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Key conditions of alpha-tocopherol encapsulation in gum Arabic dispersions

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ABSTRACT

Alpha-tocopherol or TOC is among substances that has medicinal capabilities. However, alpha-tocopherol is vulnerable to surrounding milieu settings. This leads to the necessity to shield it against unforeseen alterations during the storing or handling procedures. Encapsulation is presented as a procedure which can shield active agents from adverse changes by means of coating with polymers. In this study, gum Arabic (GA), a biopolymer derived from Acacia species, was used as the encapsulation matrix. Encapsulation process was done at different concentrations of GA dispersions (10%, 20%, 30% and 40%) and at various pH levels (5.4, 6.4, 7.4 and 8.4). To evaluate the key conditions of TOC encapsulation in GA dispersion we analysed TOC encapsulation efficiency (*EE*) and rate of release (*RR*) from GA dispersions as well as loading capacity (*LC*) of GA for TOC. The *EE*, *RR* and *LC* were determined by measuring the TOC concentration in the GA dispersions using UV Visible spectrophotometry at 291 nm. Results disclosed that the key conditions for achieving a high *LC* by GA with high efficiency of TOC encapsulation were in a dispersion of 20% GA at pH range of 6.4 and 7.4. The best *EE* of TOC and *LC* of GA were 48% and 2.8%, respectively, with a TOC average *RR* of 1.05-1.09 ppm/day. The results indicate that gum Arabic is a potential matrix to encapsulate alpha-tocopherol.

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INTRODUCTION

TOC or vitamin E is a nonpolar substance that inhibits the oxidation process and important for well-being (Niki and Traber, 2012). It is recognized to have the ability to prevent lumps and cancer growth (e.g. liver cancer). It is also associated to skin healthiness (Ben-Shabat *et al.*, 2013; Lai *et al.*, 2014; Uchihara *et al.*, 2017). TOC generally originates in a vegetable at diverse compositions. Besides γ-tocopherol antioxidant compound that has a high concentration in red blood cells and serum is TOC. (Péter *et al.*, 2015).

The low stability of TOC and the ease of degradation by oxidation process and irradiation during storage causes the use of TOC in the pharmaceutical industry to be constrained. The oxidation reactions are highly catalyzed by the environmental acidity and also by the presence of several metal ions such as Zinc and Copper besides other ions including nitrite, NO_2^- (Singh *et al.*, 1998). The presence of enzymatic substances such as ω-hydroxylation and TOC peroxidase can accelerate the oxidation reaction (Sontag and Parker, 2007). One way to overcome these shortcomings is by

encapsulation (Chawda *et al.*, 2017; Ghaheh *et al.*, 2017; Aboudzadeh *et al.*, 2018). Encapsulation can shield the particular compounds from fall-off issues due to heat, oxidation, microorganisms and other destructive aspects. Furthermore, encapsulation can intensify the versatility and management of the compounds so that it can improve the nutritional value. The substances for encapsulation matrix can be GA, one of many biocompatible polymers. GA is identified to inhibit oxidation reactions and also accelerate the TOC stability and bioavailability in the metabolism system (Al-Ismail *et al.*, 2016).

GA is an exuded substance from the Acacia trees, particularly Accacia seyal and Acacia senegal. It is a heteropolysaccharide that comprises of (1 to 3) components of β-D-galactopyranosyl. L-arabinosyl, L-rhamnosyl, D-galactopyranosyl and D- glucopyranosyl uronic acid as side chains elements (Ali *et al.*, 2012; Nayak *et al.*, 2012). Some of its properties, which are non-toxic, water-soluble, biocompatible, and tasteless, making it usable as drug carrier resources (Dragostin *et al.*, 2017). Encapsulation with GA increases drug stability, thereby increasing the storage life (Mosquera *et al.*, 2012). Besides, GA also has other properties that support the function as drug carriers such as anti-inflammatory anti-coagulation and anti-microbial. It is also not easily impaired and does not induce weight problems (Ballal *et al.*, 2011; Stefański and Postek-Stefańska, 2014; Nasir, 2013).

Our investigation explored the key conditions that support the proficiency of GA for TOC encapsulation. The study was conducted by analyzing the encapsulation efficiency (*EE*) of TOC and the TOC rate of release (*RR*) from GA dispersion along with the loading capacity (*LC*) of GA at altered pH levels. Our new finding was that increasing the GA composition steered to an increase in the TOC encapsulation efficiency in GA and an extension of the release time. However, there was a reduction in the loading capacity. Alterations to the pH affected the GA loading capacity as well as TOC encapsulation efficiency and release progression of the GA matrices. At a GA concentration of 20% and pH levels of 6.4 and 7.4, the optimum loading capacity of GA was 2.8% while the encapsulation efficiency was 48%. The average rate of release in these conditions was around 1.05-1.09 ppm/day.

MATERIALS AND METHODS

Reagents and Chemicals

The materials used was gum Arabic (Sigma Aldrich), $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$, CH_3COONa anhydrous, 0.889 g CH_3COOH, HCl, NaOH, absolute ethanol, alpha-tocopherol (Merck) and demineralized water (Brataco).

Preparation of 0.1 M phosphate buffer solutions (PBS) and 0.1 M acetate buffer solutions (ABS) at several pH

A solution of 1.98 g $Na_2HPO_4.2H_2O$ in 500 mL demineralized water was prepared to make up 0.1 M solution A. Similarly solution B was set by dissolving 1.56 g $NaH_2PO_4.2H_2O$ to make 0.1 M in 500 mL demineralized water. For PBS pH 6.4 as much as 27.8 ml of solution A was added to 72.2 mL of B solution then shaken to homogeneous. Similarly, for PBS pH 7.4 and pH 8.4, a total of 80.2 mL and 98 mL of solution A was added to 19.8 mL and 2 mL of solution B, respectively. To prepare ABS a mixture of 6.986 g CH_3COONa anhydrous and 0.889 g CH_3COOH was dissolved in 1 L demineralized. All solution was adjusted to the final desired pH using dilute HCl or NaOH as needed.

Preparation of TOC standard curve

A solution of 1.578 g TOC in 100 mL absolute ethanol (20 ppm) was used as a stock solution. Solutions of TOC with a concentration of 11 ppm; 9.5 ppm; 8 ppm; 6.5 ppm; 5 ppm and 3.5 ppm were prepared from the stock solution. An 8 ppm solution was subjected to scanning λ_{max} of TOC on a UV Visible spectrophotometer. All solutions absorbance were measured at λ_{max}, i.e. 291 nm. A plot of absorbance versus concentration was composed to build the standard curve of TOC.

TOC Encapsulation in GA

The encapsulation of TOC was done in accordance with the method by Al-Ismail (Al-Ismail *et al.*, 2016). A series of GA dispersion in a buffer solution with concentration (C_{GA}) from 10% to 40% (w/v) were formulated under various pH condition. TOC was added to each GA dispersion so that the initial concentration of TOC was 0.1 mg/mL (C_o) and agitated for 10 minutes. The dispersions were homogenized by ultrasonic apparatus (40 kHz) for 15 minutes at 30 ^{o}C. Once the dispersions had settled down, two layers were formed, the thick bottom layer and the watery top layer. The watery top layers or the supernatants containing the unencapsulated TOC were separated out for *LC* and *EE* evaluation. The thick bottom layers or the GA residues were stored in bottles covered with aluminium foil and kept in a freezer at -18 ^{o}C until use for release evaluation.

TOC encapsulation efficiency (*EE*) and GA loading capacity (*LC*) evaluation

EE and *LC* were evaluated from the unencapsulated TOC concentration in the supernatants. The concentration of unencapsulated TOC (C_t) was deter-

mined using UV Visible spectrophotometry at 291 nm. The GA loading capacity was calculated with Equation (1), while the TOC encapsulation efficiency was using Equation (2).

$$LC = (\frac{c_0 - c_t}{c_{GA}}) \times 100\% \quad (1)$$

$$EE = [1 - (\frac{c_t}{c_0})] \times 100\% \quad (2)$$

TOC rate of release (*RR*) evaluation

The TOC rate of release (*RR*) was evaluated by the TOC concentration released from GA during storage as follows: the GA residue obtained in the encapsulation procedure was dispersed in buffer solutions (1:5 w/v) and kept in closed bottle concealed by aluminum foil and placed in an incubator at 4 °C. The TOC released to the buffer solution was evaluated at 0,1,2,3 to 10 days. Each dispersion was homogenized under ultrasonic for 5 mins and followed by centrifugation at 4500 rpm for 15 mins. After the dispersion was settled down, the supernatant was separated from the GA residue, and the absorbance was analyzed at 291 nm. The procedure was repeated for every GA residue and pH.

RESULTS AND DISCUSSION

The encapsulation efficiency, rate of release as well as loading capacity were evaluated by TOC concentration in the supernatant. TOC concentrations were analysed using a standard TOC curve established in Figure 1.

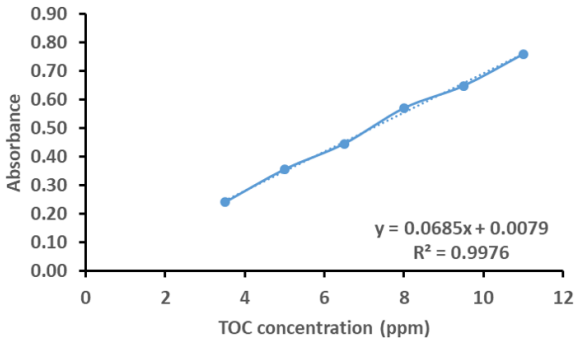

Figure 1: Standard curve of TOC

The TOC encapsulation efficiency, as well as GA loading capacity, were governed through the hydrophilicity properties of GA polymeric networks (Aliabadi *et al.*, 2007). Both of them were also affected by the interplay amongst the polymeric matrices and the TOC. The loading capacity (*LC*) is defined by the maximum concentration of TOC that can be borne by the GA polymeric matrices. Figure 2 displays that the *LC* was reducing as the concentration of GA increased at all pH environments. It was assumed that this behaviour is caused by steric barriers from the GA branch chain (Dragostin *et al.*, 2017) that disrupted the construction of supramolecular assemblies in the GA polymeric networks. This state of assemblies affected the interaction of the TOC with GA. In acidic conditions, the GA dispersibility decreased, thereby decreasing its solubility. The actions owed to the GA polyelectrolytic properties, in which the solution viscosity diminished in electrolytes settings as a result of the charges screening and low pH influence, which caused un-dissociation of the carboxylic group. The GA will be deposited, thereby weakening the interaction with the TOC and consequently leading to low loading capacity. The very acidic environment (pH<4) tend to provoke GA hydrolysis (Li *et al.*, 2015). In a basic environment (pH> 8), carboxylic groups will be entirely ionized to COO-. The charge formation creates repulsion forces amongst the GA acidic groups resulted in the destabilization of GA's 3D structures, hence reducing the loading capacity. It is bearing in mind the data in Figure 2, it was deduced that pH alterations had a great influence on the loading capacity.

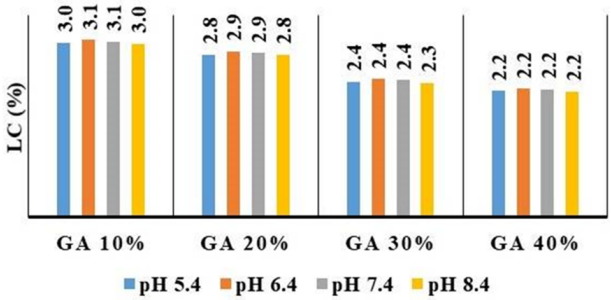

Figure 2: GA Loading Capacity (*LC*) of GA at various pH

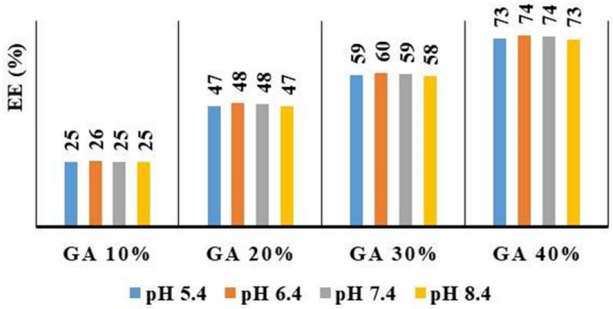

Figure 3: TOC Encapsulation Efficiency (*EE*) at various GA concentration and pH

Encapsulation Efficiency (*EE*) is another important factor besides the *LC* that should be explored in

Table 1: Rate of release (*RR*) at various GA concentrations and pH

	Average release rate (ppm/day)			
GA concentration	pH 8.4	pH 7.4	pH 6.4	pH 5.4
40%	1.34	1.07	1.06	1.35
30%	1.32	1.15	1.15	1.29
20%	1.29	1.09	1.05	1.27
10%	1.16	1.02	1.02	1.09

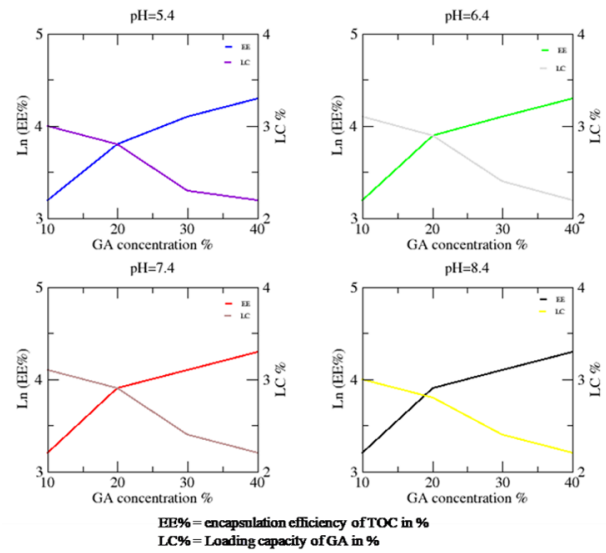

Figure 4: Association of GA Concentration and pH to TOC Encapsulation Efficiency(*EE*) and GA Loading Capacity (*LC*)

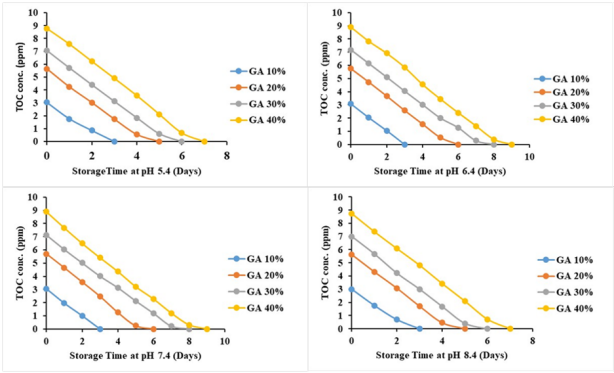

Figure 5: Release of TOC from various GA concentration and pH during 10 days of storage

the encapsulation process (Peng *et al.*, 2016). The *EE* indicates how much TOC has been captivated in these procedures. Figure 3 shows that the higher GA concentration, the higher the efficiency at the entirely pH settings. But, increasing the pH for all the GA concentrations did not affect significantly on the *EE*. The highest EE was ~ 74% at 40% GA and pH 6.4. Furthermore, Figure 3 revealed that the concentration of GA had a superior effect on the *EE* than the pH.

Based on the data in Figure 2 and Figure 3, the association of the GA concentration and the pH to the TOC encapsulation efficiency and the GA loading capacity could be defined, as in Figure 4. For convenience, the *LC* were displayed in logarithmic scale. The data showed that the behaviour of the TOC's *EE* and GA's *LC* both were alike for 10% to 40% concentrations of GA at the pH range of 5.4 to 8.4. While the *LC* decreased, the *EE* increased with an increase in the GA concentration at the entire pH level. Furthermore, the relationship disclosed that the optimum concentration of GA for generating a high *LC* and *EE* was 20% at dispersions condition of pH 6.4 and 7.4.

The study on the release of TOC from GA dispersion is displayed in Figure 5. Results showed that rising the GA concentration in the matrix served to extend the release time of the TOC at every pH levels. GA is a heteropolysaccharide polymer that included mostly 1,3-linked β-D-galactopyranosyl units as well as some of L-rhamnose, L-arabinose and D-glucuronic acid. The branch chains contain two to five 1,3-linked β-D-galactopyranosyl units attached to the polymeric backbone thru 1,6-linkages. Selected studies describe that GA consists of combinations between glycoproteins and polysaccharides. This caused the increased concentration of GA assisted to extend the release of TOC (Patel and Goyal, 2015). The complex structure of GA became more complicated when the concentration was increased. This was due to the interactions between the existing branch groups, which resulted in the prolonged release of TOC from the GA matrices.

The average rate of release (*RR*) calculated at various GA concentrations and pH levels are presented in Table 1. In neutral and weak acid environments, the GA leant to be more constant (Yao *et al.*, 2013). The acid groups, i.e. the (COOH) groups, were not ionized, and in the existence of hydrogen, some interactions were generated between the carboxylic groups (COOH) and the hydroxyl groups (-OH) from TOC. As a result, the release of TOC lasted longer. In alkaline conditions, where the carboxylic groups of GA were ionized to COO-, there was still some hydrogen interaction among GA matrix and TOC, but

it was weaker. This was the consequence of repulsion force between the TOC's ring portion and GA's COO- besides the repulsion of the acid groups from extra branches; hence, the TOC release happened at a faster rate. As the pH level approached neutral at 6.4 to 7.4, the release lasted up to 9 days. The average rate of release was 1.07 - 1.09 ppm/day. Meanwhile, in acid and basic environments of pH 5.4 and 8.4 respectively, the release was brief and only took 7 days. The average rate of release (RR) was 1.25 and 1.28 ppm/day. In view of the optimal *EE* and *LC* that were at GA concentration of 20% and pH levels 6.4 and 7.4, it can be established from Table 1 that the rate of release (*RR*) of the optimum encapsulation preparation was found to be around 1.05-1.09 ppm/day.

CONCLUSION

The key conditions for optimal TOC encapsulation in GA and loading capacity of GA for TOC were achieved at the GA concentration of 20% and pH levels 6.4-7.4. Under these settings, the encapsulation efficiency of TOC (*EE*) and the loading capacity of GA for TOC (*LC*) were 48% and 2.8%, respectively. The rate of release (*RR*) average value was 1.05-1.09 ppm/day. The research suggested that gum Arabic is a prospective substance to encapsulate alpha-tocopherol.

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