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Oleic Acid-PVA Based Amphiphilic Polymer Micelles for Vitamin D Encapsulation

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Abstract: In this study, oleic acid-PVA based amphiphilic polymer micelles were prepared for vitamin D encapsulation. The amphiphilic polymer encapsulations were characterized using Fourier transformed infrared spectroscopy (FTIR) and nuclear magnetic resonance spectroscopy (^{1}H-NMR). The goal of the study was to create micelles by using a lipophilic and biocompatible polymer. An oleic acid-substituted polyvinyl alcohol polymer was created through an acidic esterification reaction. The chemical structure of the polymer was disclosed by FTIR. To calculate the polymer's substitution ratio, ^{1}H-NMR was used. Micellization was used to encapsulate vitamin D. Scanning electron microscope (SEM) analysis was used to determine the crucial micelle concentration and the size of the oleic acid-modified PVA. Ultraviolet–visible (UV) spectroscopy was used to analyze the release of vitamin D at various pH levels. As a result, vitamin D can be enclosed in PVA polymer that has been substituted with oleic acid.

Keywords: Encapsulation, Vitamin D, Oleic Acid, Drug Release, Micelle

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1. INTRODUCTION

The process of covering solid, liquid, or gaseous components with a film coating material is referred to as encapsulation (1,2). The outer material is referred to as the shell, the shield, or the wall, and the inside substance is referred to as the core, corona, or active substance. It is frequently employed in a variety of industries, including medicine, nanotechnology, paint, cosmetics, and many more. Typically, one of the generated blocks is found to be hydrophilic, while the other is hydrophobic. Amphiphilic polymers can also be used to encapsulate molecules with a lipophilic structure (3).

Drug delivery systems that facilitate the transport of low-resolution drugs in the body have high interest. Such systems often allow the drug to be transported within the body and released in the targeted area (4,5). Polymeric carriage systems are used due to their easy adaptation in the body, protection of the drug from external factors, formation of phase separation, facilitation of distribution and easy movement within the vessels (6). In addition, drug transportation systems reduce side effects, inactivate body areas where unnecessary, and increase bioavailability, in addition to their easier production and lower costs (7).

Due to their capacity for absorption by the body, biodegradable polymers have become necessary as medication carriers in controlled-release technologies. Natural or artificial, biodegradable polymers can be broken down in vivo either enzymatically or non-enzymatically. They generate by-products that are biocompatible and toxicologically safe and can be further removed through regular metabolic function (8). Researchers currently have access to a wide variety of degradable polymers with a variety of breakdown rates thanks to the field of biodegradable polymers' rapid development (9). Polyvinyl alcohol (PVA), polylactic acid (PLA), and polylactic-co-glycolic acid (PLGA) are the most commonly utilized and researched biodegradable polymers. These polymers have been used in the formulation of numerous micro- and nanoparticulate systems.

The following benefits (16,17,18) should be provided by the encapsulation technique: the encapsulation should not have any negative consequences on the drug's stability or biological activity, the drug should not be affected during the final phase of the microsphere process, the effectiveness of the drug's encapsulation should not be subpar, and the yield of microspheres should be sufficient (up to 250 nm, ideally 125 nm). Additionally, the medication release profile and microsphere quality should be repeatable within the predetermined ranges. The microspheres must be manufactured in a powder that flows freely and exhibit neither agglomeration nor adhesion.

The coacervation technique (19,20), interfacial polymerization (21,22), and "in situ" polymerization (23,24) are the most frequently used techniques for microencapsulation.

Vitamin D, is a fat-soluble vitamin. Some types of vitamin D, such as ergocalciferol, play an important role in terms of their effects in the in vivo metabolism of calcium and phosphorus. Vitamin D has an important role in human health, from bone fractures to cardiovascular disease, neuromuscular problems, and diabetes. D vitamins could also be preferred as provitamin for the body's needs. Vitamin D precursors turns into Vitamin D by UV rays, which makes calcium binding faster (25).

In this study, PVA is modified with oleic acid, and optimum conditions for the encapsulation of vitamin D have been studied.

2. MATERIALS AND METHODS

2.1. Material

PVA (Mw ~150,000) (89% Hydrolyzed), oleic acid, sulfuric acid, ethanol, and citric acid were purchased from Sigma Aldrich. Vitamin D (98 %) was obtained from Merck.

2.2. Characterization techniques

A Shimadzu UV-Vis spectrophotometer 2450 (Kyoto Japan) was used for the spectrophotometric analysis. Using a Perkin-Elmer Spectrum 100 ATR-FTIR spectrophotometer, synthetic oligomers' chemical structures were determined (WA). A Varian Unity Inova Spectrometer (CAL USA) running at 400 MHz was used to generate ^{1}H-NMR spectra. SEM images of the resultant composites were captured using a Philips XL30 ESEM-FEG/EDAX. In order to prepare specimens for SEM, liquid nitrogen was used to solidify them, break them, and then cover them in platinum.

2.3. Synthesis of oleic acid modified polyvinyl alcohol

The oleic acid modified PVA (OA-PVA) was prepared according to previous studies (3,26). Briefly, PVA (600 g, 4 mmol) dissolved in 200 mL of distilled water and oleic acid (0.34 g, 1.2 mmol) were put into to a three-necked glass flask equipped with a nitrogen inlet, a magnetic stirrer, a reflux condenser, and a thermometer. 1 mL of H_2SO_4 was added with 250 rpm stirring at 80 °C in an oil bath through a dropping funnel. The mixture was refluxed at a temperature of 80 °C for 24 hours. The obtained product was precipitated with ethanol. The oleic acid-modified PVA was filtered and dried overnight at room temperature in a vacuum.

^{1}H-NMR: δ5.26-5.41(2H, 5.33 (dt, *J*=11.0, 7.4 Hz), 5.33 (dt, *J*=11.0, 7.4 Hz), 4,69 (1H, q, *J*=7.1 Hz), 3.57-3.6 (3H, 3.59 (q,*J*=7.4 Hz), 3.6 (tt, *J*=6.2,2.7 Hz), 3.6 (qt, *J*=6.2,2.6 Hz), 2.19-2.31 (2H, 5.33 (dt,*J*=11.0, 7.4 Hz), 2.25(t,*J*=7.4Hz), 1.91-2.03 (4H, 1.97(q, *J*=7.4 Hz), 1.97 (q, *J*=7.4 Hz), 1.97(q, *J*=7.4 Hz), 1.97 (q, *J*=7.4 Hz)), 1.35-1.84 (14 H, 1.42(tt, *J*=7.4,7.0 Hz), 1.42 (tt, *J*=7.4, 7.0 Hz), 1.43 (tt, *J*=7.4,7.0 Hz), 1.43 (tt, *J*=7.4, 7.0 Hz), 1.56 (tt, *J*=7.7,7.4 Hz), 1.56 (tt, *J*=7.7,7.4 Hz), 1.62 (qd, *J*=7.5, 6.2 Hz), 1.62 (qd, *J*=7.5, 6.2 Hz),1.71 (t, *J*=2.6 Hz), 1.71 (t, *J*=2.6 Hz), 1.77 (dd, *J*=7.1, 2.7 Hz), 1.77 (dd, *J*=7.1, 2.7 Hz), 1.77 (dd, *J*=7.1, 2.7 Hz), 1.77 (dd, *J*=7.1, 2.7 Hz), 1.11-1.34 (21H, 1.17 (d, *J*=6.2 Hz), 1.23 (tt, *J*=7.0, 6.9 Hz), 1.23 (tt, *J*=7.0, 6.9 Hz), 1.23(q, *J*=7.0 Hz), 1.23 (q, *J*=7.0 Hz), 1.23 (q, *J*=7.0 Hz), 1.23 (q, *J*=7.0 Hz), 1.23 (q, *J*=7.0 Hz), 1.24 (tt, *J*=7.0, 6.9 Hz), 1.24 (q, *J*=7.0 Hz), 1.24 (q, *J*=7.0 Hz), 1.25 (tt, *J*=7.7, 6.9Hz), 1.24 (q, *J*=7.0 Hz), 1.24 (q, *J*=7.0 Hz), 1.25 (tt,7.7,6.9 Hz), 1.25 (tt, *J*=7.7,6.9 Hz), 1.28 (tt, *J*=7.0,6.9 Hz), 1.28 (tt, *J*=7.0,6.9 Hz), 1.28 (h, *J*=7.0 Hz), 1.28 (h, *J*=7.0 Hz), 0.81-1.02 (6H, 0.86 (t, *J*=7.0 Hz), 0.96 (t, *J*=7.5 Hz).

2.4. Vitamin D encapsulation by using OA-PVA and determination of optimum capsulation parameters

Vitamin D encapsulation was performed by the direct dissolution method at optimum critical micelle concentration (CMC), resulting in the self-assembling of the drug and polymer to form polymeric micelles. In the encapsulation study, 0.6 g oleic acid-modified PVA was dissolved in 50 mL distilled water, stirred for 2 h, and 1 mL of vitamin D was added to the solution. After 1 h, 4 mL of 1M citric acid were added. The resulting mixture was stirred for 2 h at 500 rpm (27). The obtained capsules were filtered and dried.

The CMC is the minimum quantity of wall material necessary for micelle formation during the manufacturing of a capsule. Both SEM analysis and visual inspection revealed that different amounts of OA-PVA (0.2, 0.4, 0.6, 0.8, and 1 g) were used to make capsule walls, and varying amounts of vitamin D (0.5, 1 and 1.5 mL) were placed into the micelle that served as the cores of the capsules to determine the optimal encapsulation parameters.

2.5. Decomposition of nanocapsules at various pH values

The release studies were carried out at different pHs to monitor the pH dependence of vitamin D encapsulated wall material for the critical micelle concentration and the optimum vitamin D amount. The solutions were stirred for 20 minutes, followed by changing pH values. Capsule deterioration has been revealed by means of changes in its color with a UV–Vis spectrophotometer in addition to visual inspection.

3. RESULTS

3.1. Synthesis and characterization

Oleic Acid Substituted Polyvinyl Alcohol was prepared via Fisher esterification according to Figure 1.

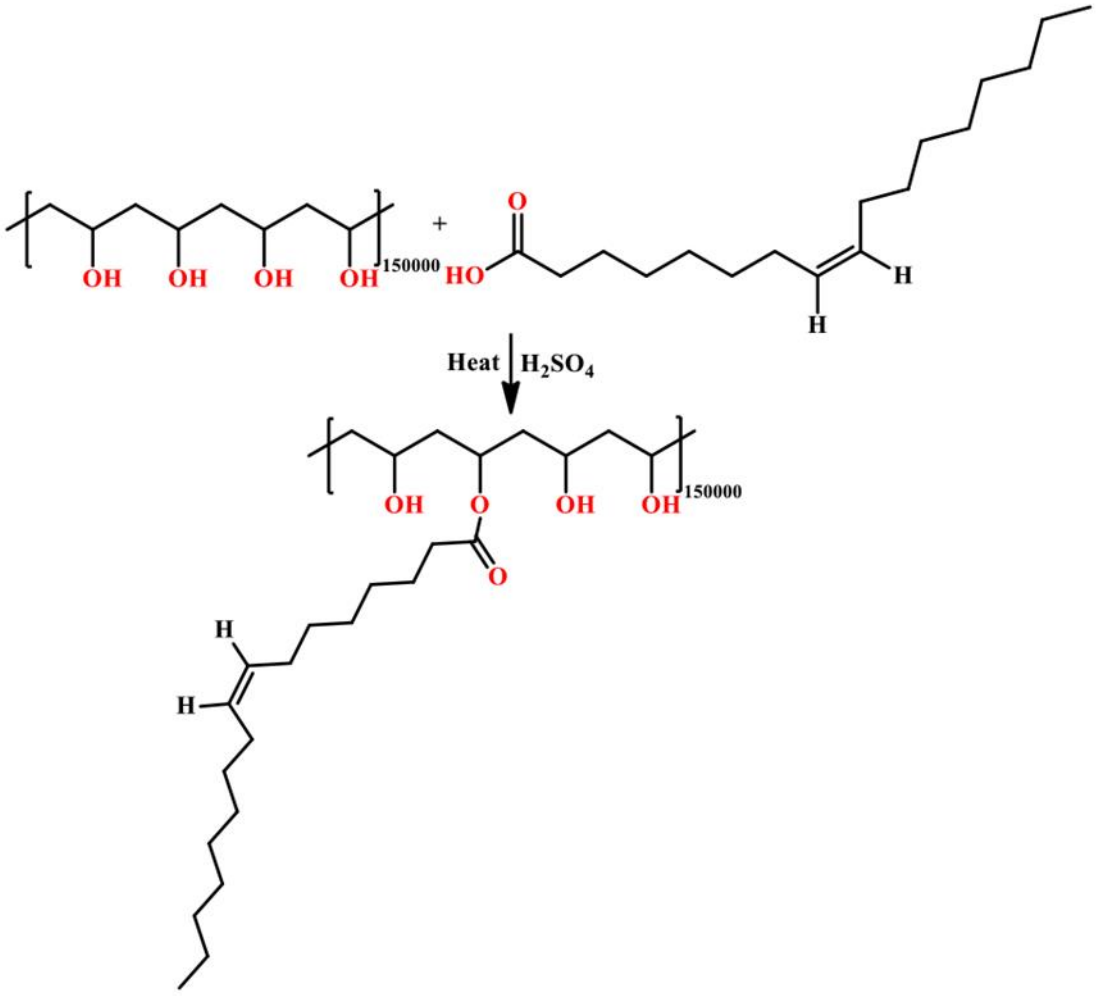

Figure 1: Synthesis of oleic acid substituted polyvinyl alcohol

In Section 2.3, the ^{1}H-NMR spectrum of oleic acid modified polyvinyl alcohol can be seen. The double bond proton signals for the oleic acid substituted polyvinyl alcohol appeared at 5.33 ppm and at 4.69-4.75 ppm for oleic acid bonded C-H. Additionally, the spectrum exhibits OH protons signals of PVA at 3.60 and 3.57 ppm. At 2.25 ppm, the first protons of oleic acid and, at 1.96 and 1.25 ppm, methyl protons (-CH_2-) of oleic acid can be seen. In addition, the methyl protons of PVA appear at 1.72, 1.76, and 1.50 ppm. Moreover, the terminal methyl protons of the oleic acid are seen at 0.86 ppm. The obtained results were in accordance with the literature (28). The substitution was calculated by comparing the areas of the peaks at 4.69-4.75 ppm oleic acid bonded C-H and OH protons signals of PVA at 3.60 and 3.57 ppm. The ratio is calculated as 1: 4. The substitution range is close to the calculated value and matches the literature (29). The results of ^{1}H-NMR analysis demonstrated that the expected structure was synthesized successfully.

Additionally, the FTIR analysis supports the ^{1}H-NMR results. Figure 2a demonstrated that the carbonyl stretching of PVA's acetate groups emerged at 1693 cm^{-1} and that the typical CH_2 stretching band for PVA appeared at 2915 cm^{-1}. Moreover, at 3307 cm^{-1} typical strong hydroxyl bands for PVA can be seen. Crystallization-sensitive band of PVA at 1090 cm^{-1} Similar results could also be found in the literature for the PVA (30). Figure 2b showed that the characteristic carbonyl strength band of oleic acid at 1711 cm^{-1}

and the peaks at 2900 and 2850 cm^{-1} were attributed to the asymmetric CH_2 stretch and the symmetric CH_2 stretch, respectively, in-plane and out-of-plane. The peak at 3005 cm^{-1} belongs to CH=CH strength of oleic acid. The results are consistent with the literature (31). Figure 2c showed that oleic acid modified polyvinyl alcohol FTIR. The key parameter of the stretching vibration of carbonyl groups in esters (C-O-C=O) was observed at 1644 cm^{-1} (figure 2c). The results are consistent with the data of Zagonel et al. (32). The emergence of the ester peak 1644 cm^{-1}, and the double bond peak at 3005 cm^{-1} demonstrates successful binding.

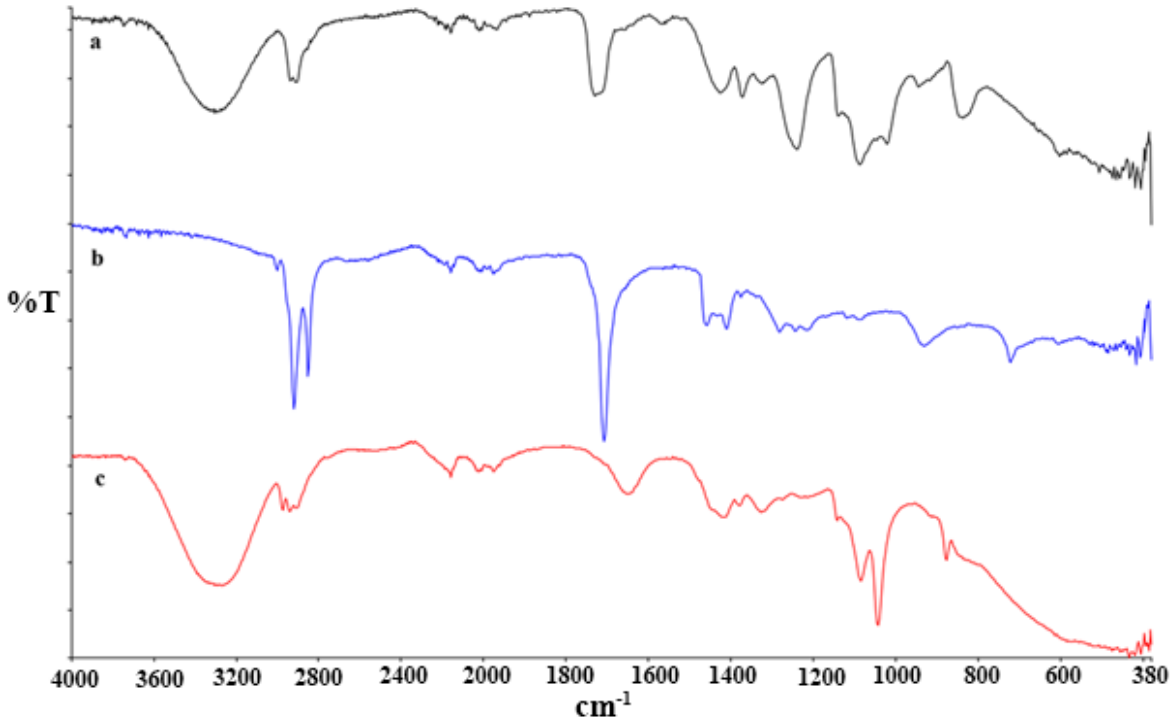

Figure 2: FTIR spectra a. PVA b. oleic acid c. oleic acid substituted polyvinyl alcohol

The FTIR spectrum of the encapsulated vitamin D at the optimum amount of vitamin D and polymer content, which are determined with critic micelle concentration and oil amount study (Figure 3). Figure 3 shows the FTIR spectrum of vitamin D with bands of 2915-2917 cm^{-1} free CH_3 stretching, 1730 cm^{-1} ester stretching, and 1088 cm^{-1} carbonyl stretching (33). Comparing the FTIR spectra of vitamin D and the capsule according to Figure 3, the vibration band at 965 cm^{-1} due to sulfoxide (S=O) and sulfhydryl groups for vitamin D disappeared in the FTIR spectrum of the capsule. It is seen that all the vitamin D was encapsulated in the core and only the polymeric peaks at the wall appeared in the spectrum (34). The absence of a vitamin D band indicates that capsulation has been achieved successfully.

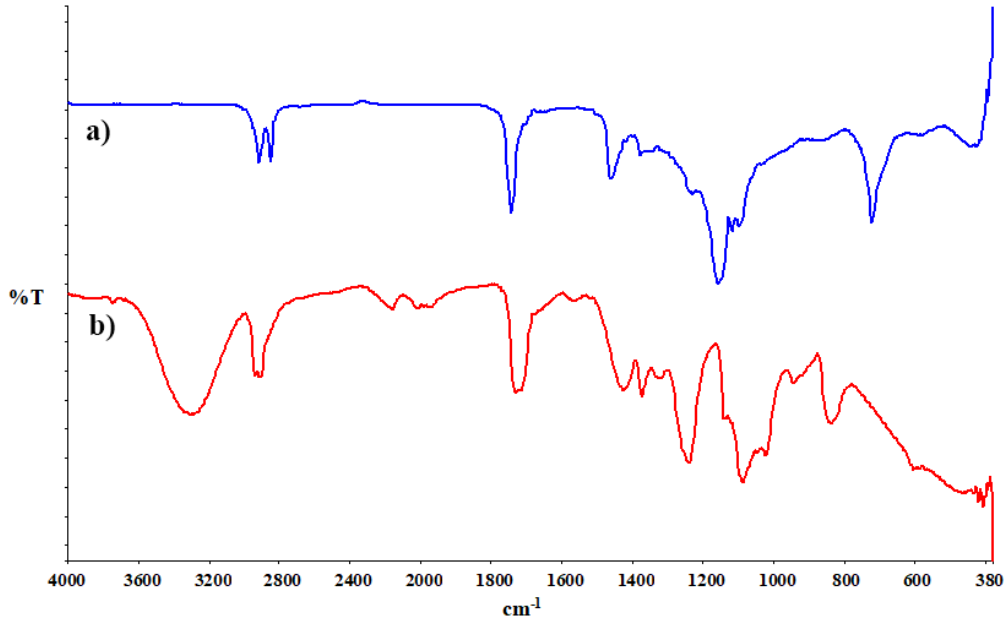

Figure 3: FTIR spectra of a) vitamin D and b) vitamin D-oleic acid modified polyvinyl alcohol capsule

The encapsulation of vitamin D was prepared under acidic conditions. When the pH dependence of encapsulation was examined (Figure 4a), it was concluded that encapsulation does not occur at a basic pH and only occurs at an extreme acidic pH (35). In addition, when the SEM images were examined, it was determined that the capsule size was homogeneous and about 6.5 nm (Figure 4b). When the size of the obtained capsules was compared with the literature, it was determined that 10 times smaller capsules were synthesized (36).

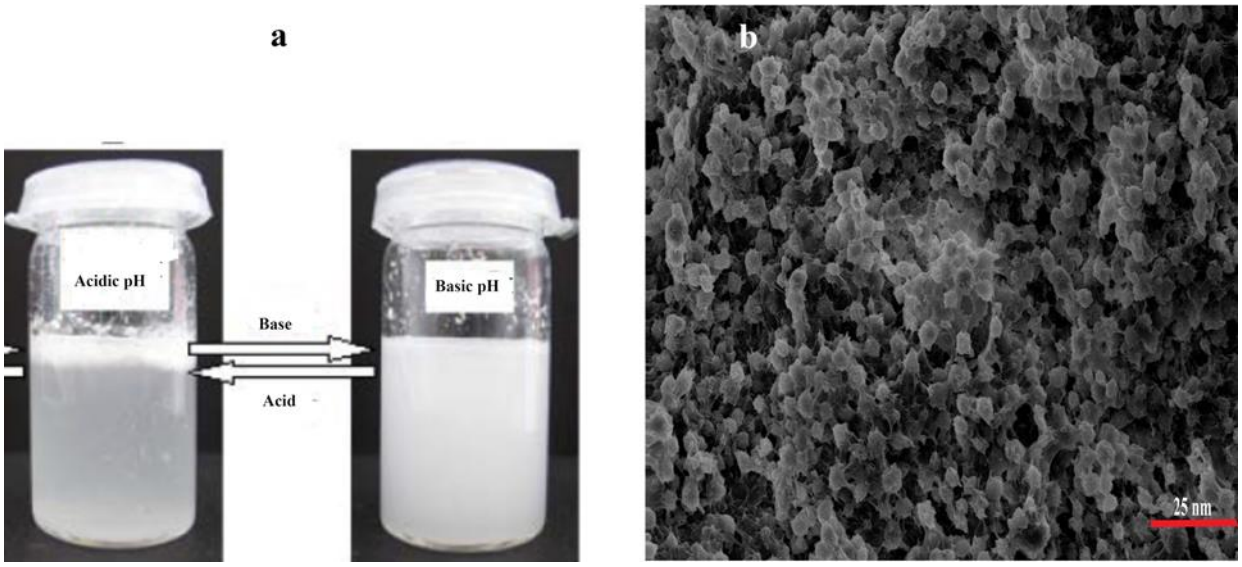

Figure 4: a. Encapsulation condition image b. SEM images of vitamin D- oleic acid modified polyvinyl alcohol capsules

The minimum oleic acid modified polyvinyl alcohol polymer that is needed to achieve critical micelle concentration for the encapsulation of vitamin D was determined. It was visually found that the encapsulation did not occur at the concentration of OA-PVA under 0.6 g/50 mL water. This result is also supported by the literature (37). In addition, when the SEM images were examined, it was seen that the capsules were agglomerated at a concentration of 1 g/50 mL, whereas the capsules were not formed at a concentration of 0.4 g/50 mL at 50.000x magnification.

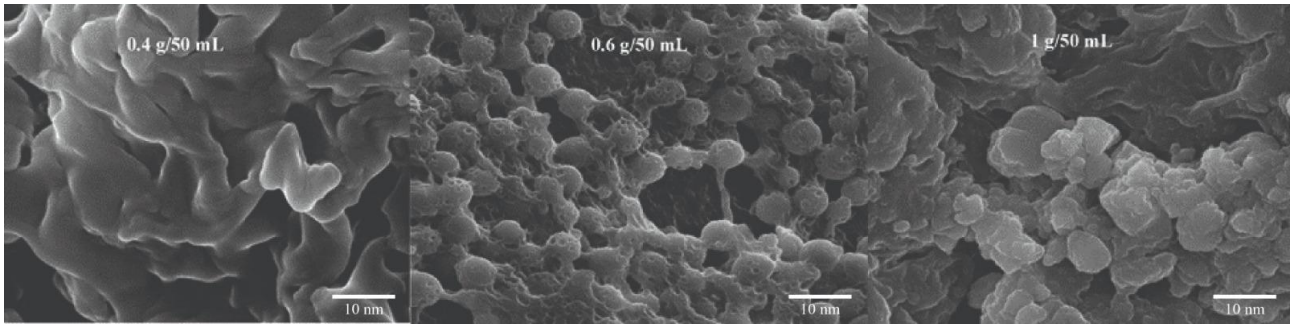

Figure 5: SEM images of capsules obtained with various amount of OA-PVA

To investigate the effect of vitamin D concentration on encapsulation, different concentrations were added to of 0.6 g of OA-PVA, and the encapsulation was visually inspected. The encapsulations of 0.5 mL and 1 mL of vitamin D were performed without issues, but when the vitamin D was further increased, the emulsion could not be formed completely with the OA-PVA. The untreated vitamin D remained on the surface. As a result, the upper limit for micellization of vitamin D was determined to be 1 mL.

3.2. Decomposition of nanocapsules at various pH values

The decomposition of capsules due to the pH changes was investigated by UV spectroscopy. The decomposition of capsules release profiles of vitamin D (Figure 6) from micelles prepared with OA-PVA polymer showed significant differences with pH. It was observed that OA- PVA polymer stabilized the vitamin D at acidic pH, but the capsules were deteriorated at pH 6 and above. The oil phase containing vitamin D was separated above pH 6. This observation was also compatible with the literature (38).

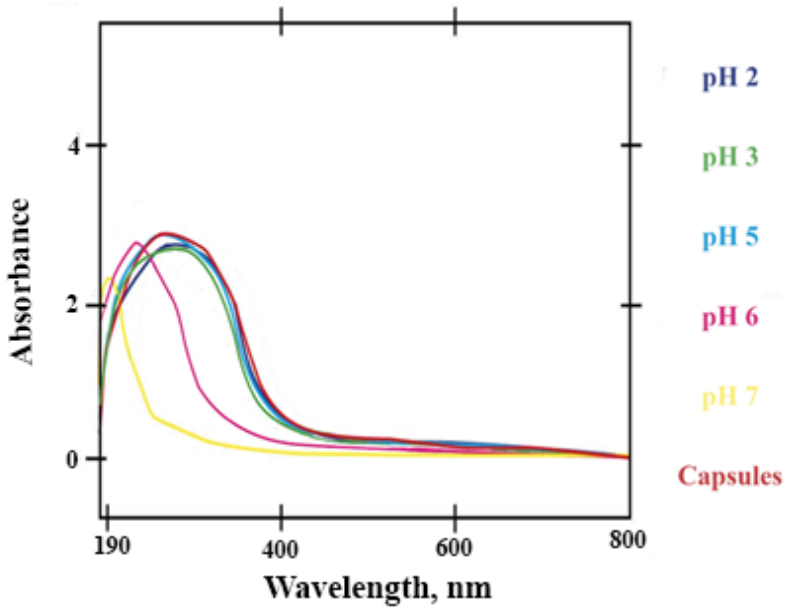

Figure 6: UV spectrum of capsules at different pH

4. CONCLUSION

OA-PVA amphiphilic polymer was prepared with Fisher acidic esterification. ^{1}H-NMR and ATR-FTIR confirmed the expected structures. The vibration band belonging to sulfoxide (S=O) and sulfhydryl groups of vitamin D at 965 cm^{-1} disappeared in the FTIR spectrum of the capsule. The absence of vitamin D bands indicates that capsulation has been achieved successfully. Herein, vitamin D encapsulation with the obtained polymer is reported for the first time. The optimum conditions for encapsulation were determined visually and by SEM. In light of the results obtained, it was concluded that the optimum capsulation conditions were 0.6 g / 50 mL polymer solution, 1 mL vitamin D, and an extremely acidic environment. The decomposition of vitamin D capsules at various pH values was investigated. It was observed that oleic acid-substituted PVA polymer stabilized the capsule at acidic pH. Considering all of the results, it could be stated that oleic acid substituted polyvinyl alcohol polymer can be used for encapsulation applications of vitamin D.

5. CONFLICT OF INTEREST

There is no conflict of interest.

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