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pH Sensitive Hydrogel Based Acrylic Acid for Controlled Drug Release

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Hydrogels, due to their unique potentials such as high-water content and hydrophilicity are of interest for the controlled release of drug molecules. The present study aims to create a controlled-release system through the preparation and characterization of hydrogels based on pH-sensitive polymers such as poly (acrylic acid). Poly (acrylic acid), p(AA), hydrogel has been synthesized by radical polymerization in solution of AA as monomer, N,N'-Methylene bis acryl amide, (MBA), as cross linking agents and ammonium persulfate (APS) as initiator and N,N,N′,N′-tetramethylmethylenediamine (TEMED) as accelerator. The investigated hydrogel was characterized by FT-IR spectroscopy and the porosity of hydrogels was determined by scanning electron microscope (SEM). The effect of pH on the swelling behavior of the hydrogel was studied in two different media (pH = 1.1 and pH = 7.4). The release percent of vitamin B12 was investigated by UV-Vis spectrophotometer. In addition, release of B12 was investigated in the presence of folic acid (B9). In the same pH, in the presence of different amounts of folic acid (B9), results showed that the samples involving the low release of vitamin B12 were arranged as follows: folic acid (25%) > folic acid (50%) > folic acid (75%).

Keywords: Acrylic acid, Hydrogel, pH sensitive, B12, B9

INTRODUCTION

Hydrogels, as water swollen polymeric networks, are formed by cross-linking reaction of hydrophilic polymer chains [1]. Since hydrogels are strongly hydrophilic, they have shown in biomedical applications such as drug delivery [2-3]. Smart hydrogels are one of the main types of hydrogels that can response selectively to the variety of external conditions such as electromagnetic field [4-5] and chemical environment [6], *etc*. [7-9]. Since different tissues and cellular compartments in the body undergo high pH variations, pH-sensitive hydrogels are widely studied to improve controlled drug delivery systems [10-12]. A superior drug delivery carrier must release the drug at the exact site, right dose and proper time [13].

Vitamin B12 is an organometallic compound containing a corrin ring and a cobalt atom in the center that plays an important role in different biochemical reactions *e.g.* reduction of disulfide groups and maintaining biological compounds such as glutathione [14]. Vitamin B12 deficiency may cause various diseases [1]. A controlled release of vitamin B12 increases its concentration in the liver and blood serum level that helps prevention or treatment of cobalt deficiency into the body. Moreover, studies have shown that keeping vitamin B12 of the body at a constant level of B12 in the body can decrease the symptoms of some diseases such as ease carpal tunnel syndrome [15-16].

Folic acid and Vitamin B12 play an important role in DNA metabolism [17]. Also they are essential for the synthesis of methionine and S-adenosyl methionine [18]. Deficiencies in folic acid and Vitamin B12, therefore, can lead to: elevated important risk factors for cancer and cardiovascular disease [19]. For these reasons, in this study, we investigate releasing vitamin B12 in the absence and presence of folic acid in different pH values.

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EXPERIMENTAL

Materials and Equipment

The monomer, AAc (99%, Sigmae Aldrich), the crosslinker, MBA (99%, Acros), the initiators, APS (99%, SigmaeAldrich) and the accelerator TEMED (98% Acros) were used in hydrogel preparation. All chemicals were used as received without further purification.

Preparation of Hydrogels

Polymeric hydrogel was synthesized from AA *via* free radical polymerization reaction techniques in mild conditions (40 °C) [20-21]. In the synthesis of p(AA) hydrogels, 5 ml AA (0.0725 mol), 0.055 g MBA (0.5%) and 10 µl TEMED were mixed with 4 ml pure water and to this solution a separately prepared APS solution of 0.165 g APS in 1 ml water (1 mol% of AA) was added and vortexed homogeneously. The mixture was placed into plastic straws (~4 mm in diameter), and these plastic straws were immersed in a 40 ºC water bath controlled by a thermostat for 4 h to complete polymerization and crosslinking. Finally, the obtained 3-D hydrogels were cut in equal shapes, washed with approximately of 2000 ml of water for 12 h. The wash water replenished every 2 h to remove unreacted species (monomer, crosslinker, accelerator and initiator). After the cleaning procedure, hydrogels were dried in an oven to a constant weight at 40 °C and kept in sealed containers for further use.

Selected FT-IR (KBr, cm^{-1}): (p(AA)): 3530 (br, strong), 2935 (m), 2865 (w), 1739 (vs), 1462 (s), 1426 (m), 1257 (m), 1172 (s), 1118 (w), 937 (w), 805 (m), 642 (w), 491 (w).

Characterization

Morphology of swollen p(AA), hydrogel was investigated with scanning electron microscopy (SEM) *via* MIRA3 FEG SEM (Tescan, Czech Republic) and an accelerating voltage of 10 keV. The sample was swollen and quickly frozen in liquid nitrogen. The hydrogel was freeze-dried at -50 ºC for 3 days to preserve their porous structure without any collapse. After that, the dried samples were deposited onto an aluminum stub and sputter-coated with gold for 60 s to enhance conductivity. FT-IR spectra of the hydrogel were taken as KBr pellets using a Bruker FT-IR spectrophotometer.

Evaluation of Swelling Behavior

Swelling properties of p(AA) hydrogel were determined by immersing known amounts of dried gel matrices, 0.003 g, within 100 ml distilled water. Swollen gels removed from water at regular intervals and dried superficially with filter paper, weighed and placed in the same bath. The measurements were continued until a constant weight was obtained for each sample. The swelling percent ratio (S%) was calculated as:

$$S\% = [(M_t - M_d)/M_d] \times 100$$

where M_t and M_d are the weights of swollen and dried hydrogels, respectively. The swelling value (S_{max}%) of hydrogels was also investigated at different pHs (1.1 and 7.4).

Drug Loaded by Equilibration

The method of soaking or equilibration was employed for vitamin B12 loading. In this method, dry hydrogel was placed in the drug solution of vitamin B12, prepared in buffer solution of pH 7.0. Then, the completely swollen hydrogel loaded with the vitamin B12 was placed in an oven at 30 °C for drying overnight.

Selected FT-IR (KBr, cm^{-1}): (Vitamin B12): 3433 (br), 3211 (w), 2929 (w), 2861 (w), 2140 (w), 1674 (vs), 1576 (m), 1556 (w), 1418 (vs), 1227 (m), 1160 (m), 1076 (w), 1000 (w), 876 (s), 715 (m), 624 (w).

Selected FT-IR (KBr, cm^{-1}): (p(AA)-B12): 3470 (br, strong), 2974 (w), 2937 (w), 2870 (w), 2684 (w), 2553 (w), 2351 (w), 2210 (w), 2030 (w), 1739 (vs), 1552 (m), 1495 (s), 1260 (s), 1176 (s), 1118 (w), 949 (w), 804 (s), 636 (m).

Release of Vitamin Drug

The completely dried hydrogel samples were soaked in 25 ml of buffered solution of pH 1.1 and 7.4. The release of vitamin B12 (or folic acid or mixture of them) from the loaded hydrogels was carried out at 37 °C in buffered solution of pH 1.1 and 7.4. Aliquot of 2 ml was withdrawn from the medium at predetermined time and analyzed using spectrophotometer at 361 nm.

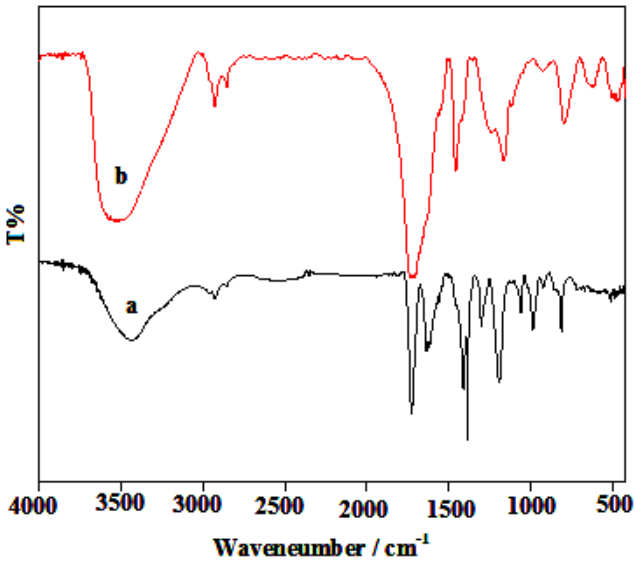


Fig. 1. FTIR spectra of a) acrylic acid, b) p(acrylic acid) hydrogel.

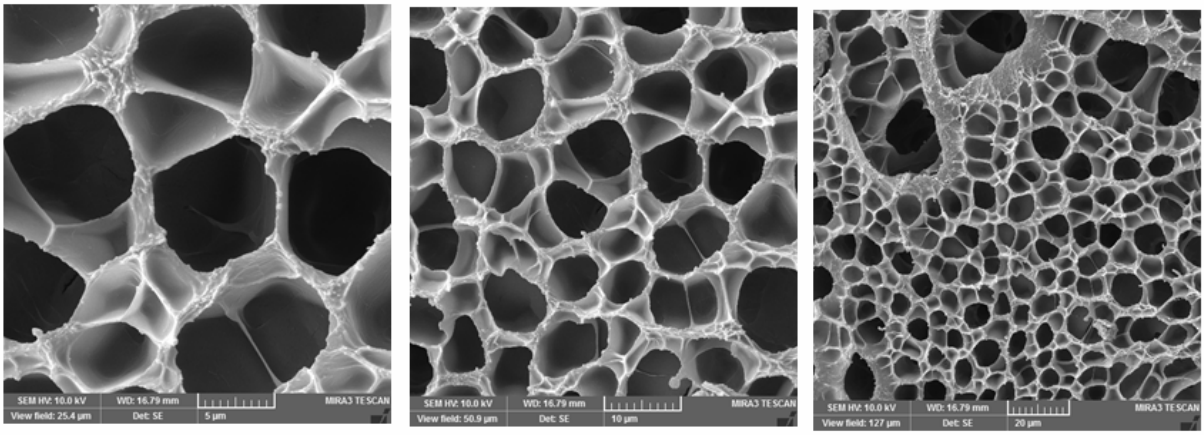


Fig. 2. SEM images of p(AA) hydrogel.

RESULTS AND DISCUSSION

Characterization

The FT-IR spectra of AA and p(AA) hydrogel are presented in Figs. 1a and b, respectively. Figure 1a indicates the O-H stretching band in 3530 cm^{-1} region and the C-H stretching at 2935 and 2865 cm^{-1} regions, respectively [22]. Also, the bands at 1739 and 1462 cm^{-1} are assigned to C=O and COO^- stretching band of acrylic acid, respectively [23]. In addition, the NH band of MBA is overlapped by OH peak at about 3200 cm^{-1} [24] so the related peak is disordered.

Scanning electron microscopy technique was used to analyze the morphology of hydrogels. The SEM image of p(AA), shown in Fig. 2, indicates the formation of homogeneous and highly porous material.

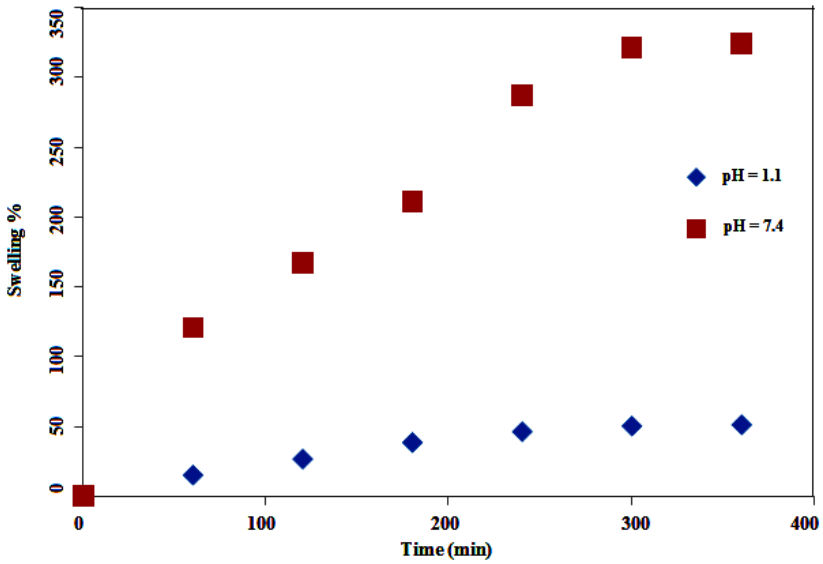
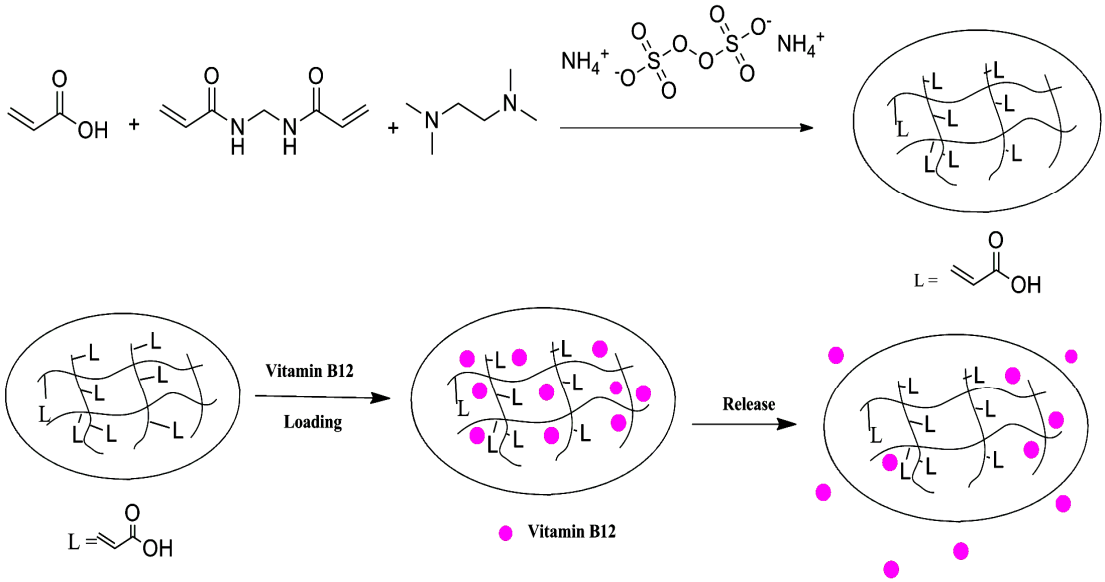


Fig. 3. The effects of pH of the medium on the swelling(S_{max}%) of p(AA).



Scheme 1. Schematic representation of drug loading and release by hydrogels

Evaluation of Swelling Behavior

The effects of pH on hydrogels swelling were investigated by insertion of known amount of hydrogels in aqueous solutions at different pHs (1.1, 7.4). Swelling percent (S%) of hydrogels were calculated by using Eq. (1)

$$S = \frac{m_t - m_0}{m_0} \quad (1)$$

Figure 3 indicated an increase in the equilibrium swelling value of the p(AA) hydrogels from approximately 51% to

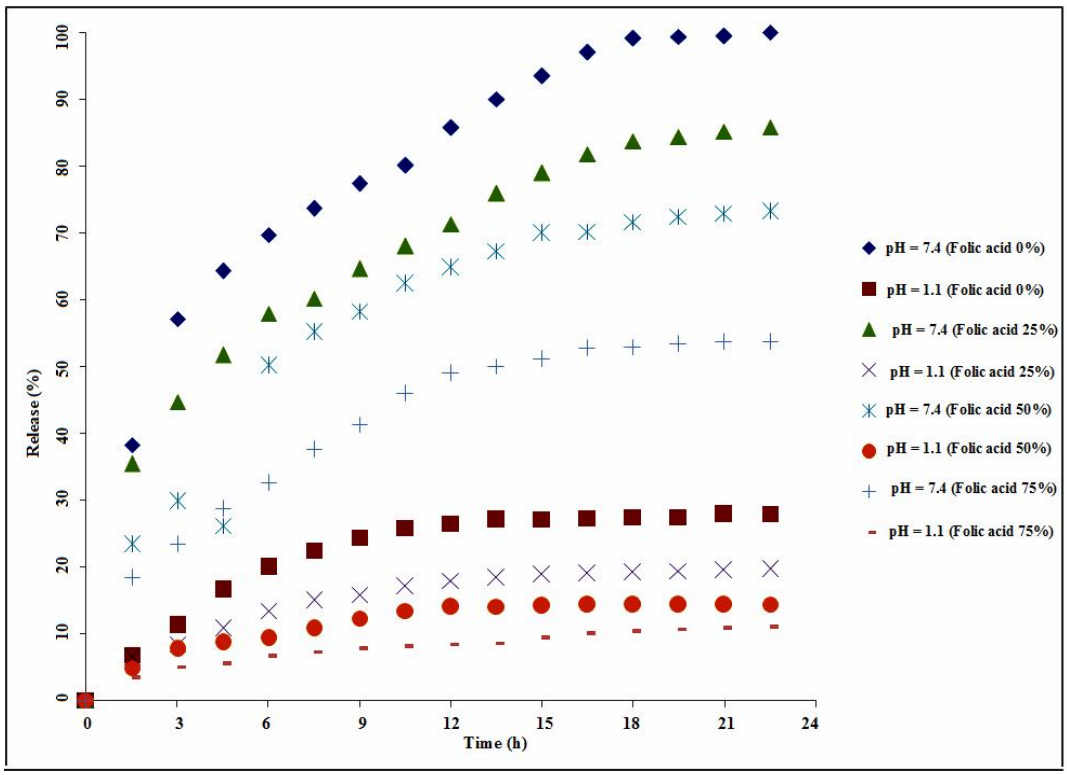


Fig. 4. Dynamic release of vitamin B12 from hydrogel as a function of time in minute at pH = 7.4 and pH = 1.1 in the presence and absent of folic acid.

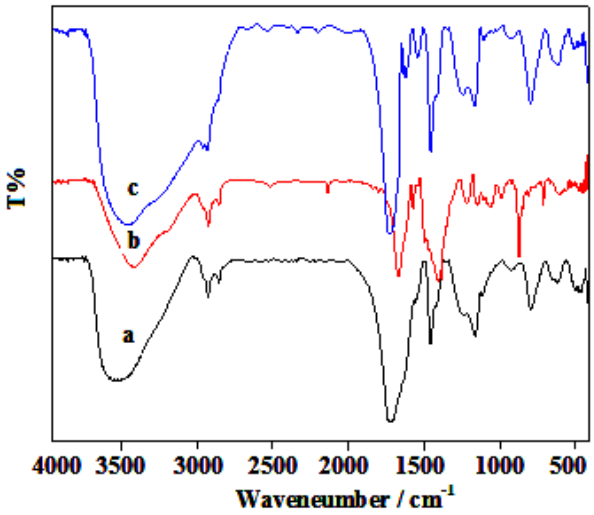


Fig. 5. FTIR spectra of a) p(AA), b) B12, c) p(AA)-B12.

323%, while the pH of swelling medium increases from 1.1 to 7.4. Due to the acidic nature of p(AA) hydrogels at low pH values (pH = 1.1) are protonated and no charge is created in the aqueous media [25]. Thus, H-bonding interactions among COOH groups within the hydrogel matrix [26] finally prevent the movements of polymeric segments and strongly prevent the solvent entry which eventually result in minimum swelling. However, in higher pH (*i.e.* pH = 7.4), charge-charge repulsion between -COO^- groups, in length of the macromolecular chains leads to the expanding of the hydrogel network and higher swelling ratios [27]. Consequently, the maximum water absorbency increases. Thus, the P(AA) hydrogel is pH-sensitive, which makes it promising material for the controlled release of drug molecules. A similar result has been reported by Zhang *et al*. [28] and Chang *et al*. [29] for the chitosan-*g*-PAA/montmorillonite hydrogel and St-*g*-PAA/SA-DS hydrogel, respectively.

Drug Release Study from p(AA) Hydrogel

The simplest method for loading of vitamin B12 in hydrogel is insertion of dried hydrogel into the saturated vitamin B12 solution [30]. Drug diffusion in to the hydrogel depends on the hydrogels porosity, drug size and chemical properties of both hydrogel and drug. When the vitamin B12 loaded hydrogel is placed in vivo, the drug will then freely diffuse from hydrogel matrix into the neighboring tissue. (As shown in Scheme 1)

The graph illustrates a rapid decrease in concentration of the vitamin B12 over time before reaching a plateau. It seems that a large amount of vitamin B12 is released when it contacts with the release medium that is referred to “burst release”. The initial fast release of vitamin B12 mostly takes place through dissolution and diffusion of the drug entrapped near to the surface or at the surface of hydrogels [31]. The second vitamin B12 release, which is slower compared to the first one, involves the diffusion of the drug loaded within the inner part of the polymer matrix through the water channels of the pores [32].

Since the hydrogel system reported here has a fair pH-dependent swelling, Fig. 4, it is anticipated that it shows its minimum and maximum release in acidic and neutral media, respectively. To verify this hypothesis, the dynamic release of hydrogel was considered in pH 1.1 and 7.4. The results (Fig. 4) clearly confirm that the hydrogel system releases the maximum amount of vitamin B12 in pH 7.4 and the minimum amount of the drug in pH 1.1.

Moreover, our results exhibit an initial burst release in the first hour of the process for both media (pH 1.1 and 7.4) that followed by an almost constant release of vitamin B12 from the hydrogels for 24 h. Similar release behavior was observed in performing the pH-dependent release of vitamin B12 from P(MPEG-PLA-co-IA-MEGMA) hydrogels, [33] and from carboxymethylcellulose-gpoly(acrylic acid)/ OMMT nanocomposite hydrogel [34].

These studies have indicated that the release behavior has an apparent pH-responsiveness and is increased as the pH of the release medium is increased.

The observed behavior should be due to electrostatic repulsion among the similarly charged -COO^- groups produced from different sources. The main sources could be ionization of -COOH groups in the medium of pH 7.4, macromolecular chains inside the drug-loaded hydrogel that undergo extensive chain relaxation and a significant extensive swelling facilitates migration of vitamin B12 out of the hydrogel with subsequent drug release. Similar phenomenon has also been reported in the literature [34-35, 14].

Nevertheless, in the medium pH 1.1, the H-bonded compact hydrogel structure restricts the movement of polymeric segments within the gel, thus resulting in the minimum release [36].

Folic acid, is an effective target-specific ligand for tumor cells [37]. To take advantage of specificity of folic acid, various polymers including chitosan [38], have been conjugated with folic acid and used as carriers for anti-cancer drugs. In this work, the vital role of vitamin B12 and B9 (folic acid), such as interdependence of vitamin B12 and folic acid on methylation reaction as well as in other metabolic processes [39], and the importance of these two materials together, motivated us to study the effect of folic acid on B12 release in different concentrations at pH = 1.1 and 7.4. According to the results, with increasing the folic acid ratio to B12, Fig. 4, the releasing of Vitamin B12 is decreased, due to intermolecular interaction, such as hydrogen bonding, between functional groups of folic acid and Vitamin B12.

FT-IR Spectrum Analysis of Vitamin B12 Loaded Hydrogel

The interactions between B12 and polymer matrix was studied using FT-IR spectroscopy to confirm the vitamin B12 loading.

The characteristic peaks of vitamin B12 were observed at 1547 cm^{-1}, 1572 cm^{-1} and 2130 cm^{-1} are assigned to the breathing modes of the corrin ring and cyanide stretching frequency, respectively [36,40]. In addition, the bands at 1674 cm^{-1} and 2140 cm^{-1}, (Fig. 5), are referred to the amide C=O stretching mode of the propionamide side chains of the corrin ring and the cyanide stretching frequency in cyanocorrinoids of B12, respectively [36].

In p(AA)-B12, due to the hydrogen bonding of the carboxylic groups, the frequency of amide band is shifted to lower frequency at about 1636 cm^{-1}. The shift of amide stretching band to lower frequencies can be ascribed to the hydrogen bonding of the carboxylic groups. Moreover, upon binding of vitamin B12 to the hydrogel matrix, the cyanide band was observed at 2030 cm^{-1} as a consequence of the weakening of cobalt-cyanide bond (Fig. 5). New bands near 1739 cm^{-1} were observed in the spectra of B12 bound in the hydrogels (Fig. 5). These bands are attributed to the carboxylic acid groups surrounding B12 [41].

CONCLUSIONS

In summary, a pH-responsive hydrogel based on acrylic acid was synthesized by radical polymerization. This hydrogel, showing an excellent pH-dependent swelling behavior, was employed to enhance the drug-release ability.

It displays a minimum swelling in pH 1.1 and maximum in pH 7.4. The release of vitamin B12 was studied by the traditional dissolution test and demonstrated low release in the acidic media of pH 1.1 as similarly observed in the stomach and showed a high release in the medium of pH 7.4. In addition, in the same pH, in the presence of different amount of folic acid (B9), results indicated that the release rate of vitamin B12 is in the following order: folic acid (25%) > folic acid (50%) > folic acid (75%).

ACKNOWLEDGEMENTS

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