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Synergistic Effect of Different Amounts of Selenomethionine and Encapsulation of Vitamin E and Its Influence in Controlling Vitamin E Release

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ABSTRACT

The series homologues of tocopherols and tocotrienols that make up vitamin E vary in terms of methyl group location and number of bonds. The body uses vitamin E for several purposes. Unfortunately, vitamin E is unstable and will degrade quickly in water, absorb less readily and have a poor level of bioavailability when exposed to heat, light or air. Selenomethionine, the typical essential trace element form of selenium (Se) produced from animal products, has therefore been added to this study. Freeze-dried technique was used to prepare encapsulation of vitamin E with different amounts of selenomethionine. The effect of different amount of selenomethionine on physicochemical properties and vitamin E release were investigated. The results reveal that when the amount of selenomethionine injected increases, the solubility of vitamin E increases, but the influence on particle size decreases. As a result, the particle dispersion followed the same patterns. After integrating selenomethionine, the encapsulation's zeta potential remains stable. The results of FT-IR and TEM research corroborate this. After adding different amounts of selenomethionine to the surface particles, the surface of the encapsulation was smooth with periferous structure, indicating that the air bubble created after freeze-drying process was impacted by the addition of selenomethionine to the surface particles. Furthermore, the release analysis reveals that the formulation with 0.03 % selenomethionine enhances vitamin E release in simulated intestinal fluid (SIF) better than in simulated gastric fluid (SGF) model, which is consistent with a prior study. It was concluded that adding varying amounts of selenomethionine varied the encapsulation and vitamin E release characteristics. In conclusion, the formulation with 0.03 % selenomethionine is consistent in terms of encapsulation stability and release performance.

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1. Introduction

Vitamin E is made up of several homologues of tocopherols and tocotrienols (α , β , δ and λ) that differ in bonding and methyl group locations [1-4]. Vitamin E plays a role in the lipid phase of food preservation and as a dietary supplement for health benefits [1,5]. Vitamin E inhibits cholesterol formation, protects against oxidative stress and is resistant to UV-induced damage (immunology) and certain malignancies, according to its physiological and pharmacological activities [5-8]. When not esterified, it has the capacity to break the creation of harmful free radicals by transferring phenolic H and electrons [1,9]. Vitamin E, on the other hand, is susceptible to oxygen, heat and light, which causes it to degrade faster, have a lower solubility in water and have limited absorption and bioavailability [1,5,10]. Selenomethionine, the most common essential trace element form of selenium (Se) obtained from animal sources, was used to make an attempt [11-14]. Selenomethionine has a higher water solubility, is easier to absorb and is highly accessible in the human body [15]. Selenomethionine, like selenium, requires patience through the usage of proteins, polyphenols, polysaccharides, melatonin, ATP and monosaccharides [16].

Furthermore, maltodextrin is a carbohydrate produced from starch that has long been utilized in the food sector to preserve and protect volatile chemicals. Maltodextrin can also be used to stabilize emulsions. Maltodextrins are only detected after partially hydrolyzing cornflour with acids or enzymes, and they have the potential to form a matrix that is necessary for wall material formation [17]. Aside from that, due to its odour, colour and tastelessness, maltodextrin is the ideal alternative for the major ingredient currently [18]. Protein-based materials, on the other hand, such as sodium caseinate, are often utilized in emulsions because of their capacity to act as an emulsifying agent. When the colloidal calcium phosphate (CCD) is removed from natural casein, individual casein proteins 1, 2 and 3 are produced. To boost their water solubility, caseins were transformed to sodium caseinate [17].

The use of encapsulation techniques in the pharmaceutical and food industries has recently gained widespread attention. Small particles of core materials are placed inside a wall material to form capsules in this technique [19]. Bioactive substances (polyphenols, micronutrients, enzymes, antioxidants and nutraceuticals) were encapsulated to prevent them from interacting with harsh environments and to control their release at specific locations. Encapsulation strategies were shown to modify physicochemical parameters such as particle size, size distribution, surface area, shape, solubility, efficiency and release mechanisms in general. It may also have an impact on product delivery [20].

In the meantime, the drying method was chosen to preserve or reduce the amount of food items required. The freeze-dried method was employed to encapsulate vitamin E in this investigation. According to Farias *et al.*, [1], freeze-drying has the ability to enhance the shelf life of items. This is owing to the fact that the freeze-drying process was carried out without the use of air and at a lower temperature. The lack of air in the processing will minimize product degradation owing to reduced oxygen interaction (lower oxidation), which will lessen chemical modification while keeping the product intact with a restricted amount of free oxygen throughout preparation. Lower temperatures, on the other hand, will aid in the preservation of the product's structure, texture, appearance and/or flavour. High temperatures will cause products to lose their function, especially for bioactive substances that are sensitive to heat, such as vitamin E. Other drying procedures, such as spray drying, may necessitate air at a high temperature (170-190 °C), causing initial oxidation of the oil and reducing the yield of the powdered product as a result of product deposition in considerable amounts on the outlet pipe and chamber wall during processing [21]. As a result, it may require a significant

number of samples during preparation, which will presumably affect the cost and efficiency of the entire process.

The effect of varying amounts of selenomethionine added was investigated in this study. Our prior study's condition for preparing encapsulation was changed, and a full analysis was also provided. The freeze-drying process was used to encapsulate vitamin E with maltodextrin, sodium caseinate and various quantities of selenomethionine. The reference was the control, which was done without the addition of selenomethionine. Each formulation was tested for water solubility, particle size, size distribution and zeta potential. Meanwhile, changes in their distribution, structure and morphology were observed using Fourier Transmission Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM) and Variable Pressure Scanning Electron Microscopy (VPSEM). A release study of vitamin E in a simulated solution model was also carried out to see how the addition of selenomethionine affected vitamin E delivery.

2. Methodology

2.1 Materials and Reagents

Sigma-Aldrich and Acros, respectively, provided maltodextrin (DE 13 - 17) and sodium caseinate. Calbiochem in the United States provided the L-selenomethionine. A local firm provided the vitamin E. (Super Vitamins Sdn. Bhd, Malaysia). The liquid vitamin E obtained was taken from local palm oil and contains 22.9 % tocopherol and 77.1 % tocotrienols, according to the report. Other compounds were utilised without further purification because they were analytical grade.

2.2 Preparation of Vitamin E Encapsulation

In a nutshell, the sample preparation conditions for encapsulated vitamin E were slightly altered [22]. In a mixture of 27.83 % maltodextrin, 9.28 % sodium caseinate and distilled water, an equivalent amount of vitamin E was added. S1, S3, S6, S9 and S12 were created by adding different amounts of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %) to the final combination. Selenomethionine was not used in the preparation of the control sample. To homogenize the mixture, each formulation was gently stirred using a hotplate (IKA® WERKE C-MAG HS 7, Germany). The final product was left to emulsify for 6 mins. To shorten freeze-drying time, the emulsified formulation was frozen for roughly 24 h. To generate a dried sample, the solidified mixture was freeze-dried for 5 h at -41°C, 4 x 10⁻⁴ mbar in a freeze-dryer (ALPHA 1 - 2 LD plus, CHRIST, Germany). The freeze-drying technique was used to create all six encapsulations. Emulsified samples were frozen first before evaporation in this method. Sublimation was applied to remove the water from the sample. The evaporation process then begin. The resulting moisture evaporated during this process, and the product naturally was encapsulated and form the pore on the surface particles. This porous structure was held in place by the solid network, leaving a void on the surface particles. The porous shape is thought to be caused by voids left by ice crystals or air bubbles during the freezing process [1]. The dried samples were gathered and ground into a powder. This powder sample was kept at -20 °C in zip-plastic in the dark sample vial until it was tested on the following day.

2.3 Characterization of Vitamin E Encapsulation

2.3.1 Water solubility of vitamin E encapsulation

The solubility of vitamin E encapsulation in aqueous systems was measured using a modified version of [22-24]. The first 40 mg of sample powder was dissolved in distilled water with an

equivalent amount of 0.4 % w/v and gently swirled on a hotplate (IKA ® WERKE C-MAG HS 7, Germany) at 750 rpm until all solid samples were totally dissolved. According to the reference study, the final mixture is soluble if the average time for solubilization is less than 5 mins. The time taken for solubilization was measured in minutes in this investigation.

2.3.2 Particle size, size distribution and zeta potential

The first powder samples were combined after being dissolved in distilled water. To eliminate numerous scattering effects from distribution, the final mixture was sonicated for 10 mins. Following separation, an equal amount of 1mL of sample was pipetted out and put into a vial for particle size, distribution and stability characterization with the Malvern Zetasizer Nano-ZSP (UK). Mean diameter size, polydispersity index (Pdl) and zeta potential were found as results. The particle dispersion was determined using the Pdl, whereas the zeta potential value shows the stability of encapsulation in the solvent. All zetasizer conditions were reported to be at 25°C with a refractive index of 1.33. A small Pdl value suggests a smaller size distribution, whereas a large Pdl value indicates a bigger distribution. The zero number denotes a lower Pdl value, while 1 denotes a higher Pdl value [25]. Unwanted particles or non-formed materials were removed from a solution by the sonication process and settle to the bottom of the flask. The top suspension was discharged and the zeta analyzer was applied to measure the sample.

2.3.3 FT-IR, TEM and VPSEM observation

The first powder sample (with and without extra selenomethionine) was placed on the scanner with potassium bromide (KBr) and the final powder was scanned from the range of 4000 - 400 cm⁻¹, respectively, for Fourier Transmission Infrared Spectroscopy (FT-IR) analysis (PerkinElmer). The resultant FT-IR spectra were recorded and grafted. Swiping with wet tissue soaked in methanol gently removed the used sample from the scanner [26].

A High Resolution-Transmission Electron Microscope 120 Kv was used to conduct the TEM study (HR-TEM 120Kv, HT7700 Hitachi, Japan). In distilled water, an equal amount of 5 mg powder sample (with and without selenomethionine) was dissolved. To remove undesirable particles, the mixture was sonicated for 15 mins. An equal volume of 10 uL liquid sample was pipetted out of the final combination and dried on the grid. HR-TEM accelerated at 80 Kv was used to observe the encapsulation image [26].

In a raster scan pattern, Variable Pressure Scanning Electron Microscopy (VPSEM, HITACHI, S-3400N, Japan) scans the sample surface with variable pressure (- 10 to 3000 Pa) and a higher-energy electron beam than regular SEM. The usage of electron emitters increased the output of tungsten filament by 10 - 20000 times. The detectors will generate the specimen's image [27]. About 5 mg of powder sample was carefully placed on the carbon tape, with great care used to avoid any sample surface damage. Before applying the gold coating, the tape was applied on the iron. The gold was thoroughly coated for about 20 mins before the samples were placed on the detector to observe morphology [26].

2.4 Release Study of Vitamin E in Model Simulated Gastrointestinal Tract (GIT)

Different pH values were used to create a simulated GIT model, including 1.2 for the stomach and 7.4 for the intestines, respectively. With minor modifications, the approach employed in this investigation was based on [22,28].

Gastric stage: By dissolving an equal amount of 0.2 % NaCl in 36.5 % HCl, simulated gastric fluid (SGF) was created. HCl was used to modify the pH of the resultant solution to 1.2. In a 25 mL simulated fluids model, the initial 150 mg of encapsulation powder was applied. The samples were shaken at 37 °C with a total speed of 100 rpm in a shaker incubator. The samples will be collected and separated using a centrifuge (MED. Instrument Centrifuge MPW-352) at 2500 rpm for 15 mins for each time interval (30 mins). The discharged sample was replaced with the same amount of simulated fluid. At 285 nm, the samples were analyzed using a UV-Vis spectrophotometer (Jenway, UK).

Small intestine stage: 0.6 g of KH_2PO_4 and 3.5 g of K_2HPO_4 were homogenized to create simulated intestinal fluid. To achieve a pH of 7.4, the solution was adjusted using NaOH. For the small intestine stage, the same procedure was used as for the gastric stage. No digestive enzymes were used in any of the simulations. The following Eq. (1) was used to compute vitamin E release:

$$\text{Cumulative release} = \text{Mt}/\text{Ma} \times 100 \quad (1)$$

Where, Mt denotes the quantity of vitamin E released from encapsulation into simulated solution (SGF and SIF) over time, t, and Ma denotes the amount of vitamin E initially encapsulated in the encapsulation. Before dissolving in simulated solution, the amount of vitamin E encapsulation (Ma) was determined by treating it with 100 % ethanol and reading it using a spectrophotometer (SGF and SIF). After then, a standard curve was created. The proportion of vitamin E may be calculated using the equation.

2.4.1 Kinetic model determination

The graph for determining kinetic release as log cumulative percentage drug release vs log time was plotted using Korsmeyer models [29]. The Eq. (2) is as follows:

$$\log \text{Mt}/\text{M}^\infty = k (\log t)^n \quad (2)$$

Where, $\log \text{Mt}/\text{M}^\infty$ stands for log cumulative percentage drug release, and $\log t$ stands for log time. The release rate constant is denoted by "k," and the release exponent is denoted by "n." The 'n' value was used to characterize the release mechanism, with $n = 0.5$ indicating Fickian diffusion, $0.45 < n < 0.89$ indicating Non-Fickian drug transport, $n = 0.89$ indicating Case II (relacational) drug transport and 'n' greater than 0.89 indicating Super case II transport [29].

Higuchi's model was stated as cumulative percentage drug release versus square root of time, with the Eq. (3) as follows:

$$f_t = Q = kH (\sqrt{t}) \quad (3)$$

Where, " \sqrt{t} " is the square root of time and "kH" is the Higuchi dissolution constant.

$Q_t = k_0t$ for the graph of cumulative amount of drug released vs time and $\log C = \log C_0 - kt/2.303$ for the graph of log cumulative percentage of drug remaining versus time are the release kinetics equations for the zero and first order models, respectively [29].

2.5 Statistical Analysis

Each sample had at least three repetitions of each experiment, and the results were presented as an average and standard deviation. Duncan's multiple range test (DMRT) with a significance level of $R = 0.05$ was used to calculate the significant difference for each associated finding using ANOVA SPSS v16.

3. Results and discussion

3.1 Water Solubility for Vitamin E Encapsulation

Water solubility test is the saturation concentration of a solute dissolved in water [22,30]. The results for water solubility in this study were summarized in Table 1. The average time for each formulation to solubilize was less than 5 mins. It agrees with the findings of a study from [22-24], which showed that the microcapsules were totally solubilized if the solubilization period was less than 5 mins. In comparison to the control sample, the selenomethionine-added sample had a greater solubility. It can be seen in Table 1. The solubility of vitamin E was considerably affected ($p < 0.05$) when varied quantities of selenomethionine were applied. In comparison to the others, sample S12 was significantly soluble in water. This is due to the high amount of selenomethionine supplied, which raises the solute saturation and lowers the hydrophobicity of vitamin E in water [31]. However, changing the sample preparation condition, on the other hand, had no effect on the solubility of vitamin E in water.

Table 1

The characteristic of control and other five formulations

Sample	Amount of selenomethionine (%)	Water solubility (min) ¹	Particle size (μm) ²	Polydispersity index (Pdl) ³	Zeta potential (mV) ⁴
Control	-	1.36 ± 0.04^f	0.23 ± 0.01^a	0.45 ± 0.12^a	-58.0 ± 2.89^a
S1	0.01	1.02 ± 0.01^e	0.53 ± 0.42^a	0.64 ± 0.17^{abc}	-56.4 ± 3.96^{ab}
S3	0.03	0.56 ± 0.02^d	0.30 ± 0.03^a	0.52 ± 0.13^{ab}	-39.3 ± 1.49^d
S6	0.06	0.47 ± 0.01^c	0.87 ± 0.64^a	0.88 ± 0.21^c	-50.8 ± 1.40^c
S9	0.09	0.34 ± 0.02^b	0.56 ± 0.19^a	0.80 ± 0.11^{bc}	-53.0 ± 1.40^{bc}
S12	0.12	0.24 ± 0.03^a	0.68 ± 0.12^a	0.82 ± 0.21^{bc}	-57.1 ± 1.93^{ab}

Note: 1^{abcde}Means with different superscripts within a column were significantly different ($p < 0.05$, $n=3$). 2^aMeans with same superscripts within a column were not significantly different [$p > 0.05$, $n=3$]. 3^{abc}Means with different superscripts within a column were significantly different [$p < 0.05$, $n=3$]. 4^{abcd}Means with different superscripts within a column were significantly different [$p < 0.05$, $n=3$]. S1-S12 indicate the sample tested with the different amount of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %, respectively)

3.2 Particle Size, Polydispersity Index (Pdl) and Zeta (ξ) Potential

Table 1 presents the findings of the zetasizer analysis obtained from this study. From the table, the average particle size for all formulations was less than 1.0 m, with sample S3 having the smallest particle size among the other samples (S1, S6, S9 and S12). In comparison to other formulations, sample S3 has a sufficient amount of selenomethionine covering the core material in encapsulation. When the droplets formed smaller, only a small portion of the surface will be covered. However, Donsi *et al.*, [32] found that the encapsulation was prone to coalescence to create the larger particle until all the surfaces were entirely covered. It is possible that this is connected to other samples (S1, S6, S9 and S12). The addition of different amounts of selenomethionine, on the other hand, had a less significant ($p > 0.05$) effect on the size obtained in this study. Particle size reduction would aid in

improving the dissolving rate of core material and increasing the drug's bioavailability in the body [33]. The surface-to-volume ratio of a product can be increased by reducing particle size to the nano level. As a result, the material activity is changed as mechanical, electrical and optical properties change [20].

In the case of particle dispersion, Table 1 shows that sample S3 distributes the best, followed by other formulations (S1, S9, S12 and S6). It has similar pattern to the results in particle size. Nonetheless, the addition of different amounts of selenomethionine had a substantial ($p < 0.05$) effect on particle distribution in this study. The study concludes that particle dispersion influenced the self-ability of microcapsules to dissolve in water, resulting in improved sample dissolution in the body. It agrees with Holowka and Bhatia's work [34], which classified Pdl ranges into three categories. The first category was below 1.0, which resulted in complete molecular weight uniformity, while the second category was between 1.0 and 1.5, which represented statistically uniform polymers, and the third category was over 2.0. This category includes polymers that are statistically dispersed and have complex chemistry in relation to human physiology. The results in Table 1 fall into the first category, indicating that the particle in the solution is totally uniform.

In term of zeta analyser, formulation S3 has a lower zeta potential value as compared to other formulations (S6, S9, S1 and S12). All formulations contain a moderately negative charge (-50 to -58mV), except for formulation S3, which is less than -50mV (Table 1). The formulation was stabilized by steric repulsion (negative-negative charge between wall materials) rather than electrostatic repulsion, most likely due to deprotonation of the ammonium group (NH_3) at pH values higher than their pKa values, which explains the ability to form thick hydrophilic layers around the droplets [2]. In contrast, protonation of carboxylic groups on the polysaccharide resulted in a reduction in negative charge in formulation S3 ($-\text{COO}^-$). However, it was hypothesized in this study that the lower charge value for formulation S3 (below -50mV) was due to their small particle size (0.30 ± 0.03^a) and distribution (0.52 ± 0.13^{ab}). As a result, this investigation discovered that differing quantities of selenomethionine have a substantial ($p < 0.05$) effect on the formulation's electrical charge.

3.3 Fourier Transmission Infrared Spectroscopy (FT-IR)

The chemical structure [16] of vitamin E encapsulation was determined using FT-IR. Figure 1 depicts the results obtained from this study. In general, it has been seen that some wavelengths have vanished and others have shifted forwards or backwards. Other wavelengths remained constant across all formulations. Stretching vibrations of a single bond, either C-C, C-N, or $-\text{CH}_2-$ of four or more methylene groups from encapsulation, are represented by the peak from $603 - 1016 \text{ cm}^{-1}$, whereas stretching vibrations of the C-O bond are represented by the peak from $1078 - 1149 \text{ cm}^{-1}$. The bending vibration of $-\text{OH}$, which contains the phenolic hydroxyl group from encapsulation, can be detected at the peak $1244 - 1498 \text{ cm}^{-1}$, while the bending vibration of $-\text{NH}$, which contains the benzene ring, can be found at the peak $1514 - 1641 \text{ cm}^{-1}$. Stretching vibration of double or triple bond of C=O from encapsulation is responsible for the peak between 1720 and 2112 cm^{-1} , whereas stretching vibration of $-\text{CH}$ of methyl group and C-C bond is responsible for the peak between 2924 and 2926 cm^{-1} . The $-\text{OH}$ and $-\text{NH}$ stretching vibrations of intermolecular hydrogen bonds from encapsulation may be observed at the peak $3280 - 3564 \text{ cm}^{-1}$, respectively [5,16]. From these findings, vitamin E was successfully encapsulated in the encapsulants and remained stable after being mixed with various concentrations of selenomethionine. The structure demonstrates that selenomethionine was efficiently combined with other encapsulants, and that the addition of selenomethionine had no effect on the stability of the encapsulant's chemical properties, preserving the encapsulation's chemical qualities.

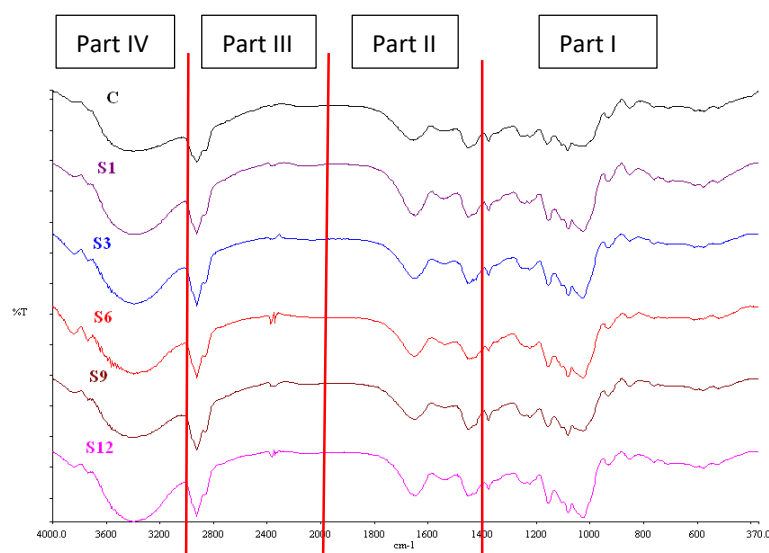
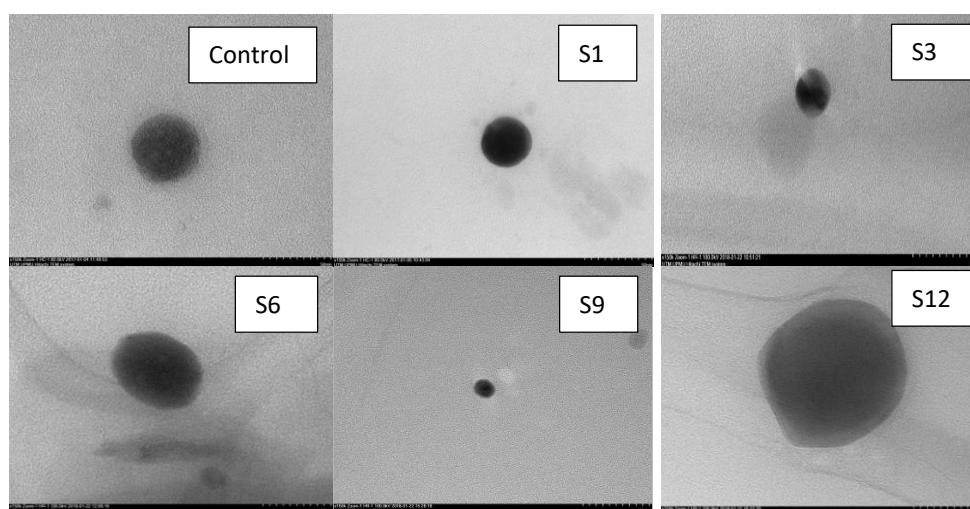


Fig. 1. FTIR spectra for encapsulation of vitamin E

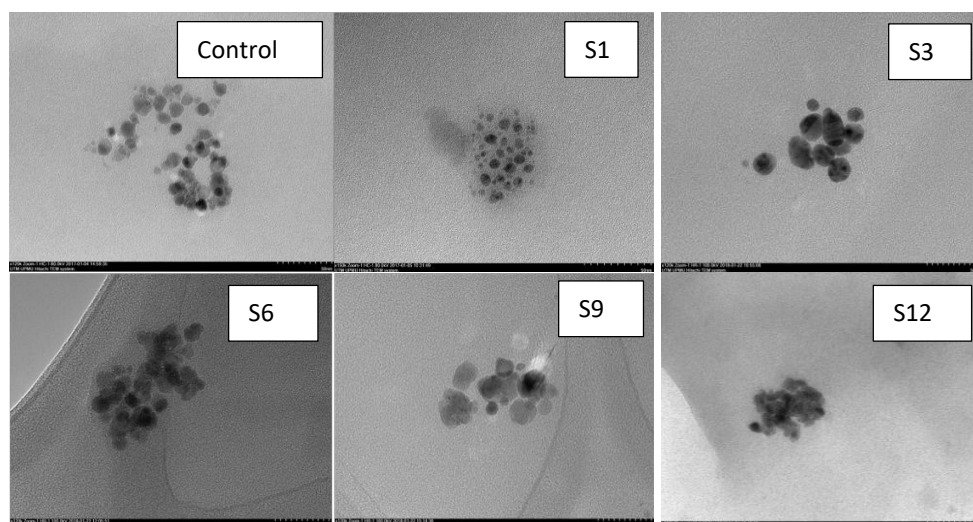
Note: S1-S12 indicate the sample tested with the different amount of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %, respectively), meanwhile, 'C' represents the control sample

3.4 Transmission Electron Microscopy (TEM)

The distribution of vitamin E in the capsule was studied using TEM [16]. The image obtained from TEM examination is shown in Figure 2. When compared to the control and other formulations, it can be shown that formulation S12 was entirely aggregated. However, when compared to the others, formulation S3 appeared to be the most spherical. This is due to the significant amount of selenomethionine incorporated in the formulation, which results in a high wall material strength against pH solvent, resulting in very good distribution when compared to the others (instead of their size, distribution and charge characteristic). In comparison to other formulations, the thick layer of wall material obtained for formulation S3 can be confirmed from Figure 2. According to a study [5], the image of vitamin E encapsulation revealed that the capsule with more layers has radiopaque qualities as a result of the thick layer of the capsule. Because the TEM instrument's radiation may not be able to pass through the wall material, the image will be darker than the shell capsule.



A. Single image (Magnification 150 k, 50 nm)



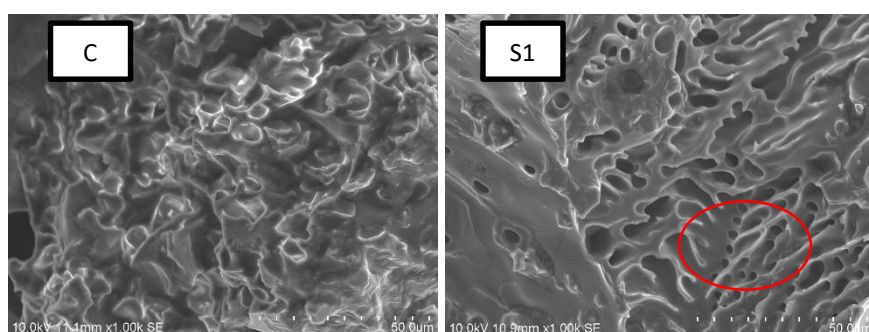
B. Overall (Magnification 120 k, 50 nm)

Fig. 2. TEM image for encapsulation of vitamin E with and without selenomethionine

Note: S1-S12 indicate the sample tested with the different amount of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %, respectively)

3.5 Variable Pressure Scanning Electron Microscopy (VPSEM)

VPSEM images revealed the image of surface morphology for vitamin E encapsulation (Figure 3). For all formulations, the magnification was set to 1000 x and the image was resolved at a diameter of 50 m. The working distance (WD) to spot the image was similarly different, ranging from 10.9 - 11.9 mm. The image for surface morphology for the control sample was uneven, reasonably smooth and fragile, whereas the formulation with selenomethionine has a poriferous structure, as seen in Figure 3. Farias *et al.*, [1] observed the creation of pores caused by cavities left by the ice crystals or air bubbles during the freeze-drying process. The number of pores on the surface rose and became smaller from control to formulation S12, as seen in the image (as the amount of selenomethionine increases). This is due to the addition of various amounts of selenomethionine, which will cover some surface area, reducing the space generation for ice crystals or air bubbles, resulting in pore size reduction and an increase in pore number. The saturation solubility and dissolving rate of a medication can be improved by reducing particle size and pore size, which increases the drug's bioavailability in the body [33]. Because of changes in mechanical, electrical and optical properties, reducing particle size increases the surface-to-volume ratio, which increases reactivity [20]. As a result, it may aid in increasing vitamin E levels in the body. Furthermore, their morphology was critical in determining release patterns since it controlled vitamin E release and distribution from encapsulation to the GIT.



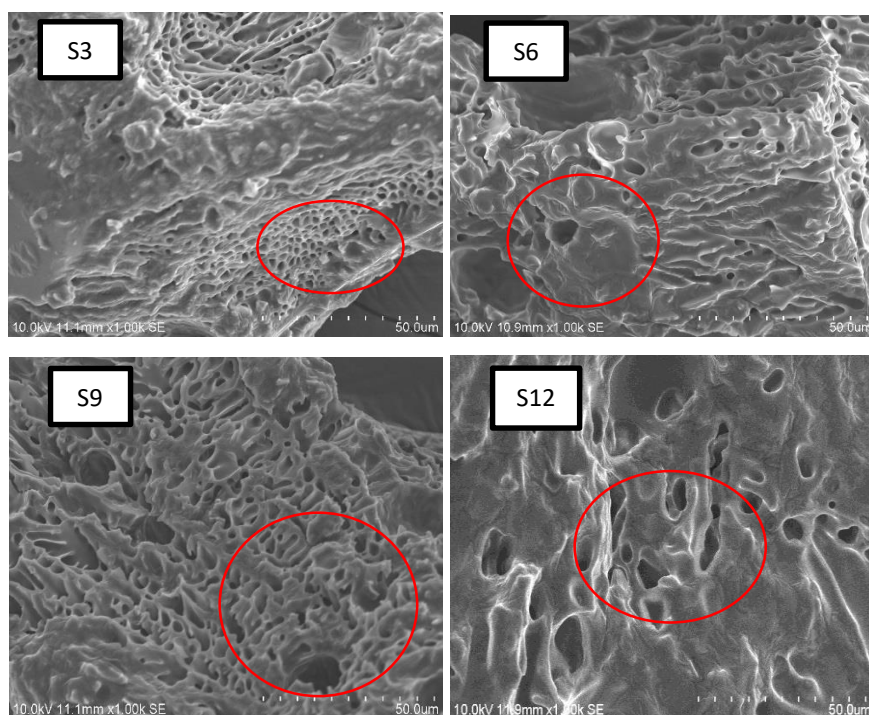


Fig. 3. VPSEM image for encapsulation of vitamin E

Note: S1-S12 indicate the sample tested with the different amount of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %, respectively), meanwhile, 'C' represents the control sample, the circle is referred to void formation and the dark color from the void is vitamin E

3.6 Release Study

3.6.1 In simulated gastric fluid (SGF) at pH 1.2

Figure 4 shows the percentage of vitamin E released into SGF models at pH 1.2. After being exposed to an acidic solution for 3 h, approximately 0 - 68 % of vitamin E was released from the capsule. After 90 mins in the acidic solution, all formulations showed an increase in vitamin E release. After 120 mins in solution, the release of vitamin E was gradually reduced for samples S9 (58 %) and S12 (51 %) while other formulations remained increased after 180 mins in SGF solution, with the exception of sample S3, which declined to 50 % at $t = 180$ mins. The encapsulation's resistance to acidic solution is demonstrated by the reduced vitamin E release. The decrease in drug dissolution rate is most likely owing to the stability of chemical bonding between organic groups and the oxide network, which leads to structural flexibility due to altered chemical reactivity and a reduction in cross-linking [35]. Furthermore, past research has suggested that the pH solution may affect the release performance of bioactive substances. The protonation of the carboxyl group to generate -COOH occurred in an acidic pH solution. More steric repulsive energy was produced in this situation [36]. As a result, more vitamin E is released into the solution. According to El-Rahman and Al-Jameel [33], lowering particle size helps to improve the dissolution rate of core material, which appears to boost the drug's bioavailability in the body. Instead of having a moderate outcome that was generally acceptable compared to others, sample S3 looked to have a decent release in SGF solution because their attributes were better than others. In SGF solution, the greatest rate of vitamin E release for sample S3 is just 52 percent every 150 mins. According to the study [28], the goal for multifunctional nanocapsules for drug delivery systems is for the encapsulants to be able to keep the core materials

resistant to strong acidic conditions while preserving their effectiveness against acid-damaged components.

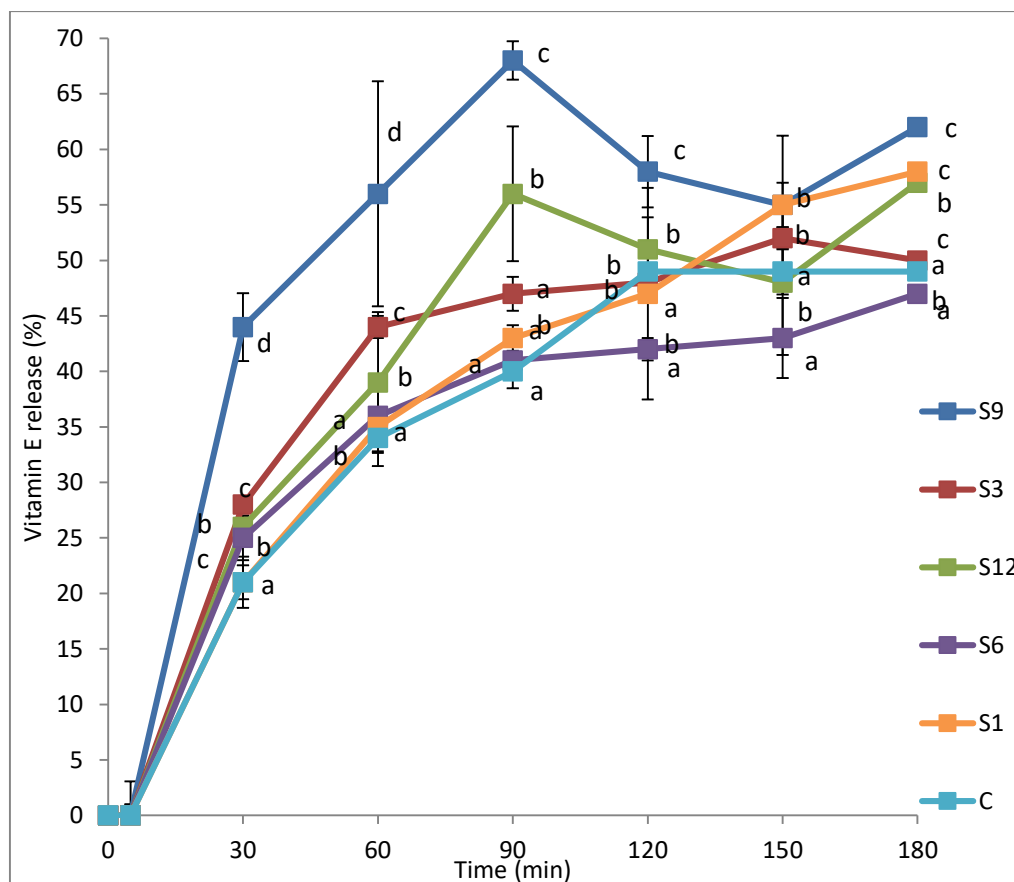


Fig. 4. Vitamin E release in simulated gastric fluid (SGF) at pH 1.2

Note: S1-S12 indicate the sample tested with the different amount of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %, respectively), meanwhile, 'C' represents the control sample. ^{a,b,c,d}_p > 0.05, not significant

The chemical reaction of release's mechanism is proposed. The carboxyl group (COO^-) from maltodextrin, sodium caseinate and selenomethionine protonated hydrogen ion (H^+) from solution to create carboxylic acid weak acids (COOH). Hydrogen ions (H^+) were used to simulate the acidic state of this study. Other groups and atoms on the back chain of hydrocarbons, such as carbon (C), ammonium (NH_3) and hydrogen (H), could not react to hydrogen ion (H^+) from acidic solution because there was no unpaired electron or free electrophile that hydrogen ion (H^+) could attack to create a stable hydrogen bond. The functional group connected to the back chain alters the chemical characteristics of hydrocarbons, causing them to become polar molecules that dissolve in water. The carboxyl group (R-COO^-) was shown to be the functional group for weak acids in this study, and this group will combine with free hydrogen ions (H^+) from acidic solution to generate a full carboxylic acid (COOH) [37] (Figure 5).

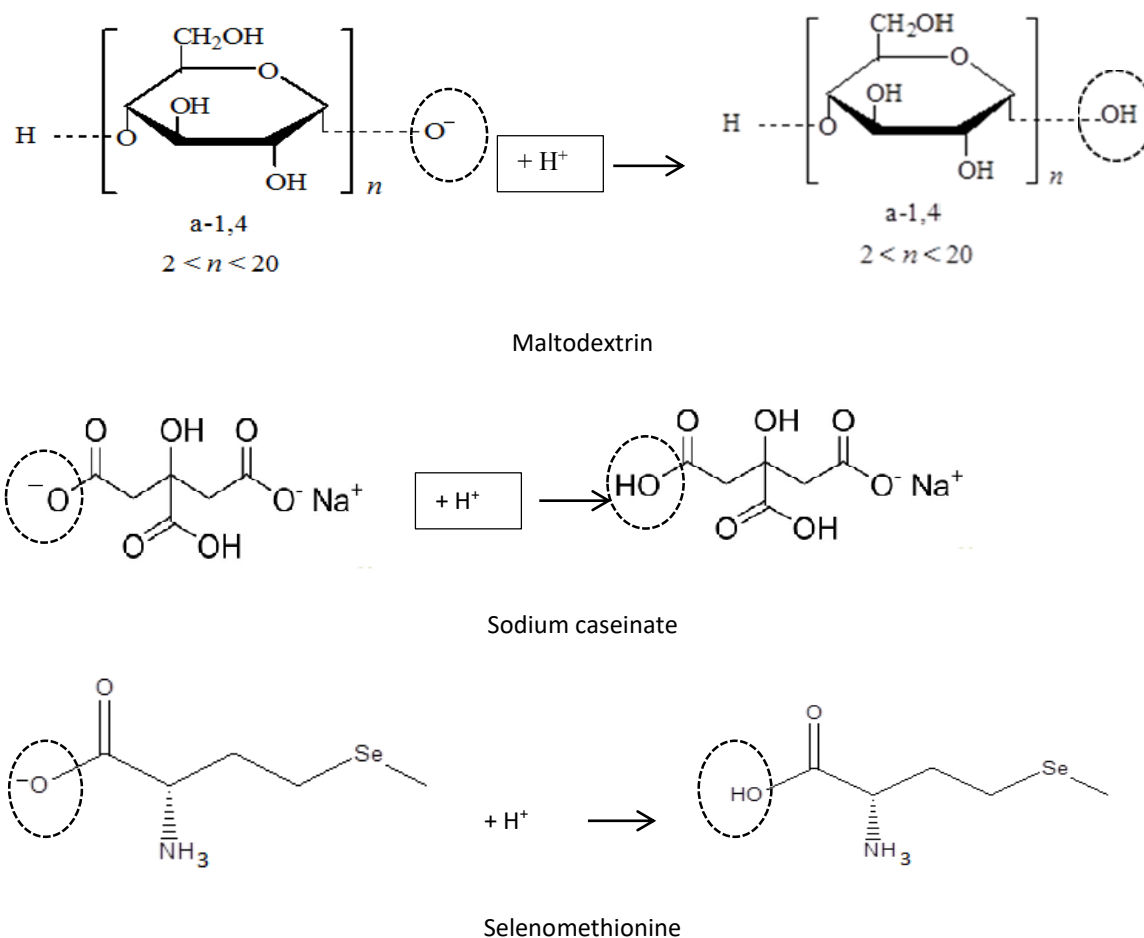


Fig. 5. The conversation of molecular form of materials inside stomach

The data collected from in vitro release is applied to kinetic testing to determine the order of the drug transport mechanism in a kinetic analysis. Table 2 summarizes the outcomes collected. Table 2 shows that the plot for Peppa's model is fairly linear in comparison to others. This is supported by their regression coefficient, which is close to one. The obtained slope ('n') is in the range of $0.45 < n = 0.89$, indicating that the medication was released by a Non-Fickian transport (anomalous) mechanism [38].

3.6.2 In simulated intestinal fluid (SIF) at pH 7.4

After dissolving each encapsulation powder in SIF at pH 7.4, the profile of vitamin E release from each encapsulation powder is shown in Figure 6. This data shows that the proportion of vitamin E released in alkaline solution is higher than in acidic solution, as the release ranges from 88 - 99 % after 30 mins. Aside from that, after 3 h in alkaline solution, the release of all formulations gradually decreased. After 5 mins in solution, sample S3 released 62 % of more vitamin E than sample S12 (56 %), sample S6 (48 %), sample S1 (44 %) and sample S9 (42 %). After 30 mins in the solution, the release of vitamin E was gradually increased for all formulations. Except for formulation S9, which increased significantly at $t = 180$ mins, but vitamin E release from all formulations gradually reduced after 180 mins in the solution. After 180 mins in an alkaline solution, the encapsulation becomes unstable. According to Fennema [36], if partial hydrolysis of peptide bonds occurs, the formulation may clump

together. Acosta [39] investigated the relationship between particle size, bioavailability and solubility of bioactive substances in intestinal fluid and discovered that tiny particle size boosted absorption, bioavailability and solubility considerably.

Table 2

Kinetic model for vitamin E release in SGF and SIF

Sample	SGF					SIF				
	Peppas kinetic data		Zero order kinetic data	First order kinetic data	Higuchi order kinetic data	Peppas kinetic data		Zero order kinetic data	First order kinetic data	Higuchi order kinetic data
	Regression coefficient (r)	Slope 'n'	Regression coefficient (r)	Regression coefficient (r)	Regression coefficient (r)	Regression coefficient (r)	Slope 'n'	Regression coefficient (r)	Regression coefficient (r)	Regression coefficient (r)
C	0.9805	0.7766	0.8552	0.8962	0.9892	0.9892	-	0.9525	0.8895	0.9892
							0.1390			
S1	0.9880	0.7957	0.9155	0.9667	0.9332	0.9332	-	0.9883	0.9150	0.9855
							0.1577			
S3	0.9540	0.7823	0.7439	0.7902	0.9458	0.9458	-	0.9941	0.9460	0.9944
							0.1432			
S6	0.9597	0.7561	0.7824	0.8320	0.9853	0.9853	-	0.9559	0.8195	0.9899
							0.1430			
S9	0.9194	0.8140	0.6007	0.6034	0.8495	0.8495	-	0.7781	0.7230	0.8379
							0.1405			
S12	0.9645	0.7954	0.7746	0.8000	0.9697	0.9697	-	0.9687	0.8580	0.9914
							0.1614			

Note: S1-S12 indicate the sample tested with the different amount of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %, respectively), meanwhile 'C' represents control sample

Furthermore, earlier research has shown that at alkaline pH, the NH_3 group is deprotonated to generate NH_2 , resulting in increased steric repulsion. As a result, the protein molecule swells and unfolds [36]. Aside from that, it was linked to the charge interaction on the surface, as well as the number of pores and their size. As a result, the vitamin E capsule will progressively dissolve into the SIF solution. However, an ANOVA test found that varying concentrations of selenomethionine had a less significant ($p > 0.05$) effect on vitamin E release in alkaline solution, particularly in the initial release (5 mins), but have a substantial effect on release after 5 mins in solution. To the best of the authors' knowledge, no research has been done on the interaction of vitamin E release and increasing the amount of selenomethionine added, but the study expects that other factors, such as the ratio

of core material to wall, particle size, distribution, charge, encapsulation efficiency (EE) and pore structure, will influence vitamin E release.

According to Figure 7, the ammonium group (NH_3) from the structure selenomethionine will deprotonate hydrogen ion ($-\text{H}^+$) from the structure to generate a weak base of ammonium group in alkaline solution (NH_2). The alkaline condition of the human small intestine was represented by the hydroxyl group (OH^-). Maltodextrin and sodium caseinate, for example, will not react with hydroxyl groups (OH^-) from alkaline solution because their charges do not interact (no positively charged structure to be attacked by negatively charged hydroxyl group (OH^-) [37]. As a result, both structures were given the label "no reaction," meaning that the molecular structure and the hydroxyl group (OH^-) from alkaline solution have no interaction.

According to Table 2, all plots for all models are found to be fairly linear. The study discovered that vitamin E release in SIF was better matched to the Higuchi model than other models based on R^2 values. Their R^2 is similarly close to one. Despite the fact that the R^2 value for the peppas model is nearly same, the 'n' slope for this model is extremely low. It demonstrates that the process of vitamin E release does not correspond to Peppas's model. The Higuchi model was widely used for modified release of pharmacological dosage forms with pores structure, according to [29]. As a result, the Higuchi order model is the best fit for vitamin E release %.

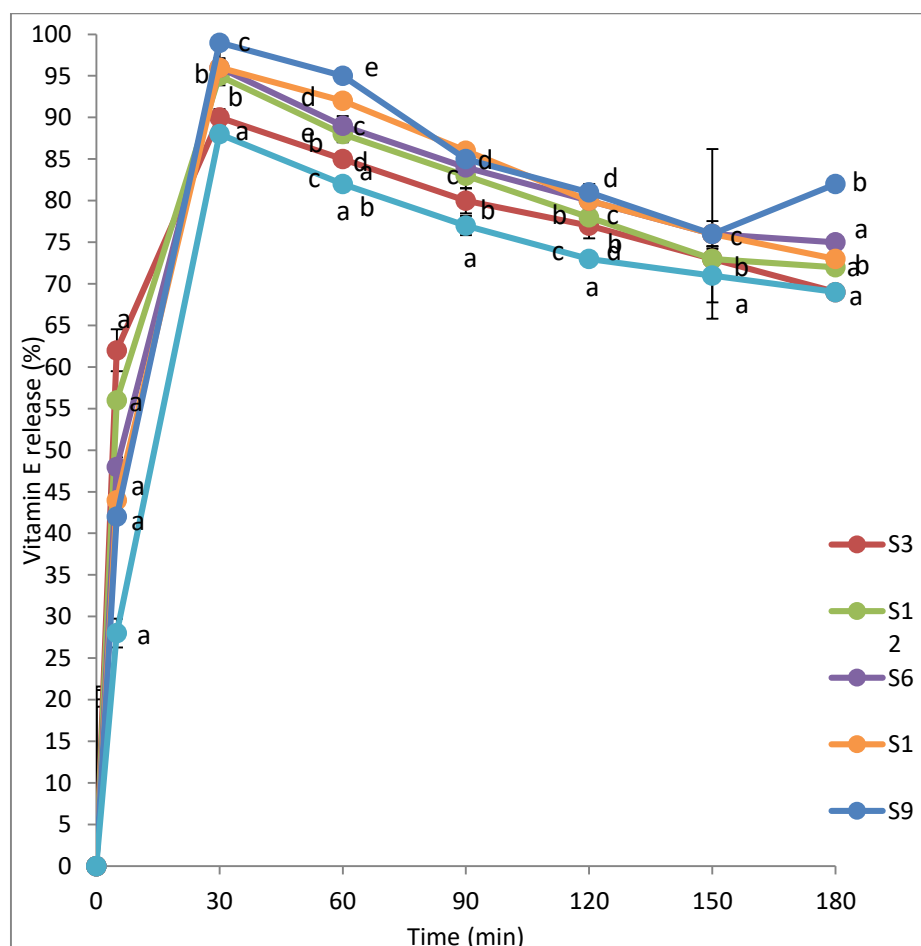


Fig. 6. Vitamin E release in simulated intestinal fluid (SIF) at pH 7.4

Note: S1-S12 indicate the sample tested with the different amount of selenomethionine (0.01, 0.03, 0.06, 0.09 and 0.12 %, respectively), meanwhile, 'C' represents the control sample. Means with same superscripts within a column were not significantly different ($p > 0.05$, $n=3$)

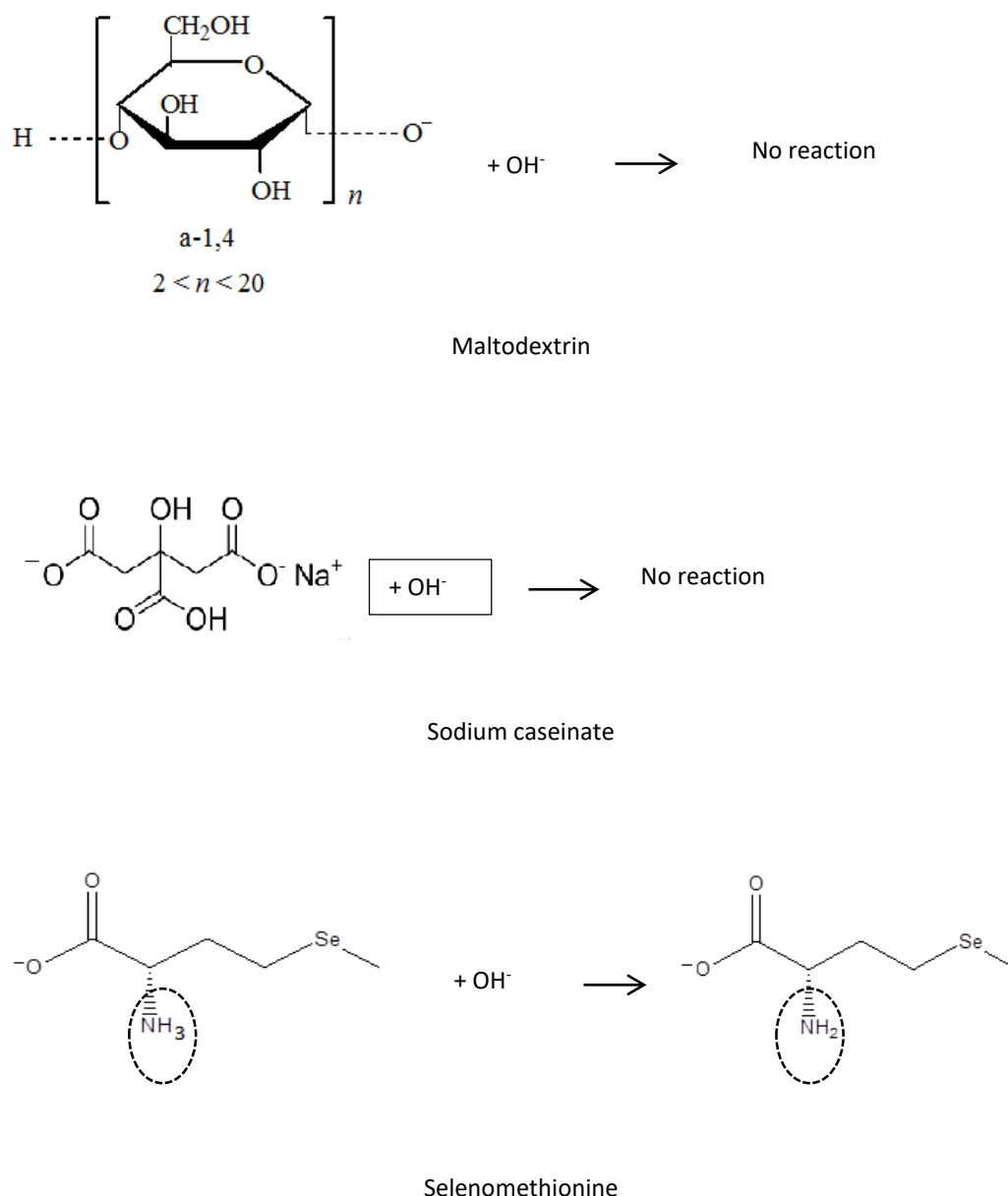


Fig. 7. The conversation of molecular form of materials inside small intestine

4. Conclusions

Using the freeze-drying process, vitamin E was successfully encapsulated in various quantities of selenomethionine. Vitamin E solubility in aqueous systems has been improved. The amount of selenomethionine in the encapsulation had no effect on the size of the particles produced. The amount of selenomethionine impacting the encapsulation was confirmed by the distribution and zeta potential value, however the encapsulation was steady. The addition of selenomethionine was able to maintain the stability of encapsulation, according to FT-IR spectra, while TEM images showed the encapsulation was stable against aggregation after varied amounts of selenomethionine were added. VPSEM images on the other hand, revealed a smooth, fragile morphological surface with a periferous structure that will aid in the release of vitamin E into the solution model. The release of vitamin E was improved after adding 0.03 % selenomethionine, according to a release study. According to the findings, encapsulating vitamin E with 0.03 % selenomethionine increased vitamin E delivery in

simulated intestinal fluid, but decreased release in the simulated gastric fluid model. It demonstrates that adding 0.03 % selenomethionine to a mild alkaline diet will help with vitamin E release while also protecting against acidic circumstances.

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