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REVIEW

VitD3-loaded solid lipid nanoparticles: stability, cytotoxicity and cytokine levels

Murat Demirbilek[a], Nelisa Laçin Türkoglu[b], Selçuk Aktürk[c] and Cem Akça[d]

[a]Advanced Technologies Application and Research Center, Hacettepe University, Ankara, Turkey; [b]Science and Technology Application and Research Center, Yildiz Technical University, Istanbul, Turkey; [c]Department of Physics, Mugla Sitki Koçman University, Mugla, Turkey; [d]Department of Metallurgical and Materials Engineering, Yildiz Technical University, Istanbul, Turkey

ABSTRACT

Vitamin D3 (VitD3) has several beneficial effects on many metabolic pathways such as immunity system, bone development. The aim of the study, encapsulation of VitD3 with solid lipids, determine encapsulation efficiency and biocompatibility of nanoparticles. Therefore, VitD3-loaded solid lipid nanoparticles (SLNPs) were developed by optimising ratios of VitD3, stearic acid, beeswax and sodium dodecyl sulphate (SDS). Thermal stability, degradation profile, crystallinity rate, encapsulation efficiency and release profile of SLNPs were determined. Cytotoxicity of SLNPs on HaCaT, L929 and HUVEC cells were investigated. Negatively charged and VitD3-loaded nanoparticles with diameters between 30 and 60 nm were obtained. SLNPs containing up to 5.1 mg VitD3 per 10 mg powder samples were obtained. Cell proliferations were stimulated after exposure with VitD3-loaded SLNPs. Besides, inflammatory response after exposure to VitD3-loaded SLNPs was evaluated via determining IL10 and TNF-alpha levels on THP-1 cells. According to the results, no inflammatory response was observed.

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Introduction

Solid lipid nanoparticles (SLNPs) have attracted attention as an alternative carrier system for 20 years to the traditional colloidal carriers, such as emulsions, liposomes and also polymeric micro and nanoparticles. SLNPs combine the advantages of traditional systems and avoid some of their major disadvantages (Ramteke et al., 2012). SLNPs utilise lipids as drug carriers where drugs are homogeneously dispersed within lipid matrix and loaded into the core of lipid shell or outside the lipid sphere. SLNPs have a very low cytotoxicity on eukaryotic cells. Although they prevent degradation of molecules while they are travelling through the biologic environment, encapsulation of lipophilic molecules can be carried out easily with SLNP (Wang et al., 2009). In addition, preparation process of SLNPs can easily be scaled up and they are suitable for prolonged drug release (Muèller et al., 2000).

The fatty acids and emulsifier selected for SLNPs play an important role on the encapsulation rate of drug and drug delivery period. Besides, the concentration of lipids and emulsifiers strongly influence the quality of SLNP nanoparticles.

VitD3 is a naturally occurring form of vitamin D and plays an important role in bone mineralisation and skeletal growth. Furthermore, it was shown that there is a correlation between blood plasma levels of VitD3 and some diseases such as cancer, cardiovascular diseases, autoimmune disease and infections (Grossmann and Tangpricha, 2010). The main source of vitamin D3 prior to oral supplements was the natural synthesis in the skin upon exposure to ultraviolet light. Given the current lifestyle of most people, the main source of VitD3 has become nutritional supplementation. The recommended daily allowance for normal children and adults is 400 IU/day (Bothiraja et al., 2016). In recent years, researchers try to improve stability, solubility and bioavailability of VitD3 in human body (Jakobsen and Knuthsen, 2014), and nowadays, vitamin D is one of the most common compounds in the pharmaceutics.

Encapsulation of active agents constitute a barrier against harmful chemical and environment such as free radicals or UV. Encapsulation is also effective to improve bioavailability of drugs such as prolong shelf time and control release (Gonnet et al., 2010). For example, Maryam Mohammadi et al. encapsulated VitD3 (Mohammadi et al., 2014), James Heyes et al.

CONTACT Murat Demirbilek muratscaffold@gmail.com Advanced Technologies Application and Research Center, Hacettepe University, Ankara, Turkey

encapsulated plasmid DNA with cholesterol (Heyes et al., 2007) for control release. Lipid encapsulation of vitamins or other drugs is not limitation to oral, transdermal, nasal or injection administrations. M. Videira et al. capsulated paclitaxel with glyceryl palmitostearate for use by the inhalation route. (Videira et al., 2012). Carla Vitorino and et al. encapsulated olanzapine and simvastatin with tripalmitin and oleic acid for use by the transdermal administration (Vitorino et al., 2014). Especially, transdermal application of lipid carriers has an occlusive effect on surface of skin because of film formation. This effect increase skin hydration and increase dermal penetration of drugs (Liu et al., 2010b). Particle size also effective for dermal penetration. Transdermal delivery of VitD3 to body is an alternative route in order to keep the therapeutic plasma levels especially for people who suffer from fat malabsorption and to overcome its variable oral bioavailability (Gonnet et al., 2010).

The purpose of the present study was to prepare VitD3-loaded SLNPs by optimising the VitD3, stearic acid, beeswax, surfactant ratios and to evaluate encapsulation, drug release and biocompatibility of VitD3-loaded SLNPs.

Materials and methods

Materials

Sodium dodecyl sulphate, glyceryl trimiristate, Tween-80, stearic acid, beeswax, phorbol myristate acetate (PMA) were purchased from Sigma Aldrich, USA. All cell culture media, foetal calf serum (FCS), L-glutamine, plates, flasks and sterilised pipets were purchased from Gibco, Thermo Fisher Scientific, USA.

Methods

Preparation of VitD3-loaded SLNPs

In this study, different lipid compositions were prepared and drug encapsulation efficiency, drug release rates were evaluated. Solid lipid nanoparticles were prepared using organic solvent emulsification/evaporation technique. The lipid phase is composed of stearic acid and beeswax at different ratios. SDS was used as surfactant. VitD3 was added to the optimised formulations at three different concentrations (0.125, 0.25 and 0.5 g/mL). Solid lipids were dissolved in 1 mL chloroform. Emulsifier phase was stirred at 1500 rpm, dissolved lipid phase was added dropwise into 50 mL emulsifier phase. After pipetting, emulsion was stirred at 600 rpm by magnetic stirrer in order to evaporate organic phase. Emulsions were centrifuged at 5000 rpm for 30 min. The supernatants were taken away and centrifuged at 11 000 rpm for 1 h, and finally, supernatants were discarded and nanoparticles were collected. SLNPs were frozen at −30 °C for 24 h. The frozen pellets were dried in a freeze dryer for 48 h. The SLNP powders were collected and stored at 2–4 °C (Potta et al., 2011).

Table 1. SLNP Formulations, containing stearic acid, bees wax and SDS were optimised.

Formulations	SDS (%)	Bees wax (g)	Stearic acid (g)	VitD3 (g)
F1	0.5	0.05	0.2	–
F2	0.5	0.1	0.15	–
F3	0.5	0.15	0.1	–
F4	0.5	0.05	0.2	0.125
F5	0.5	0.05	0.2	0.25
F6	0.5	0.05	0.2	0.5
F7	0.5	0.1	0.15	0.125
F8	0.5	0.1	0.15	0.25
F9	0.5	0.1	0.15	0.5
F10	0.5	0.15	0.1	0.125
F11	0.5	0.15	0.1	0.25
F12	0.5	0.15	0.1	0.5

The obtained blank nanoparticle formulations were optimised according to the results of the characterisation studies. At three different concentrations, VitD3 was added to the lipid phase in the optimised formulations. VitD3-loaded nanoparticles were formed as described earlier. All formulations were presented at Table 1.

Characterisation of SLNPs and VitD3 loaded SLNPs

Transmission electron microscopy (TEM) imaging of the nanoparticles

Transmission electron microscopy (TEM) images of the SLNPs were taken. SLNP samples were diluted prior to analysis. Then, the samples were imaged using a Philips CM-100 TEM (FEI Co., Hillsboro, OR) operated at 80 kV, spot 3, 200 μm condenser aperture and 50 μm objective aperture. Images were captured on Kodak SO-163 electron image film (Eastman Kodak Co., Rochester, NY). Magnifications of 11 500X and 21 000X were used for all samples.

Particle size and zeta potentials

The particle size (*z*-average size), zeta potentials, polydispersity index (PDI) of the nanoparticles were determined by photon correlation spectroscopy using Nano ZSP zeta sizer (Malvern Instruments Corp, U.K.). Measurements were performed in distilled water and repeated three times.

Thermal properties of the SLNPs

Thermal properties of the SLNPs were determined by differential scanning calorimetry (DSC,

Perkin-Elmer Corp., USA). A heating rate of 10 °C/min was employed and heating range was 0–100 °C. An empty aluminium pan was used for measurement as reference and 10 mg sample was used. Analysis was carried out under nitrogen atmosphere.

Crystallinity rates of SLNPs

Crystallinity rate of the nanoparticles were determined by X-ray diffractometer (Rigaku, Japan) with Cu Kα radiation ($\lambda = 0.1541$ nm). The scan range was $2\theta = 15°–70°$.

Degradation rate of SLNPs

Degradation rate of SLNPs (containing VitD3) were measured by a turbidimeter. 0.4 mg/mL SLNPs ($n = 3$) were suspended in PBS and incubated at different temperatures (4 °C, 37 °C and 40 °C) in a shaking incubator for 8 weeks. Turbidimetric absorbances of samples were recorded weekly.

Determination of VitD3 encapsulation rate and VitD3 release profiles

Encapsulation rate (ER) of VitD3 was determined by measuring the amount of VitD3 in the supernatants obtained after centrifugation. Each sample was repeated six times. ER of VitD3 was calculated by the following equation:

$$\mathrm{ER}(\%) = (\text{Amount of total VitD3–Amount of residual Vit D3})/(\text{Amount of total Vit D3})$$

where amount of total VitD3 is the amount of VitD3 added to the lipid phase and amount of residual VitD3 represents VitD3 amount in the final suspension.

The amount of VitD3 in suspensions was determined by MS/MS/Q-TOF mass spectrometer (2.1 mm and 50 mm (1.8 μm) Zorbax C18 column). Two different solvents were used as mobile phase; 40 mM, 1% formic acid solution and methanol was used as mobile phase A and B, respectively. Methanol gradient was performed; (0.5 min, mobile phase was consisting of 5% A, 95% B, then 2% of mobile phase A, 98% of mobile phase B for 2 min and finally 5% of mobile phase and 95% of mobile phase B 95% for two 2 min). Ten millilitres of sample was injected and flow rate was set as 500 μL/min. At the positive mode, *m/z* 385,3456 were determined.

VitD3 release rates from SLNPs were evaluated via Franz cells. 0.01 g of SLNP were suspended in 20 mL PBS and then placed in the donor phase of the Franz cell. The receiver phase was 20 ml PBS. Cellulose membrane (pore size of 15 000 Da) was placed between two phases. Franz cells were then placed in a shaking incubator at 30 °C, 100 rpm. One millilitre sample was taken at 1, 2, 3, 24, 48, 72 and 120 h intervals from the receiver phase. Same volume of PBS was added to the receiver phase to compensate the volume loss. The amount of VitD3 released from SLNP was determined by MS/MS/Q-TOF with the same method mentioned earlier.

Evaluation of in vitro immunocompatibility and cytotoxicity of SLNPs

Cytotoxicity of SLNPs on different cell lines; keratinocytes (HaCaT), endothelial (HUVEC) and fibroblast (L929) was determined via XTT assay. Each SLNP concentration was repeated six times. Cells were cultured with their appropriate cell medium (RPMI-1640 with 10% FCS, 1% L-glutamine was used for HaCaT, THP-1 and L929 cell lines. M199 media containing 10% FCS and 1% L-glutamine were used for HUVEC cell line. The cells were incubated at 37 °C in 5% carbon dioxide incubator. All cells were cultured in 25-cm^2 tissue culture flask.

For XTT assay, cells were seeded onto 96-well plates (3×10^3 cells per well) and incubated overnight. The medium was aspirated and fresh medium containing SLNPs with different concentrations as 100, 50, 25, 1 and 0 μg/mL were added to wells. The plates were incubated at 37 °C in 5% carbon dioxide incubator for 24 h. After incubation, the medium was aspirated and 100 μL fresh medium containing 20 μL of XTT solution (1 mg/mL in PBS) was pipetted to the each well, incubated at 37 °C for 4 h and absorbance at 450 nm were recorded (Mutlu et al., 2016).

To evaluate immunocompatibility of SLNPs, the effect of SLNPs to pro/anti-inflammatory cytokines released from monocyte-macrophage cells (THP-1) were determined. Each SLNP concentration was repeated six times. For this purpose, THP-1 cells (5×10^3 cell/mL) were seeded on 24-well plate and cultured in RPMI-1640 medium and 200 nM PMA. THP-1 cells were cultured for three days in the medium and followed for the transformation of monocytes to macrophages. Then cells were washed with RPMI-1640 medium in order to remove non-adherent cells. SLNPs were diluted in RPMI medium (1 μg/mL, 50 μg/mL and 100 μg/ml) and pipetted on transformed cells and incubated for two days. The media from each well were collected, centrifuged at 5000 rpm for 15 min. Levels of IL-10 and TNF-alpha in media were determined via commercial human IL-10 and TNF-alpha kits.

Statistical analysis

Six sample for each cell culture and VitD3 release experimental group were used. Numerical data were analysed using GarphPat 6 programme and Coloum analysis, non-parametric *t*-test. Statistical significance was considered at $p < .005$.

Results and discussion

In this study, VitD3 was encapsulated with solid lipids. Crystallinity, degradation profile, VitD3-loading capacity of nanoparticles were correlated. Cytotoxicity and *in vitro* immunocompatibility of the optimised formulations were determined.

Transmission electron microscopy (TEM) imaging of the nanoparticles

The size and shape of nanoparticles were characterised by TEM images, TEM photographs were given at Figure 1. According to TEM photographs, round-shaped nanoparticles in 30–60 nm size range were obtained.

Particle size and zeta potentials

Particle size and zeta potentials of SLNPs ($n = 3$) were examined by Zeta Sizer and results were presented in Table 2. The zeta potential of the nanoparticles is between −30 and +30, indicating the stability of the particles. (Liu et al., 2010a). As seen on the Table 2, zeta potentials of SLNPs were between -9.37 ± 2.04 and -28.13 ± 1.21. The PDI values of SLNPs were found to be in 0.32 ± 0.04 to 0.56 ± 0.05 range. Both zeta size and TEM results indicated that size distribution of nanoparticles was not in broad range. Besides, it was seen that the increasing stearic acid ratio decreases the nanoparticle diameter. Praveen et al. have produced stearic acid nanoparticles with particle sizes between 116.3 ± 4.3–137.6 ± 6.23 nm (Gaur et al., 2013). In contrast to these results, Noorma et al. have produced beeswax-glyceryl monostearate blend SLNPs and they have found that particle size increased when the ratio of glyceryl monostearate increased (Rosita et al., 2014).

Zeta analysis of VitD3-loaded SLNPs was also performed. Loading nanoparticles with VitD3 has not changed PDI values and zeta potentials of nanoparticles. However, zeta size of SLNPs has increased about 50 nm following VitD3 loading. Chi-Hsien Liu et al. have loaded vitamin K1 to solid lipid nanoparticles and they have found that zeta size of their nanoparticles has increased 0.25–5.0% (Liu et al., 2010a). Controversially, Iscan et al. have expressed that loading vitamin K has no effect on the zeta size of lipid nanoparticles (İşcan et al., 2005).

Table 2. Zeta size and zeta potential of the SLNP formulations were determined.

Formulations	Zeta potention (mV)/STD	Conductivity (mS)/STD	Zeta size (nm)/STD	PDI/STD
F1	-9.37 ± 2.04	25.63 ± 4.27	195.00 ± 7.21	0.46 ± 0.09
F2	-21 ± 1.86	21.80 ± 0.95	188.00 ± 5.20	0.56 ± 0.05
F3	-17.00 ± 1.85	21.63 ± 1.56	208.00 ± 5.20	0.37 ± 0.01
F4	-14.33 ± 1.45	23.87 ± 2.50	154.00 ± 11.53	0.42 ± 0.07
F5	-18.83 ± 1.00	18.17 ± 1.53	124.67 ± 7.23	0.54 ± 0.05
F6	-25.33 ± 3.11	17.60 ± 1.48	119.67 ± 2.08	0.40 ± 0.03
F7	-16.33 ± 2.49	18.67 ± 1.04	147.67 ± 7.77	0.35 ± 0.03
F8	-23.77 ± 1.04	16.90 ± 0.36	118.67 ± 4.16	0.32 ± 0.04
F9	-28.13 ± 1.21	15.08 ± 3.46	236.00 ± 5.29	0.43 ± 0.03
F10	-20.6 ± 2.66	17.32 ± 2.69	220.82 ± 4.61	0.28 ± 0.01
F11	-21 ± 1.62	20.61 ± 2.02	215.05 ± 6.67	0.33 ± 0.06
F12	-26.7 ± 3.08	20.61 ± 2.02	245.86 ± 5.92	0.40 ± 0.04

Nanoparticles of 118 0.67 ± 4.16 to 245.86 ± 5.92 nm in size were obtained.
Zeta potentials were in the range -9.37 ± 2.04 to -26.7 ± 3.08 ($n = 3$).

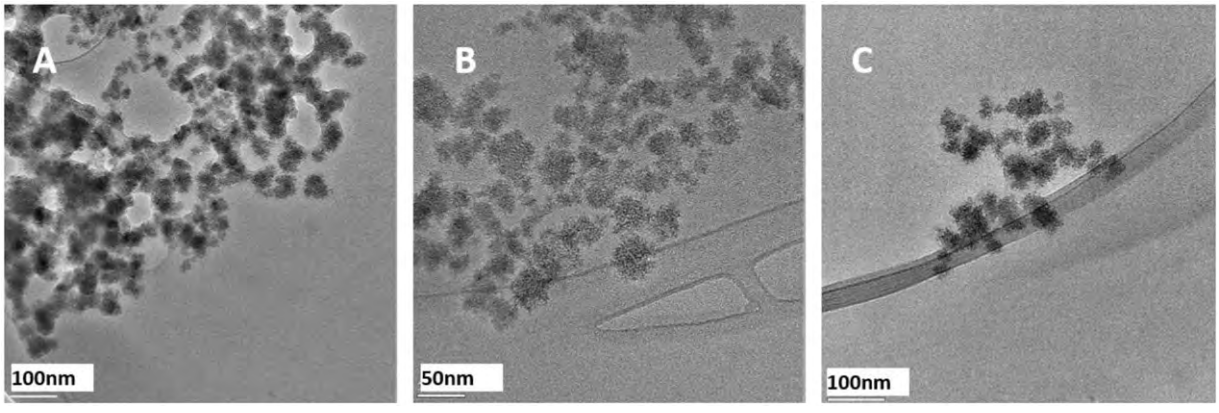

Figure 1. Physical characterisation of F4 (A), F5 (B) and F6 (C) formulations were performed by using TEM photographs. Round shape, in 30–60 nm size range nanoparticles, were obtained.

Table 3. Melting temperature and enthalpies were determined of bare bees' wax, stearic acid and SLNP formulations by using DSC experiments.

Formulations	Melting temperature (°C)	Melting enthalpy (J/g)	Onset temperature (°C)
F1	53.12	330.45	50.45
F2	53.48	356.01	51.66
F3	52.53	365.84	49.32
Stearik asit	58.1	177.6	52.84
Beeswax	62.44	161.55	52.45

The melting temperatures of SLNPs were lower compared to individual melting temperatures of both bulk beeswax and stearic acid as expected.

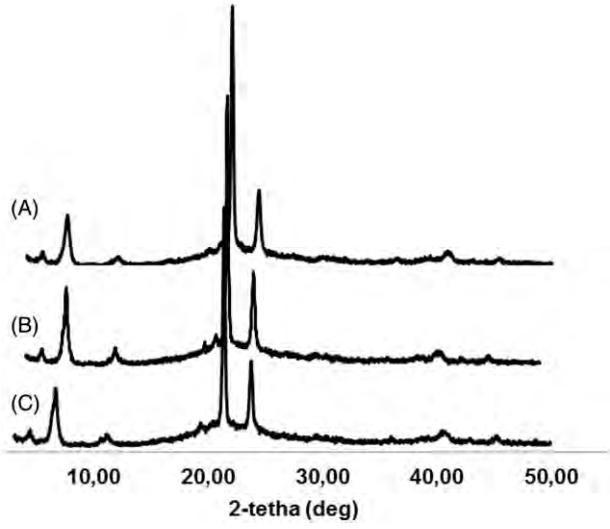

Figure 2. Crystallinity of SLNPs were determined by XRD patterns. Decrease stearic acid concentration slightly decreased crystallinity of the nanoparticles.

Thermal properties of the SLNPs

DSC analysis was performed to obtain the thermographs of the SLNPs and details of results were presented at Table 3. The melting temperatures of SLNPs were lower compared to individual melting temperatures of both bulk beeswax and stearic acid as expected. Gomes et al have produced solid lipid nanoparticles and reported that melting point of nanoparticles was slightly higher than the bulk lipid however melting enthalpy of their nanoparticles were extremely lower than the nanoparticles in this study (Gomes et al., 2014).

Crystallinity rates of SLNPs

X-ray diffraction (XRD) patterns of SLNPs were presented in Figure 2. The diffraction patterns of F1, F2, F3 displayed characteristic peaks of stearic acid and bees wax. The diffractogram of F1, F2 and F3 exhibited a diffraction peaks at about $2\theta = 4°$, 6°, 11°, 21° and 23°. These peaks were consistent with the peaks associated with the stearic acid (Akanda et al., 2015). The F1, F2 and F3 diffractograms displayed peaks at about $2\theta = 21°$, 24° (Lim et al., 2015) and these peaks were consistent with the peaks associated with the beeswax (Attama et al., 2006).

Crystallinity ratio of the nanoparticles was calculated, according to the area under the curves (Karimi and Taherzadeh, 2016). It was found the crystallinity rate of F1, F2 and F3 were 48.1%, 44.6% and 44.4%, respectively.

Degradation and VitD3 release profiles of SLNPs

One of the important issue that affects the drug release profile is the degradation rate of a nanoparticles (Souto and Müller, 2006). Degradation studies were performed at three different temperatures as 4°, 37° and 40 °C and repeated three times for each temperature. As seen in Figure 3, increasing the temperature has resulted in increase of the degradation rate of the nanoparticles; at the same time, the increase in wax concentration increased the degradation rates (Moreno-Sastre et al., 2016).

Encapsulation rate of F4, F5, F6 were calculated as 43.3 ± 0.8; 63.01 ± 1.0; 60 ± 1.4. F7, F8 and F9 were calculated as 45 ± 0.8; 59.8 ± 2.2; 52.09 ± 0.2. F10, F11 and F12 were calculated as 55 ± 0.9; 72.8 ± 1.1; 77.79 ± 1.2, respectively. Compared to the encapsulation rates, in terms of drug-loading capacity, F12 formulation was the most effective. The amount of VitD3 in formulation of F12 were calculated as 5.83 mg ±0.08/10 mg particles. These results have indicated that beeswax ratio had a positive effect to encapsulation capacity. The encapsulation efficiency was the lowest in F4 formulation as 1.62 ± 0.01/10 mg (Park et al., 2003).

F6, F8 and F12 formulations were selected for the release studies due to high VitD3 encapsulation rates. The released VitD3 amount was determined by liquid chromatography/tandem mass spectrometry (LC/MS/MS) (Cong et al., 2008). Release rates from F6, F8 and F12 formulations were presented in Figure 4. As seen from the graphic, F6, F8 and F12 released $45.25 \pm 5.47\%$, $59.93 \pm 3.64\%$ and $88.31 \pm 2.97\%$ of encapsulated VitD3 within 120 h. It can be concluded that release rate of VitD3 was increased related to increasing amount of beeswax. Genç et al encapsulated vitamin B-12 with compritol and they achieved 93% encapsulation efficiency; however, 80% of encapsulated vitamin B-12 released within 3 h, at pH 7.4 (Genç et al., 2015). In another study, Ma et al were prepared silymarin-loaded stearic acid nanoparticles and 95% of silymarin was released in one week (Ma et al., 2016).

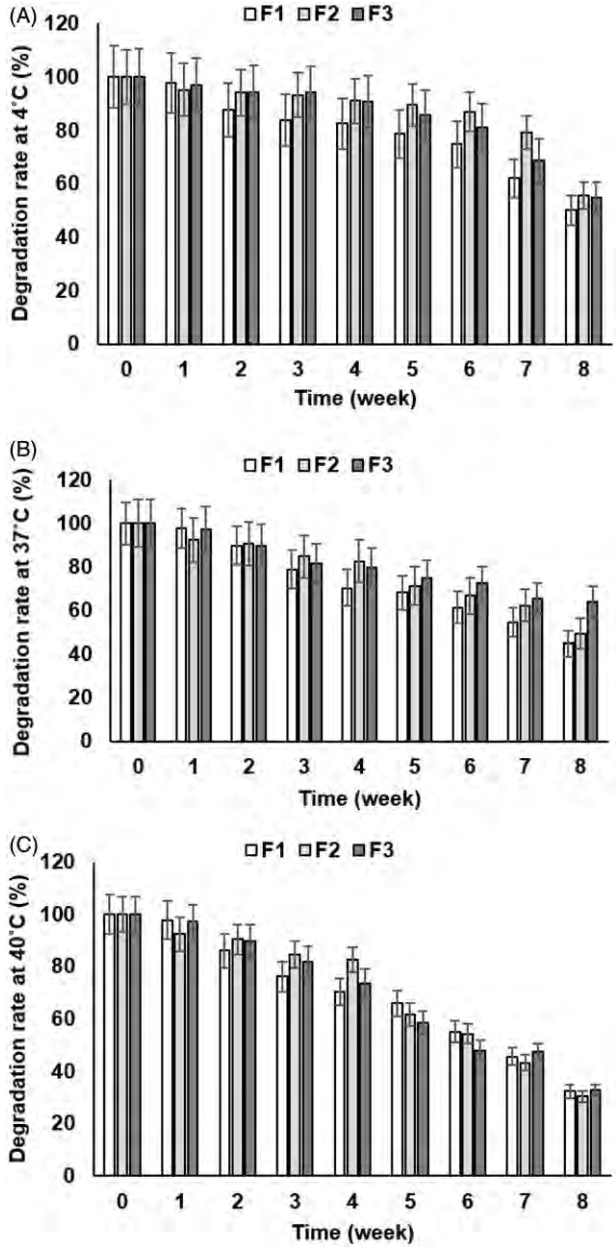

Figure 3. Degradation profiles of SLNPs. Increasing temperature and wax concentration increased degradation rate of the nanoparticles ($n = 3$).

Cytotoxicity of the SLNPs

In this study, cytotoxicity of SLNPs on HaCaT, HUVEC, L929 were determined by XTT test. Data were presented at Figure 5. Since SLNPs are made of biological materials, they are generally well tolerated by the body. However, the potential toxicity of emulsions should be taken into account. Numanoglu U. have stated that a cytotoxicity problem would not occur if appropriate emulsifiers are used (Numanoglu and Tarımcı, 2006). In this study, 0.5% SDS was used as surfactant and no cytotoxicity effect was observed on cells (Weyenberg et al., 2007). But, Dong et al have

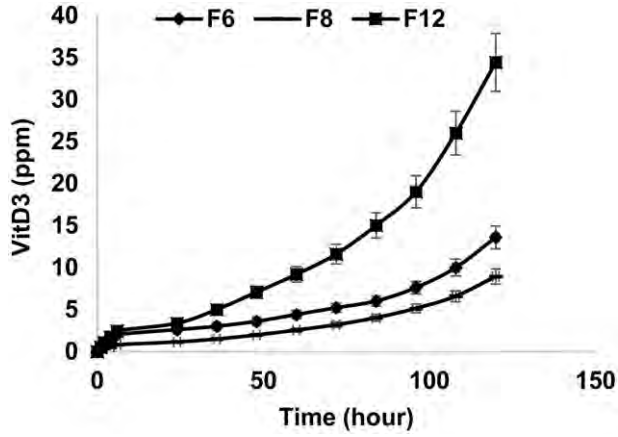

Figure 4. VitD3 release profiles of SLNPs were determined by mass spectrometer. In formulation, beeswax ratio has a positive effect to encapsulation capacity. ($n = 6$).

demonstrated that SDS and sodium dodecylbenzene sulphonate (SDBS) were toxic to astrocyte cells (Dong et al., 2009). The viability of L929, HUVEC and HaCaT cells was found as 105.06% ± 10.18, 99.81% ± 15.5 and 130.57% ± 15.58, respectively. All cell lines were interacted with 100 μg/mL of F83 formulation prior to the experiment. When 100 μg/mL of F5 formulation was evaluated, cell viability of L929 cells was found as 101.76% ± 11.58, HUVEC cells was 99.1% ± 7.5, cell viability of HaCaT cells was found as 130.8% ± 9.6 (Huang et al., 2009). According to the cytotoxicity results, SLNPs containing VitD3 were not found cytotoxic on HaCaT, HUVEC, L929 cell lines. Furthermore, depending on increasing VitD3 content, cell proliferation was stimulated. Especially, cell proliferation of HaCaT was stimulated more on VitD3 loaded formulations than other cell lines.

Cytokine levels

Cytokines involve in many stages of the immune response and have both positive and negative regulators. They regulate the type and magnitude of immune response. Cytokines can be released from lymphocytes, platelets, endothelial cells and activated macrophages against bacterial products, toxins or biomaterials. Particularly IL-10 and TNF-alpha shares many common biological properties and both of them are released from activated macrophages, lymphocytes. IL-10 is an anti-inflamatory cytokine, TNF-alpha is pro-inflammatory cytokine (Park and Barbul, 2004). Vitamin D inhibits the proliferation and conversion of T lymphocytes and also suppresses release of pro-inflammatory cytokines from macrophages (Bhalla et al., 1984). In this context, the interaction between the nanoparticles with monocyte-macrophages allows

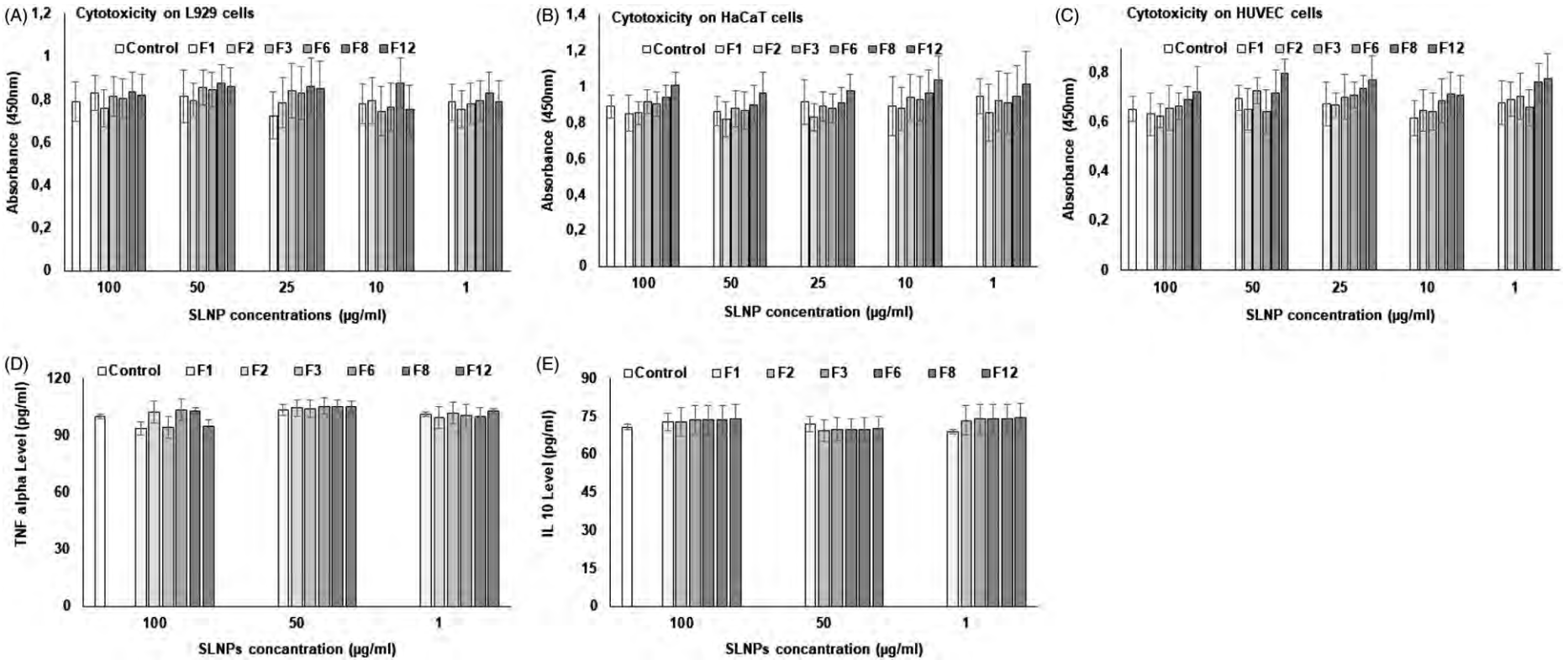

Figure 5. Cytotoxicity of SLNPs on L929 (A), HaCaT (B) and HUVEC (C) cell lines were determined. SLNPs containing VitD3 were not found cytotoxic on HaCaT, HUVEC, L929 cell lines. Furthermore, depending on increasing VitD3 content, cell proliferation was stimulated. Especially, cell proliferation of HaCaT was stimulated more on VitD3-loaded formulations than other cell lines. Effects of SLNPs on pro/anti-inflammatory cytokine levels were determined by using THP-1 cell lines. SLNPs did not increase the secretion level of TNF-alpha (D) and also IL-10 (E), compared to the control group. ($n = 6$).

us to determine the *in vitro* immunocompatibility of SNPs (Birkedal-Hansen, 1993). In this study, nanoparticles were interacted with monocyte–macrophage cell line and then TNF-alpha and IL-10 secretion levels were determined. As seen in Figure 6, SLNPs did not increase the secretion level of TNF-alpha and also IL-10, compared to the control group. According to the results, TNF-alpha and IL-10 levels in all nanoparticle formulations were found similar to the control group.

Conclusions

This study has clearly demonstrated that beeswax-stearic acid blend formulation is beneficial for the preparation of stable solid lipid nanoparticles. Round, in the size of 30–60 nm, nanoparticles were obtained. It was seen that, increased beeswax concentration increased nanoparticle degradation rate, increased VitD3 encapsulation efficiency, increased VitD3 release rate due to reduced crystallinity. The SLNPs were immunocompatible and were not cytotoxic to keratinocytes (HaCaT), endothelial (HUVEC) and fibroblast (L929) cell lines. Consequently, VitD3 loaded SLNPs were considered to be ideal delivery system due to its encapsulation efficiency, drug release rate and biocompatibility.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Akanda MH, Rai R, Slipper IJ, Chowdhry BZ, Lamprou D, Getti G, Douroumis D. Delivery of retinoic acid to LNCap human prostate cancer cells using solid lipid nanoparticles. Int J Pharm 2015;493:161–71.

Attama A, Schicke B, Müller-Goymann C. Further characterization of theobroma oil–beeswax admixtures as lipid matrices for improved drug delivery systems. Eur J Pharm Biopharm 2006;64:294–306.

Bhalla AK, Amento E, Serog B, Glimcher LH. 1,25-Dihydroxyvitamin D3 inhibits antigen-induced T cell activation. . J Immunol, 1984;133:1748–54.

Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. J Periodontal Res, 1993;28:500–10.

Bothiraja C, Pawar A, Deshpande G. Ex-vivo absorption study of a nanoparticle based novel drug delivery system of vitamin D3 (Arachitol Nano^{TM}) using everted intestinal sac technique. J Pharm Invest, 2016;46:425–432.

Cong TT, Faivre V, Nguyen TT, Heras H, Pirot F, Walchshofer N, Sarciron ME, Falson F. Study on the hydatid cyst membrane: permeation of model molecules and interactions with drug-loaded nanoparticles. Int J Pharm 2008; 353:223–32.

Dong L, Witkowski CM, Craig MM, Greenwade MM, Joseph KL. Cytotoxicity effects of different surfactant molecules conjugated to carbon nanotubes on human astrocytoma cells. Nanoscale Res Lett, 2009;4:1517.

Gaur PK, Mishra S, Purohit S. Solid lipid nanoparticles of guggul lipid as drug carrier for transdermal drug delivery. BioMed Res Int, 2013;2013:750690.

Genç L, Kutlu HM, Güney G. Vitamin B12-loaded solid lipid nanoparticles as a drug carrier in cancer therapy. Pharm Dev Technol, 2015;20:337–44.

Gomes MJ, Martins S, Ferreira D, Segundo MA, Reis S. Lipid nanoparticles for topical and transdermal application for alopecia treatment: development, physicochemical characterization, and in vitro release and penetration studies. Int J Nanomed 2014;9:1231.

Gonnet M, Lethuaut L, Boury F. New trends in encapsulation of liposoluble vitamins. J Control Release, 2010; 146:276–90.

Grossmann RE, Tangpricha V. Evaluation of vehicle substances on vitamin D bioavailability: A systematic review. Mol Nutr Food Res, 2010;54:1055–61.

Heyes J, Palmer L, Chan K, Giesbrecht C, Jeffs L, Maclachlan I. Lipid encapsulation enables the effective systemic delivery of polyplex plasmid DNA. Mol Ther, 2007;15:713–20.

Huang YC, Royappa AT, Tundel S, Tsukamoto K, Sharma V. Biocompatibility of polyglycidol with human peripheral blood mononuclear cells. J Appl Polym Sci, 2009;111: 2275–8.

İşcan Y, Wissing S, Müller R. Solid lipid nanoparticles (SLNTM) for topical drug delivery: incorporation of the lipophilic drugs N, N-diethyl-m-toluamide and vitamin K. Pharmazie, 2005;60:905–9.

Jakobsen J, Knuthsen P. Stability of vitamin D in foodstuffs during cooking. Food Chem, 2014;148:170–5.

Karimi K, Taherzadeh MJ. A critical review of analytical methods in pretreatment of lignocelluloses: Composition, imaging, and crystallinity. Bioresour Technol 2016;200: 1008–18.

Lim JH, Kim J, Ko JA, Park HJ. Preparation and characterization of composites based on polylactic acid and beeswax with improved water vapor barrier properties. J Food Sci 2015;80:E2471–7.

Liu CH, Wu CT, Fang JY. Characterization and formulation optimization of solid lipid nanoparticles in vitamin K1 delivery. Drug Dev Ind Pharm, 2010a;36:751–61.

Liu D, Ge Y, Tang Y, Yuan Y, Zhang Q, Li R, Xu Q. Solid lipid nanoparticles for transdermal delivery of diclofenac sodium: preparation, characterization and in vitro studies. J Microencapsul, 2010b;27:726–34.

Ma Y, He S, Ma X, Hong T, Li Z, Park K, Wang W. Silymarin-loaded nanoparticles based on stearic acid-modified Bletilla striata polysaccharide for hepatic targeting. Molecules, 2016;21:265.

Mohammadi M, Ghanbarzadeh B, Hamishehkar H. Formulation of nanoliposomal vitamin D3 for potential application in beverage fortification. Adv Pharm Bull, 2014;4:569.

Moreno-Sastre M, Pastor M, Esquisabel A, Sans E, Viñas M, Bachiller D, Pedraz J. Stability study of sodium colistimethate-loaded lipid nanoparticles. J Microencapsul, 2016;33:636–45.

Muèller RH, Maèder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm, 2000;50:161–77.

Mutlu EC, Bostan MS, Bahadori F, Kocyigit A, Oner ET, Eroglu MS. Lecithin-acrylamido-2-methylpropane sulfonate based crosslinked phospholipid nanoparticles as drug carrier. J Appl Polym Sci, 2016;133:44105.

Numanoglu U, Tarımcı N. Characterization of solid lipid nanoparticles (SLNTM) and their pharmaceutical and cosmetic applications. Ankara J Fac Pharm, 2006;35:211–35.

Park JE, Barbul A. Understanding the role of immune regulation in wound healing. Am J Surg, 2004;187:S11–S16.

Park SN, Lee HJ, Lee KH, Suh H. Biological characterization of EDC-crosslinked collagen–hyaluronic acid matrix in dermal tissue restoration. Biomaterials 2003;24:1631–41.

Potta SG, Minemi S, Nukala RK, Peinado C, Lamprou DA, Urquhart A, Douroumis D. Preparation and characterization of ibuprofen solid lipid nanoparticles with enhanced solubility. J Microencapsul, 2011;28:74–81.

Ramteke K, Joshi S, Dhole S. Solid lipid nanoparticle: a review. IOSR J Pharm, 2012;2:34–44.

Rosita N, Setyawan D, Soeratri W, Mrtodihardjo S. Physical characterization of beewax and glyceryl monostearate binary system to predict characteristics of solid lipid nanoparticle (SLN) loaded para methoxy cinnamic acid (PCMA). Int J Pharm Pharmaceut Sci, 2014;6:939–45.

Souto E, Müller R. The use of SLN® and NLC® as topical particulate carriers for imidazole antifungal agents. Pharmazie 2006;61:431–7.

Videira M, Almeida AJ, Fabra À. Preclinical evaluation of a pulmonary delivered paclitaxel-loaded lipid nanocarrier antitumor effect. Nanomed Nanotech Biol Med, 2012;8:1208–15.

Vitorino C, Almeida A, Sousa J, Lamarche I, Gobin P, Marchand S, Couet W, Olivier JC, Pais A. Passive and active strategies for transdermal delivery using co-encapsulating nanostructured lipid carriers: in vitro vs. in vivo studies. Eur J Pharm Biopharm, 2014;86:133–44.

Wang JJ, Liu KS, Sung K, Tsai CY, Fang JY. Skin permeation of buprenorphine and its ester prodrugs from lipid nanoparticles: lipid emulsion, nanostructured lipid carriers and solid lipid nanoparticles. J Microencapsul, 2009;26:734–47.

Weyenberg W, Filev P, Van Den Plas D, Vandervoort J, De Smet K, Sollie P, Ludwig A. Cytotoxicity of submicron emulsions and solid lipid nanoparticles for dermal application. Int J Pharm, 2007;337:291–8.