **Fig. 1 (a)** shows the schematic representation for synthesis of hydrophobic OM-coated SPIONs by thermal decomposition method.

(ii) Nanoformulation by polymeric encapsulation

The hydrophobic OM-coated SPIONs and curcumin (Cur) are encapsulated inside novel mixed micelles via nanoprecipitation method. Typically, 100 mg of TPGS:F127 (mixed at specific mass ratios – refer **Table 1)** and SPIONs : Cur (5:1) are mixed in 5 ml THF to form an organic matrix solution (OMS), which is then added drop-wise into 30 ml of aqueous stabilizer solution (containing F127:TPGS at specific ratios – refer **Table 1**) under magnetic stirring. Then, the above mixture is stirred for 8 h to evaporate THF to form a mixed micelles delivery system (MMDS) by encapsulating SPIONs and Cur. The schematic representation of the nanoformulation by encapsulation of hydrophobic SPIONs and curcumin within TPGS/F127 mixed micelles is shown in **Fig. 1 (b)**.

Characterizations

The morphologies (size/shape) of the as-prepared hydrophobic SPIONs and MMDS are investigated through transmission electron microscopy (TEM). The average hydrodynamic sizes of the MMDS are determined via dynamic light scattering (DLS) technique. For determining the encapsulation efficiency (EE in %) of Cur, 1 mg of MMDS is dissolved in 1 ml of methanol to destroy the micelles and then, the absorbance of Cur (after the release from the MMDS) is observed at 422 nm by using UV-vis spectrophotometer and compared with Cur standard curve. Similarly, for determining the EE of SPIONs (in terms of iron - Fe), 1 mg of MMDS is digested by using concentrated HCl and subsequently mixed with potassium thiocyanate to form an iron thiocyanate complex, whose absorbance is observed at 474 nm by using UV-vis spectroscopy for comparison with Fe standard curve. The EE is calculated from the following equation:

EE (in %) = 100* ((Mass of Cur/Fe encapsulated inside micelles)/Mass of Cur/Fe initially added)

Moreover, for drug release studies, 1 mg/ml solutions of free Cur (in methanol) or chosen MMDS (in water) is taken in a dialysis bag (12kDa) and immersed in a beaker containing 100 ml of PBS (pH 7.4) with 0.5% Tween while maintained the temperature at 37 ^{0}C under magnetic stirring (100 rpm). Next, 1 ml of dissolution medium is taken at pre-determined intervals (0-12 h) and the same amount of fresh PBS is added. Then, the concentration of released Cur is measured via UV-vis for each time interval.

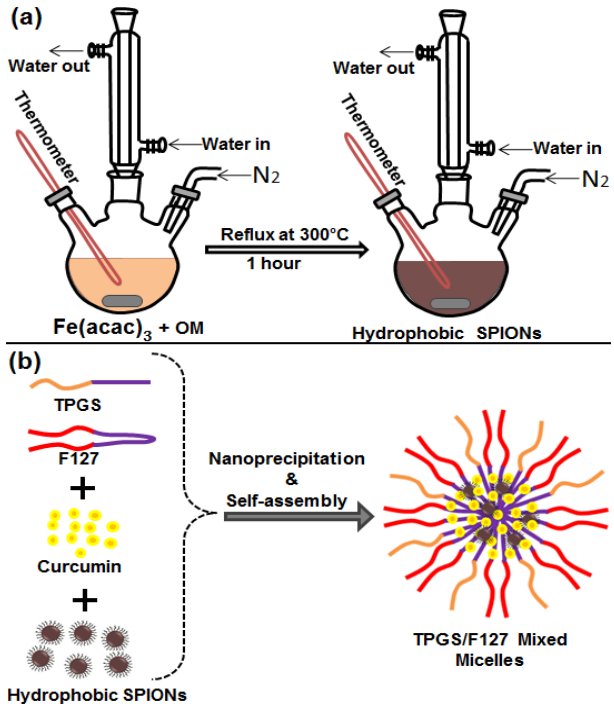

Fig. 1. Schematic representation for (a) synthesis of hydrophobic OM-coated SPIONs by thermal decomposition method, and (b) Nanoformulation by encapsulation of hydrophobic SPIONs and curcumin within TPGS/F127 mixed micelles.

Results and discussions

The phase purity and nature/amount of surface coatings of SPIONs are characterized by using (X-ray diffraction) XRD and Fourier-transform infrared spectroscopy (FTIR)/thermogravimetric analysis (TGA), respectively as reported in our previous publication [**17**]. It has been noted that the as-synthesized hydrophobic SPIONs have demonstrated magnetite (Fe_3O_4) phase as per the XRD results. Moreover, the presence of OM on the surface of the SPIONs is confirmed by the corresponding adsorption peaks in FTIR spectra. In addition, the amount of OM is found to be ~12% in TGA results and the magnetic saturation (Ms) value is determined to be ~ 62 emu/g (via vibration sample magnetometer (VSM)) for the as-synthesized SPIONs, which are reported in our previous publication [**17**].

Fig. 2 displays the TEM image of the hydrophobic SPIONs. It can be seen that the as-prepared SPIONs are spherical in shape and monodispersed with average particle sizes of ~9 nm.

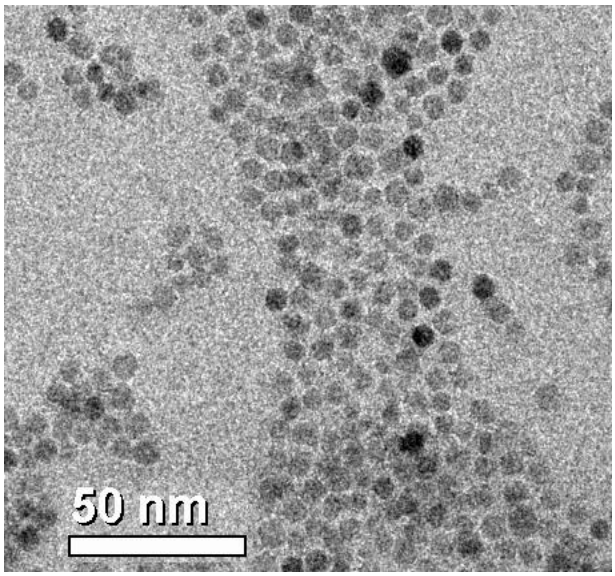

Fig. 2. TEM image of OM-coated hydrophobic SPIONs.

Table 1 shows all the nanoformulations, which are prepared by co-encapsulation of the hydrophobic OM-coated SPIONs and the model drug - Cur (at 5:1 ratio) inside the mixed micelles made of TPGS:F127 (in OMS at different mass ratios). The mean hydrodynamic diameter (Dh) values of MMDS samples prepared in the presence of aqueous stabilizer solutions of TPGS (i.e., MMDS1 – MMDS5), F127 (i.e., MMDS6 – MMDS10) and TPGS:F127 (MMDS11 – MMDS13) are approximately in the range of 84-90, 138-159 and 83-92 nm respectively. It can be noted that Dh of the MMDS samples which are prepared by using TPGS/TPGS:F127 are comparatively smaller than those samples prepared by using F127 as aqueous stabilizers. This could be mainly due to the lower hydrophilic-lipophilic balance (HLB) value of TPGS as compared to F127.

Moreover, lower Dh values of 84.63, 138.63 and 83.6 nm are obtained for MMDS3, MMDS6 and MMDS11 when the ratio of TPGS:F127 in OMS is maintained as 50:50, 100:0 and 75:25 for the corresponding aqueous stabilizers TPGS, F127 and TPGS:F127, respectively. In addition, larger Dh values of 90.60, 158.03 and 92.41 nm are respectively attained for MMDS4, MMDS9 and MMDS13, which are prepared using 25:75 ratio of TPGS:F127 in OMS for the corresponding aqueous stabilizers (i.e. TPGS, F127 and TPGS:F127). Nevertheless, the Dh values of all the MMDS samples are below 160 nm, which are highly suitable for biomedical applications.

Table 1. TPGS/F127 based Mixed Micelle Nanoformulations.

Sample Name	Aqueous Stabilizer Solution	TPGS : F127 Ratio in OMS (w/w)	D_h (nm) (n=3)	Yield (in %)	Cur EE (in %)
MMDS1	TPGS	100:0	90.37	77.24	56.93
MMDS2	TPGS	75:25	84.93	61.22	69.45
MMDS3	TPGS	50:50	84.63	92.31	94.79
MMDS4	TPGS	25:75	90.60	87.82	85.16
MMDS5	TPGS	0:100	89.73	75.32	87.19
MMDS6	F127	100:0	138.63	95.51	61.34
MMDS7	F127	75:25	156.23	84.29	22.95
MMDS8	F127	50:50	147.73	88.46	50.38
MMDS9	F127	25:75	159.00	89.74	32.19
MMDS10	F127	0:100	158.03	93.91	74.73
MMDS11	TPGS/ F127 (75:25)	75:25	83.60	85.58	64.36
MMDS12	TPGS/ F127 (50:50)	50:50	91.00	92.41	79.71
MMDS13	TPGS/ F127 (25:75)	25:75	91.97	90.71	73.74

Besides, the yields of nanoparticles are generally above 75% (except for MMDS2). Moreover, the highest yields of nanoparticles (i.e. 92.31, 95.51, and 92.41 %) are obtained for the MMDS3, MMDS6 and MMDS12 when the ratio of TPGS:F127 in OMS is 50:50, 100:0 and 50:50 for the corresponding aqueous stabilizers TPGS, F127 and TPGS:F127 respectively.

In addition, the encapsulation efficiencies (EE) of Cur are determined approximately in the ranges of 56.9 – 94.7, 22.9 – 74.7 and 64.3 – 79.7 % for the samples of MMDS1 – MMDS5, MMDS6 – MMDS10 and MMDS11 – MMDS13 respectively. The highest EE% of Cur is obtained for MMDS3 when ratio of TPGS:F127 in OMS is 50:50 for the corresponding aqueous stabilizer TPGS.

From the above studies, it can be noted that the nanoformulation of MMDS3 (50:50 TPGS:F127 in OMS) has shown relatively lower Dh value, higher nanoparticle yield and maximum EE%, and therefore, the MMDS3 is chosen for further investigations. **Fig. 3** shows the TEM image of the MMDS3 and it clearly confirms the encapsulation of SPIONs and Cur inside the TPGS/F127 mixed micelles.

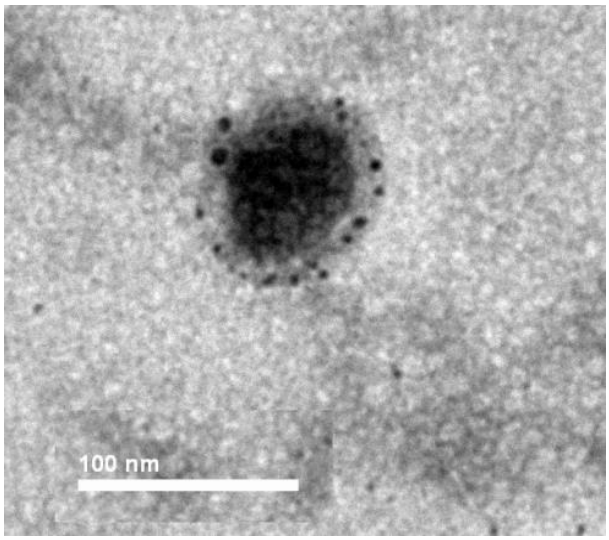

Fig. 3 TEM image of MMDS3 confirms the encapsulation of hydrophobic SPIONs and Cur inside the TPGS/F127 mixed micelles.

Moreover, the EE of iron (Fe) in MMDS3 is determined as 56.32%. Besides, in drug release studies, free Cur has shown very rapid release behavior as compared to very slower cumulative release rate of Cur from the mixed micelles based nanoformulation (i.e., MMDS3) - as shown in **Fig. 4**. Only ~10% of the loaded Cur is released from the MMDS3 during 12h, while ~68% of Cur is quickly released into PBS in free Cur experiments for the same time period.

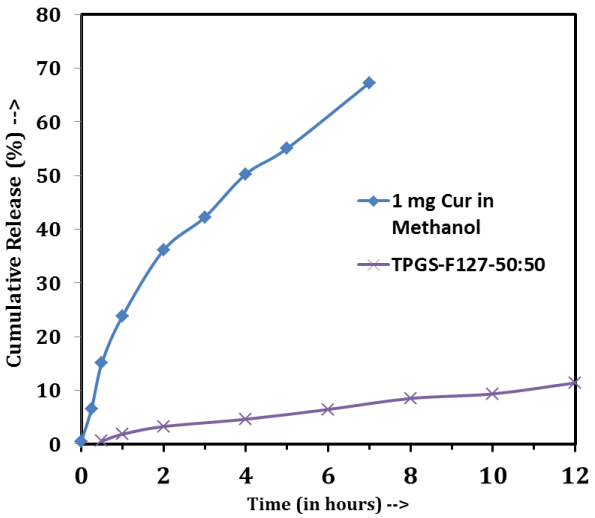

Fig. 4. Drug release profile of free Cur and TPGA:F127-50:50 nanoformulation (i.e., MMDS3).

Conclusions

To summarize, we have synthesized OM-coated hydrophobic SPIONs with size ~9nm, by thermal decomposition method. Then, novel TPGS/F127 mixed micelles based nanoformulations are successfully developed by co-encapsulating the as-synthesized hydrophobic SPIONs and a model chemotherapeutic drug (curcumin). Among all, 50:50 TPGS:F127 mixed micelle based nanoformulation has shown comparatively lower Dh value (i.e., 84.63 nm), better encapsulation efficiency (~94.7% for curcumin and 56.3% for SPIONs), high nanoparticle yield (above 90%) and good drug release profile. This indicate that the as-prepared TPGS:F127 mixed micelle based nanoformulation (with SPIONs and curcumin) are suitable candidates for further biomedical applications in cancer treatments.

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