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Modified montmorillonite nanolayers for nano-encapsulation of biomolecules

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Abstract

Vitamin B6 was nano-encapsulated in between modified montmorillonite nanolayers. Results indicated that electrostatic interaction forces dominate the adsorption onto different sites of the nanolayers. The successful nano-encapsulation was achieved when the interlayers spaces of the nanolayer were saturated with cations of Na^+ or Ca^{2+} resulted in adsorption of vitamin B6 in between nanosheets. At these conditions, controlled pH-responsive desorption properties were detected and vitamin B6 was released mostly from the interlayer spaces. The presented modified montmorillonite could be used for nanoencapsulation of drugs and biomolecules with high protection of carrying materials during storage and even through the digestion process.

Keywords: Materials science, Nanotechnology

1. Introduction

The food and pharmaceutical industries have been facing challenges for development and implementation of new techniques that can produce enhanced and safe pharmaceutical or food supplement carriers while also being economically and technically efficient and environmentally friendly [1, 2]. For this goal nanoencapsulation has been developed to pack substances especially biomolecules using different materials such as nano-emulsification systems, nanocomposites, and nano-structures [3,

4, 5]. It will provide the final product a desired functionality with the improvement of drug or food supplement stability during storage and even digestion process [6].

Nanoencapsulation showing stimuli-responsive characteristics were considered for delivery of different drugs and biomolecules to the specific part of the body. Administration of different nanomaterials for this goal is mainly dictated by the safety profile of the materials, desired therapeutic target, the availability, and price of the nanomaterials [7, 8]. Such increasing attention towards nanocarriers has usually increased the concern for its safety for the human body in addition to its final functionality limiting its attractiveness for the industry [9]. Meanwhile, montmorillonite has been presented as a promising nanocarrier of proteins [10], vitamins [11, 12], antibiotics [13], and anticancer drugs [14] due to its particular properties such as biocompatibility [15, 16, 17], high adsorption capacity [18], low toxicity [19], and its low price [20].

Montmorillonite is known as a new smart carrier introducing new mechanisms of adsorption enabling highly controlled delivery procedures such as pH-responsive in addition to drug protection from chemical denaturation [21, 22, 23]. All these functional characteristics are due to the cation exchange property which enables the montmorillonite for adsorption of the different biomolecules [23, 24]. Adsorption characteristics of different biomolecules onto montmorillonite has been studied for food and nutrition applications or drug delivery applications [12, 21] such as promethazine hydrochloride [25], tetracycline [26], timolol maleate [27], vitamin B6 [11], and vitamin B12 [12, 23]. In all these studies biomolecules are adsorbed onto different sites of the montmorillonite such as in the interlayer spaces, the external surfaces, and the edges of montmorillonite [28]. Such adsorption processes are dependent on the charge density of the montmorillonite and the adsorbate in addition to the cation exchange capacity of the montmorillonite.

However, there is not still a systematic study describing a successful method for adsorption of biomolecules or drugs in between nanosheets with controlled release mechanism only from the interlayer spaces which minimize the release from other sites of nanosheets. In this process of encapsulation, the biomolecules are adsorbed in the interlayer spaces of the montmorillonite which improves its stability [12, 21, 23] in the harsh chemical condition in the body before the biomolecule being desorbed [8, 29]. In this study, it is aimed to use montmorillonite nanolayers for nano-encapsulation of a biomolecule using the cation exchange mechanism with controlled smart release properties only from the interlayer spaces. It is mainly targeted to limit adsorption of the biomolecule to different parts of nanolayers except for the interlayer spaces and to get the highest amount of release just from interlayer spaces in a pH-responsive reaction. For this purpose, vitamin B6 was selected with diverse electrostatic properties when placed in different pHs. The results of this study provide great advances in the application of montmorillonite with advanced

adsorption and stimuli-responsive delivery properties for food and pharmaceutical applications.

2. Experimental

2.1. Materials

Vitamin B6 (pyridoxine hydrochloride), hydrochloric acid (HCl, 37% w/w, Merck), sodium hydroxide (NaOH, analytical reagent; Merck), sodium chloride (NaCl, analytical reagent; Merck), and calcium chloride, ($CaCl_2$, analytical reagent; Merck) was used as received. Deionized water (the water was purified to a resistivity of ≥ 18.2 MΩ cm) was used in all experiments. The bentonite sample was provided by Salafchegan bentonite mine (Iran).

2.2. Purification and modification of montmorillonite

Montmorillonite was modified in five different procedures (Fig. 1 and Table 1). In the first method, only <2.5 μm particles of montmorillonite were separated from bentonite by centrifuge force and named NC_1. In the second method, NC_1 was reacted with 0.1 M HCl at 40 °C for 10 minutes and then centrifuged, washed with plenty of water and named NC_2. In the third method, NC_1 was reacted with 0.1 M NaOH at 40 °C for 10 minutes and then was washed and called NC_3. In the fourth
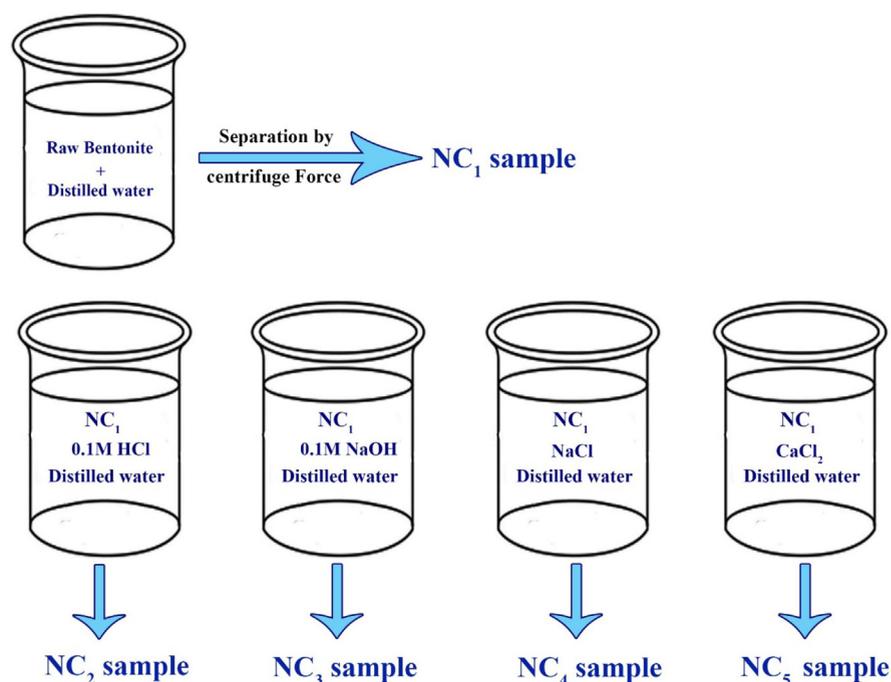
Fig. 1. The schematic illustration of montmorillonite modification process.

Table 1. Details of nanoclay purification parameters.

Sample	Particle size (μm)	CEC (meq/100g)
NC_1	2.5	85
NC_2	2.5	108
NC_3	2.5	106
NC_4	2.5	84
NC_5	2.5	86

and fifth method, NC_1 sample was soaked in water containing NaCl or $CaCl_2$ and then was centrifuged and washed and named NC_4 or NC_5, respectively [12, 30].

2.3. Kinetic of adsorption vitamin B6 on the montmorillonite

For kinetics experiments, a solution of 1 g/L vitamin B6 in deionized water was prepared and then montmorillonite samples (50 mg) were added to above-mentioned solution (5 mL) and shacked for different duration of 10, 20, 30, 45, 60, 90, 120, 180, 270, 360, 480, 960, 1440, 2880, and 4320 minutes at room temperature (25 °C). Then the mixtures were centrifuged and the concentration of the supernatant was measured by UV-visible spectroscopy at the detection wavelength of 291 nm. The amount of adsorbed vitamin was measured through mass balance. In order to investigate the effect of pH on adsorption process, adsorption kinetics were done at different pH of 3, 6.5, and 9.5.

2.4. Adsorption isotherms of vitamin B6 on montmorillonite

The solutions (5 mL) of 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, and 2.25 g/L vitamin B6 in deionized water at different pH were prepared and the nanoclay samples (50 mg) was added to them. The mixtures were shaken for 90 minutes to reach maximum level at 25 °C. After reaching the equilibrium the mixture was centrifuged and supernatants concentrations were measured by UV-visible spectroscopy at a detection wavelength of 291 nm. The amount of adsorbed vitamin was measured through mass balance. The adsorption isotherms were calculated by plotting the amount of vitamin B6 adsorbed at equilibrium versus the equilibrium solution concentration.

2.5. In vitro release of vitamin B6 from montmorillonite

In vitro release of vitamin B6 from montmorillonite was analyzed at two pH of 1.2 (representing the gastric condition) by mixing 250 mL of 0.2 M HCl and 147 mL of 0.2 M KCl and pH 7.4 (representing the intestinal condition) by mixing 250 mL of 0.1 M KH_2PO_4 and 195.5 mL of 0.1 M NaOH as buffer solutions. The release of vitamin B6 was analyzed by a dialysis membrane bag containing 160 mg of

montmorillonite-vitamin B6 complex and 10 mL of buffer solution. The bag was then immersed in 120 mL of the same buffer solution in a 250 mL balloon. The balloon containing dissolution media and dialysis bag was immersed in a water bath and rotated with a speed of 100 rpm and the temperature was set to 37°C. At 30 minutes of time intervals, 5 mL of the dissolution media was taken and the concentration of vitamin B6 was measured by UV-visible spectroscopy and the same volume was replaced with a fresh dissolution media.

2.6. Characterization

Normal XRD analysis in the range of 2θ from 3 to 80° for determination of impurities in nanoclay samples was done using Philips PW1800. Low angle X-ray diffraction (LXRD) analysis was carried out in the range of 2θ between 2 and 10° using Rigaku D/max-1200 with Cu Kα radiation. Unico UV-2100 spectrophotometer was used to measure vitamin B6 concentration in solution and calculated using the standard curve. The cation exchange capacities of the samples were measured using ammonium acetate method [31].

3. Results and discussion

Fig. 2 compares the XRD patterns of raw bentonite samples along with purified montmorillonite. The major phases in the raw bentonite samples are montmorillonite, quartz, illite, cristobalite and calcite. These are common minerals which exist in bentonite deposits [32]. No indication of quartz was found in purified montmorillonite sample after purification however other form of SiO_2 like cristobalite existed in little amount. Interestingly, montmorillonite 001 reflection was sharpened after purification showing improvement of purity of the montmorillonite [30].

The purified montmorillonite samples were analyzed using SEM to analyze their morphological characteristics (Fig. 3). A layered structural formation was observed for all raw and purified samples.

Fig. 4 represents the kinetics of adsorption of vitamin B6 on different purified and modified montmorillonite samples at 25 °C at different pH of 3, 6.5, and 9.5 with the initial concentration of vitamin B6 at 1 g/L. Results showed that the adsorption process reached to the equilibrium rapidly in the first 20 minutes for all adsorbents and after that, the adsorption was stopped. The maximum amount of adsorption was occurred at pH of 6.5 for all adsorbents. This phenomenon could be described as follows: vitamin B6 is a water-soluble vitamin which exists in protonated and neutral form at low pH. The mixture of protonated, deprotonated, and neutral forms will exist at neutral pH and by increasing the pH, the deprotonated species of vitamin B6 will form (Fig. 5) [11]. At pH of 6.5 protonated form of the vitamin was reacted as a cation and adsorbed in interlayer spaces of montmorillonite nanolayers through
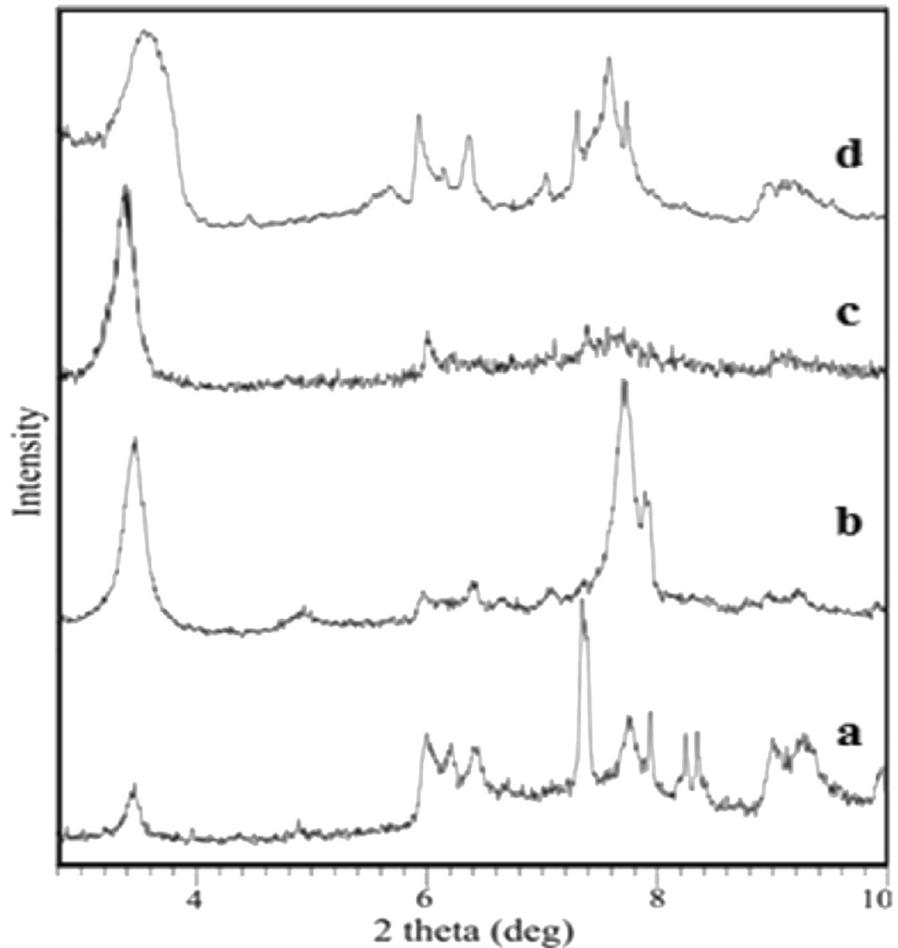
Fig. 2. XRD pattern of samples, a: row bentonite, b: NC_1, c: NC_2, and d: NC_3.

a cation exchange reaction mechanism except for the sample NC_2 (Fig. 6). It was proved by the XRD results that the basal spacing of all montmorillonite samples (except for NC_2) after adsorption of vitamin was increased (Fig. 6) [20]. Considering the size of vitamin B6 around 1.5 Å its intercalation to the montmorillonite nano-layers resulted in such increase in the basal spacing after adsorption. In addition, at this pH, deprotonated forms of vitamin B6 molecules are exchanged with the OH^- groups existed on the surface of the montmorillonite nanolayers [33] resulting the excess amount of vitamin adsorbed. No changes in the basal spacing of the NC_2
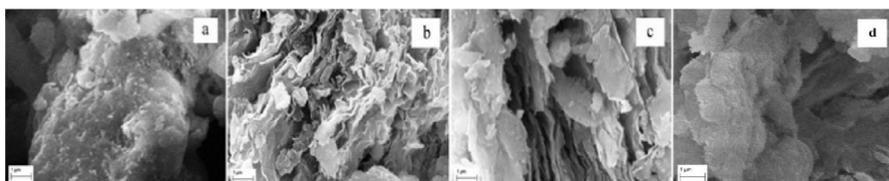
Fig. 3. SEM image of sample. The scale bar in all images is 1 nm. a: row bentonite, b: NC_1, c: NC_2, d: NC_3.
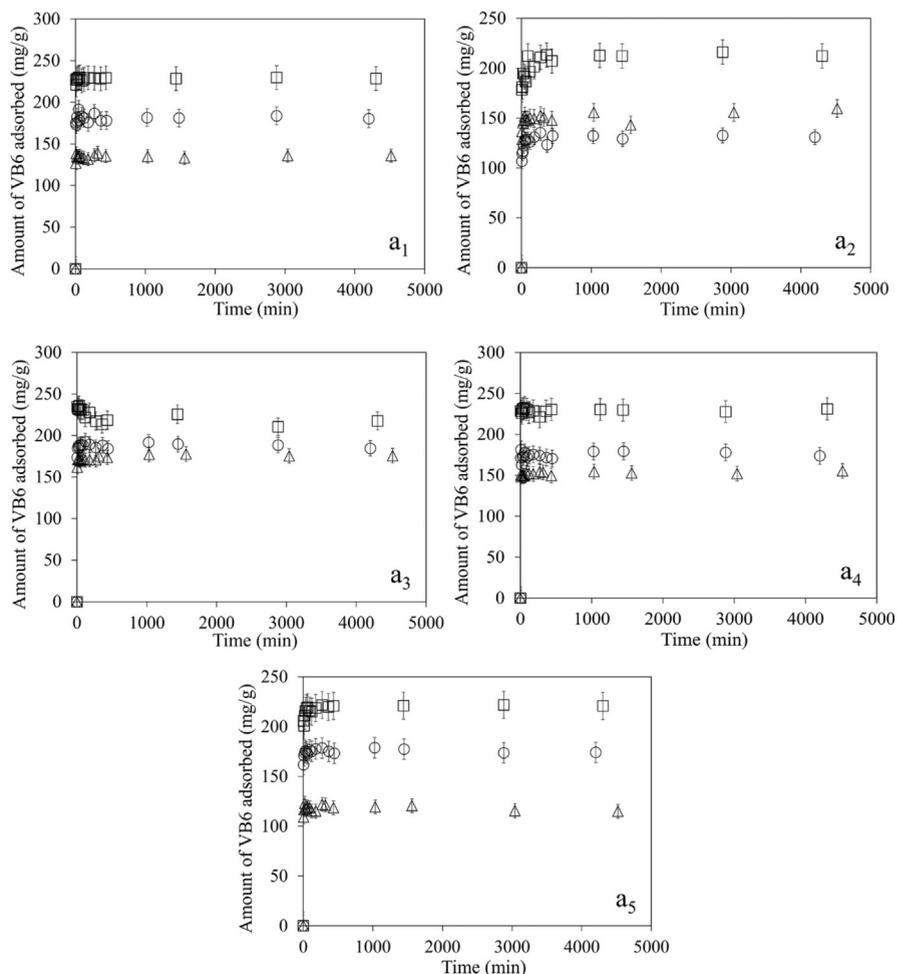
Fig. 4. Kinetics of adsorption of vitamin B6 on montmorillonite samples (a1: NC1, a2: NC2, a3: NC3, a4: NC4, and a5: NC5), Symbols describe the pH of adsorption, ($\triangle$: pH = 3, $\square$: pH = 6.5, $\bigcirc$: pH = 9.5).

sample after adsorption of the vitamin was detected at any conditions (Fig. 6). In fact, the surface and edges of the NC_2 sample nanolayers were saturated with H^+ cations and therefore, there was less tendency to adsorb in the interlayer spaced through the cation exchange mechanism (Fig. 7).

By reducing the pH to 3, the amount of vitamin adsorbed on the montmorillonite nanolayers reduced for all samples. At this pH, the vitamin existed in its protonated form and adsorbed mostly in the interlayer spaces through a cation exchange mechanism with a reduction in the adsorption on the external surfaces of nanolayers due to the repulsion electrostatic force (Figs. 4 and 6) [34]. Such condition is much more critical for sample NC_2 with more positively charged nanolayers leading to almost no adsorption in the interlayer spaces (Fig. 6b).

When the pH of the solution was increased to 9.5, the edges charge of montmorillonite became more negative [33] and the montmorillonite interlayer cations created
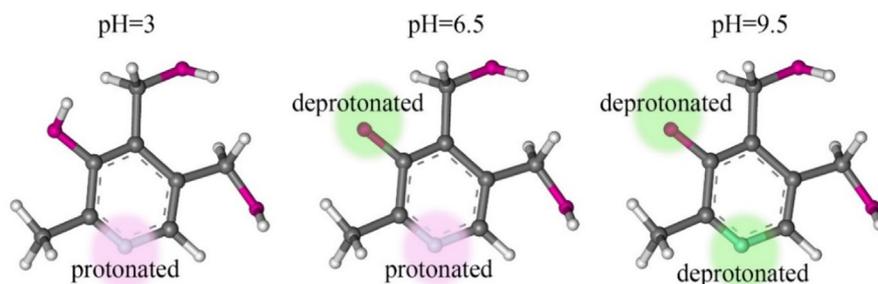
Fig. 5. Schematic description of structure of vitamin B6 at different pH.
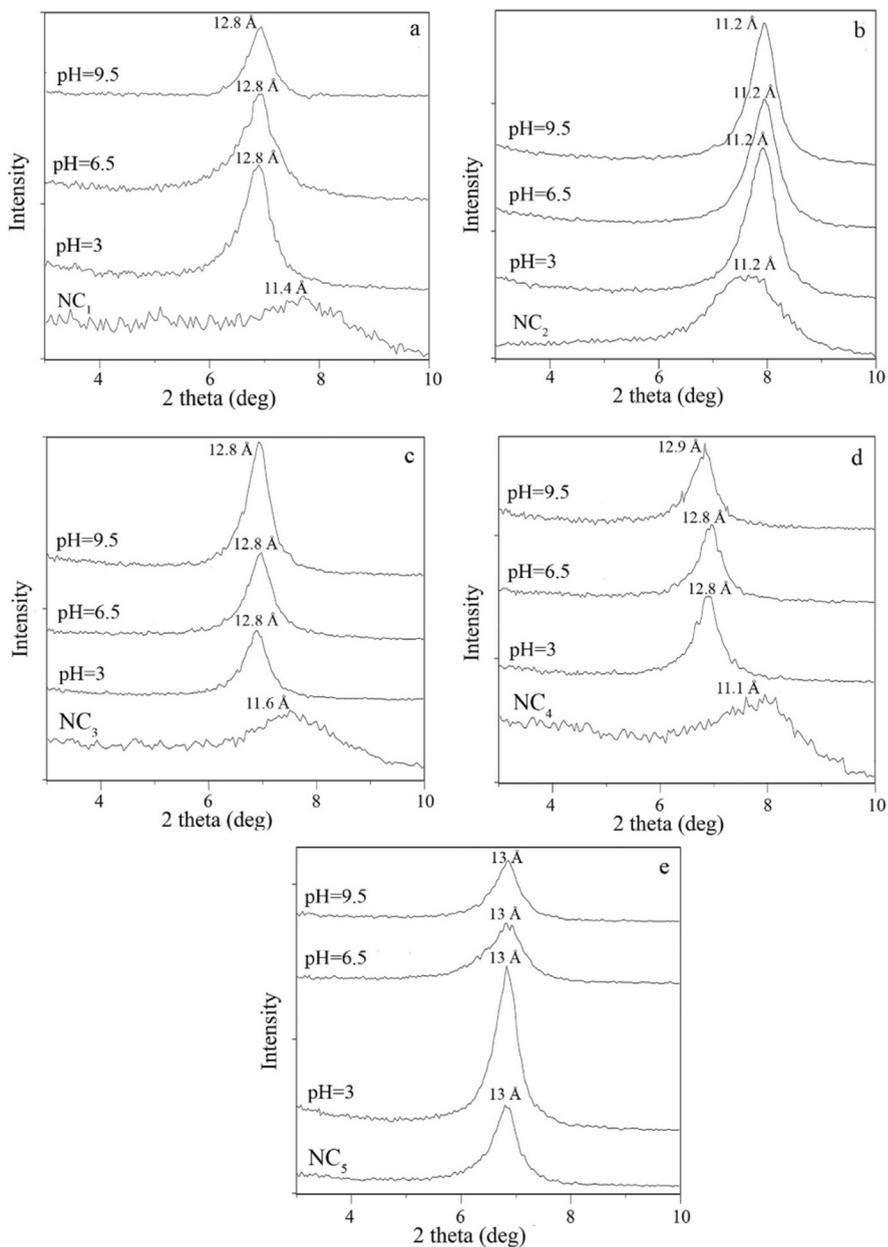
Fig. 6. XRD patterns of montmorillonite samples before and after adsorption vitamin B6 at different pH: a: NC_1, b: NC_2, c: NC_3, d: NC_4, e: NC_5.
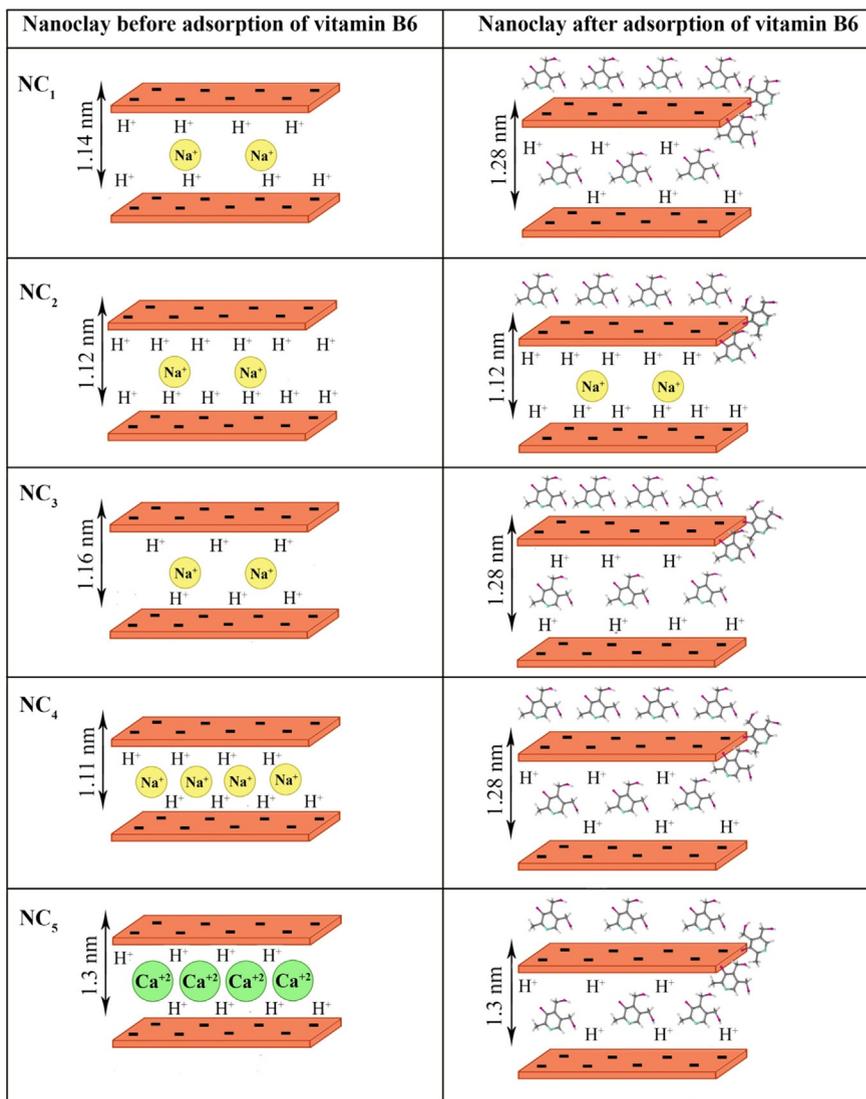
Fig. 7. Schematic description of adsorption of vitamin B6 onto surfaces and interlayers of different montmorillonite samples.

a bridge between the negative surface of the montmorillonite and deprotonated form of the vitamin B6. Then, the vitamin B6 could gradually migrate to the interlayer spaces of the montmorillonite nanolayers [12]. Such migration happened for all samples except NC_2 which again was due to the high concentration of H^+ on the nanolayers (coming from the purification and modification process) resulting repulsion electrostatic forces and limits the migration of the vitamin-cation complex to the interlayer spaces.

The result of the adsorption isotherm (Fig. 8 and Table 2) showed that the maximum amount of adsorption of vitamin B6 onto montmorillonite nanolayers happened at
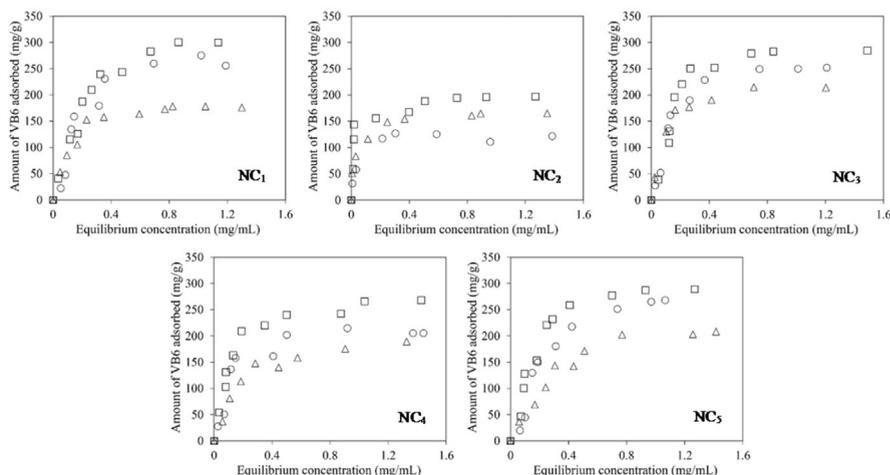
Fig. 8. Adsorption isotherm of vitamin B6 on different montmorillonite samples. Symbols describe the pH of adsorption, $\triangle$: pH = 3, $\square$: pH = 6.5, and $\bigcirc$: pH = 9.5.

Table 2. Calculated parameters of Langmuir and Freundlich isotherm models for adsorption of vitamin B6 onto montmorillonite samples.

Adsorbent	pH of adsorption	Langmuir model			Freundlich model		
		C_m (mg g^{-1})	K_l (L mg^{-1})	R_L^2	n_f	K_f (mg g^{-1})	R_F^2
NC$_1$	3	188.68	1.20	0.99	2.88	85.88	0.86
NC$_1$	6.5	384.62	0.43	0.98	1.95	101.60	0.88
NC$_1$	9.5	303.03	0.69	0.99	1.47	69.69	0.76
NC$_2$	3	169.49	3.10	1	4.58	106.11	0.95
NC$_2$	6.5	200	4.16	1	4.77	127.07	0.83
NC$_2$	9.5	128.21	3.39	1	4.22	73.60	0.85
NC$_3$	3	227.27	1.38	0.99	2.48	103.17	0.84
NC$_3$	6.5	322.58	0.70	0.96	1.95	106.50	0.73
NC$_3$	9.5	285.71	0.71	0.97	1.83	88.04	0.83
NC$_4$	3	217.39	0.48	0.99	2.15	68.77	0.84
NC$_4$	6.5	285.71	1.06	1	2.93	121.92	0.82
NC$_4$	9.5	217.39	1.44	0.99	2.19	79.42	0.78
NC$_5$	3	243.90	0.39	0.94	1.79	58.37	0.91
NC$_5$	6.5	312.5	0.71	0.98	3.18	137.03	0.80
NC$_5$	9.5	322.58	0.44	1	1.25	55.21	0.80

pH 6.5 which could be due to the adsorption of the deprotonated form of the vitamin on the external layers and the protonated form to the interlayer spaces through the cation exchange mechanism. Interestingly, the lowest amount of adsorption happened at pH of 3 which at this condition, the dominant adsorption mechanism

is considered to be the cation exchange as the vitamin existed in its protonated form as described earlier.

Due to the results achieved the encapsulation of vitamin B6 in the interlayer spaces of montmorillonite nanolayers could occur through two different mechanisms, the cation exchange and the cationic bridge adsorption on the edges of the nanolayers followed by migration of vitamin-cation complex to the interlayer spaces. A set of in-vitro release experiments were planned and tested to analyze the release properties of nano-encapsulated vitamins whether the desorption was mainly controlled by release of vitamins from the interlayer spaces. Fig. 9 shows the kinetics of desorption of vitamin B6 from different samples at two pH of 1.2 (simulated gastric condition) and pH of 7.4 (simulated intestinal condition). The amount of vitamin B6 desorbed from all samples was less at pH of 1.2 compared to that at the pH of 7.4.

At pH of 7.4 two different desorption trend were detected for different samples, one was for samples NC_1, NC_2, and NC_3 in which the highest amount of vitamin was released when the adsorption was done at pH of 9.5 and second was for samples NC_4 and NC_5 in which the highest amount of vitamin was released when the adsorption was done at pH of 3. At pH 3 the main adsorption mechanism was described to be the cation exchange and the highest amount of vitamins adsorbed in the interlayer spaces as it is presented in the protonated form and react like cations. Hence, higher amount of desorption at this conditions for samples NC_4 and NC_5 should have been release from the interlayer spaces of montmorillonite showing a successful nanoencapsulation. However, for NC_1, NC_2, and NC_3 samples, at pH of 9.5, the vitamin mostly has adsorbed on the external surfaces of the nanolayers. Hence, the desorption has occurred mainly from the external surfaces of the montmorillonite showing that the encapsulation was not the main mechanism in the delivery process.

In addition, the trend of desorption from NC_4 and NC_5 samples was much more uniform with a steady trend starting from the beginning to the end of desorption process (360 minutes) compared to samples NC_1, NC_2, and NC_3 in which the adsorption trend was not very much uniform and continued for 100 minutes and then almost stopped. The montmorillonite samples after desorption tests were analyzed with the XRD analysis and the results indicated that at pH of 1.2 (Fig. 10) for all adsorbents except NC_2 the basal spacing of the montmorillonite was slightly reduced. On the other hand, after desorption of vitamin B6 at pH of 7.4 (Fig. 11), the basal spacing of montmorillonite reduced much more for all samples. Considering the amount of vitamin desorbed from different samples and based on the adsorption mechanism described earlier, it could be suggested that for samples NC_4 and NC_5
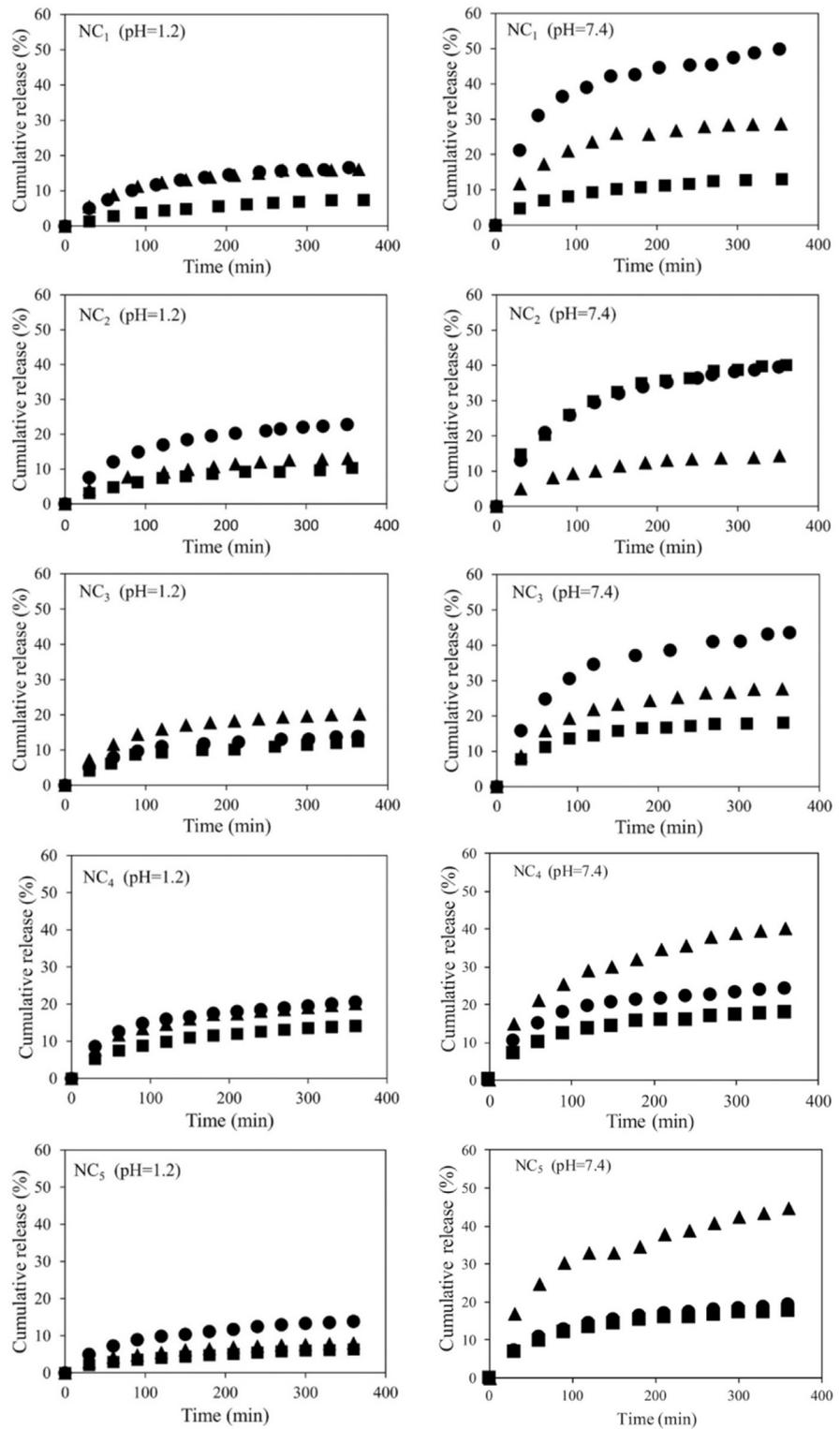
Fig. 9. Kinetics of desorption of vitamin B6 from montmorillonite samples at two simulate desorption media of gastric (pH: 1.2) and intestinal (pH: 7.4) when the adsorption pH was set to: ▲:3, ■:6.5, and ●:9.5.
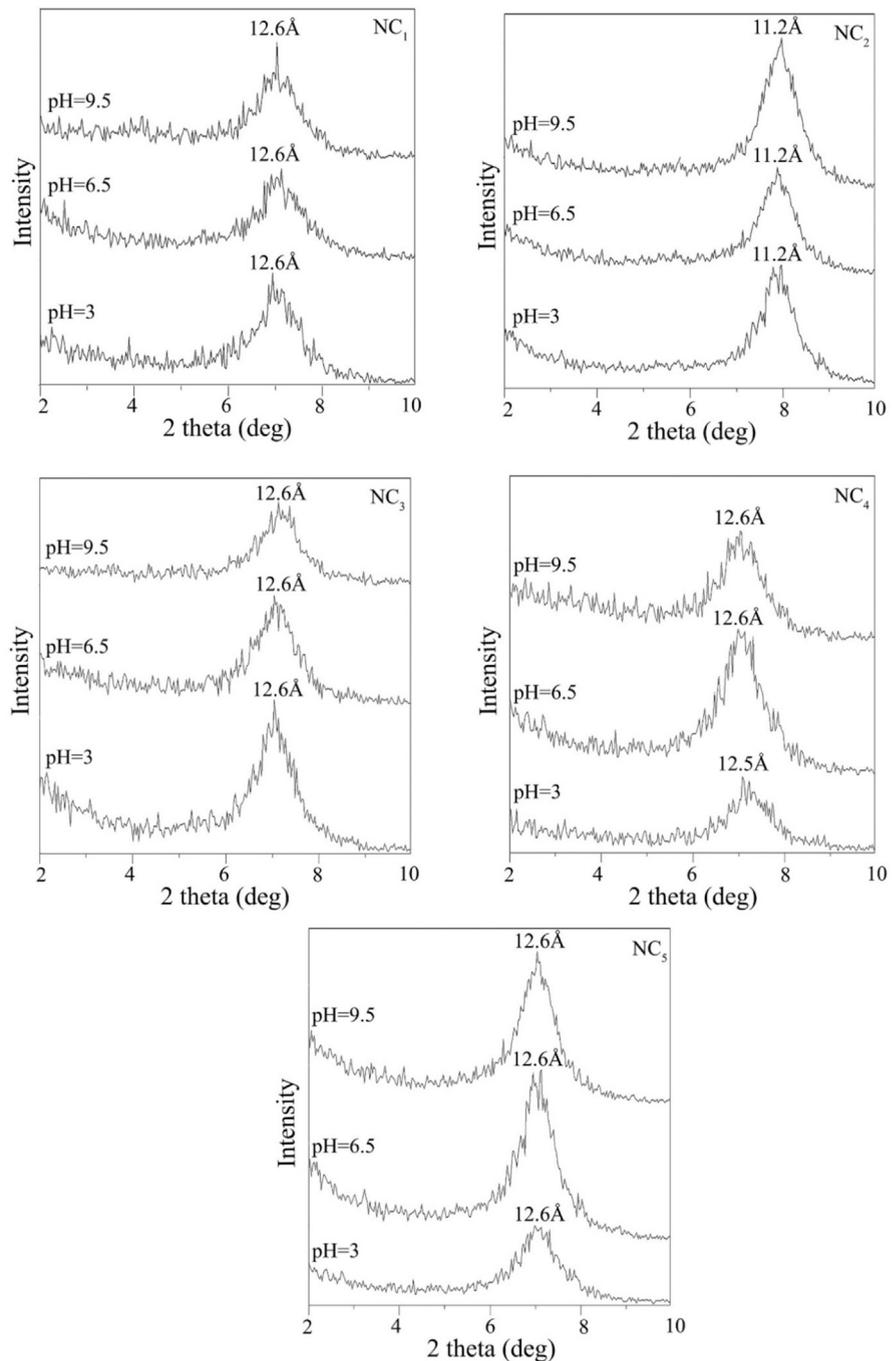
Fig. 10. XRD pattern of montmorillonite samples after desorption of vitamin B6 at pH of 1.2 when the adsorption was taken place at different pH of 3, 6.5, and 9.5.

the more percentage of the vitamin was desorbed from the interlayer spaces of the nanolayers. This is an important evidence showing a successful nano-encapsulation of the vitamin in the interlayer spaces of the montmorillonite nanolayers.
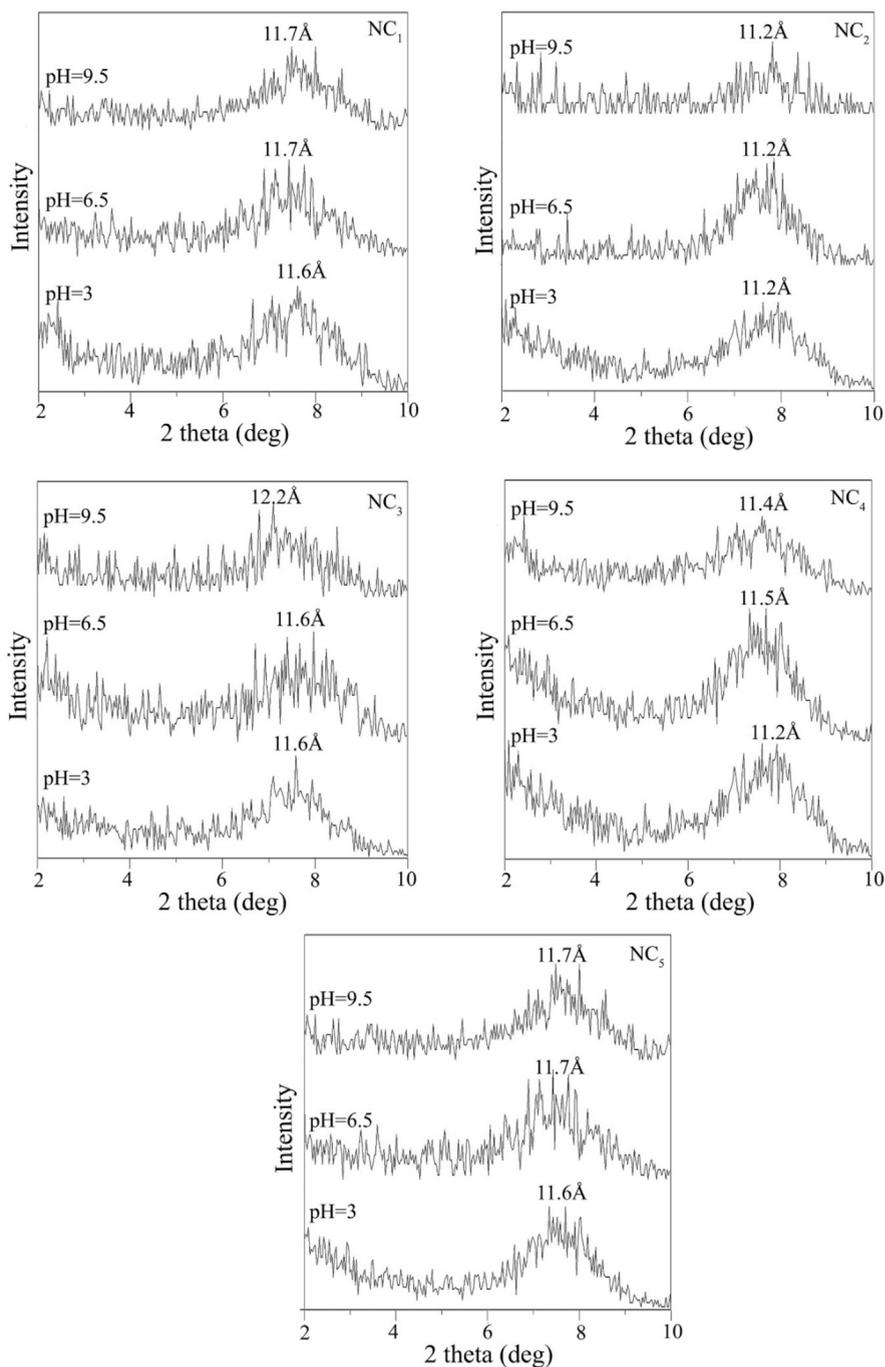
Fig. 11. XRD pattern of montmorillonite samples after desorption of vitamin B6 at pH of 7.4 when the adsorption was taken place at different pH of 3, 6.5, and 9.5.

4. Conclusions

In summary, the montmorillonite nanolayers were developed in order to encapsulate vitamin B6 in the interlayer spaces. The results showed that the nano-encapsulation

of vitamin B6 onto montmorillonite nanolayers was mainly controlled by the electrostatic attraction forces which was modified by the adsorption pH. In addition, the nano-encapsulated vitamin showed pH-responsive delivery properties. Finally, results of this study indicated that the montmorillonite nanolayers could be modified for development of nano-engineered systems for carrying of food supplements and drugs.

Declarations

Author contribution statement

Mozhgan Akbari Alavijeh, Zahra Ramazani Afarani: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Mehdi Nasiri Sarvi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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