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Production of novel vitamin D_3 loaded lipid nanocapsules for milk fortification

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ABSTRACT

Lipid nanoparticles are carriers to improve stability, solubility, and efficacy of bioactive compounds. In this paper, novel vitamin D_3 loaded lipid nanocapsules (LNC) were produced by phase inversion method. The produced nanocapsules were characterised by particle size, polydispersity index, zeta potential, encapsulation efficiency, and encapsulation load. LNC showed sizes in the range of 31.43 to 36.66 nm. Optimum LNC formulation was selected for further analysis (such as morphological study, analysis of chemical structure, release study, and sensory evaluation). Transmission electron microscopy revealed that particles had approximately spherical shape. The Fourier transform infrared spectra indicated that no adverse reactions occurred between vitamin D_3 and lipid nanocapsules. About 9.6% of vitamin released in gastric simulated solution (pH: 1.2), which indicated that LNC can protect vitamin against acidic conditions. Sensory evaluation revealed the potential application of produced vitamin D_3 loaded LNC for development of fortified milk.

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Lipid nanocapsules; Nanoencapsulation; Phase inversion method; Physicochemical properties; Vitamin D

Introduction

Vitamin D refers to a family of structurally related compounds that exhibit anti-rachitic activity. Members of the D family are derived from the cyclopentaneperhydrophenanthrene ring system, which is similar to other steroids, such as cholesterol. However, in comparison to cholesterol, vitamin D has only three intact rings. Naturally occurring members of the vitamin D family differ from each other only in the structure of their side chains.[1] Two major chemical forms of this vitamin are vitamin D_3 (cholecalciferol or activated 7-dehydrocholesterol) and vitamin D_2 (ergocholecalciferol or activated ergosterol). Vitamin D_2 is synthesised by ultraviolet (UV) irradiation of ergosterol, and vitamin D_3, the preferable form of vitamin D, is a compound formed in the skin.[2] Vitamin D is a liposoluble nutraceutical compound, and its major functions are to increase the intestinal absorption of calcium, regulate phosphorus balance, and promote normal bone formation and mineralisation.[3] Numerous studies have shown the relationships between vitamin D level and body organ states. The presence of adequate level of vitamin D can prevent bone disease, cancer, diabetes, and multiple sclerosis especially in adults.[4] Due to low water solubility, vitamin D cannot be dissolved completely into aqueous foods or beverages, and therefore it is hardly found in products such as skim milk and low fat dairy products, which are important sources for calcium and phosphate.[5] Furthermore, liposoluble vitamins like vitamin D_3 are very sensitive to oxidation, UV light, or processing conditions.[4,6]

Nowadays, nanoencapsulation technology has been receiving increasing attention. It is a process to entrap bioactive ingredients into nanocarriers for preservation against undesirable conditions, make them soluble in aqueous system, and control their release.[3,7] Furthermore, the encapsulation techniques through controlled release and small doses administration are able to reduce the hyper vitaminosis

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syndrome and the possible side effects.[8] Lipid-based nanocarriers are beneficial for the delivery of poorly water soluble compounds. Examples for such lipid nanocarriers are nanoemulsions, solid lipid nanoparticles (SLNs), nanostructure lipid carriers (NLCs), and lipid nanocapsules (LNCs).[6,9] Lipid nanocapsules are new generation of nanocarriers and are characterised by a hybrid structure between polymeric nanocapsules and liposomes. In comparison to liposomes which are leaky, unstable in biological fluids, and are manufactured by organic solvents, LNCs are prepared by solvent-free and soft-energy procedures which show a great stability. This nanocarrier is formulated with generally recognised as safe (GRAS) ingredients, and due to their very small size (in the range of 25–100 nm) and capacity to encapsulate lipophilic and hydrophilic bioactive compounds, LNC can be an excellent alternative to liposomes, emulsions, or micro-emulsions.[10] LNCs consist of three main components: an oily phase, an aqueous phase containing NaCl, and a non-ionic surfactant (Fig. 1). The oily core is composed of medium-chain triglycerides surrounded by a membrane made from a mixture of lecithin and a pegylated surfactant. Each component has different influences on formulation and stability of LNC (Table 1). LNC production is based on the phase-inversion temperature phenomenon of an emulsion.[11,12] It should be noted that there is not any research work on application of LNC for encapsulation of food bioactives.

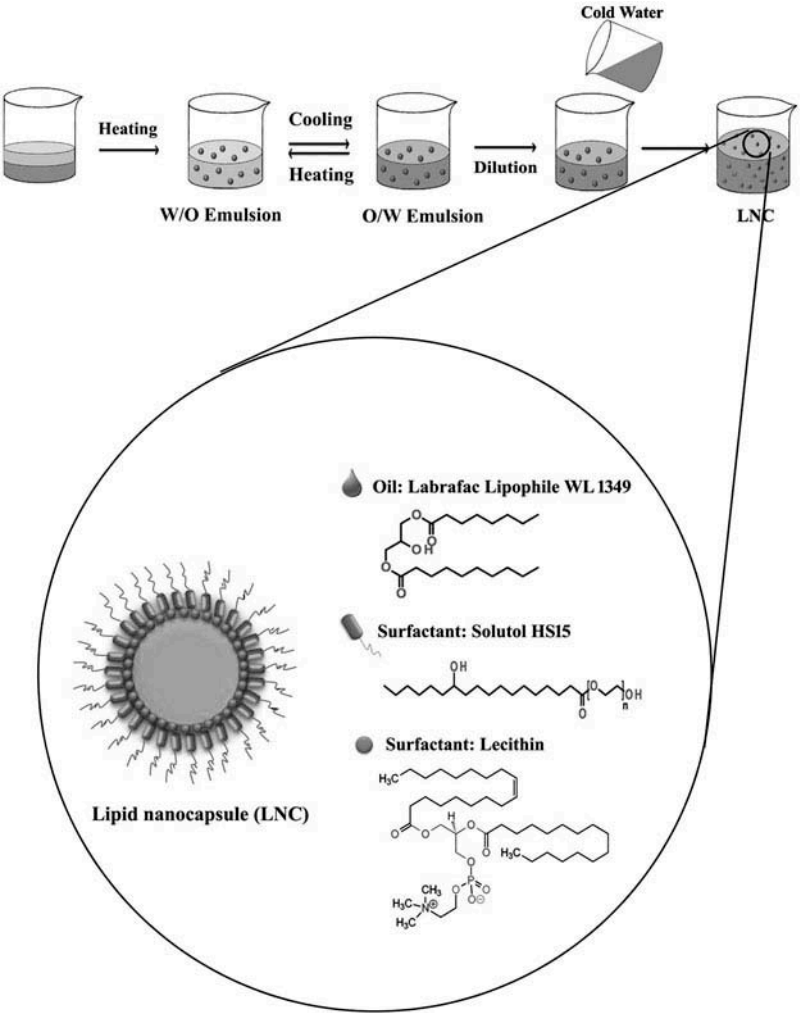

Figure 1. Schematic production method of lipid nanocapsules by phase-inversion temperature (PIT) technique and chemical structure of LNC and its components.

Table 1. Factors affecting formulation and stability of LNC prepared by PIT method.[20]

Factor	Effect
Solutol®	Major influence on LNC formation and stability
Temperature cycles	Favouring LNC formation and improving the quality of LNC dispersion
Labrafac®	Influence on LNC size
NaCl	Decrease of PIT
Lipoid®	Stabilising the LNC rigid shell and favouring the freeze-drying process

The purpose of this study was to develop lipid nanocapsules for vitamin D_3 entrapment by phase inversion method. Furthermore, physicochemical properties and the potential application of developed nanocarriers for milk fortification were investigated.

Materials and methods

Materials

Vitamin D_3 (40 IU/μg) was purchased from Roche Company (Village-Neuf, France). Labrafac® WR 1349 (capric and caprylic acid triglyceride) was supplied by Gattefossé (Lyon, France), and Polyethylene glycol hydroxyl stearate (Solutol® HS 15) was provided by BASF (The Chemical Company, Ludwigshafen, Germany). Soybean lecithin, sodium chloride (NaCl), and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany).

Preparation of vitamin D_3 loaded LNC

The three basic components of LNC formulation are an oil phase, an aqueous phase, and a nonionic surfactant. The oil phase contained triglycerides of capric and caprylic acids that are known under the commercial name of Labrafac® WR 1349. Solutol® HS 15 is the hydrophilic surfactant that is derived from polyethylene glycol (PEG), and it is a mixture of free PEG 660 and PEG 660 hydroxystearate. Lipoid® that is composed of 69% phosphatidylcholine soybean lecithin was also applied as an emulsifier. The aqueous phase contains of MiliQ® water and sodium chloride.

LNCs were prepared according to phase-inversion temperature (PIT) process. The preparation method involved two steps: (i) formation of W/O emulsion by mixing all components (i.e., Labrafac, lecithin, Solutol HS15, NaCl, water, and vitamin D_3) under magnetic stirring and heating from room temperature to about 85°C (above the PIT). Cooling the solution to 60°C (below the PIT) led to the formation of an O/W emulsion. Several temperature cycles crossing the phase-inversion zone (PIZ) between 85°C and 60°C were then carried out. (ii) This was followed with sudden dilution with cold water (0°C) which led to irreversible shock and breaking of the micro emulsion system and forming of stable nanocapsules (Fig. 1). In this work, concentrations of Labrafac were 16.8% and 20.5%, and the percentages of vitamin to total lipid phase were 4% and 8% (w/w). The formulation codes were tabulated in Table 2.

Particle size, zeta potential, and polydispersity index

The average diameter, polydispersity index, and zeta potential of the nanocapsules were determined by photon correlation spectroscopy (PCS) with a Zetasizer (NanoSizer 3000, Malvern Instruments,

Table 2. Formulation code of vitamin D loaded LNCs.

Formulation code	Lipid percent	Vitamin D ratio to total lipid phase (%)
1L	16.8	4
2L	20.5	4
3L	16.8	8
4L	20.5	8

Malvern, UK). Photon correlation spectroscopy is a standard method for determination of particle size and distribution.[13]

Encapsulation efficiency and load

Encapsulation efficiency (EE) and encapsulation load (EL) were determined using centrifugation method.[14] A 500 µl LNC dispersion was placed in a Millipore tube with cutoff of 10 kDa (Millipore, Bedford, MA, USA) and ultra-centrifuged (Sigma, Germany) at 10,000 rpm for 10 min at room temperature. The amount of unloaded vitamin D_3 in the filtrate phase was determined by HPLC at wavelength of 265 nm (HPLC; Shimadzu, Japan, equipped with a C18-ODS column and UV (FPD-6AV) detector). Methanol by isocratic flow and the flow rate of 1.2 ml/min was considered as the mobile phase. EE and EL were calculated by Eqs. (1) and (2):

$$EE\% = \frac{(W_T - W_F)}{W_T} \times 100 \tag{1}$$

$$EL\% = \frac{(W_T - W_F)}{W_L} \times 100 \tag{2}$$

where W_T is the total weight of applied vitamin D_3, W_F is the amount of free (unloaded) vitamin in filtrate phase, and W_L is the weight of the used lipid in preparation of LNC.

Morphological study

Morphology of the produced LNC was observed using transmission electron microscopy (TEM; CM120, Philips Germany). The sample was prepared by depositing 10 µL of nanocapsule dispersions on carbon-coated grids. Then, it was left to dry for 1 h before imaging.

Fourier transform infrared spectroscopy

To Certificate encapsulation of vitamin D_3 in the nanocarrier and formation of any possible interaction between vitamin and nanocarriers, Fourier transform infrared spectroscopy (FTIR, 8400S; Shimadzu, Japan) was applied. The infrared spectra were detected at 4 cm^{-1} resolution in the frequency range between 800 cm^{-1} and 4000 cm^{-1} using KBr Pellet method.

Vitamin D release and kinetic modeling

Vitamin D_3 release profiles from the nanocarriers were studied in gastric (pH: 1.2) and intestinal (pH: 7.4) simulated solutions applying dialysis bag method at 37°C.[14,15] The dialysis bag retains the nanocarriers but allows transfer of the dissolved/released vitamin molecules into the release media.[16] For this purpose, 2 ml of vitamin loaded nanocarrier solution was sealed into dialysis bag (Sigma, Canada) with a 12 kDa cutoff. The bag was then placed into 48 ml gastric buffer for 2 h. It was subsequently subjected to 48 ml intestinal buffer for 6 h. At specified intervals, the amount of released vitamin was determined by HPLC method at wavelength of 265 nm.

To study release kinetic of vitamin D_3 in the gastric and intestinal media, release data were modeled by Higuchi, Rigter-Peppas, Quadratic, and Weibull models (Eqs. 3–6)[14,17]:

$$C = kt^{0.5} \tag{3}$$

$$C = kt^n \tag{4}$$

$$C = \left(k_1 t^2 + k_2 t\right) \times 100 \tag{5}$$

$$\ln[-\ln(1 - C)] = a_w \ \ln t - \ln b_w \tag{6}$$

where C is vitamin concentration at time t, k is kinetic constant, and n is release exponent. Encapsulant release from spherical carriers with $0.43 \leq n$ is controlled by Fickian diffusion mechanism and $n \geq 0.85$ is commanded for dissolution phenomenon, and $0.43 < n < 0.85$ is governed by combination of two mechanisms. The shape parameter, a_w, in Weibull model characterises the curve as either exponential ($a_w = 1$), sigmoid, S shaped, with upward curvature followed by a turning point ($a_w > 1$) or parabolic with a higher initial slope and after that consistent with the exponential ($a_w < 1$).[18]

Sensory properties

Milk is a complete nutritional source for humans and is widely marketed and consumed. Therefore, milk was chosen as a high calcium content food for the investigation of potential application of LNC for fortification. For this purpose, milk was fortified with 4μg/100 mL (160 IU) vitamin D_3. Sensory evaluation of enriched milk samples was performed based on both oral and non-oral features by trained panelists. Three milk samples, i.e., blank, vitamin D_3 loaded LNC enriched milk, and direct vitamin D_3 enriched milk, were provided at temperature about 7°C and assessed using hedonic scale of 1–7. Parameters such as taste creamy flavour, after taste, yellowness, creamy aroma homogeneity, thickness, and total acceptance were used for descriptive analysis of milk samples.[14]

Statistical analysis

All measurements were performed in three replications. Analysis of variance (ANOVA) and comparison of the mean were accomplished by SPSS software (Version 14) by Duncan's multiple test. All experiments were tested using complete randomised design, while sensory analysis was performed by randomised block design.

Results and discussions

Particle size analyses

Particle size and distribution (polydispersity index) are two most important characteristics which reveal the homogeneous nature of produced nanodispersions and play important roles in the physical stability, solubility, release rate, turbidity, and chemical stability.[8,19] Furthermore, it has been reported that bioavailability of encapsulated compounds increases by decreasing particle diameter.[20] Particle size and polydispersity index of LNC are affected by several factors such as proportions of the constituents, viscosity of the lipid phase, and production conditions.[21] In this study, the effects of lipid and vitamin D_3 percentage on the average particle size of LNCs were investigated. As Table 3 revealed, all the prepared LNC have the mean particle size distribution in the range of 31.43–36.66 nm. The percentage of Labrafac had a significant effect ($P<0.05$) on the average size of LNC, which showed an increase in particle size with raising oil proportion. This

Table 3. Particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency (EE), and encapsulation load (EL) of produced nanocarriers.

Formulation code	Particle size (nm)	Zeta potential (mV)	PDI	EE (%)	EL (%)
1	31.95 ± 0.39^{b}	-6.01 ± 0.75^{c}	0.249 ± 0.011^{ab}	96.56 ± 0.34^{b}	4.09 ± 0.014^{a}
2	36.66 ± 1.24^{a}	-2.29 ± 1.11^{a}	0.206 ± 0.001^{b}	98.81 ± 0.11^{a}	3.29 ± 0.003^{c}
3	31.43 ± 0.34^{b}	-5.08 ± 0.53^{bc}	0.276 ± 0.040^{a}	93.25 ± 0.67^{d}	3.66 ± 0.057^{b}
4	35.58 ± 0.06^{a}	-3.67 ± 0.305^{ab}	0.243 ± 0.002^{ab}	95.43 ± 0.45^{c}	3.02 ± 0.030^{d}

Values in each column for LNC followed by different letters are significantly different ($p < 0.01$).

phenomenon could be due to reducing the surfactant ratio in formulation by increasing the oil concentration. The proportion of hydrophilic surfactant (Solutol) had a major influence on the average diameter and size distribution of the LNC. It has been previously reported that diameter of nanocarreirs decreased when Solutol proportion increased due to its stability properties.[22] Particle size reduction protects the droplets against aggregation.[22,23] Barras et al. (2012) designed and characterised flavonoid loaded lipid nanocapsules by phase inversion method. They reported that the Solutol proportion as hydrophilic surfactant had a major influence on the particles' average diameter and their size distribution, and therefore diameter decreased when Solutol proportion increased.[24] Statistical results revealed that no significant difference was obvious between particle size means by changing vitamin D_3 concentration.

Zeta potential

Zeta potential (ZP) characterises the electric charge at the particle surface and gives information about repulsive forces between particles or droplets.[21] It is an important and useful indicator to predict physical stability of colloidal systems. This factor even influences the release kinetic and the biological fate of nanoparticles.[12,16,19]

As can be seen in Table 3, all of the LNCs formulations had weak negative surface charges (in the range of -2.29 to -6.01 mv) which was due to the negative contribution of phospholipids molecules and the presence of PEG dipoles in their shell.[12] The results showed that absolute values of zeta potential of LNC significantly ($p<0.05$) increased by decreasing oil phase which could be due to increase of Solutol proportion at the surface of nanocarriers. The results also showed that the vitamin concentration did not have any significant ($p>0.05$) effect on the zeta potential of LNC.

Polydispersity index

Polydispersity index shows particle size distribution, which is between 0 and 1. PDI values of 0.1–0.25 show a fairly narrow size distribution where values greater than 0.5 indicate broad distribution. This factor has an important effect on physical stability of emulsion and should be as low as possible (preferably lower than 0.5).[19] All produced vitamin D_3 loaded LNC formulations had PDI value between 0.206 and 0.276, which indicates their narrow size distribution. No significant differences were observed in PDI values of different formulations ($P>0.05$).

Encapsulation efficiency

Encapsulation efficiency (EE) is defined as the ratio of encapsulant (core) in nanoparticles to initial encapsulant added to the lipid phase. It is affected by nanocarrier ingredients, production method, and solubility of encapsulant in lipid. It can influence some properties of the nanocarriers such as release profile leakage during storage.[19] The EE values of LNC were in the range of 93.25–98.81% (Table 3) which demonstrated that high amount of vitamin D_3 was incorporated into the nanocarriers. One of the important features of lipid nanocapsules is their ability to encapsulate high amounts of lipophilic bioactive compounds. The results also showed that the EE of LNC is significantly affected by different amounts of vitamin as well as Labrafac concentration ($P<0.05$). The LNC formulations prepared with higher amount of Labrafac showed higher encapsulation efficiencies. It could be due to good solubility of vitamin D_3 in the oily core. On the other hand, higher lipid concentration led to greater particle size, which provide more capacity for vitamin incorporation.[25] Yuan et al. (2007) prepared NLC from mixtures of solid and spatially incompatible liquid lipids by melt-emulsification method and reported that EE improved by increasing liquid lipid content into the formulations of NLC.[26] However, increasing of vitamin content led to a reduction of EE. This phenomenon could be attributed to capacity constraints of developed nanocarriers to accept more vitamins in their structures.

Encapsulation load

Encapsulation load (EL) is defined as the mass ratio of encapsulant to lipid phase. This factor is mainly influenced by the ratio of encapsulant to shell, polymorphic state of the lipid, solubility of the encapsulant in the melted lipid, and types of lipids.[19,26] Results tabulated in Table 3 showed that produced LNC formulations had encapsulation load from 3.02% to 4.09%. By increasing oil concentration, encapsulation load decreased significantly. This phenomenon could be attributed to decrease of oil to vitamin ratio. Moreover, increase of vitamin content led to a reduction of EL. As previously described, adding higher amount encapsulant than capacity of nanocarriers led to reduction of EL. It should be noted that having a high encapsulation load is always favourable, while encapsulation load more than 50% is not proper due to increase of risk of bioactive leakage.[27] For future analysis (such as morphological analysis of chemical structure, release study and sensory evaluation), the formulation with the lowest size and polydispersity index as well as highest zeta potential (formulation code of No. 1) was selected as the optimum formulation.

Morphological characterisation

The result of TEM imaging of optimised LNC is depicted in Fig. 2. It was obvious that particles had approximately spherical shape. Similar particle shape was reported for miltefosine lipid nanocapsules by Eissa et al (2015).[28] The average sizes of nanocarriers in TEM image were in agreement with PCS results.

Fourier transform infrared spectroscopy analysis

Fourier infrared absorption spectroscopy was carried out to explain chemical interactions. FTIR analyses the vibrational motions of the molecules and can be used for determination of fatty acids in different ways.[29] FTIR analysis of all the ingredients used in the LNC formulation (Labrafac, Solutol, lecithin, and vitamin D_3), blank, and vitamin D_3 loaded LNC were studied for the checking of probable interactions between LNC and vitamin in the final formulation (Fig. 3).

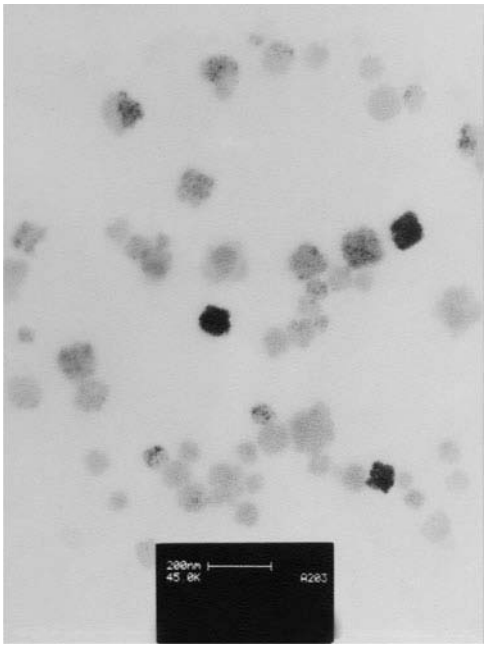

Figure 2. TEM morphology of formulation No. 1.

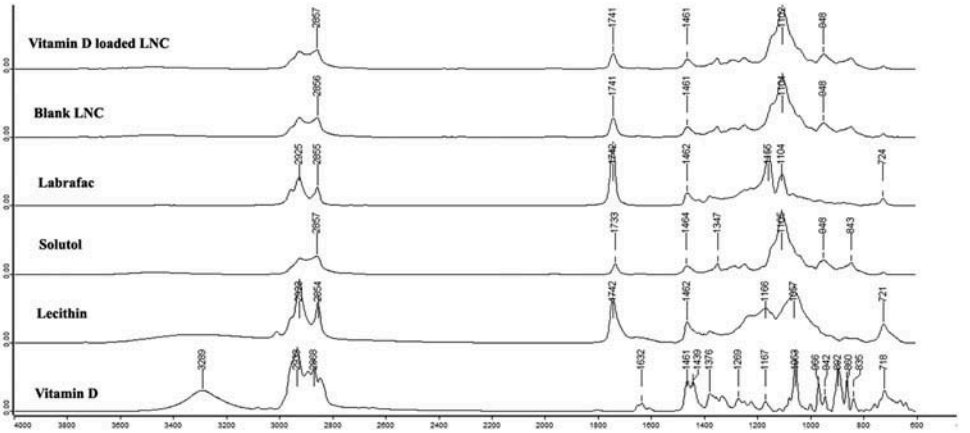

Figure 3. FTIR spectra for vitamin D loaded LNC, blank LNC, Labrafac, Solutol, lecithin, and vitamin D.

A series of bands were observed in the spectrogram of vitamin D_3, namely hydrogen bond O–H stretching (3289 cm^{-1}), alkyl C–H stretches (2938 cm^{-1} and 2868 cm^{-1}), C=O stretching (1632 cm^{-1}), C-O group (1167 cm^{-1}), CH_2 group (718 cm^{-1}), and several other picks in the wavenumber range of 835–966 cm^{-1} indicating C–H bending. Labrafac spectrum showed peaks around 724 cm^{-1} corresponding to aliphatic C–H bonds, at 1104 cm^{-1} for C-O groups, stretching vibration of C=O ester groups, and symmetric stretching vibration of CH_2 at 1742 $cm^{-1,}$ 2855 $cm^{-1,}$ and 2925 cm^{-1}, respectively.

In the spectra of blank LNC, a band at 1104 cm^{-1} was observed which denoted to the C-O groups. Several peaks at 948 cm^{-1} (absorption bands of C-C groups), 1741 cm^{-1} (ester groups), and also 2857 cm^{-1} (symmetric stretching vibration of CH_2) were observed, which were also detected in the spectra of all ingredients. All of these peaks were present without any deviation in the spectra of vitamin D_3 loaded LNC formulations. Therefore, it could be confirmed that no chemical interaction occurred between ingredients, and vitamin was compatible with applied lipids.

Release study and kinetic modeling

In vitro vitamin D_3 release experiments were performed in simulated gastric (pH: 1.2) and intestine (pH: 7.4) media. The release profiles of vitamin D_3 loaded LNC were depicted in Fig. 4. As can be seen, about 9.6% of vitamin D_3 release was happened after 2 h in simulated gastric fluid at 37°C, and ultimately over the next 6 h in the intestine media, about 16.2% of the vitamin D_3 was released from the nanocapsules. Solubility of bioactive compound in the release medium and core oil as well as the partitioning between them were considered as the key factors in release behaviour. The core of LNC is liquid and thus ensures a homogenous dispersion of the bioactive compound throughout the whole particle. Therefore, the rate of release will only be affected by the extent of compound-oil interaction and sustained or fast release rates could be achieved by changing the oil used in the core materials.[30] The low vitamin D_3 release percentage from LNC could be due to its lipophilic properties, and therefore its localisation in the oily core is more probable than at the particle surface. Another reason can be the rigid external shell of LNC which was resistant to digestion and acted as a barrier for the diffusion of vitamins during *in vitro* release experiments. It is generally assumed that compounds are released by several processes such as (i) diffusion through the particle matrix, (ii) degradation, and (iii) diffusion through micro channels that are formed by erosion.[31] In a previous study, it has been reported that the LNCs can provide considerable

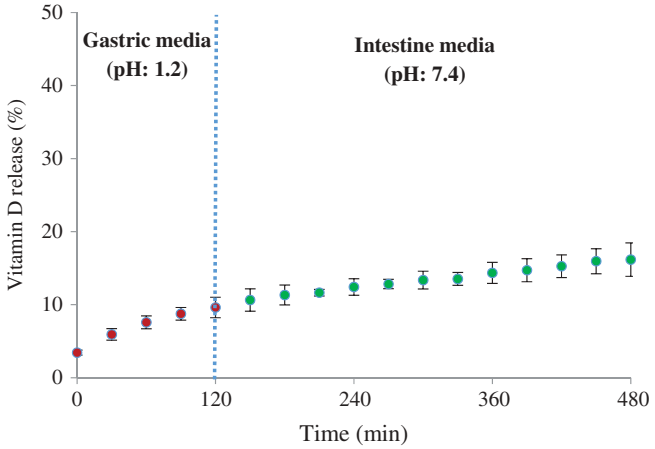

Figure 4. Release profiles of vitamin D loaded LNC at gastric media (pH: 1.2) and intestinal media (pH: 7.4).

bioactive encapsulation capacity and also exhibit sustained release functions at the site of action.[12]

Vitamin D_3 release from LNCs was also kinetically studied by Higuchi, Rigter-Peppas, Quadratic, and Weibull models. Table 4 listed the model parameters and their corresponding correlation coefficients. By comparison of k values of Higuchi model, it was obvious that release rate in gastric was faster than intestinal media. Based on shape factor values ($a_w > 1$) of Weibull model, it is indicated that the releases were S shaped, with upward curvature followed by a turning point. The Rigter-Peppas was the best model based on its correlation coefficients. In this model, *n* is an exponent parameter which is used to describe different release mechanisms of bioactive compound. The value of $n < 0.45$ indicated that the release of bioactive compound govern by diffusion mechanism; $n > 0.89$ is attributed to the dissolution of particles, and $0.45 < n < 0.89$ is due to the combination of both Fickian diffusion and dissolution mechanisms.[32] Therefore, the result indicated that the release phenomenon in LNC is mainly governed by combination of both Fickian diffusion and dissolution mechanism for intestinal media and diffusion mechanism for gastric media as well as whole release process.

Sensory properties

Milk is a good source of calcium, and its nutritional value enhances when it contains suitable amount of vitamin D. Sensory results of blank milk, fortified milk with vitamin D_3 loaded LNC, and

Table 4. Model parameters of vitamin D release from LNC.

Model		Whole release process	Intestinal media	Gastric media
Higuchi	k	0.008	0.003	0.02
	R	0.952	0.923	0.98
Rigter–Peppas	N	0.363	0.808	0.349
	K	0.017	0.001	0.005
	R	0.998	0.995	1
Quadratic	k_1	−1.036E-8	−1.549E-9	−2.307E-8
	k_2	7.99E-06	2.37E-06	4.69E-06
	R	0.84	0.99	0.966
Weibull	a_ω	1.026	1.011	1.021
	b_ω	21.494	108.96	12.64
	R	0.879	0.778	0.986

Parameters of models were obtained by nonlinear regression. 'R' is correlation coefficient, and n, k, k_1, k_2, a_ω, and b_ω are parameters of the models.

Table 5. Sensory results of fortified milk with vitamin D loaded LNC, direct fortification, and blank samples.

	Properties						
Sample	Creamy flavour^{NS}	After taste^{NS}	Yellowness^{NS}	Creamy aroma^{NS}	Homogeneity^{NS}	Thickness^{NS}	Total acceptance^{NS}
Blank milk	4.55 ± 0.38	3.23 ± 1.01	3.78 ± 0.19	4.00 ± 0.33	4.00 ± 0.00	5.23 ± 0.83	5.56 ± 0.83
Direct vitamin fortification	5.11 ± 0.19	3.23 ± 0.50	3.67 ± 0.19	4.78 ± 0.19	4.67 ± 1.20	5.00 ± 0.33	5.34 ± 1.34
Vitamin loaded LNC	4.55 ± 0.19	3.45 ± 0.70	3.45 ± 0.38	3.56 ± 0.50	4.23 ± 0.96	5.45 ± 0.70	4.89 ± 0.70

NS: Not significant at 0.05.

fortified milk using direct vitamin addition were depicted in (Table 5). Significant differences were not observed between blank sample and fortified samples for all characteristic features. Generally, sensory evaluation results indicated acceptability of fortified milk with vitamin D_3 loaded LNC.

Conclusion

Lipid nanocapsules are novel semi-solid particles with structure similar to lipoproteins and can be characterised as a hybrid between polymeric nanoparticles and liposomes with high stability against physical stress. The aim of this work was to investigate feasibility of LNC for encapsulation of vitamin D_3. LNC was produced by phase inversion temperature, and vitamin D_3 loaded LNC was produced in a small size. Moreover, high encapsulation efficiency values were achieved (more than 90%). The result of TEM imaging showed that particles had an approximate spherical shape. The FTIR results did not indicate any adverse reaction between vitamin D_3 and lipid nanocapsules. It was found that vitamin D_3 release from produced LNC was prolonged over a significant period, and Rigter-Peppas was the best model to describe release profile. Finally, sensory evaluation indicated the acceptability of fortified milk with vitamin D_3 loaded LNC. The result of this paper showed that LNC can give a new hope for the efficient delivery of bioactive components in different food and beverages.

Acknowledgements

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References

1. Norman, A.W.; Henry, H.L. *Vitamin D*, in *Handbook of Vitamins*, 4, Editor. CRC Press: Boca Raton, Florida, **2007**.
2. Bender, D.A. *Nutritional Biochemistry of the Vitamins*. Cambridge University Press: New York, **2003**.
3. Domingues, N.J.A. *Carrier Systems for Vitamin D*. **2013**.
4. Abbasi, A.; et al. *Stability of vitamin D3 encapsulated in nanoparticles of whey protein isolate*. Food Chemistry **2014**, *143*, 379–383.
5. Livney, Y.D.; et al. Nanoencapsulation of Hydrophobic Nutraceutical Substances within Casein Micelles. In *XIVth International Workshop on Bioencapsulation*, **2006**; 1–4.
6. Mohammadi, M.; Ghanbarzadeh, B.; Hamishehkar, H. Formulation of Nanoliposomal Vitamin D3 for Potential Application in Beverage Fortification. Advanced Pharmaceutical Bulletin **2014**, *4*, 569–575.
7. Fathi, M.; Martín, Á.; McClements, D.J. Nanoencapsulation of food ingredients using carbohydrate based delivery systems. Trends in Food Science & Technology **2014**, *39*(1), 18–39.
8. Hirsjärvi, S.; et al. Evaluation of Surface Deformability of Lipid Nanocapsules by Drop Tensiometer Technique, and Its Experimental Assessment by Dialysis and Tangential Flow Filtration. International Journal of Pharmaceutics **2012**, *434*, 460–467.
9. Kalepun, S.; Manthina, M.; Padavala, V. Oral Lipid-Baseddrugdeliverysystems. Acta PharmaceuticaSinica B **2013**, *3*(6), 361–372.
10. Varshosaz, J.; Hajhashemi, V.; Soltanzadeh1, S. Lipid Nanocapsule-Based Gels for Enhancement of Transdermal Delivery of Ketorolac Tromethamine. Journal of Drug Delivery, **2011**, 1–7.

11. Vrignaud, S.; et al. Design, Optimization and in Vitro Evaluation of Reverse Micelle-Loaded Lipid Nanocarriers Containing Erlotinib Hydrochloride. International Journal of Pharmaceutics **2012**, *436*(1–2), 194–200.
12. Huynh, N.T.; et al. Lipid Nanocapsules: A New Platform for Nanomedicine. International Journal of Pharmaceutics **2009**, *379*, 201–209.
13. Yegin, B.u.A.; Lamprecht, A. Lipid Nanocapsule Size Analysis by Hydrodynamic Chromatography and Photon Correlation Spectroscopy. International Journal of Pharmaceutics **2006**, *320*, 165–170.
14. Fathi, M.; et al. Hesperetin-Loaded Solid Lipid Nanoparticles and Nanostructure Lipid Carriers for Food Fortification: Preparation, Characterization, and Modeling. Food and Bioprocess Technology **2012**, *6*(6), 1464–1475.
15. Fathi, M.; Varshosaz, J. Novel Hesperetin Loaded Nanocarriers for Food Fortification: Production and Characterization. Journal of Functional Foods **2013**, *5*(3), 1382–1391.
16. Das, S.; et al. Formulation Design, Preparation and Physicochemical Characterizations of Solid Lipid Nanoparticles Containing a Hydrophobic Drug: Effects of Process Variables. Colloids and Surfaces B: Biointerfaces **2011**, *88*(1), 483–489.
17. Barzegar-Jalali, M.; et al. Kinetic Analysis of Drug Release from Nanoparticles. Journal of Pharmacy and Pharmaceutical Sciences **2008**, *11*(1), 167–177.
18. Fathi, M.; et al. Novel Caffeic Acid Nanocarrier: Production, Characterization, and Release Modeling. Journal of Nanomaterials, **2013**, 1–9.
19. Tamjidi, F.; et al. Nanostructured Lipid Carriers (NLC): A Potential Delivery System for Bioactive Food Molecules. Innovative Food Science & Emerging Technologies **2013**, *19*, 29–43.
20. Hategekimana, S.; et al. Encapsulation of Vitamin E: Effect of Physicochemical Properties of Wall Material on Retention and Stability. Carbohydrate Polymers **2015**, *124*, 172–179.
21. Zhou, H.; et al. Characterisation and Skin Distribution of Lecithin-Based Coenzyme Q10-Loaded Lipid Nanocapsules. Nanoscale Res Lett **2010**, *5*, 1561–1569.
22. Lamprecht, A.; Bouligand, Y.; Benoit, J.-P. New Lipid Nanocapsules Exhibit Sustained Release Properties for Amiodarone. Journal of Controlled Release **2002**, *84*(1–2), 59–68.
23. Heurtault, B.A.; et al. The Influence of Lipid Nanocapsule Composition on their Size Distribution. European Journal of Pharmaceutical Sciences **2003**, *18*(1), 55–61.
24. Barras, A.; et al. Formulation and Characterization of Polyphenol-Loaded Lipid Nanocapsules. International Journal of Pharmaceutics **2009**, *379*, 270–277.
25. Teng, Z.; Luo, Y.; Wang, Q. Carboxymethyl Chitosan–Soy Protein Complex Nanoparticles for the Encapsulation and Controlled Release of Vitamin D3. Food Chemistry **2013**, *141*, 524–532.
26. Yuan, H.; et al. Preparation and Characteristics of Nanostructured Lipid Carriers for Control-Releasing Progesterone by Melt-Emulsification. Colloids and Surfaces B: Biointerfaces **2007**, *60*(2), 174–179.
27. Fathi, M.; Mozafari, M.R.; Mohebbi, M. Nanoencapsulation of Food Ingredients Using Lipid Based Delivery Systems. Trends in Food Science & Technology **2012**, *23*(1), 13–27.
28. Eissa, M.M.; et al. Miltefosine Lipid Nanocapsules for Single Dose Oral Treatment of Schistosomiasis Mansoni: A Preclinical Study. PLoS One **2015**, *10*(11).
29. Bunaciu, A.A.; Aboul-Enein, H.Y.; Hoang, V.D. Vibrational Spectroscopy Used in Milk Products Analysis: A Review. Food Chemistry **2016**, *196*, 877–884.
30. Abdel-Mottaleb, M.M.A.; Neumannc, D.; Lamprecht, A. In Vitro Drug Release Mechanism from Lipid Nanocapsules (LNC). International Journal of Pharmaceutics **2010**, *390*, 208–213.
31. Lamprecht, A.; Bouligand, Y.; Benoit, J.-P. New Lipid Nanocapsules Exhibit Sustained Release Properties For Amiodarone. Journal of Controlled Release, **2002**, *84*, 59–68.
32. Fathi, M.; et al. Hesperetin-Loaded Solid Lipid Nanoparticles and Nanostructure Lipid Carriers for Food Fortification: Preparation, Characterization, and Modeling. Journal of Food Bioprocess Technology **2012**, *6* (6), 1464–1464