



### Research Article

## ENHANCEMENT OF THE SOLUBILITY OF HERBAL POLYPHENOLIC COMPOUNDS IN THE PRESENCE OF LACTIC ACID BUTYLETHER PEG (200)

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### ABSTRACT

Polyphenolic compounds found in some plants are very important for human health because of their antioxidant and some disease healing properties. However polyphenolic compounds have a disadvantage that their bioavailability is low due to their low solubility in water. In this study, a new PEG derivative, lactic acid ester of butylether PEG 200 (PEG-ester) has been synthesized and then it has been interacted with curcumin and morin to improve poor water solubility of these polyphenolic compounds. PEG ester, was found to be miscible with water and significantly improved the water solubility of curcumin and morin. The characterization of emulsion systems formed with PEG-ester in the presence of curcumin and morin were investigated. The amount of curcumin and morin in emulsion systems was carried out by UV/VIS spectrophotometric measurements. Interactions were characterized by FTIR measurements. Solubility and stability studies were performed.

**Keywords:** Curcumin, morin, emulsion, water solubility, lactic acid ester of butylether PEG (200).

### 1. INTRODUCTION

Many natural phenolic compounds found in plants have very beneficial effects on human health as well as their antioxidant capacity. Several studies have shown that polyphenols, which contain more than one phenolic group, have an important role in the reduction of lipid oxidation in tissues with antioxidant activities. It has also been shown to be effective in reducing the risk of developing some diseases. Majority of these are cancer, diabetes, heart diseases, cognitive disorder and neurological diseases [1-3].

Turmeric (*curcuma longa*) is an important plant in Asian public health. Turmeric is a tropical plant used by people against various diseases. In the South Asian region, especially in India, it is cultivated extensively. It has a wide range of uses ranging from taste and coloring agent in curry sauce to healing a lot of diseases and health problems in traditional medicine. In recent years, numerous researches with this tropical plant root have shown that its medicinal power is related to curcumin. Curcumin is an important polyphenolic compound which is the main active ingredient

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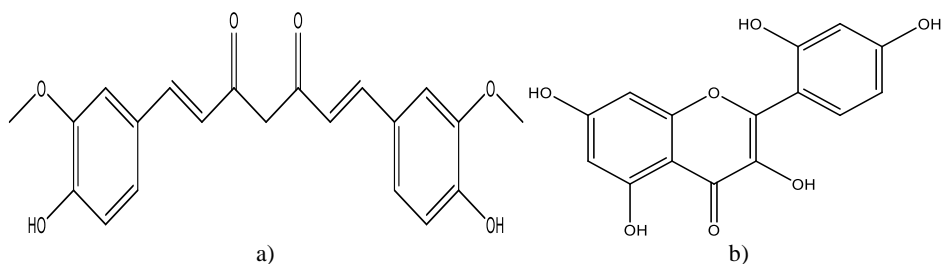
of turmeric [4]. In recent years, numerous studies have been carried out which characterize the pharmacological properties of curcumin. Curcumin, which has been in use for a long time in eastern medicine, has been shown to have strong pharmacological effects and it has been shown to inhibit inflammation and arthritis, which are believed to be the result of serious metabolic disorders caused by oxidative stress in cells, and also to prevent the development of diseases such as cancer, parkinson's and Alzheimer's [5, 6]. Curcumin has been proven to have many therapeutic effects [7, 8], mainly antioxidant, anticancer [9, 10]. A study showed that curcumin has a strong anti-inflammatory effect and modulates the severity of acute lung injury [11]. For thousands of years, it has been widely used among the public because of its therapeutic effects against many diseases [12-14]. It has also shown potential in the treatment of diabetes [15] and Alzheimer's [16-18] disease. When the neutral pH value is exceeded, curcumin undergoes a rapid degradation in aqueous medium [19].

Morin is a yellow (yellow-brown when it comes into contact with air) polyphenolic bioactive compound found in mulberry, bilberry, blackcurrant, cranberry and some Central and South American plants and fruits (eg Osaga Orange). One of the most important features of morin is the inhibition of the formation of amyloid polypeptide, which is known to cause Alzheimer's disease, and the elimination of formed amyloid fibers [20]. In a study, an albumin based nanoparticle system has been prepared and the degradation kinetics, solubility and stability of morin have been investigated [21]. In another study, morin was found to be very unstable at pH 9 and degradation rates were evaluated [22].

Curcumin and morin are practically insoluble in water. Although they are alkaline soluble, they undergo rapid hydrolytic degradation at pH values above neutral. This low solubility in water limits the practical application of these valuable polyphenolic substances [23]. There have been many studies for improving solubility of substances that are less soluble and therefore have little bioavailability so far. One of the substances used for enhancing solubility of these products is polyethylene glycol (PEG). PEG is one of the commonly used compound as dispersing and solubilizing agent in the food and pharmaceutical industries. They can also be used to modify the viscosity of liquids. PEGs with various molecular weights are water-soluble polyether alcohols. PEGs are also used as intermediates in organic synthesis. PEG, albeit to a limited extent, provides an increase in the water solubility of less soluble substances. In a study recently we have carried out, it was found that using PEG derivatives instead of PEG was more effective for enhancing of the water solubility of substances [24].

Emulsions are mixtures of two liquids that are insoluble in each other that is, one liquid is dispersed in another. Water, on the other hand, is a phase of an emulsion which is important with many features such as surface tension and bonding capacity [25, 26]. Emulsions which are extensively used in the food industry, contribute to human health by providing protection of functional components against environmental conditions. Emulsions are emphasized in the protection, transportation and easy use of functional food components [27].

In this study, a new PEG derivative, lactic acid ester of butylether PEG 200 (PEG-ester), was synthesized in order to increase the solubility of curcumin and morin. The curcumin and morin emulsions formed by using this PEG-ester were prepared and characterized, and their interaction mechanisms and stability were investigated. Curcumin and morin structures are shown in Scheme 1.



**Scheme 1.** Structures of polyphenols a) Curcumin b) Morin

## 2. EXPERIMENTAL

### 2.1. Materials and methods

#### 2.1.1. Materials

Curcumin (97%), Morin (2',3,4',5,7-Pentahydroxyflavone, 97%) was purchased from Merck (Darmstadt, Germany). Polyethylene glycole monooleate and tween 20 were obtained Sigma-Aldrich (Taufkirchen Germany). Lactic acid (Merck), Poly (ethylene glycol) butylether (PEG 200) (Aldrich) and P-toluene sulfonic acid (Aldrich), Acetic acid, sodium acetate, methanol and sodium hydroxide were supplied from Fluka (Fluka AG, Buchs, Switzerland). All the chemicals used in the study were of analytical reagent grade. The solutions have been prepared by using ultrapure water from Millipore Milli Q System (resistivity equal to 18 M $\Omega$ ). Phosphate buffer solutions with different pH values were prepared by using phosphoric acid, and then adjusted with sodium hydroxide.

#### 2.1.2. Esterification of Poly(ethylene glycol) butyl ether with lactic acid

10 g of Poly(ethylene glycol) butyl ether and 4.37 g (48.54 mmol) of Lactic acid and catalytic amount of p-toluene sulfonic acid were mixed and transferred into Dean-Stark apparatus. The reaction mixture was heated removing of water at 180 °C. Then, the viscose and yellow reaction content was cooled and put into 50 mL of hexane. The phase separation was observed and the PEG- lactic acid ester (PLE) was removed by using separating funnel. The product was dried under vacuum at 40 °C for removing of hexane. The yield was found about 12.50 g. The PLE is soluble in water and common organic solvents such as ethanol, acetone, ethyl acetate and DMF.

#### 2.1.3. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR Spectrophotometer (Nicolet) was used to analyze the structure of the synthesized PEG butyl ether-lactic acid ester and the interactions between curcumin, morin and PEG ester.

#### 2.1.4. UV/VIS measurements

UV/VIS measurements were performed with a UV-Vis Spectrophotometer (Perkin-Elmer Lambda 25) spectrometer. Measurements were performed at 425 nm for curcumin and 325 nm for morin solutions.

#### 2.1.4. Preparation of the Calibration Curve

For the calibration curve of curcumin, 5 mg of curcumin and morin were dissolved in 5 mL of ethanol to prepare the stock solutions. Dilute standard solutions were prepared from the stock solution for UV/VIS measurements.

#### 2.1.5. Preparation of the emulsion systems

The PEG-ester emulsions were prepared in different compositions with a total of 10 mL each, and placed in beakers. 5 mg of the compound (curcumin, morin) was added to each and stirred with a mechanical stirrer at room temperature for 5 minutes at 600 rpm. Resulting homogeneous mixture filtered through 0.45 mm membrane filter for UV/VIS measurements for the determination of curcumin and morin contents.

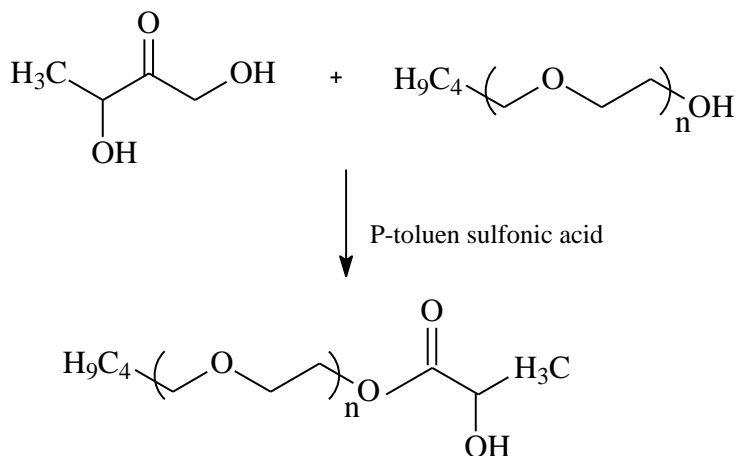
#### 2.1.6. Polarized Optical Microscopy (POM)

POM studies were performed with Leica DM2500P equipped with a LTSE350 Liquid Crystal Prosystem TMS 94 Hot Stage.

### 3. RESULTS AND DISCUSSION

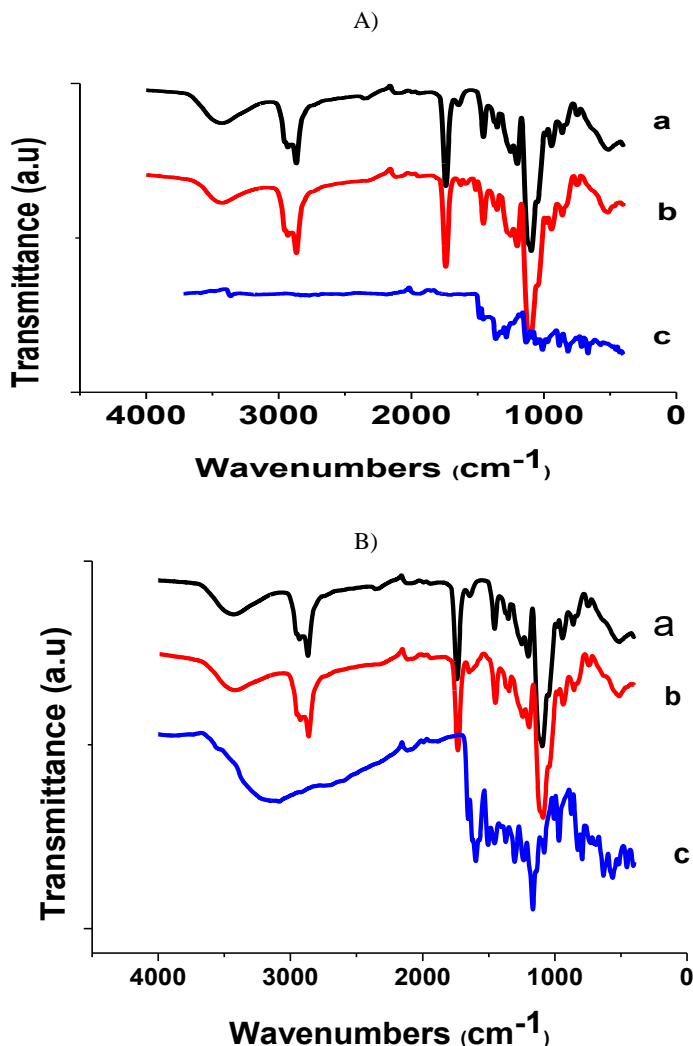
#### 3.1. Synthesis of PEG-ester

PEG based polymeric lactate ester was synthesized starting from poly (ethylene glycol) butyl ether ( $M_n \sim 206$ ) and lactic acid esterification reaction in the presence of catalytic amount of PTSA at 180 °C. The reaction was proceeded to remove water from reaction medium.



**Scheme 2.** Synthesis of PEG-ester

The characterization of PEG-ester was performed by using FT-IR spectroscopy technique. According to the Fig.1 butyl ether PEG lactate represent ester  $-\text{C}=\text{O}$  stretching vibration at  $1739.77\text{ cm}^{-1}$ . Also,  $-\text{OH}$  peak at  $3435.36\text{ cm}^{-1}$ ,  $-\text{C}-\text{O}-$  ether peak at  $1097.92\text{ cm}^{-1}$  and strong aliphatic  $\text{C}-\text{H}$  stretching vibration peak at  $2867\text{ cm}^{-1}$  were observed.



**Figure 1.** FTIR spectra A) PEG-ester (a), the mixture of PEG-ester and curcumin (b), curcumin (c) B) PEG-ester (a), the mixture of PEG-ester and morin (b), morin (c)

### 3.2. Interactions PEG ester with morin and curcumin by FTIR measurements

Interactions between curcumin and PEG lactic acid ester and its emulsion system with both curcumin and morin were investigated by FTIR (Fig.1-A and Fig. 1-B). The OH band at 3435 cm<sup>-1</sup> was observed both PEG-lactac ester and its emulsions. The peaks at 1627.99 cm<sup>-1</sup> and broad peak at 1652 cm<sup>-1</sup> are attributed to the groups -C=C- and/or -C=O for curcumin and morin respectively. Also, the peak which was observed at 1597 cm<sup>-1</sup> in Fig.1-A-b belongs to the symmetrical aromatic ring stretching vibrations (-C = C-) for curcumin emulsion. According to IR spectrum of curcumin, the bands at 1425.32, 1231.90 and 1275.27 cm<sup>-1</sup> were found to shifted to 1455, 1201.52 and 1249.75 cm<sup>-1</sup>, respectively. On the other hand, the original morin FTIR peak

of 1598, 1505, 1303, 1166, 826, 793 and 562-686  $\text{cm}^{-1}$  disappear in the PEG-morin emulsion. On the other hand, the stretching vibration of  $\text{-C-O-C-}$  band for morin shifts from 1310 to 1353  $\text{cm}^{-1}$ .

Except for hydroxyl peak, curcumin and morin IR bands are also present in the mixture formulation, but high wavelength shifts, disappears some peaks or reduction in intensities indicate that the interaction may be due to non-covalent hydrogen bonds, possibly with OH and C = O and enol groups.

### 3.3. Calibration graphs for curcumin and morin

Standard solutions of 0.5 mg / mL of curcumin and morin dissolved in ethanol were prepared. The standard solutions were diluted and the UV absorbances were measured at 425 nm for curcumin and 325 nm for morin. Obtained calibration graphs are shown in Fig. 2-a and Fig. 2-b. The curcumin and morin content of the prepared emulsions were determined by using the calibration graph equations. The linear calibration plot was obtained in the concentration of curcumin from 0.001 mg/mL to 0.016 mg/mL with the linear regression equation  $y = 46.638x + 0.0109$  ( $R^2 = 0.9971$ ) and in the concentration of morin from 0.025 mg/mL to 0.30 mg/mL with the linear regression equation  $y = 0.301x + 0.216$  ( $R^2 = 0.9980$ ).

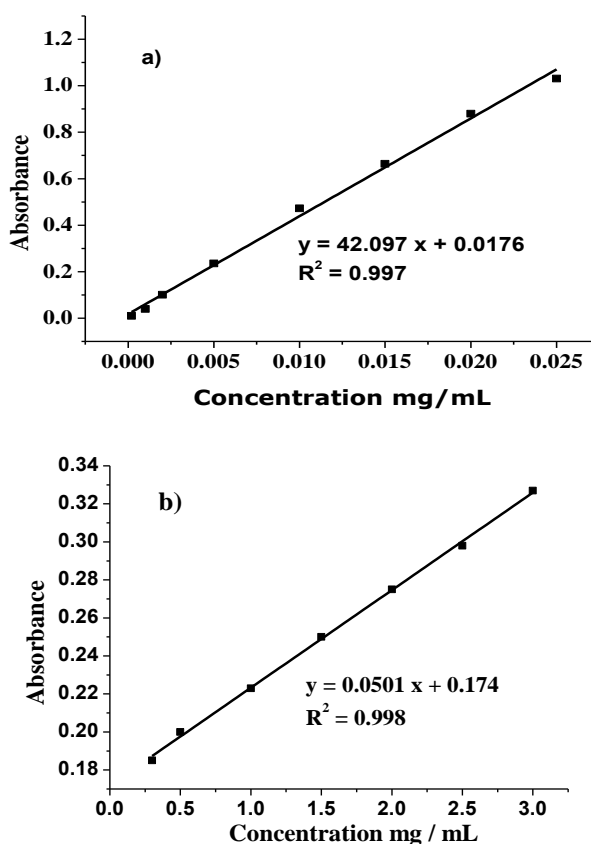
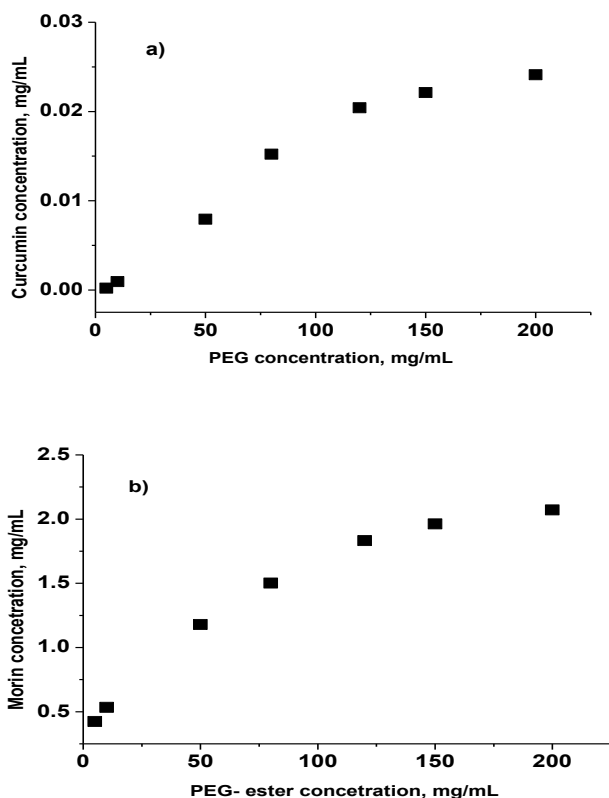


Figure 2. Calibration graphs of curcumin (a), and morin (b).

### 3.4. Water solubility of curcumin and morin in the presence of PEG -ester

Synthesized PEG-ester was used for solubility enhancement of both curcumin and morin. To investigate the solubility of curcumin and morin in water in the presence of PEG, solutions in various curcumin/PEG-ester ratios (and also morin/PEG-ester ratios) were prepared. In a total of 10 mL solution, 0.005 g of polyphenolic substance (curcumin, morin) was kept constant and a series of solutions were prepared in which PEGester amounts were increased. Eight samples containing a fixed amount of polyphenolic substance and different amounts of PEGester were prepared. PEGester amounts were selected as 5, 10, 50, 80, 100, 120, 150, 200 mg / mL. The amount of curcumin and morin dissolved in the prepared solutions were determined by using UV/VIS spectrophotometric measurements. Absorbance measurements were performed at 425 nm for curcumin and 325 nm for morin. The amounts of curcumin and morin were determined by using the obtained calibration curve equations. Solubilities obtained with various amounts of PEG-ester are given in Table 1. The solubilities of curcumin and morin against different concentrations of PEG-ester in 10 mL of water were plotted in Fig. 3. Fig. 3 shows a significant increase in the solubility of curcumin and morin at a concentration of about 100 mg /mL PEG-ester. The increase in solubility is slowed down after 100 mg/mL PEG-ester concentration. Therefore, 100 mg/mL was chosen as the optimum PEG-ester concentration for the 0.005 g polyphenolic substances (curcumin and morin).



**Figure 3.** Solubility of curcumin (a) and morin (b) at different PEG-ester concentrations in 10 mL of water.

**Table 1.** Solubility obtained at various PEG-ester concentrations for curcumin and morin.

PEG-ester (mg/mL)	Curcumin (mg/mL)	Morin (mg/mL)
5	0.0002	0.4243
10	0.0009	0.7347
50	0.0079	1.3895
80	0.0149	1.4801
100	0.0151	1.5743
120	0.0204	1.9328
150	0.0221	2.0442
200	0.0245	2.1718

### 3.5. Effect of pH

The influence of pH on the water solubility curcumin and morin was investigated. Phosphate buffer solutions were used to investigate the effect of pH on the water solubility of curcumin and morin. Curcumin and morin degrade above pH 7. Thus buffer solutions between pH 2 and 7 were used to study the pH effect. The solubility of curcumin and morin was determined by UV/VIS measurements in emulsions prepared using buffer solutions with these pH values instead of water. Each solution contains 1000 mg PEG-ester and 2.5 mg curcumin (or morin). Table 2 shows the solubility of curcumin and morin in different pHs of these solutions. The table also shows that the solubility is independent of pH. The PEG-ester has a nonionic property. The increase in the solubilities of the polyphenolic substances obtained in this study may be due to the hydrogen bonding interaction between the polyphenols and the PEG-ester.

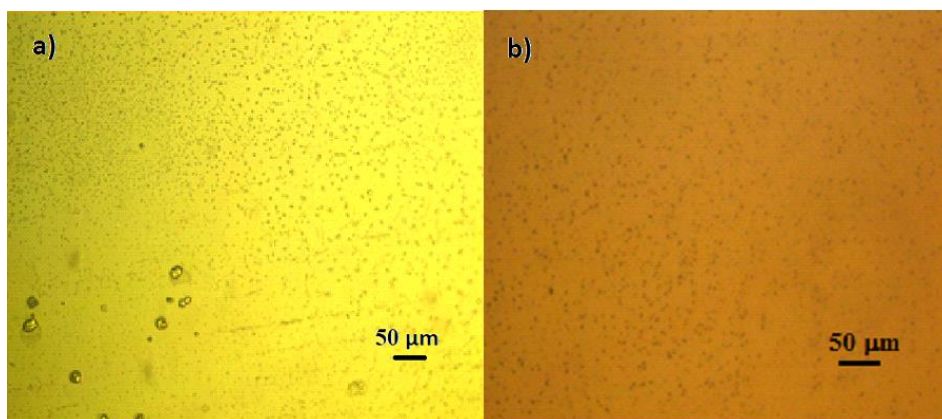
**Table 2.** Solubility of curcumin and morin at different pHs

pH	Curcumin (mg/mL)	Morin (mg/mL)
2	0.0116	1.2344
3	0.0137	1.0420
4	0.0125	1.0604
5	0.0127	1.1146
6	0.0125	1.0927
7	0.0103	1.2191
Water	0.0151	1.5743

### 3.6. Characterization of the emulsion systems by Polarized Optical Microscopy (POM)

POM observation was performed to characterize the emulsion systems. Examples of POM photographs are shown in Fig. 4. POM micrographs reveal that the particles are dispersed in a very smooth and fairly even manner in the emulsions. Particle sizes vary between 10 and 40  $\mu\text{m}$ .





**Figure 4.** POM micrographs of the curcumin (a) and morin (b) emulsions with PEG-ester prepared by the described procedure at room temperature.

### 3.7. Stability of the Emulsions

The curcumin and morin emulsions were prepared by using 1000 mg PEG-ester, 5 mg curcumin (or morin) in a total 10 mL of solution. Then, they were allowed to stand at room temperature ( $20^{\circ}\text{C} \pm 2$ ). The contents of the curcumin and morin emulsions were analyzed every day for one week and then once a month for two months. There was no decrease in the content of the curcumin and the morin at the end of the period showing that the amount of curcumin and morin remained constant in these emulsions.

## 4. CONCLUSION

It is possible to make more useful polyphenolic compounds of plants in human nutrition by increasing the low water solubility of these compounds, because of the bioavailability of these valuable products of plants will be increased. With this aim, PEG-ester (Lactic acid butyl ether PEG-200), which was synthesized for the first time in this study, was able to be easily miscible in water and increased solubility of polyphenols. In the presence of 100 mg/mL PEG-ester, the solubility of curcumin (0.0151 mg/mL) increased by 1300 fold compared to raw water (0.011  $\mu\text{g/mL}$ ). The solubility of morin also in the presence of 100 mg/mL PEG ester showed a 52-fold increase (1.58 mg/mL) compared to the raw water (28.7  $\mu\text{g/mL}$ ). Moreover, emulsions that were stable for at least two months were obtained using PEG-ester. A lot of polyphenolic compounds like curcumin and morin are very important effective substances of plants and fruits due to their antioxidant properties and preventive and curative effects in many diseases. Unfortunately, their bioavailability is low due to their low water solubility. The PEG-ester with water, without flocculation and agglomeration, formed a stable emulsion, resulting in a significant increase in the solubility of curcumin and morin in water. As a result, new synthesized PEG-ester can be an alternative agent to increase the water solubility of the polyphenolic compounds. PEG-ester is biocompatible and has important properties such as ability to mix easily with water in any ratio, to form very stable emulsion.

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