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Preparation and characterization of α-tocopherol nanocapsules based on gum Arabic-stabilized nanoemulsions

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Abstract The preparation of water dispersed α-tocopherol nanocapsules through solvent-displacement technique using gum Arabic (GA) as natural stabilizing and emulsifying biopolymer, for a first time was aimed in current research. The effects of GA concentrations on physicochemical and biological characteristics of prepared nanocapsules, namely, mean particle size, size distribution, zeta potential, rheological properties, turbidity, in vitro antioxidant activity and cellular uptake were evaluated, subsequently. The result indicated that the mono modal size distributed water dispersible α-tocopherol nanocapsules could be successfully attained using selected technique in sizes ranged from 10.01 to 171.2 nm and zeta potential of − 13.5 to − 47.8 mv. The prepared nanocapsules showed the dilatant rheological properties and acceptable radical scavenging (antioxidant activity). The cellular uptake of samples were increased up to 12 times more than microsized α-tocopherol. Consequently, the prepared water dispersed nanosized α-tocopherol can effectively be used in water based food and beverage formulations as nutrition enhancer or natural preservatives.

Keywords α-Tocopherol · Gum Arabic · Nanoencapsulation · Nanoemulsions · Stabilizer

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Introduction

Nanotechnology has been found various applications in aqueous based food, pharmaceutical and cosmetic systems, especially for their lipid-based active compound ingredients, such as α-tocopherol for resolving their low water-solubility or water-insolubility problems. Moreover, size reducing of these ingredients into nano-ranges can increase their cellular uptake and bio-availabilities, considerably (Tan and Nakajima, 2005). Thus, Incorporation of lipophilic antioxidants into edible nanosized delivery systems has developed into a main field in the food industries (Gomes et al., 2017).

Since the nano-encapsulation of lipophilic bioactive compounds can be gained in nanoemulsion systems, the nanoencapsulated-lipophilic active compounds known as nanoemulsions, mostly (Jo and Kwon, 2014; Trau and Renneberg, 2003). The main destabilizing mechanism of these nanoemulsions is Ostwald ripening which mostly occurs in heterogeneous dispersions with wide dispersed phase' size distribution (PDI), due to moving the smaller particles towards the larger ones and sticking over onto them. Therefore, the homogeneous systems in terms of their dispersed phase size distributions are less prone to Ostwald ripening destabilization phenomenon (Anarjan et al., 2014). The stabilization of nanoemulsions can be occurred either electrostatic or steric repulsions, in which the natural biopolymers such as proteins or polysaccharides can provides both for nanoemulsions (Anarjan and Tan, 2013a; 2013b). The previous researches reported that using the biopolymers as stabilized and emulsifier could improve the chemical stability of active compounds noticeably, compared to small molecular synthetic emulsifiers. They also found that the stabilizer nature and concentration can affect the nanoemulsions' mean particle sizes, size

distribution and chemical stabilities, considerably (Anarjan and Tan, 2013a; 2013b; Mao et al., 2009).

Gums are a large group of polysaccharides or protein-polysaccharide compounds with great ability in producing the most viscose products in less concentrations. The use of gums as stabilizers or emulsifies have been received the most attentions in recent researches, as they can produce the homogeneous nanoemulsions with acceptable less dispersed phase' particles sizes, as well as high chemical and physical stabilities (Anarjan and Tan, 2013a; 2013b). Then, they can provide the nanocapsules with the most desirable characteristics such as less mean particle size, narrow size distribution and the highest chemical and physical stabilities (Weissmueller et al., 2016).

Gum Arabic is the most widely used amphiphilic polysaccharide to stabilize beverage emulsions The emulsifying activity of gum Arabic at oil–water interfaces can be attributed to the hydrophobic protein fraction that is covalently linked to hydrophilic polysaccharide structures. The hydrophobic part anchors the gum Arabic molecules to the droplets surfaces, while the hydrophilic part provides stability against droplet aggregation through steric and electrostatic repulsion (Anarjan and Tan, 2013a; 2013b; Anarjan, et al., 2012).

While α-tocopherol was previously encapsulated in various nanoemulsion systems (Byun et al., 2011), using gum Arabic as stabilizer and emulsifier are limited. Thus, the aim of present study is the encapsulation of α-tocopherol in gum Arabic stabilized nanoemulsions via solvent displacement technique and the evaluation of used stabilizer concentration effects on characteristics of gained products.

α-Tocopherol nanocapsules could successfully be produced using gum Arabic as stabilizer and emulsifier via solvent-displacement technique with mean particle sizes ranged from 10 to 171 nm depending on used gum Arabic concentrations. The gum Arabic with polysaccharide-protein nature is absorbed to the lipophilic droplets of α-tocopherol, as the protein part of gum Arabic acts as emulsifier, and its polysaccharide part stabilizes the newly produced nanoparticles via steric stabilization mechanism (İbanoğlu, 2002). Thus, the existence of protein-polysaccharide complex in the gum Arabic creates longer and more appropriate stability of the lattice structure through the repelling forces of the electrostatic charges extant on the molecules' surfaces, as well as the steric keeping away activities (Anarjan and Tan, 2013a; 2013b; İbanoğlu, 2002).

Materials and methods

Materials

α-Tocopherol was purchased from Merck Co. (Darmstadt, Germany). Gum Arabic and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), were supplied from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). HT-29 (HTB38, human colon carcinoma cell lines) and modified McCoy's 5a medium (ATCC 30-2007) were acquired from the American Type Culture Collection (ATCC, Manassas, VA). Penicillin, streptomycin, fetal bovine serum (FBS), and trypsin 0.25% were obtained from Grand Island Biological Company (GIBCO, Grand Island, NY). Phosphate-buffered saline (PBS) was acquired from Sigma (St. Louis, MO). All analytical grade solvents were sourced from Dr. Mojallali (Dr. Mojallali Chemical Complex Co. Tehran, Iran). All chemicals were used without further purification.

Preparation of water-dispersed nano-encapsulated α-tocopherol

The nano-encapsulated α-tocopherols were prepared according to Anarjan and Tan (2013a) with some modifications. Gum Arabic was gradually dissolved in 50 ml of double distilled water in various concentrations (0.05, 0.1 and 0.15%), under magnetic stirring for 24 h in order to completely hydration, and 0.05 g α-tocopherol was also dissolved in 10 ml acetone and was slowly added to gum Arabic solution under stirring by a high-speed homogenizer (SilentCrusher M, Germany), rotating at 10,000 rpm, using a syringe pump in volume rate of 2 ml/min. The acetone was then removed from system in a vacuum rotary evaporator (Laborota 4002-Digital) at 80 rpm, 50 °C and 30 min. The samples were volume up to 50 mL by addition of deionized water. The ratio of solvent to aqueous phases was set at 1:5 for all samples.

Analytical methods

Particle size, polydispersity (PDI) and zeta potential measurements

Mean particle size, PDI and zeta potential of the samples were determined based on dynamic light scattering, in a Zetasizer (Nano-ZS, UK), 1 day after the samples preparation. All samples were diluted (1:10) with double distilled water to prevent the effects of multiple scattering. The measurements' temperature was set at 25 °C. The dynamic light scattering technique measures the time-dependent oscillation of scattered light in dispersed nanoparticles due to Brownian movement, which depends

on their dimensions. PDI, changes from 0 to 1, is a dimensionless guesstimate that describes the homogeneity of nanocapsules, in which the smaller values correspond to a narrower and finer particle size distribution. The zeta-potential determinations were performed by the measurement of the electrophoretic mobility distribution of particles using the laser Doppler velocity technique on undiluted samples after sample preparation. The zeta potential was calculated using the Smoluchowski equation from the measured velocity values (Anarjan et al., 2012; Yin et al., 2009). The results were reported as the mean of three reading for each replications of samples.

Turbidity measurement

The absorbance of all the samples were measured as proposed by Anarjan et al. (2014) at a wavelength of 600 nm, using an UV–visible Spectrophotometer (Model Ultraspec 2000, made in England), 1 day after sample preparation. Double distilled water was used as blank sample. All measurements were replicated.

Rheological measurements

Dynamic shear rheological properties, namely, storage modulus (G′), loss modulus (G″), and complex viscosity (η*) of the samples were evaluated at room temperature using an Anton Paar Physica rheometre (MCR301, Austria) equipped with a concentric cylinder measurement system with a radius ratio of 1.0846. 1 mL of homogeneous sample, was poured into the central cylinder of the device and the cylinder was returned back to its place. Then, the probe was inserted into the central cylinder containing the sample and the analyses were ordered to the device via the related software. The shear tension and viscosity were measured as a function of the shear velocity (0.1–100 1/s) for the determination of the flow behavior of the samples and eventually the flow behavior of the samples was determined. The reliability test was carried out in a shear range from 0 to 100 1/s (Anarjan and Tan, 2013a; 2013b).

Determination of α-tocopherol concentration

Quantitative measurement of a-tocopherol concentration was performed using a high performance liquid chromatography (HPLC, CT- 10A VP, Shimadzu, Kyoto, Japan), equipped with an SPD-10AV UV–Vis detector, a LC-10AT pump system, a CT0-10A oven, at 295 nm and a Nova-Pak C18 (3.9 9 300 mm) Waters HPLC column, after complete extraction of α-tocopherol by hexane. An isocratic mobile phase of methanol: water (99:1 v/v) at 1.0 mL/min, was used in this measurements. The oven temperature was set at 40 °C. The calibration of the peak area versus α-tocopherol concentration was linear in the concentration range of 50–500 mg/L. The results were expressed in mg/L (Anarjan et al., 2014). The measurements were replicated for all samples.

α-Tocopherol cellular uptake (an in vitro assay)

The uptake of prepared nano-encapsulated α-tocopherol, by human colon carcinoma HT-29 cell line, as model human colon epithelial cells, was evaluated for estimation the samples' bioavailabilities. The cells were seeded in McCoy's 5a medium, containing 1% (v/v) penicillin, 10% (v/v) FBS, at 37 °C and atmosphere of 95% air and 5% CO_2. 3 days after seeding process, the cells were washed by PBS and incubated with supplemented- McCoy's 5a medium with 10 mL α-tocopherol nanocapsules. After extra 48 h incubation of cells with the supplemented culture medium, in mentioned condition, the cells were again washed by PBS, 3 times and detached by trypsin, and re-suspended in 10 mL of culture media. An aliquot of this cell suspension was used for the determination of the cell number. The remaining cell suspension was added to 2 mL of water/ethanol (1:1, v/v) mixture (Anarjan et al., 2012). The α-tocopherol of the rest cell suspensions was extracted using hexane/methanol (1:1, v/v), and quantified by HPLC, as mentioned in previous section. All measurements were replicated for each samples.

Antioxidant activity measurement

The DPPH stable radical was used for in vitro antioxidant activity determination of samples. The DPPH solution (0.1 mM) was prepared via solving 3.9 mg DPPH in 100 ml methanol and used as the blank sample. The solution was kept for 30 min in the dark in order to reaction to be completed. 2 ml of the samples was added into 2 ml of DPPH solution and was incubated in the dark. After 30 min, the absorbance of each solution was determined at 517 nm. Methanol solvent was used as blank (Anarjan et al., 2012). All measurements were performed twice to establish their correctness.

The antioxidant activity was obtained using Eq. 1:

$$\text{antioxidant activity} = \left(\left(A_{\text{blank}} - A_{\text{sample}}\right)/A_{\text{blank}}\right) \times 100 \quad (1)$$

where, A_{blank} is the light absorption by the blank sample and A_{sample} is the light absorption by the samples.

Statistical analysis

The physicochemical and biological characteristics of prepared nanocapsules were subjected to various one-way

variance analyses (one-way ANOVA) using the Minitab v. 16 Statistical Package (Minitab Inc, State College, PA). All experiments and measurements were performed in duplicate, and Tukey's multiple comparison test was used for the determination of significant differences ($P < 0.05$) between the responses.

Results and discussion

Particle sizes and particle size distribution

The size characteristics of gained nanoparticles were shown in Table 1 and also Fig. 1.

The results indicated that the mean particle sizes of produced nanoparticles raised with increasing of the gum Arabic concentrations. Moreover, the homogeneity of system decreased considerably at high gum Arabic concentration uses. Thus, the mono-modal system changed to multi-modal ones by increasing the stabilizer concentrations. According to the particle sizes obtained for the samples, it can be concluded that the minimum sized nanoparticle can be produced at the lowest concentration of the gum Arabic uses.

The particle size growth with gum Arabic concentrations can be related to the formation of surrounded thicker gum Arabic layer around the lipophilic active compound (α-tocopherol) during stabilization step of nanoparticles, since more stabilizer molecules are available at higher concentrations of gum Arabic uses. However, more increasing of stabilizer molecules led to formation of stabilizer micelles (without α-tocopherol core) which would possess less mean sizes as seen in Fig. 1 for the nano-capsules produced at the highest concentrations (the picks with smaller sizes) (Anarjan and Tan, 2013a; 2013b; Tan et al., 2016). Furthermore, due to uneven distribution of stabilizer onto the nanoparticles at higher stabilizer uses, the size of obtained nano-capsules varied considerably, leading to producing the heterogeneous dispersion systems from the particle sizes view point (Anarjan and Tan, 2013a; 2013b; Mao et al., 2009; Shu et al., 2018).

Generally, water-dispersed nanocapsules with smaller sizes are more favorable because these particles hold higher solubility and greater bioavailability (Anarjan et al., 2014). However, the heterogeneous colloids are less

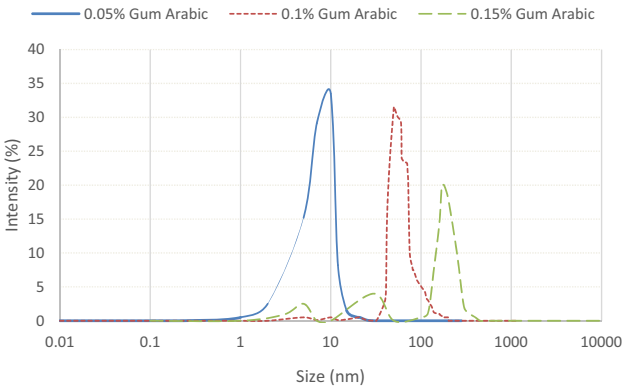

Fig. 1 Size distribution of nano-encapsulated α-tocopherols

physical stable, since it is more prone to Ostwald ripening phenomena. Thus, the other reason why the nanoparticles have undergone such an increase in size, pertains to the presence of solvent impurities in the samples and the size growth though this ripening Phenomenon (Anarjan et al., 2013).

Most of previous researches concluded the optimum concentrations for used emulsifier molecules leading to producing the lipid based organic compound nanoparticles with the least particle sizes, in which increasing the emulsifier up to certain level decreased the mean particle sizes of gained nanoparticles, while further increase of emulsifier content affects the particle size of nanoparticles inversely (Anarjan et al., 2013; Tan and Nakajima, 2005). Therefore, increasing of the size of nanoparticles with emulsifier concentration, or its decreasing, can be observed in various studies based on selected concentrations for emulsifiers (Anarjan et al., 2013; Shu et al., 2018; Tan et al., 2016).

PDI and zeta potential

As mentioned before, the colloidal systems with smaller PDI values has more uniform nanoparticles. Beside the operation criteria, the dispersion systems with smaller PDI are more desired due to their more physical stabilities and less susceptibilities to Ostwald ripening instability mechanism (Anarjan et al., 2013, 2014). The PDI of prepared α-tocopherol nanocapsules are shown in Table 1.

The results revealed that the PDI of samples raised by increasing the natural emulsifier concentrations. Therefore,

Table 1 The mean particle size, size distribution, PDI and zeta potential nanoencapsulated α-tocopherols

Gum Arabic concentration (%)	0.05	0.1	0.15
Mean particle size (nm)	10.01 ± 2.50^{a}	49.46 ± 3.1^{b}	171.2 ± 9.50^{c}
Particle size distribution (nm)	1.712 ± 0.90^{a}	10.37 ± 2.10^{b}	37.35 ± 5.80^{c}
Polydispersity Index (PDI)	0.403 ± 0.009^{c}	0.525 ± 0.011^{b}	0.634 ± 0.008^{a}
Zeta potential (mv)	-13.5 ± 2.5^{c}	-34.4 ± 3.3^{b}	-47.8 ± 2.6^{a}

the samples became heterogeneous and consequently, physically unstable by increasing the gum Arabic concentrations. The probably created micelles in excess concentrations of emulsifier molecules can causes the observed size heterogeneity of nanoparticles in high emulsifier concentration uses (Anarjan et al., 2013). Like mean particle sizes, various decreasing or increasing trend for PDI of dispersion systems, by emulsifier concentrations have been reported in previous researches, based on either working range of emulsifier concentration or nature of emulsifier, and an optimum level for concentration have been resulted for each emulsifier, in order to get the most homogenous water dispersed organic nanoparticles (Anarjan et al., 2013; Mao et al., 2009; Tan and Nakajima, 2005).

Zeta potential is the charge at particles mobile surface and is used to determine the degree of flocculation or deflocculation in nanosystems. The greater zeta potential net values, either positive or negative, corresponds to the more stable nanoparticles stabilized through electrostatic mechanism (Anarjan et al., 2012; Yin et al., 2009). The electrostatic repulsion between particles with the same electric charge prevents the aggregation of the particles. It was reported that the nanoparticles with net zeta potential values greater than 25 would have higher physical stabilities (Yin et al., 2009). Therefore, zeta potential computation is an appropriate method for predicting the colloid systems' physical stabilities. Zeta potential is a function of biopolymer's surface charge and the environmental nature and the absorbed layers of the surface on which α-tocopherol has been dispersed. With the increase of the surface charge, the interaction between the α-tocopherol and the biopolymer increases and, as a result, the bioactive compound is better delivered (McClements, 2013).

In this study the gum Arabic nanoparticles were negatively charged, due to the high molecular weight and the large number of free hydroxyl groups in the structure of gum Arabic; when it is dispersed in water (Delahaije et al., 2015).

Gum Arabic consists of branched arabinogalactan blocks attached to a polypeptide backbone. This polypeptide backbone (protein structure) significantly increases the hydrophilicity and surface activity of gum Arabic (Phillips and Phillips, 2011; Ribeiro et al., 2016). The interfacial membrane produced by gum Arabic should provide good stability against droplet aggregation and coalescence in prepared α-tocopherol nanoparticles. According to zeta potential values of samples (Table 2), the electrostatic repulsion contributed to the stabilizing mechanism. It can also been seen that the zeta potential values increases with the increase of the gum Arabic concentration in such a manner that it increases from − 13.5 mv in the sample having the lowest amount of gum Arabic to − 47.8 mv in the sample having the highest amount of gum Arabic. Thus, particles' charge density intensifies with the increase of the gum Arabic concentration and the electrostatic stabilization of nanoparticles increases, subsequently.

Turbidity

The concentration, size and the relative refractive index of the particles are among the most affective factors on the colloidal nanoencapsulated lipid based system turbidity, as an increase in each mentioned parameters can enhance the system turbidity. In water-dispersed nanoparticles, the turbidity is a useful index for estimation of their stabilities, in which an increase in system turbidity can be related to the particle size growth as well as active compound destruction (Anarjan et al., 2014; Jafari et al., 2017; McClements, 2013; Yuan et al., 2008). The results turbidity measurement data were summarized in Table 2.

The results indicate that the turbidity has increased by increasing of the particle sizes, due to rise of emulsifier concentration, which was completely expected. Increase in the turbidity can be attributed to the increase in the particle sizes and the existence of either the light scattering free α-tocopherol droplets in the aqueous environment, or the acetone in the aqueous phase which increases the system refractive index (Mirhosseini et al., 2008). These results agreed to the Yang and McClements (2013) researches in which they produced the nanoencapsulated vitamin E using a natural gum as food-grade emulsifier isolated from the *Quillaja saponaria* Molina tree. They concluded that increasing the emulsifier caused a reduction in the droplets' size and decreased the turbidity.

Rheological properties

Figure 2 depicts the relationship between the complex viscosity and the angular frequency of the prepared various nanoencapsulated α-tocopherols. As it can be observed, the complex's viscosity was decreased with reducing of gum Arabic concentration.

Rheological properties are the most important qualitative feature of the liquid systems and they are closely correlated with the structural attributes. The type and the intensity of the interaction between the particles, the particles size and distribution and the properties of the massed particles are just some parameters influencing the rheological characteristics. The reduction in the particle size is usually followed by an increase in the viscosity. However, besides increasing the size, the coagulation of the particles in the dispersion can cause an increase in the viscosity in a certain fraction volume. The thickness and the structure of the film formed by the emulsifier can also be effective on the dispersion viscosity on the surface layer (Mozafari et al., 2008). With the increase in the concentration of the

Table 2 The turbidity variations of water dispersed nanoencapsulated α-tocopherols during 2-week storage at ambient condition

Storage time (day)	Gum Arabic concentration (%)		
	0.05	0.10	0.15
2	0.008 ± 0.0009^{aA}	0.019 ± 0.004^{bB}	0.022 ± 0.003^{cC}
6	0.007 ± 0.0002^{aA}	0.015 ± 0.0009^{bB}	0.024 ± 0.005^{cC}
10	0.007 ± 0.0002^{aA}	0.012 ± 0.001^{bB}	0.016 ± 0.005^{cB}
14	0.006 ± 0.0009^{aA}	0.010 ± 0.0003^{bB}	0.013 ± 0.009^{cB}

$^{a–c}$ are letters denoting the significant difference ($P < 0.05$) of values in columns

$^{A–C}$ are letters denoting the significant difference ($P < 0.05$) of values in rows

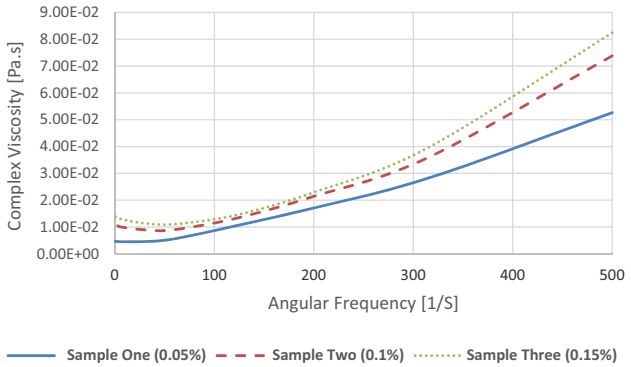

Fig. 2 Rheological characteristics of nanoencapsulated α-tocopherols

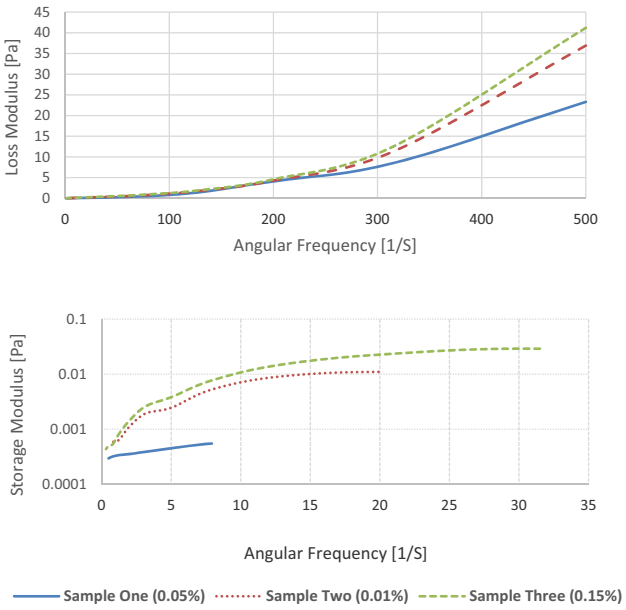

Fig. 3 The loss and storage modulus of nanoencapsulated α-tocopherols

particles, the dispersed system's viscosity also increases. Encapsulated α-tocopherol that possesses a lower viscosity is appropriate for food systems using presenting a low viscosity like in drinks (Derkach, 2009; Teixeira et al., 2017). The results indicated that the decrease in gum Arabic concentration is accompanied by a reduction in the complex viscosity in the entire samples. The complex viscosity is a complex modulus to complex frequency ratio and it is a scale indicating the total hardness of the object. The increase in gum Arabic concentration can influence the particles massing as well as the continuous phase viscosity thereby to exert an effect on the total viscosity of the particles' colloid system. On the other hand, there is always a direct and nonlinear relationship between the solutes concentration and viscosity in certain temperatures and the increase in the concentration of the dissolved substance led to an increase in the viscosity. In higher concentrations of the gum Arabic, the massing mechanism due to the formation of the binding bridge between the particles can be intensified as a result of which the particles' massing, and subsequently, the viscosity will be increased (Derkach, 2009; Kaur et al., 2017). Therefore, the encapsulated α-tocopherol in the sample prepared using less emulsifier concentrations, having less viscosity, and is appropriate for less viscose food formulation uses such as beverages.

Figure 3 displays the relationship between G′ (storage modulus), and G″ (loss modulus), respectively, with angular frequency. If the G′ (storage modulus) is found larger than G″ (loss modulus), the elastic behavior will prevail to viscous behavior (gel property) and vice versa. Results indicated that increasing of the stabilizer concentration augmented both G′ and G″, in which the G″ growth was higher than G′. Therefore, the viscous behavior prevailed to elastic behavior, giving more liquid characteristics to samples. The third sample, with the highest gum Arabic concentration, in contrast to the other ones, exhibited more viscous texture, due to its larger G′ compared to G″. Moreover, according to Fig. 3, this sample had higher ability for storing energy in elastic transformation. The previous researches' results shown that both G′ (storage modulus) and G″ (loss modulus) increase with the increase of angular frequency and they are nearly dependent on frequency. In less angular frequencies, the dispersions demonstrate a liquid-like behavior in which the loss modulus has been larger than storage modulus. The gradual increase observed in G′ and G″ values has been due to the

increase of used polysaccharide based stabilizer molecular weight (Hou et al., 2012). According to Fig. 3, the samples' loss modulus, were also increased by adding up the stabilizer in samples. Furthermore, all three samples possessed non-Newtonian behavior as dilatant fluids.

Cellular uptake

The in vitro cellular uptake of gained either encapsulated nano-sized or bulk micro-sized α-tocopherol by HT-29 intestinal cells were also investigated to make an overall estimation of their bioavailabilities. The results of the samples' cellular uptake have been summarized in Table 3. Generally, the cellular uptake reduces with increase of stabilizer concentration, due to its fiber composition that prevents perfect uptake of lipidic substances. Thus, the fiber-like nature of the gum Arabic, restrains the α-tocopherol cellular uptake. Unlike the proteins, α-tocopherol injection in filamentous stabilizers, causes a decrease in their cellular uptake. These findings are well in compliance to the results reported by previous researches (Ribeiro et al., 2006). An overall comparison of the α-tocopherol uptake by nanoparticles and macro-sized α-tocopherol, as well, indicates that in the best case, the nanoencapsulated α-tocopherol cellular uptake improved about 12 times, as compared to macro-sized ones, and this is a very promising finding in nutritional terms.

Previous researches have resulted that there is an optimum range for uptake in the particle sizes ranging from 10 to 50 nm and the uptake rate reduces in any domain above or below this range. Moreover, it was shown that the uptake rate is proportional to the particle sizes, indirectly (Anarjan et al., 2011). Possibly, the nanoparticles with smaller sizes are in a more efficient surficial interaction with the cell membranes in contrast to the larger nanoparticles. The particles with sizes larger than 1 μm have been taken via other endocytosis mechanisms like liquid phase pericytosis. Since the smaller size particles can improve the effectiveness of the particle-based oral pharmaceutical systems, the nanoparticles with smaller sizes can definitely improve the cellular uptake and they are widely applicable in chemotherapy uses (Anarjan et al., 2011; Lu et al., 2017; Ribeiro et al., 2006). According to the results obtained from the particle size and cellular uptake and considering the numerous studies performed in this regard, it can be concluded that the samples prepared with less stabilizer concentrations, are in optimum uptake range.

DPPH antioxidant activity

The scavenging ability of prepared nanoencapsulated α-tocopherol against DPPH radicals were also studied as an index of their antioxidant activities in cells. Antioxidant activity of nanoemulsions would be associated to their ability in scavenging of free radical species such as superoxide anion radical ($O_2^{\cdot-}$) or free radicals hydroxyl ($OH^{\cdot}$). The results were shown in Table 3. The antioxidant activity of samples increases with the upturn of gum Arabic. With the increase of gum Arabic concentration, the ability to donate electron to the free radicals increases and therefore the controlling power intensifies and the free radicals chain of reactions cease. It seems that beside the α-tocopherol, GA have also efficient capacity for deactivation of excited electronic states and radicals. There is increasing experimental evidence that associate the antioxidant function with its protein fraction, mainly by amino acid residues such as histidine, tyrosine and lysine, which are generally considered as antioxidants molecules (Park et al., 2005). Therefore, the third sample, with the highest gum Arabic content, has antioxidant property larger than the others.

Thus, in the present study, the water-dispersed nanocapsules of α-tocopherol were successfully produced via solvent displacement method, using gum Arabic. The nanocapsules were produced with mean particle size ranged from 10.01 to 171.2 nm, in which increasing the stabilizer concentration, led to an increase in the mean particle size, size distribution, PDI, zeta potential, turbidity, chemical instability, viscosity, storage and loss modules,

Table 3 The Cellular uptake and radical scavenging ability of nanoencapsulated α-tocopherols

Samples name	Gum Arabic concentration (%)	Cellular uptake(fmol/cell)	Radical inhibition percentage
1	0.05	1392 ± 367.2^{a}	65.10 ± 1.3^{c}
2	0.1	1327 ± 217.2^{a}	84.05 ± 1.6^{b}
3	0.15	1159 ± 168^{b}	92.67 ± 1.5^{a}
α-tocopherol (macro-size)	–	113 ± 20.7^{c}	–

[a–c]Are letters denoting the significant difference ($P < 0.05$) between the data of the column (each response)

and a decrease in either cellular uptake or antioxidant activity. Thus, the stabilizer concentration exerts a very important effect on the physicochemical and biological properties of the encapsulated nano-sized lipophilic bioactive compounds, such as α-tocopherol. The gained water dispersed nanoencapsulated α-tocopherol, showed considerable higher stability, cellular uptake and antioxidant activity, consequently, can effectively be utilized as additive in water-based food and pharmaceutical formulations as a valuable preservation and nutritional substance.

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