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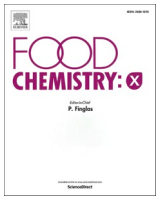

Vitamin D3 capsulation using maillard reaction complex of sodium caseinate and tragacanth gum

Fatemeh Navab [a,b,c], Atefe Rezaei [b,d], Mohammad Hossein Rouhani [b,c], Farnaz Shahdadian [b,e], Mahsa Alikord [b,d,*]

[a] Student Research Committee, Isfahan University of Medical Sciences, Isfahan, Iran
[b] Nutrition and Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[c] Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran
[d] Department of Food Science & Technology, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran
[e] Department of Clinical Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

The encapsulation of vitamin D3 (VitD3) using the Maillard reaction complex of sodium caseinate-tragacanth gum (TG) to the production of water-soluble vitamins were studied. Spray drying was used to prepare the complex. Its physicochemical properties, stability, and release characteristics were evaluated. The results showed that containing sodium caseinate- Tragacanth gum (TG) 1 % (*w/v*) and VitD3 1 % (w/v) had the highest encapsulation efficiency (71 %). The resulting microcapsules showed suitable particle size, strong negative zeta potential, and good stability with spherical morphology. Thermal and spectroscopic analyses showed proper interaction between wall and core components. In vitro, release and simulated digestion studies demonstrated the ability of microcapsules to protect VitD3 under gastric conditions and provide controlled release in the intestine. This encapsulation system shows potential for enriching food with VitD3 and increasing its stability and bioavailability.

1. Introduction

Vitamin D (VitD) is one of the most important fat-soluble vitamins, which exists in two primary forms including VitD2 (ergocalciferol) and VitD3 (cholecalciferol) (Liu et al., 2020). Unlike VitD2, which is synthesized in plants through ultraviolet radiation, VitD3 is naturally produced in human skin upon exposure to sunlight. Natural sources of VitD3 in some foods such as fish, beef liver, eggs, and dairy products are often insufficient to meet human needs and lead to the common phenomenon of VitD3 deficiency, especially among people who do not have enough sunlight and dietary VitD3 intake (Maurya & Aggarwal, 2019). VitD3 deficiency is associated with an increased risk of diabetes, hypertension, cardiovascular diseases, and rickets. Various strategies such as encapsulation, microcapsulation, and nanocapsulation have been used to address this problem in the food and pharmaceutical industries (Shah et al., 2021).

Encapsulation is a process in which bioactive substances such as vitamins, antioxidants, fatty acids, phytosterols, lycopene, lutein, minerals, and probiotic living cells are coated in a homogeneous or heterogeneous shell (Champagne & Fustier, 2007; Jafari et al., 2008; Luo et al., 2012). The edibility and biocompatibility of these shells are among the most important features of these shells in food (Mozafari et al., 2008; Nazzaro et al., 2012). In addition, the encapsulation can change the solubility characteristic, mask off-flavors, enhance sensory properties, protect bioactive components from adverse conditions, and enhance controlled release during digestion, thereby enhancing storage, stability, and bioavailability (Akhavan et al., 2018; Katouzian et al., 2017; Rezaei et al., 2018; Szente et al., 2021). Microencapsulation is a versatile technique, the core material is encapsulated in the wall material (Zhang et al., 2022). spray drying is the most widely used technique

Abbreviations: AGJ, Artificial gastric juice; ANOVA, One-way analysis of variance; DLS, Dynamic light scattering; EE, Encapsulation efficiency; EL, Encapsulation load; FTIR, Fourier transform infrared spectroscopy; GRAS, Generally recognized as safe; HCl, Hydrochloric acid; NaOH, Sodium hydroxide; PDI, Polydispersity index; SEM, Scanning electron microscopy; TG, Tragacanth gum; TGA, Thermogravimetric analyses; VitD, Vitamin D; VitD3, Vitamin D3; XRD, X-ray diffraction analysis.

* Corresponding author at: Nutrition and Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail addresses: Mahsaalikord@yahoo.com, m.alikord@nut.mai.ac.ir (M. Alikord).

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to encapsulate various bioactive compounds (Akbarbaglu et al., 2021). The cost-effectiveness, production of high particle quality, rapid operating speed, continuous production capacity, and ease of industrialization make it a dominant encapsulation technique to produce powders (Rodríduez-Huezo et al., 2004; Shao et al., 2018). Nanoparticles present a remarkable opportunity for the mineralization of pharmaceutical compounds in aqueous solutions, delivering cost-efficient solutions, straightforward handling, and impressive efficacy (Al-Toriahi et al., 2023; Hamed et al., 2023). Selecting a suitable wall materials is crucial to achieve high encapsulation efficiency and optimal release rate (Bajaj et al., 2021). Water-soluble hydrocolloids such as maltodextrin, gums, and modified starches are commonly used due to their good emulsifying ability, film-forming capacity, and low viscosity (Gałkowska et al., 2023).

The development of protein-stabilized oil-in-water emulsions has provided food and pharmaceutical industries with an alternative means of delivering hydrophobic bioactive compounds. However, emulsions used in such applications can become unstable under various circumstances, including changes in temperature, pH, and ionic strength, as well as the presence of digestive enzymes and other active surface agents (Sarkar et al., 2010). Previous studies have shown that protein-stabilized emulsions including those using soy protein isolates and whey proteins become unstable under gastric circumstances due to the reasons outlined aboves. Carbohydrate-based encapsulation is often preferred over protein-based wall materials, because the performance of protein-based walls is sensitive to pH, temperature, and ionic strength. Natural polymers, particularly tragacanth gum (TG) and casein, have received significant attention due to their biodegradable nature in the human body. Casein, as the major component of milk proteins, is widely used not only in dairy but also in non-dairy products due to its unique functional properties. Casein dissolves quickly in water when its pH differs slightly from its isoelectric point of around 4.6. In addition, casein can form complexes with a wide range of cationic and anionic polysaccharides, including guar gum, carrageenan, pectin, xanthan gum, gum arabic, chitosan, sodium alginate, and TG. Since 1961, TG has been recognized as generally recognized as safe (GRAS), with accepted concentrations of 0.2 % to 1.3 % in food products (Anderson & Bridgeman, 1985). For many years, the use of TG has been widespread in pharmaceutical, food, and cosmetic industries for its exceptional properties such as high acid resistance, thickening, stabilizing, emulsifying, suspending, and gelling ability. High functional properties can be obtained when carbohydrates and proteins are combined (Cheng et al., 2019). Combining polysaccharides with protein-stabilized emulsions, whether conjugated with the proteins or unconjugated, could enhance thermal and gastric stability (Diftis & Kiosseoglou, 2003; Diftis & Kiosseoglou, 2006; Ray & Rousseau, 2013; Xu et al., 2014). Electrostatic or covalent bonds can form protein-polysaccharide complexes; nevertheless, only covalent bonds can provide better stability in adverse conditions. On the other hand, a high degree of tolerance to pH and temperature changes, ionic strength, and the proteolytic reaction was demonstrated by the polysaccharide and protein complexes prepared by the Maillard reaction. Moreover, emulsified bioactives can be controlled in the gastrointestinal tract by choosing the right Maillard-type protein and polysaccharide complex (Sedaghat Doost et al., 2020b).

In this study, we intended to explore the properties of a novel class of complexes composed of protein, polysaccharide, and Maillard reaction products, which have been underutilized as emulsifiers and stabilizers in previous research. Specifically, our objectives were three-fold: (1) to design and synthesize a Maillard reaction product sodium caseinate-TG complex with optimal emulsification capabilities, (2) to investigate the storage and thermal stability of oil-in-water emulsions stabilized by these complexes with VitD3, and (3) to assess their performance under simulated gastrointestinal conditions and mimicking the human digestive system.

2. Materials and methods

2.1. Materials and chemicals

Liquid VitD3 (cholecalciferol, ≥98 %) was obtained from Alhavi Pharmaceutical Company (Tehran, Iran (.TG was purchased from Merck (Darmstadt, Germany). Dialysis membrane (cut-off between 12,000 and 14,000 kDa) was purchased from Sigma-Aldrich (USA). Other used substances were sodium caseinate (92 % protein), n-hexane (99 %), sodium bicarbonate (99.5–100.5 %)s, potassium chlorate (99 %), sodium chlorate (99.5 %), α-amylase (from *Bacillus licheniformis*, ≥500 U/g), hydrochloric acid (HCl) (37 %), pepsin, porcine gastric mucin (Type III), trypsin enzyme, pancreatin, phosphate buffer, and sodium hydroxide (NaOH) of analytical grade and purchased from Merck (Darmstadt, Germany).

2.2. Production of maillard complex between casein and tragacanth gum

A solution of casein and TG with pH 7 was prepared using deionized water, adjusted using HCl (1 M), and NaOH (1 M). Following hydration at 4 °C for 12 h, the samples were kept at room temperature for 24 h. Then, followed by using a freeze dryer (Dena Vacuum, Iran), dried powder of the sample was obtained. To perform the Maillard reaction, controlled conditions, including relative humidity, temperature, and processing time were considered. The dried powder was incubated for seven days in a desiccator containing saturated sodium chloride salt to create 74 % relative humidity at an incubation 60 °C (Sedaghat Doost et al., 2020).

2.3. Incorporation of VitD3 into the complex

VitD3 was added to the oil (10 % (*v*/v) of sunflower oil) and kept at 4 °C for 24 h for hydration. The complex of sodium caseinate and TG was dissolved in water, and then VitD3 was added to it with different weight ratios according to Table 1 s. Then, it was stirred at room temperature for 5 min at 24,000 rpm. Then, the resulting solution was dried by spray drying (DSD-02 Dorsa Tech. Iran), under the following condition: inlet temperature 180 °C, fluid pump rate 35 %, air flow rate 35 m^3/h, aspiration ratio 80 %, and with the use of a two-fluid flow atomizer nozzle of 0.7 mm diameter for 15 min. The spray drying yield was between 40 %–70 %. Finally, the collected powder was kept at 4 °C in dark packaging to avoid reaction acceleration (Jannasari et al., 2019).

2.4. The Encapsulation efficiency and load

Measurement of non-encapsulated VitD3 was conducted gravimetrically to assess encapsulation efficiency and VitD3 load. To disperse microcapsules, they were blended with 15 mL of hexane and shacken for 2 min at room temperature. Following the filtration of the solvent and evaporating the hexane by a rotary vacuum at 60 °C, the isolation of non-encapsulated vitamin from the surface of microcapsules was accomplished. Using weight measurement, non-encapsulated VitD3 was determined. According to the following equations, encapsulation efficiency (Eq.1) and encapsulation load (Eq.2) were calculated (Ifeduba & Akoh, 2016):

$$EE\ (\%) = [(VD_T – VD_F)/VD_T] \times 100 \quad (1)$$

$$EL\ (\%) = [(VD_T – VD_F)/W_T] \times 100 \quad (2)$$

where EE is encapsulation efficiency, EL is encapsulation load, VD_T is representative of the total mass of VitD, VD_F is representative of the mass of surface vitamin, and W_T is representative of the total mass of microcapsules.

2.5. Determination of size distribution and ζ-potential

Using dynamic light scattering (DLS) (90Plus Nanoparticle Size Analyzer, Brookhaven Instruments), the polydispersity index (PDI), average particle size, and ζ-potential of encapsulated VitD3 were measured. To determine the size, volume distribution was used at a fixed angle of 90°. Nanocarriers' size distribution was determined by the PDI. Size distributions are narrower when the PDI is lower. Also, by using the mean electrostatic mobility value, ζ- potential was calculated to determine the surface charge. All measurements were repeated three times (Yuan et al., 2017).

2.6. Scanning electron microscopy (SEM)

To prepare the samples, dried complex powder was gently sprinkled onto double adhesive tape that was adhered to an aluminum stub. Using a sputter coater, the gold deposited on the stubs to a thickness of approximately 300 Å. SEM analysis of all samples was performed with a Scanning Electron Microscope (JSM-6100, JEOL, Tokyo, Japan) at 2500× magnification and an accelerating voltage of 10 kV (Jain et al., 2016).

2.7. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectrophotometer (Jasco FTIR-680 plus) was used to assess chemical interactions between functional groups. Its wavenumber range was set between 400 and 4000 cm^{-1}. For this purpose, FTIR spectra of TG, sodium caseinate, VitD3, and TG-caseinate microcapsules loaded with VitD3 were evaluated. Pills were created by mixing the freeze-dried samples with potassium bromide (KBr) in a ratio (1:100) (Butstraen & Salaün, 2014).

2.8. X-ray diffraction analysis (XRD)

X-ray diffractometer (Asenware AW/XDM 300, China) was used to analyze crystallographic structures. An aluminum plate was loaded with 200 mg of sample powders for this experiment. It was conducted under the conditions of 0.154 nm Cu K-alpha-1 radiation, current of 30 mA, voltage of 40 kV, steps of 0.02 per second, and Bragg angle range from −10 to 118° (Salehi et al., 2021).

2.9. Thermogravimetric analyses (TGA)

Using thermal gravimetric analysis, we measured the samples thermal resistance. TGA analysis was carried out using the Mettler Toledo DSC1 fitted with STRe software (Mettler Toledo, Switzerland). Analyzing was performed at a heating rate of 10 °C/min with a temperature range of 40 to 300 °C by placing 5 mg of the sample in an aluminum container. The purge gas was Hydrogen (Jain et al., 2016).

2.10. In vitro release study

Simulated gastric (pH = 1.2) and intestinal fluids (pH = 6.8) were used to determine the VitD3 release in vitro. To produce simulated gastric fluid (pH = 1.2), HCl (1 M) was dissolved in 80 mL of NaCl (2 g) and then adding distilled water until it reached a volume of 1000 mL. Pepsin was also used for the preparation of gastric solution. The simulated intestinal solution (pH = 6.7) was prepared by adding 77 mL of NaOH (0.2 N) to KH_2PO_4 solution (6.8 g in 250 mL water), and subsequently increasing the volume until 1000 mL with distilled water was obtained (Rezaei & Nasirpour, 2019). For the final pH adjustment, NaOH (0.2 N) or HCl (0.2 N) was used. Pancreatin enzymes were also needed for the preparation of phosphate solution. Saliva simulation was prepared according to Davis et al.'s method (Davis et al., 1971). Simulated saliva contained 20 meq/L potassium chloride, 25.0 meq/L sodium bicarbonate, 2.0 meq/L sodium chloride, and 2 g/L α amylase. To evaluate the release profile, a dialysis bag with a cellulose membrane molecular weight cut-off between 12,000 and 14,000 Da (Sigma, USA) was used. Before closing two sides of the dialysis bag, a 3 mL solution of the sample with the determined concentration was added. Then, it was placed in an amount of 32 mL gastric fluid (incubated in a shaker incubator at 37 °C and 100 rpm for 2 h). For concentration measurement, 3 mL of the sample was taken from the medium every 30 min and then it was filled with 3 mL of the fresh buffer immediately. Following 2 h, the dialysis bag was taken place in 32 mL of the intestinal buffer (for a period of 6 h at 100 rpm and 37 °C incubated in a shaker incubator). A series of samplings was also collected every one h by the same sampling method. Finally, we read the absorptions of solutions with a UV–Vis spectrophotometer (CECIL CE. 2502 2000 SERIES, Bio-Quest, UK) at 264 nm (Fahami & Fathi, 2018; Hasanvand, Fathi, Bassiri, et al., 2015). According to the following equation (Eq.3), the cumulative release was calculated.

$$\text{VitD3 release (\%)} = \frac{\textit{the amount of released vit D3}}{\textit{The amount of vitD3 in complex}} \times 100 \quad (3)$$

2.10.1. In vitro digestion

The in vitro digestion was performed based on the Mao and McClements (2012) to simulate the human gastrointestinal tract (Mao & McClements, 2012). For keeping the digestive solution at 37 °C during the experiments, a water bath and incubator were used. Also, for amending the samples pH, 0.25 M HCl or NaOH was used. Following is a brief description of the digestive process:

2.10.2. Initial system

In the pre-experiment phase, we heated and shaked 20 mL samples in a constant temperature shaker for 30 min at 37 °C.

2.10.3. Mouth phase

Firstly, 20 mL of artificial saliva consisting of 0.6 g mucin (ASWS) at 4 °C nocturnal was stirred. Then, ASWS was heated at 37 °C for 10 min merging with the initial solution. Following the merging of samples with ASWS, the pH was corrected to 6.8. To mimic the digestive process in the oral cavity, stirring the mixtures was continuously performed for 10 min in a thermostatic magnetic stirring water bath at 37 °C.

2.10.4. Stomach phase

The artificial gastric juice (AGJ) was heated at 37 °C for 10 min. Later, 30 mL of the final oral solution was merged with 30 mL of the AGJ containing 0.0032 g mL^{-1} pepsin, and the PH was adjusted to 2.5. Samples were continuously stirred for 2 h at 37 °C to complete the simulated stomach digestion.

2.10.5. Small intestine phase

Before starting this phase, preparing bile salt solution was done.3.5 mL bile salt solution and 1.5 mL of artificial intestinal juice were heated at 37 °C merging with 30 mL of the final gastric solution and the complex was continuously stirred. At the same time as modifying the pH to 7.0, the amount of 0.5375 g trypsin was added to the mixture. Completing simulated intestine digestion took 2 h. The solution of NaOH (0.25 M) was used to maintain a constant pH. Continuous titration of the reaction system was conducted for 2 h at 37 °C with this solution. To protect from sunlight exposure, all samples were wrapped in tin foil.

2.11. Bioaccessibility

After digestion in the small intestine, the digested solution was centrifuged at 12,000 rpm for 15 min at 4 °C. The sample was divided into two layers after centrifugation. The upper layer was a clear layer that contained VitD3, and the lower layer was a dense, insoluble layer formed from undigested compounds. The conversion rate and bioavailability of the product containing VitD3 are calculated according

to the following equations (Eq. 4 and 5) (Zheng et al., 2015):

$$\text{Transformation rate (\%)} = (\text{D digesta/D initial}) \times 100 \quad (4)$$

$$\text{Bioaccessibility (\%)} = (\text{D complex/D digesta}) \times 100 \quad (5)$$

where D digesta is the VitD3 concentration in the sample that was collected after the small intestine phase. D initial was the initial concentration of VitD3 and D complex was the concentration of VitD3 in the transparent layer. The amount of VitD3 in the resulting samples was measured using HPLC.

2.12. Scanning electron microscopy and release kinetics

Release kinetics were analyzed using the Korsmeyer-Peppas model. The cumulative amount of VitD3 released (M_t/M_∞) was plotted against time (t) on a log-log scale. The release exponent (n) and release rate constant (k) were determined from the slope and intercept, respectively, of the linear portion of the plot ($R^2 > 0.98$). All experiments were performed in triplicate, and results are presented as mean ± standard deviation (Siepmann & Peppas, 2001).

2.13. Statistical analysis

All stages were repeated at least three times and the data were expressed as mean ± SD. The significant differences were determined by One-way analysis of variance (ANOVA) and the Duncan test method ($P \leq 0.05$) via SPSS (version 20).

3. Result and discussion

3.1. Encapsulation efficiency

The encapsulation efficiency results were examined to determine the optimal formulation (Table 1 s). The formulation "4" in Table 1 s, containing Maillard complex of casein-TG 1 % (*w/v*) and VitD3 1 % (w/v), proved the highest ample encapsulation efficiency. Thus, this formulation was selected for subsequent tests. The high efficiency in this formulation showed that the Maillard complex was formed between protein and carbohydrate and a useful matrix was also formed to trap VitD3. This may be due to the amphiphilic nature of this complex, which enables it to interact well with hydrophobic VitD3 and aqueous environment, simultaneously. This ratio between the Maillard complex and VitD3 probably strikes a balance between optimizing encapsulation and maintaining the structural integrity of the particles (Luo et al., 2012). The observed encapsulation efficiency compared to other previous studies such as Hassanvand et al. obtained encapsulation efficiency in the range of 62–78 % for VitD3 using modified starch-based nanocarriers, which was acceptable (Hasanvand, Fathi, Basiri, et al., 2015). The Maillard complex demonstrated high efficacy as an encapsulating agent for VitD3. Also, the results of Table 1 s show that increasing the concentration of Maillard complex from 1 % to 2 % (w/v) does not necessarily increase the encapsulation efficiency. This indicates that there is an optimal ratio between the complex and VitD3 for effective encapsulation. The presence of additional carrier materials not only does not help further improvement in encapsulation but also can potentially interfere with the system by forming stable particles (Gonçalves et al., 2016). Considering the characteristic of VitD3 which is sensitive to environmental factors such as heat, light, and oxygen, the relatively high efficiency of encapsulation obtained in this study is acceptable and this effective encapsulation can help protect VitD3 against these factors and potentially improve the stability and shelf life of the vitamin in various food and pharmaceutical applications (Abbasi et al., 2014).

3.2. Particle size distribution and zeta potential

The results of strongly negative zeta potential (−37 mV) in Fig. 1(a) indicate the good colloidal stability of the formed biopolymer particles. This result was obtained from a series of 10 measurements, with an average count rate of 231.4 kcps and a single clear peak in the zeta potential distribution plot. Considering that particles with zeta potential values greater than +30 mV or less than −30 mV are considered very stable due to strong electrostatic repulsion forces between particles (Cheng et al., 2019), a good colloidal distribution is observed. This high stability can help to improve shelf life and prevent the accumulation of microcapsules in different formulations. The presence of a large negative charge on the surface of the particles indicates that the Maillard complex of sodium caseinate and TG effectively covers VitD3 and creates an outer layer with a negative charge. This negative charge probably originates from the carboxyl groups of both protein-containing system components and polysaccharide components of the complex. The stability conferred by this high negative zeta potential is crucial for the long-term storage and use of encapsulated VitD3, making the particles resistant to aggregation and maintaining their dispersion in aqueous media, which is required for uniform distribution in food products and potentially increased bioavailability is essential (Oh et al., 2013). Furthermore, the strong electrostatic repulsion between the particles may help to better protect the encapsulated VitD3 from environmental factors such as oxidation and degradation, potentially extending its shelf life in various formulations.

The DLS results showed that VitD3 particles encapsulated in the Maillard complex have a multimodal size distribution. Three distinct peaks can be seen in Fig. 1 (b), with an initial peak at approximately 7.356 μm (Fig. 1 (b)), which accounts for 99.3 % of the total scattered light intensity. Also seen in Fig. 1 (b) are two smaller peaks, one about 872 nm and the other 106 nm, which contributed 0.6 % and 0.1 % to the total intensity, respectively. After calculating the PDI of 0.553, a relatively wide size distribution was observed. The z-average diameter, which represents the intensity-weighted average hydrodynamic diameter, was measured at 6.925 μm. The multimodal size distribution observed in the results indicates a complex system of encapsulated VitD3 particles. The dominant population was about 7 μm, which indicates the formation of large aggregates or clusters, which can be attributed to the interaction between the Maillard complex and VitD3 during the encapsulation process. This size range is significantly larger than conventional nanoencapsulation systems, often for particle sizes below 1 μm, which is noted for improved bioavailability and stability (Oh et al., 2013). However, microparticles can still be useful for specific food applications, potentially providing long-term release and protection of VitD3 as shown by some studies (Jafari et al., 2008). The presence of smaller particles in the range nm also indicates that part of the particles remains in the sub-micron range, which can help improve dispersion and potentially increase the bioavailability of encapsulated VitD3. Also, relatively high dispersion index and inhomogeneity of particle size distribution are not uncommon in food encapsulation systems using natural polymers (Haham et al., 2012). While larger particle sizes may limit certain applications that require nano-sized particles, they can be useful for food fortification. It is suspected that larger particles may provide better protection against processing conditions and controlled release of VitD3. Overall, the results of particle size and zeta potential indicate that good colloidal stability is formed and can lead to improved protection of VitD3 during storage and processing, as well as controlled release properties in various applications (Fathi et al., 2014).

3.3. Scanning electron microscopy

The scanning electron microscope (SEM) was employed to analyze the particle size, morphology, and elemental composition (Ben Amor et al., 2023; Ramezani et al., 2024). The results of the SEM images (Fig. 2) showed a heterogeneity of particles with a mostly spherical

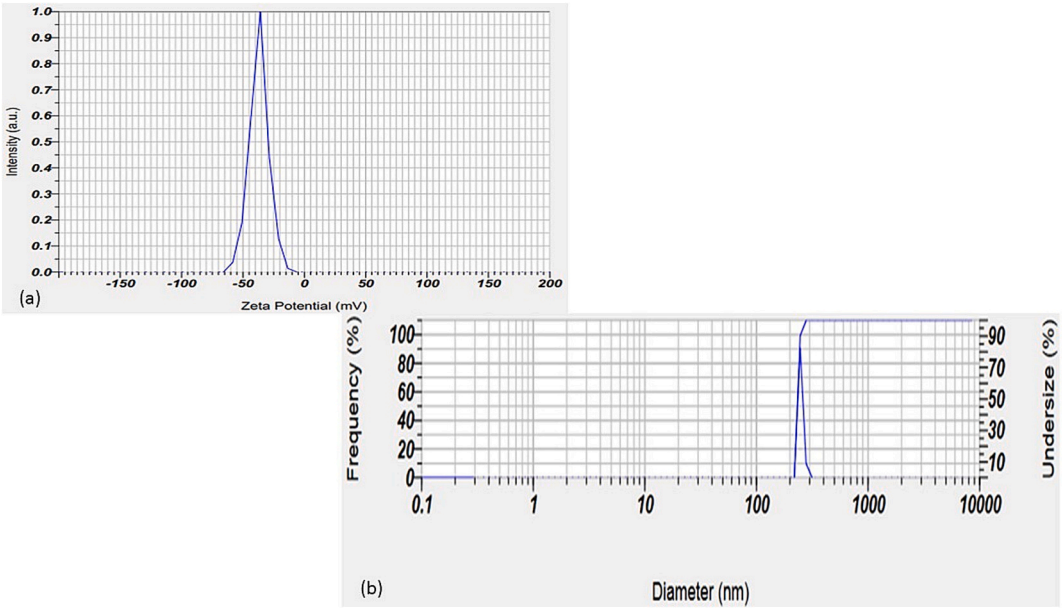

Fig. 1. Zeta potential (a) and particle size distribution (b) of vitamin D3 encapsulation.

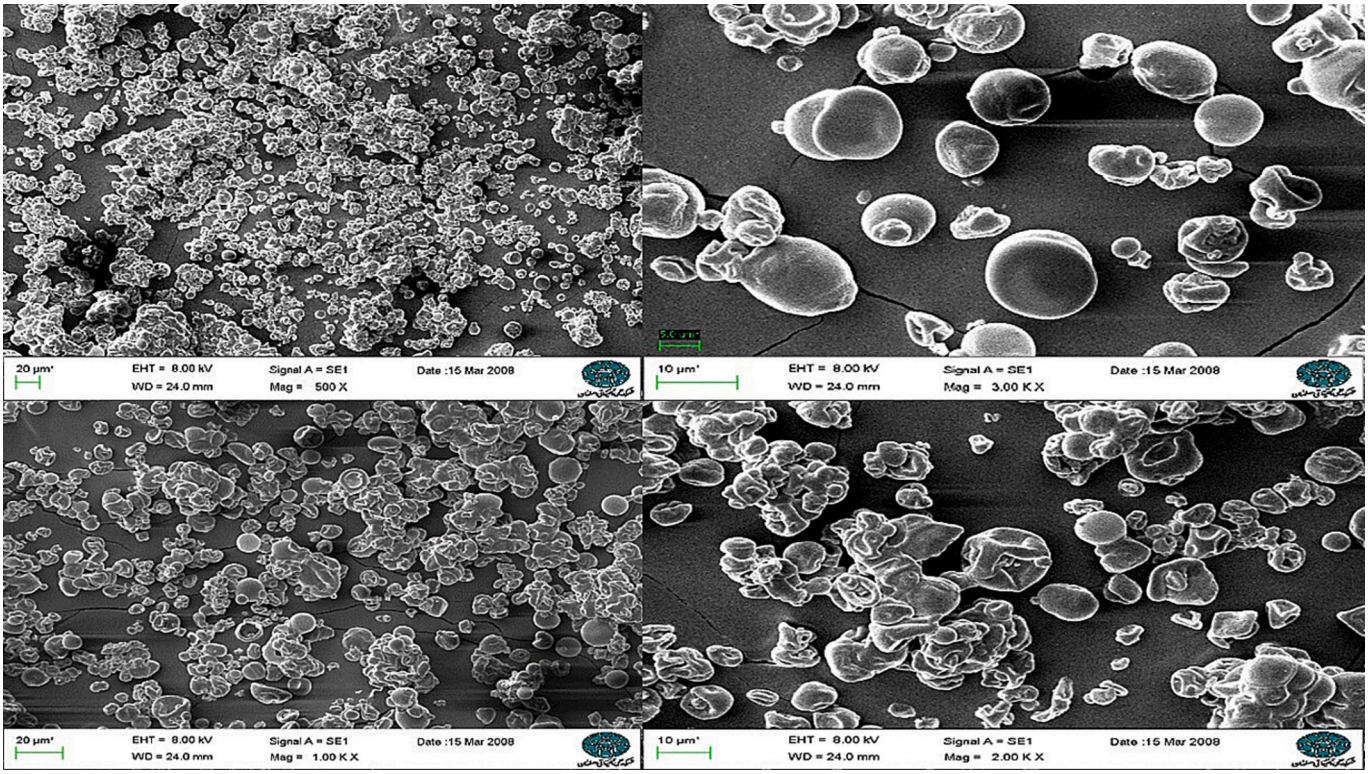

Fig. 2. SEM of vitamin D3 encapsulation.

morphology. The size of the particles is approximately 1–20 µm, most of which are in the range of 5–10 µm. At low magnifications, clusters of particles can be seen that together form larger clusters. However, at higher magnifications, single and more recognizable particles with a smooth surface and some degree of deformation or indentation are observed. Also, some particles have collapsed or flattened structures, possibly due to the spray drying process or sample preparation for SEM. Spherical morphology is typical for spray-dried particles (Gharsallaoui et al., 2007). The presence of some collapsed or sunken particles in spray-dried powders is also not uncommon and may be recognized due to the rapid evaporation due to water during the drying process. Spherical morphology may affect the release kinetics of VitD3 from encapsulation (Liu et al., 2015). Also, the smooth surface of most particles shows the uniform coverage of the Maillard complex around the VitD3 core, which can help to improve the protection and controlled release of the encapsulated vitamin. The size range (µm) corresponds to typical spray-dried particles used in food applications (Liu et al., 2015). This range could suggest advantages such as improved treatment and incorporation into food matrices compared to nanoparticles (Zuidam & Shimoni, 1970). However, it may also impact the dissolution rate and

bioavailability of encapsulated VitD3.

3.4. Fourier transform infrared spectroscopy

The spectra results reveal characteristic absorption bands for sodium caseinate, tragacanth gum, their complex, VitD3, and encapsulated VitD3. The results showed valuable insights into the molecular structure and interactions of the encapsulation system. Both sodium caseinate and TG exhibit a broad, intense band around 3380–3430 cm^{-1}, attributable to O—H stretching vibrations of hydroxyl groups and N—H stretching of amino groups (Ye et al., 2017). The peaks at 2926–2931 cm^{-1} correspond to C—H stretching vibrations of methyl and methylene groups. The region between 1000 and 1800 cm^{-1} shows several distinctive peaks such as Amide *I* band (C═O stretching) at ~1650 cm^{-1}, Amide II band (N—H bending and C—N stretching) at ~1540 cm^{-1}, C—O stretching and O—H bending vibrations of polysaccharides at ~1040 cm^{-1}. The complex formed between sodium caseinate and TG exhibits similar overall features, indicating the preservation of major functional groups. However, slight shifts in peak positions and intensities suggest molecular interactions between the two components, likely through hydrogen bonding and electrostatic interactions (Dai et al., 2017) **(**Fig. 3 & 4**)**. The VitD3 spectrum shows characteristic peaks at 3470 cm^{-1} (O—H stretching), 2938 cm^{-1} (C—H stretching), and 1744 cm^{-1} (C═O stretching). The encapsulated VitD3 spectrum retains these key features, confirming the presence of VitD3 within the complex matrix. Notably, the encapsulated VitD3 spectrum shows additional peaks and intensity changes compared to pure VitD3, particularly in the fingerprint region (1000–1500 cm-^{1}). This suggests the successful incorporation of VitD3 into the sodium caseinate-TG complex and potential interactions between the vitamin and the encapsulating materials (Luo et al., 2012). The broadening of the O—H stretching band (~3388 cm^{-1}) in the encapsulated VitD3 spectrum compared to pure VitD3 indicates increased hydrogen bonding, likely due to interactions with the polysaccharide and protein components of the complex. The preservation of key VitD3 peaks in the encapsulated form, albeit with some shifts and intensity changes, suggests that the encapsulation process successfully incorporated the vitamin while maintaining its chemical structure. This is crucial for ensuring the bioavailability and stability of VitD3 in the final product **(**Fig. 5**)**. In conclusion, the FTIR analysis provides strong evidence for the successful encapsulation of VitD3 within the Maillard complex of sodium caseinate and tragacanth gum. The spectra reveal key functional groups and their interactions, supporting the formation of a stable encapsulation system that may offer improved protection and delivery of VitD3.

3.5. X-ray diffraction analysis

The X-ray diffraction patterns presented in Fig. 6 & 7 offer valuable insights into the crystalline structure and physical state of VitD3, the Maillard complex of sodium caseinate and tragacanth gum, and the encapsulated VitD3 particles. The XRD pattern of pure VitD3 exhibits several sharp, distinct peaks, particularly in the 2θ range of 15° to 25°. These peaks are indicative of a crystalline structure, which is typical for pure, unprocessed VitD3 (Cholecalciferol) (Gomaa et al., 2014). The most prominent peaks are observed at approximately 18°, 20°, and 22°, corresponding to the characteristic lattice planes of VitD3 crystals. The XRD pattern of the Maillard complex formed between sodium caseinate and TG shows a broad, amorphous hump centered around 20°. This diffraction pattern is characteristic of amorphous materials, indicating that the complex lacks a long-range ordered structure (Wang et al., 2019). The absence of sharp peaks suggests that the interaction between sodium caseinate and TG results in a disordered, non-crystalline state, which is common for protein-polysaccharide complexes. The XRD pattern of the encapsulated VitD3 particles reveals a combination of features from both the pure VitD3 and the complex. The broad amorphous hump centered about 20° is still present, indicating the preservation of the amorphous nature of the Maillard complex. However, the sharp peaks characteristic of crystalline VitD3 are notably absent or significantly reduced in intensity.

This transformation in the XRD pattern of encapsulated VitD3

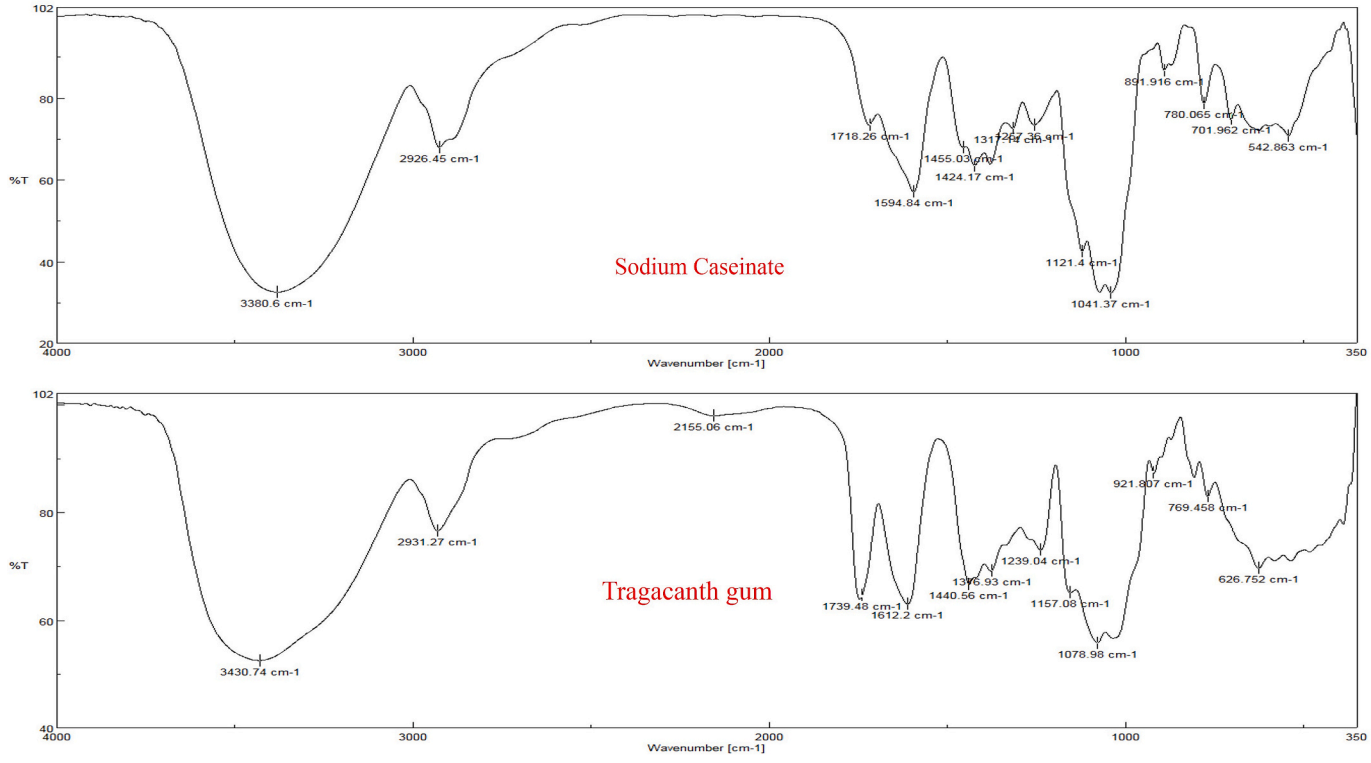

Fig. 3. FTIR of the sodium casein and tragacanth gum.

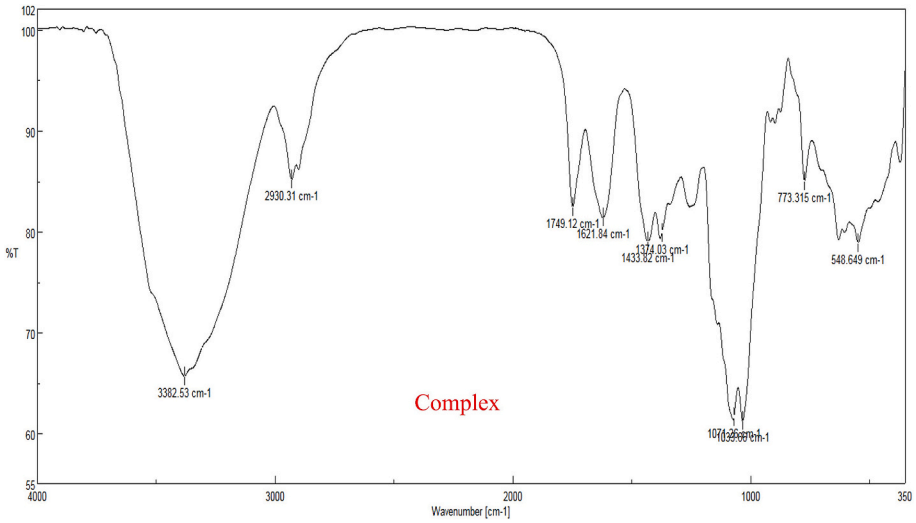

Fig. 4. FTIR of the maillard complex.

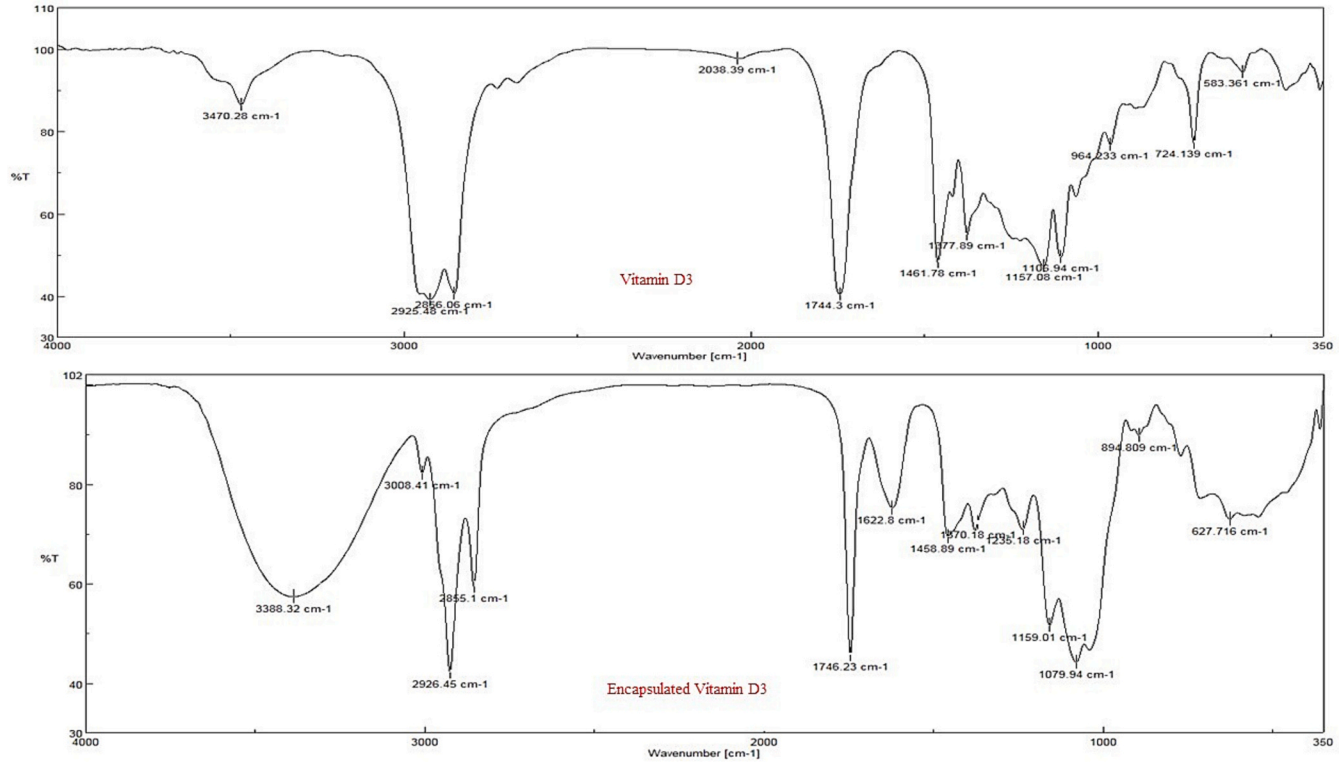

Fig. 5. FTIR of the vitamin D3 and vitamin D3 encapsulation.

compared to pure VitD3 suggests a significant change in the physical state of the vitamin upon encapsulation. The disappearance of sharp crystalline peaks indicates that VitD3 has been molecularly dispersed within the amorphous matrix of the Maillard complex (Luo et al., 2011). This molecular dispersion is highly desirable in encapsulation systems as it can lead to improved solubility, bioavailability, and stability of the encapsulated compound. The absence of crystalline VitD3 peaks in the encapsulated form suggests that the encapsulation process has successfully prevented the aggregation and recrystallization of VitD3 molecules. This is crucial for maintaining the functionality and bioavailability of the vitamin, as crystalline structures can often hinder dissolution and absorption in biological systems. Furthermore, the preservation of the amorphous hump in the encapsulated VitD3 pattern indicates that the overall structure of the Maillard complex remains intact after incorporating the vitamin. This suggests that the encapsulation process does not significantly disrupt the complex formation between sodium caseinate and TG, which is essential for maintaining the stability and functionality of the encapsulation system. In conclusion, the XRD analysis provides strong evidence for the successful encapsulation of VitD3 within the Maillard complex of sodium caseinate and TG. The transition from a crystalline to an amorphous state for VitD3 suggests improved solubility and potential bioavailability, while the preservation of the complex structure indicates a stable encapsulation system.

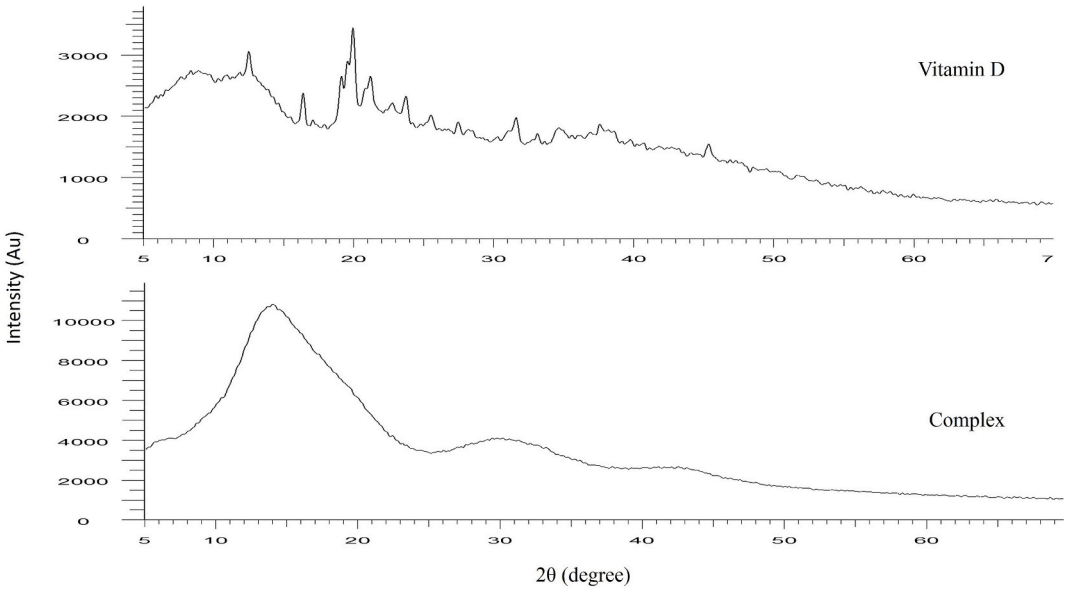

Fig. 6. XRD of the vitamin D3 and maillard complex.

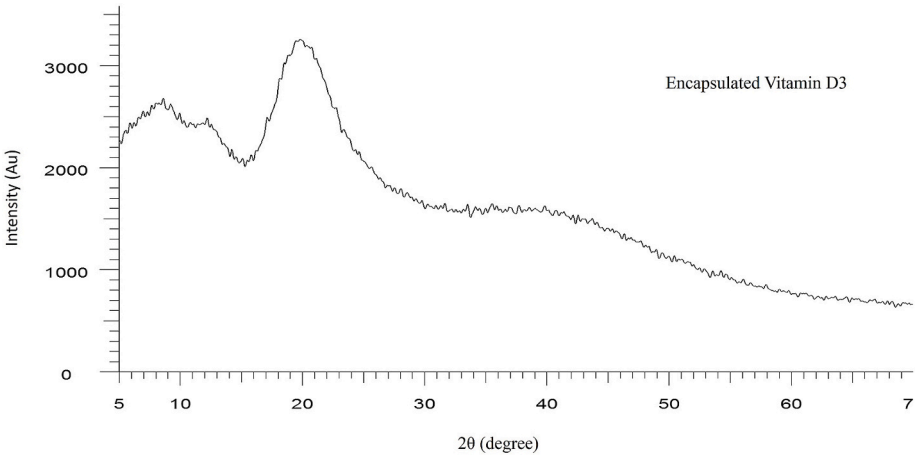

Fig. 7. XRD of the Vitamin D3 encapsulation.

3.6. Thermogravimetric analyses (TGA)

The TGA curves presented in Figs. 8s–10s offer valuable insights into the thermal stability and decomposition behavior of the Maillard complex (sodium caseinate and TG), pure VitD3, and the encapsulated VitD3 system. The complex exhibits a multi-stage decomposition profile, with an initial mass loss of approximately 10 % between 50 and 100 °C, likely corresponding to the evaporation of bound water (Hosseini et al., 2013). This is followed by a significant mass loss starting around 200 °C, attributed to the thermal degradation of the protein and polysaccharide components. The complex's thermal stability up to about 200 °C is advantageous for potential food and pharmaceutical applications. Pure VitD3 demonstrates a distinct thermal profile characterized by a sharp mass loss beginning at approximately 350 °C. This single-stage decomposition is typical for crystalline organic compounds and indicates the volatilization or degradation of VitD3 molecules (Luo et al., 2012). The onset temperature of decomposition suggests that VitD3 has good thermal stability in its pure form. The TGA curve for the encapsulated VitD3 system shows a composite behavior that combines elements from both the complex and pure VitD3 profiles, but with notable differences. The initial water loss stage (50–100 °C) is less pronounced compared to the pure complex, suggesting that the encapsulation process may have reduced the system's hygroscopicity. The main degradation stage begins at a lower temperature (around 150 °C) compared to both the pure complex and VitD3, indicating interactions between the complex and VitD3 that affect the overall thermal stability of the system (Gonçalves et al., 2018). The decomposition profile is more gradual and occurs over a wider temperature range compared to the individual components, suggesting that the encapsulation has created a more thermally heterogeneous system, potentially due to the molecular dispersion of VitD3 within the complex matrix. The absence of the sharp decomposition peak characteristic of pure VitD3 at 350 °C in the encapsulated system indicates that the vitamin is no longer present in its crystalline form. This supports the successful encapsulation and molecular dispersion of VitD3 within the complex matrix. The differences observed in the TGA profiles can be explained by molecular interactions between VitD3 and the complex components, physical state changes of VitD3 from crystalline to amorphous or molecularly dispersed, and a potential protective effect provided by the complex.

These TGA results provide strong evidence for the successful encapsulation of VitD3 within the Maillard complex of sodium caseinate and TG. The altered thermal behavior of the encapsulated system suggests potential improvements in the stability and handling properties of VitD3, which could be beneficial for various applications in food and

pharmaceutical industries. Further studies combining TGA with other analytical techniques, such as FTIR, provided additional insights into the molecular interactions and structural changes occurring during the encapsulation process and thermal treatment.

3.7. Bioaccessibility

Bioaccessibility refers to the amount of the ingested vitamin that is absorbed and functions in specific tissues (Maurya et al., 2020). The bioavailability of vitamins and other bioactive compounds in the gastrointestinal tract is reduced due to physiological conditions including pH and digestive enzymes. Meanwhile, by encapsulation of bioactive compounds, they are protected and delivered to the small intestine, where absorption mostly occurs, without degradation by stomach environments (Bao et al., 2019). In this investigation, after an entire gastrointestinal simulation, the bioaccessibility of VitD3 was examined. It has been defined using Eq calculation, based on VitD3 concentrations in VitD3 complex and total digesta. Results indicated the efficacy of encapsulation of VitD3 in enhanced accessibility. In this regard, the bioavailability of non-encapsulated VitD3 was about 35 %, but after the encapsulated VitD3, the bioavailability reached to 85 %. Moreover, following the simulated digestion of capsules, the amount of recovered capsulated VitD3 was significantly lower than the unencapsulated one (4.2 ± 0.4 and 31.0 ± 0.8 %, respectively) ($P < 0.05$). Accordingly, 95.8 % of VitD3 in capsules may be bioavailable, as opposed to only 69.0 % in unencapsulated form (Lamsen et al., 2020). Results of microencapsulation of VitD3 using carboxymethyl tara gum and gelatin A demonstrated 53 % $\pm$ 0.07 bioccessibility after in vitro digestion (Santos et al., 2021). In addition, results of a study indicated that 90 % of the VitD2 in casein micelles remained active after in vitro proteolysis, while only 67 % in unencapsulated form was observed (Moeller et al., 2018). Furthermore, results of VitD3 encapsulation with corn protein hydrolysate versus unencapsulated VitD3 demonstrated respectively 95 % and 62 % bioavailability (Lin et al., 2016). Accordingly, the encapsulation of VitD3 protected against degradation and facilitated efficient absorption and maintenance of a considerable amount of the vitamin (Table 2 s).

3.8. Release

Encapsulation is not only intended to increase the stability of compounds but also to facilitate their absorption by regulating their release. The results of the VitD3 release in the gastric and intestine environment are shown in Fig. 11s. It was not necessary to simulate the large intestine since VitD3 digestion and absorption typically take place in the small intestine. According to our findings, approximately 28 and 59 % of the vitamin was delivered to the gastric and intestinal tract after 2 and 6 h, respectively. The reason could be related to VitD3 protection against degradation in the simulated gastrointestinal fluid by the stable network structure of the complex. TG is an anionic biopolymer that resists gastric degradation due to its acidity resistance (Amani et al., 2022). As well, the pH of the stomach (3.0) promotes the interaction between biopolymers with opposite charges (Luo et al., 2012). On the other hand, since α-amylase hydrolyzes starch and amylopectin which are absent in our complex wall material (sodium caseinate and TG), VitD3 release did not occur in the oral phase and the initial concentration of VitD3 was maintained after this phase. According to a recent investigation, a considerable release rate of VitD3 before the first 20 min of the simulated gastric fluid was observed in the VitD3 and amylose VitD3 physical mixture groups (69.2 % $\pm$ 3.5 % and 72.3 % $\pm$ 5.0 %, respectively) (Liu et al., 2023). The results of VitD3 encapsulation by soy protein and carboxymethyl chitosan indicated about 35 % release after 30 min in simulated gastric fluid (Teng et al., 2013). Another study conducted on VitD3 microencapsulation by complex coacervation using carboxymethyl tara gum and gelatin A demonstrated about 50 % release of the VitD3 within the first 2 h. These findings were related to the hydrolysis of gelatin as the capsule wall due to the effects of pepsin and gastric PH (3.0). Furthermore, the results of the VitD3 release from the complex coacervation of carboxymethyl tara gum-lactoferrin demonstrated approximately 50 % release during the gastric phase. Lactoferrin is a highly sensitive protein that degrades completely during the gastric phase. According to reports, enhanced stability during this phase is due to the complex coacervation of lactoferrin with other biopolymers. Based on our findings encapsulation of VitD3 not only improved stability in the stomach but also promoted the release of VitD3 in the intestine, which, in turn, minimized the degradation of VitD3. Moreover, the resulting Maillard complexes act as efficient carriers for VitD3 protection. Thus, the Maillard complex between casein and TG containing VitD3 could be used as an additive in the food industry as well as supplements. By enhancing VitD3 uptake, these foods and supplements can help us prevent cardiovascular disease, diabetes, hypertension, and rickets associated with VitD3 inadequacy.

3.9. Scanning electron microscopy and release kinetics

To elucidate the digestion pattern and release kinetics of encapsulated vitamin D3 (VitD3), we conducted in vitro simulated gastrointestinal experiments in conjunction with scanning electron microscopy (SEM) analysis. The release profile was quantitatively assessed using the Korsmeyer-Peppas model, expressed as $Mt./M\infty = kt^n$, where $Mt./M\infty$ represents the fraction of VitD3 released at time t, k is the release rate constant, and n is the release exponent. Linear regression analysis of log ($Mt/M\infty$) versus log(t) yielded n values of 0.43 and 0.62 for the simulated gastric and intestinal phases, respectively. The gastric phase exhibited Fickian diffusion ($n < 0.45$), indicating concentration gradient-driven release, while the intestinal phase demonstrated non-Fickian transport ($0.45 < n < 0.89$), suggesting a combination of diffusion and erosion-controlled release. The release rate constants (k) were calculated as 0.28 and 0.59 for the gastric and intestinal phases, respectively (Table 3 s). This integrated approach provided valuable insights into the sustained release behavior of the encapsulated VitD3 system, with the biphasic release profile optimized for targeted delivery in different physiological environments (Yasir et al., 2010). This comprehensive analysis combines quantitative release kinetics with qualitative structural observations, providing a robust understanding of the digestion pattern and sustained release behavior of the encapsulated VitD3 system throughout the simulated gastrointestinal tract.

4. Conclusion

In this study, a novel encapsulation system for VitD3 was developed using a Maillard reaction complex of sodium caseinate and TG. The optimized formulation demonstrated high encapsulation efficiency, suitable particle size, and good colloidal stability. Spectroscopic and thermal analyses confirmed the successful encapsulation and improved stability of VitD3. The in vitro release study showed the potential for targeted intestinal delivery of VitD3.

These findings highlight the promise of this system for enhancing VitD3 stability and bioavailability in food fortification applications. Future work in food chemistry could explore its use in developing fortified dairy products, functional beverages, and baked goods with improved VitD3 retention and delivery. To fully realize these applications, further research should evaluate the system's performance in various food matrices, conduct in vivo studies to confirm enhanced bioavailability, investigate potential synergistic effects with other nutrients, and assess industrial-scale production feasibility. This innovative approach opens new avenues for addressing vitamin D deficiency through food fortification, potentially contributing to improved public health outcomes.

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CRediT authorship contribution statement

Fatemeh Navab: Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Atefe Rezaei:** Writing – review & editing, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mohammad Hossein Rouhani:** Writing – review & editing, Supervision. **Farnaz Shahdadian:** Writing – review & editing. **Mahsa Alikord:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Project administration, Investigation, Data curation.

Declaration of competing interest

The authors have no conflict of interest.

Data availability

Data will be made available on request.

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