Controlled release of vitamin U from microencapsulated brassica oleracea l. var. capitata extract for peptic ulcer treatment

Controlled Release of Vitamin U from Microencapsulated Brassica oleracea L. var. capitata Extract for Peptic Ulcer Treatment

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Abstract

Cabbage plant (Brassica oleracea L. var. capitata) contains compounds such as polyphenols, minerals, and ascorbic acid, as well as some amino acids such as glutamine, which has anti-inflammatory properties. In addition, its nutrient contains the component of vitamin U (S-methylmethionine) which is effective in the treatment and prevention of peptic ulcer disease. The aim of this study is to perform microencapsulation of Brassica oleracea L. var. capitata extract for controlled release of vitamin U for peptic ulcer treatment. Within this scope, vitamin U and some amino acids (L-methionine, L-glutamine, L-histidine, L-lysine, L-aspartic acid) were extracted from a cabbage by extraction methods and microencapsulated. The gelatin/gum Arabic and gelatin/sodium alginate polymer complexes were used as wall materials. Morphological analysis of the microcapsules showed that the microcapsules had a homogeneous, spherical shell structure. The results of HPLC analysis confirmed that vitamin U and amino acid compounds in cabbage extract are also present in the structure of microcapsules. $FTIR$ analysis confirmed the interaction between shell materials and microcapsules, and the similarities in the bands of the plant extract and microcapsules indicated microencapsulation of the plant extract successfully. In vitro release testing of the microcapsules was studied in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 7.4) for 48 h. The maximum encapsulation efficiency and release were obtained as 86.92% and 93.6% for the gum Arabic-contained microcapsule, respectively.

Keywords: Cabbage plant extract, vitamin U, microencapsulation, gelatin, gum Arabic, gelatin, sodium alginate, controlled release

Introduction

With the increase of consumers who are conscious about health, the tendency to use functional foods that provide physiological effects in the body, reduce the risk of formation of certain diseases, and have protective and therapeutic properties has also increased. Research on this subject has shown that functional foods are meeting basic nutritional needs and are effective in regulating metabolic functions and preventing and treating some diseases such as peptic ulcer with their bioactive components.

Peptic ulcer is known as a serious disease among gastrointestinal diseases. Many medicinal drugs are used for the treatment of peptic ulcer disease (**Buener et al., 2013**; **Harsha et al., 2017**). However, there is an increasing interest in plants with medicinal value as alternative treatments. Cabbage plant is an important plant in terms of nutrition due to its bioactive compounds. Cabbage (Brassica oleracea L. var. capitata) is an herbaceous and leafy plant belonging to the Brassicaceae family, which is among the important food products in terms of nutritional sources. Compounds such as polyphenols, minerals, and ascorbic acid are widely found in the cabbage plant and have beneficial and curative effects on human health. (**Carvalho et al., 2011; Lee et al., 2018; Nosek et al., 2011; Rokayya et al., 2014; Samec et al., 2017; Cvetkovic et al., 2019; Poschner et al., 2019**). The healing and therapeutic properties of the cabbage plant are due to the fact that it contains a large number of active compounds such as flavonoids, glucosinolates, vitamins, and carotenoids. It also has an important place in pharmacology due to its antibacterial, anti-inflammatory, antidiabetic, and anti-oxidative bioactivities (**Pongmalai et al., 2017; Cvetkovic et al., 2019; Gruszecki et al., 2022**).

In addition, studies have found that peptic ulcer disease is caused by the lack of a nutritional factor of vitamin U (**Carvalho et al., 2011**). Vitamin U, which is considered a functional food as a natural amino acid, is a substance that is chemically known as S-methylmethionine (DL-methionine methyl sulfonium chloride) and belongs to the group of physiologically active compounds. Vitamin U is a good source of anti-ulcer factors, as well as has pharmacological properties such as anti-inflammatory and analgesic (**Kim, 2003**). The potential of vitamin U extracted from cabbage for anti-ulcer therapies has been studied by various researchers (**Carvalho et al., 2011; Cheney, 1949; Hadda et al., 2014; Kim, 2003; Oguwike et al., 2013**). Cheney carried out experimental studies to reveal the curative effect of cabbage juice containing vitamin U on peptic ulcer treatment on thirteen patients (**Cheney, 1949**). Carvalho et al. did research to evaluate the effect of Brassica oleracea var. capitata aqueous extract on stomach ulcers of Wistar rats. In their results, they presented that cabbage juice has a therapeutic effect in the treatment of stomach ulcers (**Carvalho et al., 2011**). These beneficial compounds found in plants are sensitive to environmental and biological conditions and can show decomposition reactions easily. The microencapsulation technique is often preferred as an alternative solution to protect these active ingredients from environmental conditions (**Eghbal & Choudhary, 2009; McClements & Lesmes, 2009**). Microencapsulation is the technique of encapsulation within the wall material to protect the core material against adverse environmental factors such as moisture, oxygen, and light, thus preserving the properties of the bioactive components in foods and promoting the controlled release of the active compound in the microcapsule under desired conditions (**Bhagya Raj & Dash, 2022; Calderón-Oliver & Ponce-Alquicira, 2022; Carpentier et al., 2022; Bamidele & Emmambux, 2021; Da Silva Soares et al., 2019; Nazzaro et al., 2012; Rajam & Anandharamakrishnan, 2015**). Microcapsules consist of two parts, the core (active) component and the shell or wall (coating) material. The core material contains the active ingredient, while the shell material covers and protects the core material.

Microencapsulation process was carried out (i) to protect sensitive substances from external environmental conditions; (ii) to mask organoleptic properties of substances such as color, taste, and odor; (iii) to provide controlled release of an active (active) substance; (iv) safe use of toxic substances; (v) to ensure the continuous, targeted release of the drug (active) substance; (vi) to protect the active ingredients from enzymatic degradation in the gastrointestinal tract; and (vii) to prevent the adverse effects of the drug such as gastric irritation (**Jyothi et al., 2010; Choudhury et al., 2021; Parente et al., 2022; Calderón-Oliver & Ponce-Alquicira, 2022**).

The wall material of microcapsules has a significant influence on the microencapsulation process and affects the functional properties of the active substance (**Bastos et al., 2018; Muhoza et al., 2020; Ribeiro et al., 2020**). In general, the wall materials used in the microencapsulation technique are natural, biodegradable polymers such as gelatin, gum Arabic, chitosan, maltodextrin, starch, carboxymethyl cellulose, and paraffin (**Choudhury et al., 2021; Jayanuddin et al., 2016**). Another important situation is the choice of microencapsulation methods. The physical and chemical properties of the core and wall material are important in the selection of microencapsulation methods. Among the microencapsulation techniques, the complex coacervation method is often preferred due to its various advantages such as high encapsulation efficiency, and low concentration of materials, and can be applied to many biopolymeric materials. Complex coacervation is basically explained as the interaction of two biopolymers with opposite charges, reducing solubility and phase separation, leading to complex formation. Complex coacervation occurs mainly through electrostatic interactions between oppositely charged polymer solutions, and from this interaction two liquid phases are formed: the polymer-poor continuous phase and the coacervate dense phase, which is used to coat the polymer-rich active (core) components (**Archut et al., 2022; Ferreira & Nicoletti, 2021**). This interaction depends on the polymer concentration, polymer properties, core material, changes in pH and temperature (**Ma et al., 2009; Mancer et al., 2018; Mendanha et al., 2009; Rutz et al., 2017; Bolson Moro et al., 2021**).

When the studies were examined, S-methylmethionine (vitamin U) and different amino acids were determined in various types of tea such as green tea, black tea and, Chinese cabbage, red cabbage extracts (**Hong & Kim, 2006; Kim, 2003; Ohtsuki et al., 1987**) However, the determination of the active substance in the white cabbage plant extract and the microencapsulation studies of the plant extract containing S-methylmethionine were not found.

The aim of this study is to prepare the extracts of the dried and raw leaves of the green cabbage plant by Soxhlet and decoction extraction methods without losing their properties, and successful microencapsulation of the plant extract. The presence of vitamin U (S-methylmethionine) and amino acids in the extracts was determined by HPLC-amino acid analysis. Then, the extracts were microencapsulated with gelatin/gum Arabic and gelatin/sodium alginate polymer complexes to optimize their release kinetics. The release behavior of the microcapsules was examined in simulated gastric juice (pH 1.2) and simulated intestinal fluid (pH 7.4) conditions.

Materials and Methods

Materials

Cabbage plant (Brassica oleracea L. var. capitata) was purchased from a local grocery. Gelatin type A and gum Arabic (GA) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Sodium alginate (SA) was purchased from Sigma-Aldrich Co. (Merck Ltd., China). Glutaraldehyde (25%) was obtained from Penta (Prague, Czech Republic). Acetic acid glacial and hydrochloric acid were purchased from

VWR International (Pražská, Czech Republic). DL-Methionine methylsulfonium chloride (S-methylmethionine, vitamin U) was obtained from TCI (Tokyo Chemical Industry, Japan).). L-methionine (≥ 98%), L-glutamine (≥ 99%), L-histidine (≥ 99%), L-lysine (≥ 98%), L-aspartic acid (≥ 98%), glucorapha-nin (> 95%) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Phosphate-buffered saline was obtained from Serana (Pessin, Germany). All the reagents were analytical grade.

Extraction Process

The Soxhlet and decoction extraction (aqueous extraction) methods were used in the preparation of the extracts with minor modifications (**Kim, 2003; Oliveira et al., 2008; Pereira et al., 2019**). Prior to the extraction process, the same amount of cabbage leaves were prepared in raw (50 g) and dried (50 g) forms to compare its extraction efficiency. For the Soxhlet extraction, ethanol/deionized water (2:1) mixture was used as a solvent and the total volume of the solvent mixture was 200 mL. The leaves and the solvent were separately added into the Soxhlet apparatus and the system was heated until it got boiled. The extraction process time was kept constant for 3 h. After extraction was complete, the solvent was removed with a rotary vacuum evaporator. The experiment was done three times. Extracts were stored at 4 °C for analysis and microencapsulation.

In the decoction extraction, 50 g of raw and 50 g of dried cabbage leaves were boiled in the same volume of deionized water (200 mL) in separate beakers at 100 °C for 30 min. It was then cooled and filtered through Whatman No.1 filter paper. Both extract samples were stored at 4 °C for analysis and microencapsulation. The same amount of plant forms and the same volumes of solvents were used in both extraction processes.

Preparation of Microcapsules

The microencapsulation studies of cabbage leaf extract were performed according to the complex coacervation method with a minor modification (**Correa-Filho et al., 2019; Devi et al., 2012; Koksal & Gode, 2017**). Two different polymer mixtures were prepared as wall materials. The first polymer mixture was prepared using gelatin/sodium alginate (3.5:1) and the second polymer mixture was prepared using gela-tin/gum Arabic (1:1). The optimized weight ratios of the polymers were maintained for all experiments for coacervate formation. As a microencapsulation method, the complex coacervation method, which is a physico-chemical method, was preferred. The flowchart of microcapsule production is given in **Fig. 1**.

The core material (cabbage plant extract) was added dropwise to the gelatin/gum Arabic (GA-based) and gela-tin/sodium alginate (SA-based) polymer mixture solutions separately. The mixtures were homogenized at 50 °C, 1500 rpm for 15 min. The pH of the emulsions was reduced to 4-4.5 for the gelatin/gum Arabic solution and 3.5-3.75 for the gelatin/sodium alginate solution by adding 10% (v/v %) acetic acid. These pH values were determined according to the electrostatic interaction between the polymers and the isoelectronic points that provide the formation of complex coacervation (**Devi et al., 2012; Gomez-Estacaab et al., 2016; Koksal & Gode, 2017**). During the coacervation process, the temperature of the systems were reduced to 5-10 °C with the help of an ice water bath and the solutions were stirred 500 rpm using a magnetic stirrer for 1 h. Subsequently, 3 mL of glutaraldehyde was added to each system as a cross-linker and mixed for another hour at the same conditions. Obtained microcapsules then washed with deionized water and freeze-dried for 48 h. Final GA-based and SA-based microcapsules are then stored in the refrigerator for analysis.

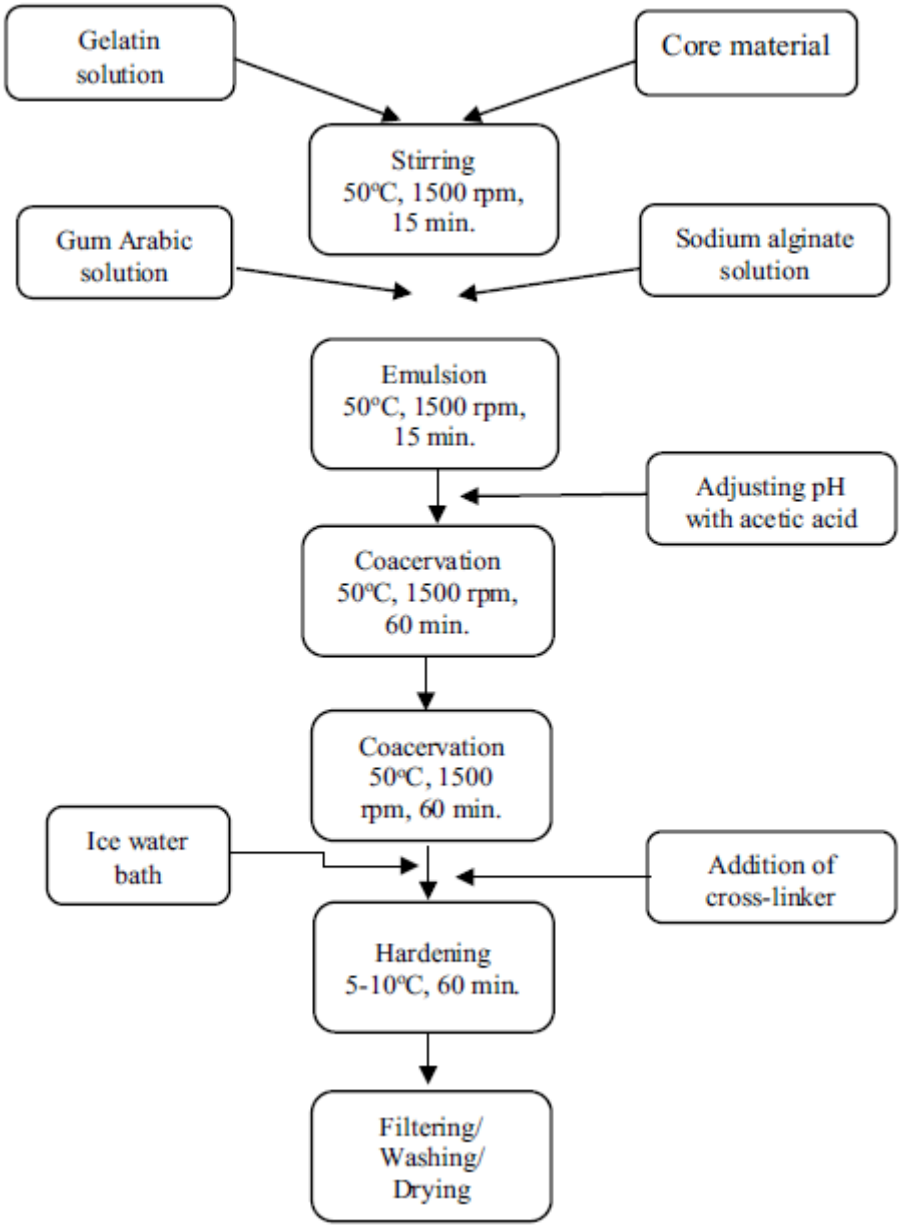

Fig. 1 Flowchart of the microencapsulation proces

For the comparison study, the chemical S-methylmethio-nine (vitamin U) was microencapsulated using the complex coacervation method at the same conditions to compare the amounts of amino acids.

The Efficiency of Encapsulation

Encapsulation efficiency (EE) was determined according to experimental studies mentioned in similar studies (**Ocak, 2012; Sittipummonkol et al., 2019**).

$$EE\% = \frac{\text{Total extract amount} - \text{Surface extract amount}}{\text{Total extract amount}} \times 100 \quad (1)$$

In this equation, the total extract amount represents the weight of extracted cabbage leaves, while the surface extract amount represents the amount of non-encapsulated extract remaining in the beakers. The amount of surface extract was calculated in the formulation based on similar studies in the literature (**Ahn et al., 2008; Aziz et al., 2014; Hu et al., 2020; Koksal et al., 2022**). Briefly, a sample of the capsule was placed in a flask containing 50 mL of n-hexane, and the mixture was gently shaken for 5 min without capsule destruction and structure intact. This procedure was repeated three times. Then, the solution was filtered with filter paper and evaporated using n-hexane rotary evaporator at 69 °C. This process was applied separately for all microcapsule samples.

Chromatographic Analysis of Extract

The high-performance liquid chromatography (Shimadzu Prominence, Germany) was used to compare the amounts of active ingredient (S-methyl methionine) and amino acids of cabbage plant extracts and microcapsules. Agilent Eclipse ACE5 C-18 (250 × 4.6 mm, 5 μm; Agilent, USA) column was used. The gradient elution was performed by using 0.1 M acetic acid (A) and methanol (B) as the mobile phase composition: 80:20 (A:B) for 1 min, 50:50 for 25 min, 20:80 for 40 min, and the flow rate was 1 mL/min.

One hundred fifty microliters of saturated sodium bicarbonate, 100 pL of 2 N NaOH, and 1 mL of Dansyl chloride were placed on the extract. The mixture was incubated at 40 °C for 45 min. After waiting for 10 min at room temperature, 50 μL of 25% NH_3 was added. It was incubated for 30 min at room temperature. Mobile phase is added to it. After filtering through a 0.45-μm filter, it was injected into $HPLC$ (**Köse et al., 2011; Mazzucco et al., 2010**).

Morphological Analysis of Microcapsules

The surface morphology of microcapsules was examined by scanning electron microscopy (SEM) (FEI Quanta FEG250, Thermo Fisher Scientific, USA) at an acceleration voltage of 20 kV with 150 X magnification.

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transform infrared spectroscopy (Nicolet IS5, Thermo Scientific, USA) equipped with iD5 attenuated total reflectance (ATR) was used to identify the functional groups of microcapsules (SA-based and GA-based), cabbage plant extract, and wall polymers. The $FTIR$ spectra were recorded in 64 scans at 2 cm^{-1} resolution in the 400-4000 cm^{-1} wavelength range using a ZnSe crystal.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis was performed using a thermogravimetric TGA Q500 (TA Instruments, USA) in a nitrogen atmosphere (50 mL min^{-1}). The tested for thermal stability in a temperature range

of 25-600 °C, at a heating rate of 5 °C min^{-1}. The data were evaluated using the Universal Analysis 2000 software. The sample's mass of 15 $\pm$ 1 mg was used for analysis.

Swelling Studies

The swelling behavior of microcapsules (SA-based and GA-based) was investigated at two different solutions of HCl buffer (pH 1.2) and phosphate-buffered saline (pH 7.4) at 25° and 37°. For the experiment, 20 mg dry microcapsules were placed in separate 20 mL of solution for 24 h. Temperature and pH values were determined using similar studies (**Mooranian et al., 2014; Negrulj et al., 2016; Zokti et al., 2016**). The swollen microcapsules were removed by blotting with filter paper to remove surface moisture and then determined gravimetrically by increasing on an electronic balance. The degree of swelling (%) of the microcapsules was calculated from the equation (**Karakurt et al., 2021**):

$$Swelling\ degreee\% = \frac{Wf - Wi}{Wf} \qquad (2)$$

Table 1 $HPLC$- amino acid analysis results of cabbage plant extracts. The data are reported as the mean value $\pm\ SD$ ($n\ =$ 3)

Samples	S-methyl methionine (ppm)	Glutamine (ppm)	Methionine (ppm)	Lysine (ppm)	Histidine (ppm)	Aspartic acid (ppm)	Glucoraphanin (ppm)
Raw cabbage plant leaf (decoction)	111.22 ± 4.2	6.94 ± 0.26	<LOD	30.91 ± 1.17	7.69 ± 0.3	28.79 ± 1.09	221.87 ± 8.43
Dried cabbage plant leaf (decoction)	1013.21 ± 38.5	52.492 ± 1.99	75.99 ± 2.88	57.99 ± 0.89	2.36 ± 0.12	68.51 ± 2.60	349.03 ± 13.26
Raw cabbage plant leaf (Soxhlet extraction)	478.92 ± 18.20	12.84 ± 0.48	26.79 ± 1.02	28.42 ± 1.08	6.07 ± 0.23	24.46 ± 0.93	475.32 ± 18.06
Dried cabbage plant leaf (Soxhlet extraction)	712.65 ± 27.08	60.97 ± 2.31	<LOD	42.84 ± 1.63	19.98 ± 0.76	44.25 ± 1.68	653.169 ± 24.82

where (Wi) is the initial weight and (Wf) is the final weight of the microcapsules in the swollen state.

In VitroRelease Study

The in vitro release study was evaluated using SA-based and GA-based microcapsules to compare their release performances. The release property was studied in simulated gastric fluid of hydrochloric acid buffer (pH 1.2) and simulated intestinal fluid of phosphate-buffered saline (pH 7.4) to mimic the human body. 0.1 g of freeze-dried microcapsule samples were taken and placed in 10 mL of prepared pH liquid media placed on an oscillating stirrer. The release profiles of the microcapsules were observed at 100 rpm for 48 h. At specified time intervals, 1 mL was withdrawn from the aliquot samples and the same volume of fresh medium was added. The release amount of the encapsulated extract was analyzed with a UV-VIS photometer (Photolab 6600-Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim,

Germany) at a wavelength of 288 nm. Each measurement was performed three times and the average value was used. In order to estimate the loading capacities of the microcapsules, desorption method was also carried out by placing the microcapsule samples in a determined amount of n-hexane for 2 days. Then, aliquots of 1 mL were withdrawn from the solutions and their absorbances were measured by UV-VIS spectroscopy (**Karakurt et al., 2021**) ($\lambda = 288$ nm, Abs $= 0.0688$ (μg mL^{-1}); $R^2 = 0.9917$).

Statistical Analysis

Statistical significance was done by one-way ANOVA analysis using the Origin 8.0 software with consideration of $p < 0.05$ and all results represent the mean value of triple measurements.

Results and Discussion

Chromatographic Analysis of Cabbage Extract and Microcapsules

The $HPLC$-amino acid analysis was performed to determine the presence and amounts of the active ingredient (S-methyl-methionine, vitamin U) and various amino acids (L-methionine, L-glutamine, L-histidine, L-lysine, L-aspartic acid) in the extracts obtained as a result of the extraction processes. **Table 1** shows the amounts of S-methylmethionine and amino acids of interest in extracts of dried and raw cabbage leaves.

The $HPLC$ analysis results showed that the dried cabbage plant extract contains higher amounts of S-methylmethionine (vitamin U) and amino acids in both Soxhlet extraction and decoction extraction methods. Moreover, higher S-methylme-thionine and amino acid compounds were observed for the extract prepared by the decoction extraction, compared to Soxhlet extraction. **Song et al.** (**2017**) gave similar results in their study for the determination of S-Methyl methionine in vegetables belonging to the Brassicaceae family, and they stated that the dried white cabbage plant has a higher rate of s-methyl methionine active ingredient in the aqueous extraction than other plants in the Brassicaceae family (**Song et al., 2017**).

The $HPLC$ analysis showed that the dried cabbage plant leaf extract prepared by the decoction extraction method contains high levels of S-methyl methionine and amino acids. For this reason, microencapsulation studies were carried out in two different polymer complexes using dried cabbage plant leaves as core material.

Table 2 HPLC-amino acid analysis results of microcapsule samples. The data are reported as the mean value $\pm SD$ ($n = 3$)

Samples	S-Methyl methionine (ppm)	Glutamine (ppm)	Methionine (ppm)	Lysine (ppm)	Histidine (ppm)	Aspartic acid (ppm)	Glucoraphanin (ppm)
S-methyl methionine microcapsule	451.29 ± 17.15	30.86 ± 1.17	55.86 ± 2.12	6.20 ± 0.24	2.57 ± 0.09	251.98 ± 9.58	103.36 ± 3.93
SA-based microcapsule	91.03 ± 3.46	12.62 ± 0.48	20.52 ± 0.78	24.95 ± 0.95	4.05 ± 0.15	71.51 ± 2.72	61.07 ± 2.32
GA-based microcapsule	104.77 ± 3.98	14.92 ± 0.57	25.54 ± 0.97	79.99 ± 3.04	3.82 ± 0.14	71.95 ± 2.73	97.66 ± 3.71

The $HPLC$ analysis of microcapsules containing S-methylmethionine and microcapsules of cabbage plant extracts were also carried out to compare the amounts of active ingredients of vitamin U and amino acids. The results of microcapsules are given in **Table 2**.

The analysis results of the microcapsules showed that the desired active ingredient of vitamin U and amino acid compounds (L-methionine, L-glutamine, L-histidine, L-lysine, L-aspartic acid) was found in both microencapsulated samples; however, the concentrations of the compounds decreased during microencapsulation. A higher amount of vitamin U and amino acid was detected in the GA-based microcapsule compared to the SA-based microcapsule.

The Efficiency of Encapsulation

The encapsulation efficiency of microcapsules in two different polymer complexes was calculated according to **Eq. 1**. The encapsulation efficiency of microcapsules containing gum Arabic and sodium alginate was 86.92% and 81.70%, respectively. Therefore, GA-based microcapsules are expected to present more efficient results for swelling and release studies compared to SA-based microcapsules, which is presented in the "**Swelling Studies" and "In Vitro Releasing Property of Microcapsules**" sections.

The Morphology of Microcapsules

The morphological analysis of the microcapsules were investigated with a scanning electron microscope (SEM). The morphological images of GA-based and SA-based microcapsules are shown in **Fig. 2**.

The SEM images did not show significant differences in the morphologies of the microcapsules in the two different wall material formulations. Microcapsules are generally in spherical shape. It was observed that the surface morphologies of the microcapsules were generally between 20 and 100 pm with homogeneous and various dimensions. The formation of microcapsules of different sizes and the aggregated appearance of the capsules may have resulted from freeze-drying and separation processes (**Aksoylu Ozbek & Gung Ergonul, 2020; Obradovic et al., 2022**).

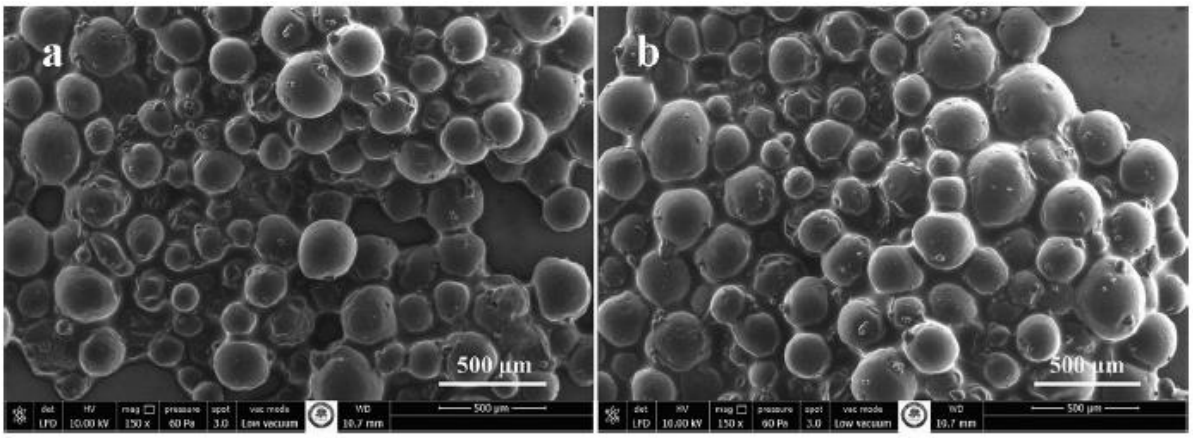

Fig. 2 SEM analysis images of microcapsule samples at 150 × magnification. **a** GA-based microcapsules. **b** SA-based microcapsules

FTIR Results

The $FTIR$ spectra of microcapsules, cabbage plant extract, the wall materials of gelatin, sodium alginate, and gum Arabic are shown in **Fig. 3**. When the IR spectra were examined, a broad band was observed in the range of 3000-3500 cm^{-1} in gelatin and gum Arabic. These bands correspond to the stretching vibration of the amino and hydroxyl groups in the structure (**Cai et al., 2019; Comunian et al., 2013; Shaddel et al., 2018**). In the IR spectrum of the GA-based microcapsule, a broad band was observed at around 3250 cm^{-1}, which supports the presence of gelatin and gum in the microcapsule. The bands corresponding to the C-H stretching vibration of the cabbage extract around 2900 cm^{-1} were observed in similar bands in both microcapsule structures. The band around 1600 cm^{-1} of the extract can be assigned to the amide group in the structure, as it was also observed by **Zhang et al.** (**2021**). The weak band seen at 2900 cm-1 in the spectrum of gum Arabic is the characteristic peak of negatively charged carboxylic groups. In the coacervation of the positively charged amino groups of gelatin and the negatively charged carboxyl groups of gum Arabic, amide formation was observed at approximately 1600 cm^{-1} in the GA-based microcapsule spectrum. This band, which is seen around 1600 cm^{-1} in the IR spectrum of the microcapsules, supported the presence of wall polymers and cabbage extract into the structure (**Comunian et al., 2013; Shaddel et al., 2018**).

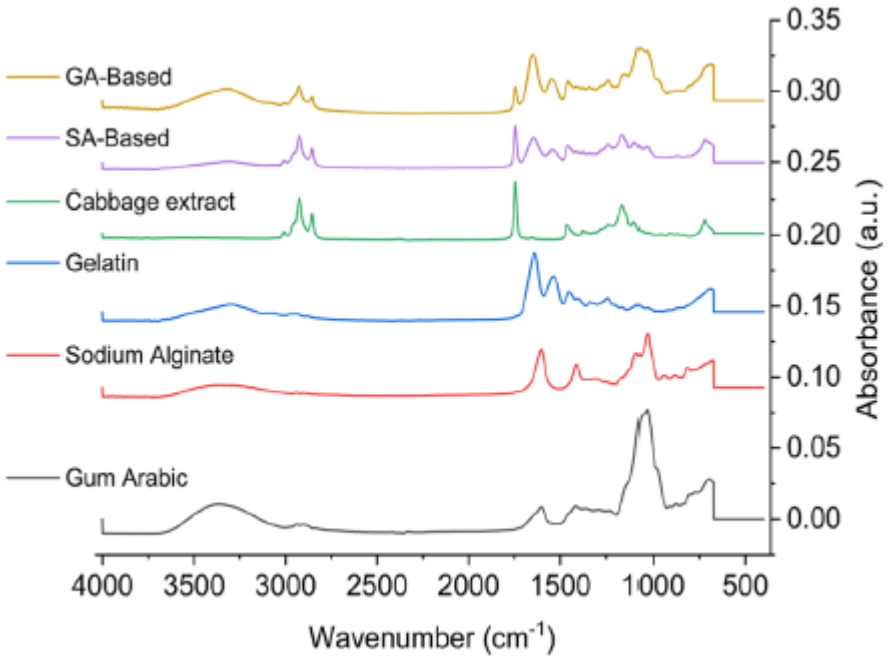

Fig. 3 $FTIR$ spectra of the GA-based microcapsule, SA-based microcapsule, cabbage extract, gelatin, sodium alginate, and gum Arabic

The amide peak observed in gelatin-sodium alginate coacervation showed a slight shift in the SA-based microcapsule spectrum. This showed that negatively charged sodium alginate groups interacted with positively charged gelatin groups. A similar observation was presented by **Devi et al.** (**2012**). C-O stretching vibrations, which appear more intensely in the IR spectra of sodium alginate around 1100 cm^{-1}, were observed in similar bands in the IR spectrum of SA-based microcapsule. This confirmed the structure of the microcapsule. The band seen around 1750 cm^{-1} in the IR spectrum of the cabbage extract supported the presence of the carbonyl group in the structure. These bands corresponding to the carbonyl group in the cabbage extract showed a slight shift in the IR spectra of both microcapsule

samples and were observed around 1720 cm^{-1}. This showed the interaction of cabbage extract with microcapsule samples and the introduction of core material into the microcapsule. The $FTIR$ analysis confirmed the interaction between the wall materials and the microcapsules, and also the similarities seen in the bands of the cabbage extract and the microcapsules confirmed the microencapsulation of the cabbage plant extract.

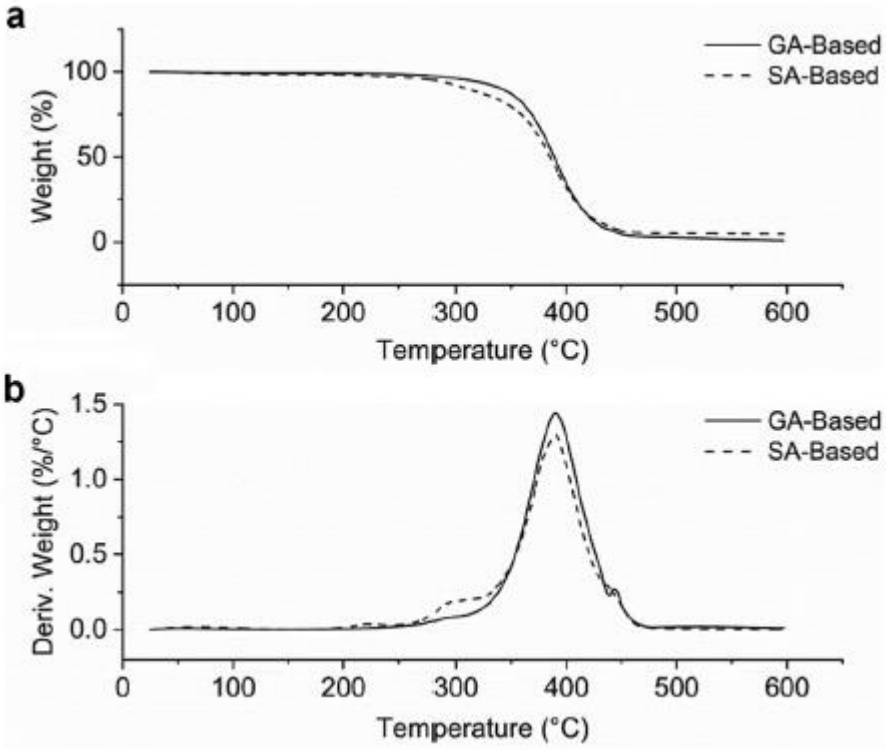

Fig. 4 Obtained TGA **a** and $DTGA$ **b** curves of measured samples GA-based and SA-based microcapsules

TGA Results

The thermogravimetric (TGA) and derivative thermogravimetric ($DTGA$) curves of measured samples are shown in **Fig. 4**. As can be seen from the corresponding figures, the SA-based sample showed three degradation steps in comparison with the GA-based sample, in which only two degradation steps were obtained.

The first degradation step was observed in a temperature range of 25-150 °C. The weights losses were 1.35 wt% for sample SA and 0.59 wt% for GA-based sample, respectively. This degradation stage can be attributed to water loss. The second degradation step was only observed for SA-based samples. This degradation step occurred in the temperature range of 150-240 °C and may be attributed to degradation of sodium alginate (**Jana et al., 2015; Reddy & Thakur, 2018; Soares et al., 2004**), which serves as shell and covers the core part consisting of gelatin. As can be seen, the almost 1.5 wt% of sodium alginate was determined by TGA analysis. The maximum degradation rate was observed at 223 °C according to $DTGA$ curve (peak maximum position). Compared to the SA-based sample, no degradation step attributed to the degradation of part of the shell was observed in the GA-based sample. It is probably due to the higher decomposition temperature of gum Arabic (**Cozic et al., 2009; Mothé & Rao, 2000; Zohuriaan & Shokrolahi, 2004**).

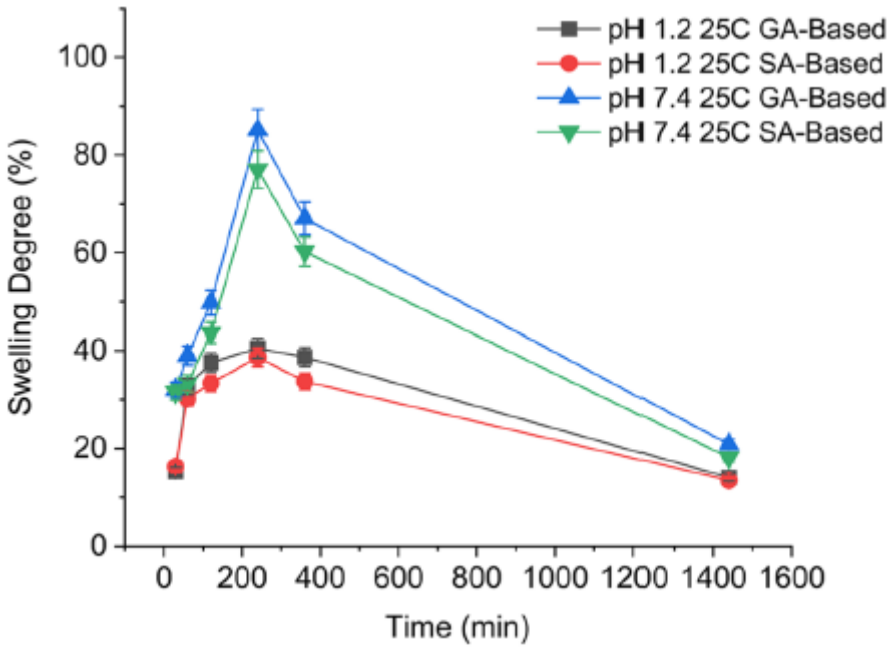

Fig. 5 Swelling characteristics of GA-based and SA-based microcapsules (pH 1.2 and 7.4) at 25 °C

GA-based degradation therefore probably overlaps with the main degradation step. The third degradation step was observed in the temperature range of 240-600 °C for SA-based sample, and in the range of 150-600 °C for GA-based sample, respectively. This degradation step can be attributed to degradation of gelatin (**Soares et al., 2004; Kuzema et al., 2015**), which represents the core part of prepared samples. As can be seen, SA-based and GA-based samples contained around 92.4 wt% and 98.31 wt% of gelatin. The maximum degradation rate was observed at 390 °C according to $DTGA$ curve (peak maximum position). Finally, the residues (or ash) at 600 °C corresponded to the amount of unburned compounds in samples. Based on the analysis, the SA-based sample contained around 4.7 wt% of unburned compounds. Amount of these compounds was smaller in the GA-based sample (around 1.1 wt%).

Swelling Studies

Characterization of swelling of microcapsules prepared with two different wall formulations was carried out. The degree of swelling is an important indicator that affects the release profiles of microcapsules. The swelling study showed that the wall materials, the pH of the medium, and the temperature significantly affect the swelling properties of the microcapsules ($p < 0.05$).

Figures 5 and **6** show that the degree of swelling increased with the increase in temperature and pH in the microcapsules prepared in both formulations (GA-based and SA-based). At pH 1.2, there was no significant change in the degree of swelling of the microcapsules at both temperatures ($p < 0.05$). Although there is limited information about pH-dependent swelling of microcapsules in related studies, it has been reported that most microcapsules show less swelling in simulated gastric fluid (pH 1.2) compared to simulated intestinal fluid (pH 7.4) conditions (**Mooranian et al., 2014; Neo et al., 2012**).

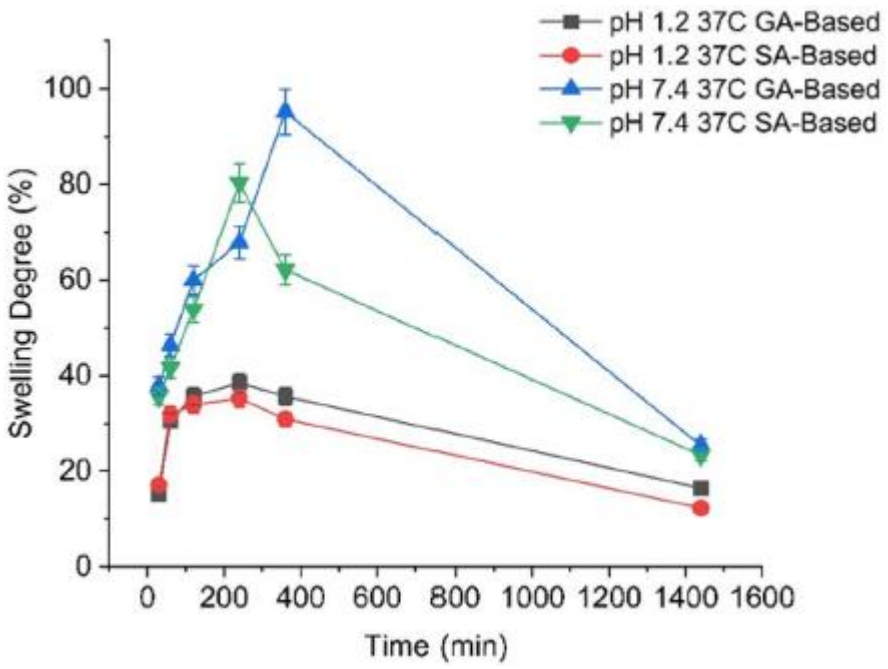

Fig. 6 Swelling characteristics of GA-based and SA-based microcapsules (pH 1.2 and 7.4) at 37 °C

At pH 7.4, however, swelling differed at both temperatures tested, with the greatest degree of swelling observed at 37 0C (body temperature). When the degree of swelling at 37 °C and pH 7.4 was compared in terms of microcapsules in both formulations, the highest swelling was observed in the GA-based microcapsules. The reason for this can be interpreted as the increase in penetration with the increase of the -OH bond in the structure of gum Arabic and the expansion taking place with it (**Goh et al., 2012; Singh et al., 2010**). The degree of swelling of the microcapsules showed that gum arabic as a coating material has a high-strength swelling feature due to its hygroscopic polymer property. GA-based microcapsules showed the highest swelling ability of 95.14% in simulated intestinal fluid at 37 °C for 360 min. On the other hand, SA-based microcapsules showed the highest degree of swelling (80.31%) in 240 min under the same conditions.

In Vitro Releasing Property of Microcapsules

In vitro release assay was performed at pH 1.2 (simulated gastric fluid) and pH 7.4 (simulated intestinal fluid) for 48 h for GA-based and SA-based microcapsules produced under optimal efficiency conditions.

The release of the core material from the microcapsule structures occurs by mechanisms such as diffusion, dissolution, surface erosion, and desorption (**Hosseini et al., 2013; Singh et al., 2010**). These mechanisms in the release profile are affected by the pH of the medium. The cumulative release profile at pH 1.2 and pH 7.4 in our study can be expressed as a two-step process. For the first 360 min, there was a burst of release followed by a slower release for GA-based and SA-based microcapsules. The initial burst release stage was attributed to the rapid diffusion of core material adsorbed on the surface of the microcapsules and close to the wall structure.

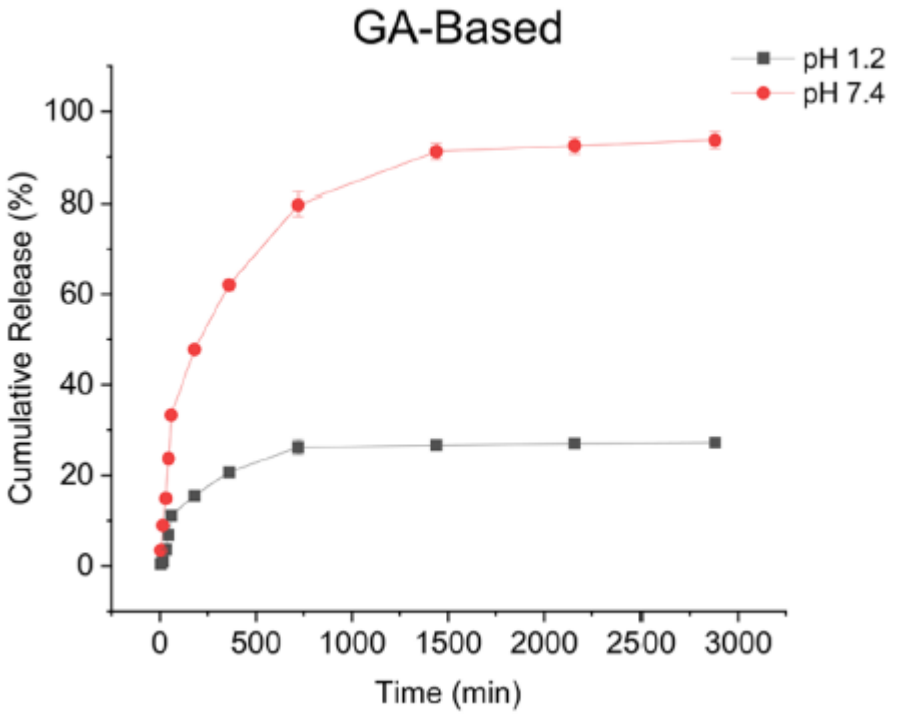

Fig. 7 In vitro release profiles of cabbage extract from GA-based microcapsules at pH 1.2 and pH 7.4

Since the dissolution rate of the polymer wall near the surface is high, the amount of extract released from the microcapsule will also be (**Anitha et al., 2011; Hosseini et al., 2013; Nishi & Jayakrishnan, 2004; Riyajan & Sakdapipanich, 2009**). At pH 1.2, 27.2% of the extract (core material) was released from GA-based microcapsules and 27.0% from SA-based microcapsules for 48 h (**Figs. 7** and **8**). At pH 7.4, 93.6% of the extract was released from GA-based microcapsules and 82.9% from SA-based microcapsules, and more than 50% of the core material was observed to be released from both microcapsule samples within 4 h. The results were also compatible with the swelling degree of the microcapsules (**Figs. 5** and **6**).

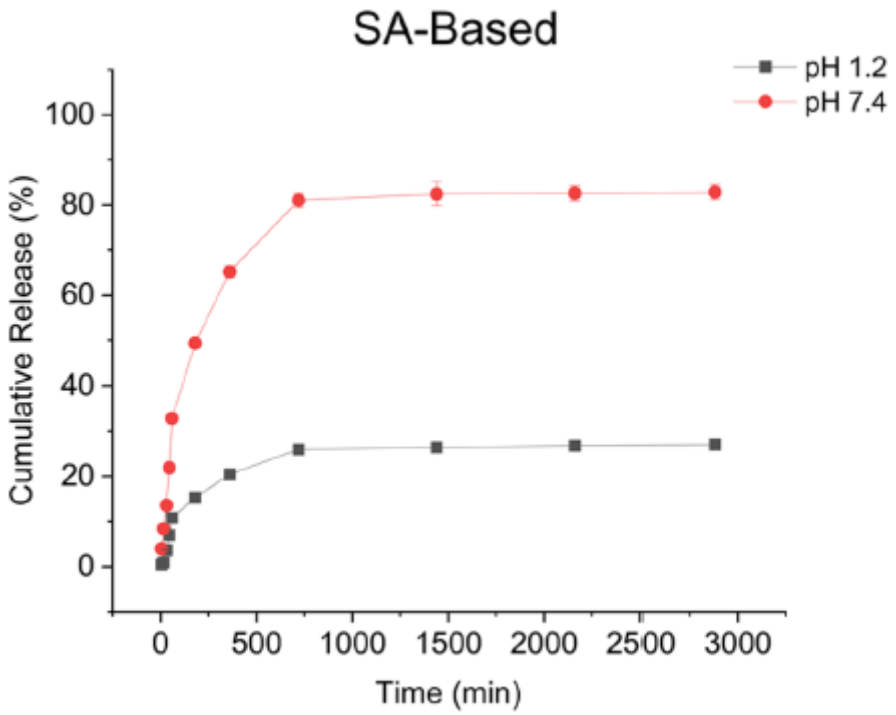

Fig. 8 In vitro release profiles of cabbage extract from SA-based microcapsules at pH 1.2 and pH 7.4

The release profiles indicated that the GA-based and SA-based microcapsules are stable under acidic conditions; however, showed that SA-based microcapsules were more unstable under alkaline conditions than GA-based microcapsules. **Nishi and Jayakrishnan** (**2004**) also reported that they observed a slower and more continuous in vitro release of conjugated gum Arabic microspheres at 37 °C PBS (pH 7.4). Based on the results obtained, it can be concluded that gum Arabic and sodium alginate as a coating material is resistant to an acidic environment, whereas gum Arabic is more resistant than sodium alginate in alkaline environments, and sodium alginate rapidly releases the core material under these conditions. The studies have shown that the pH-sensitive properties of the shell structure of microcapsules can be used to control the drug release process of microcapsules as they pass through the gastrointestinal tract. In the literature, **Omer et al.** (**2021**) reported that the microcapsules they prepared in alginate, carboxymethyl chitosan, and amine chitosan complexes to increase pH-sensitive drug encapsulation efficiency exhibited a sustained release profile under simulated column fluid (pH 7.4) conditions. **Xiao et al.** (**2018**) reported that Bovine serum albumin, protein drugs, loaded o-carboxymethylchi-tosan-gum Arabic coacervate microcapsules have enhanced stability against gastric fluid pH and are effective in intestinal targeted delivery. The release results in our study showed that the microcapsules have good pH-controlled release and sustained release properties, and thus may be an alternative therapeutic application for peptic ulcer therapy release carriers.

Conclusion

In this study, cabbage (Brassica oleracea L. var. capitata) plant extract containing the active ingredient of S-methylmethionine (vitamin U) was successfully extracted and microencapsulated by complex coacervation method using two different natural polymeric wall materials of gum Arabic and sodium alginate, combined with gelatin. $HPLC$ analysis results confirmed that the vitamin U and amino acid

compounds in cabbage extract are present in the structure of microcapsules. The morphological analysis of the microcapsules showed that GA-based and SA-based microcapsules have a homogeneous, spherical shell structure. Another object of the study, the pH-dependent targeted release profiles of GA-based and SA-based microcapsules, showed that they exhibited high and controlled release at pH 7.4. In vitro release profiles showed that GA-based microcapsules have a better and longer-term release pattern compared to SA-based microcapsules. Overall, the characterization results of the microcapsules showed that the capsules containing the cabbage plant extract have pH controlled and sustained release properties and may provide an alternative application for peptic ulcer therapy release carriers. On the other hand, the less release behavior of microcapsules at pH 1.2 compared to pH 7.4 can be improved by increasing the acid resistance of the wall materials. In order to make comparisons, extraction, microencapsulation, and characterization studies of other plants belonging to the Brassicaceae family can be carried out and the scope of the study can be expanded. In this context, encapsulation of the plant extract containing the active ingredient S-methylmethionine (vitamin U) will guide further studies.

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