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Evaluation of the antioxidant capacity of gallic acid encapsulated in liposomes

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ABSTRACT

The protection and improvement of bioavailability of phenolic compounds is a problem that can be solved by various methods including the use of liposomes. The aim of this study was to determine the effect of gallic acid concentration on the entrapment efficiency by liposomes and to determine their antioxidant capacity. Liposomes were produced at three different concentrations of gallic acid (10, 100 1000μg/mL), gallic acid was determined using the Folin- Ciocalteu method (Previous, samples were separated chromatographically), the antioxidant capacity was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) method and the determination of the empirical constant of breakdown chain speed and efficiency of entrapment of gallic acid. The results obtained indicate that the highest content of gallic acid was found in liposomes with 100μg/mL, also the increased efficiency of entrapment was associated with this concentration (98%). The ability of free radical trapping showed no difference between the liposomes with 100 and 1000μg/mL. The higher speed of chain breakage was found in the liposomes at 1000μg/mL. Results indicate that it is possible to obtain liposomes with gallic acid at very high efficiency.

KEYWORDS

Gallic acid;
Antioxidants;
Liposome;
Chain breaking rate.

INTRODUCTION

The oxidation phenomena induced by reactive oxygen species (ROS) and superoxide free radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). The action of ROS is counteracted by an antioxidant defense system; an imbalance between ROS and antioxidant compounds may result in a disintegration of the membrane cellular, damage to membrane proteins and DNA mutation, which may in turn initiate or propagate the development of many diseases such as cancer, liver damage, cardiovascular problems, aging, diabetes and atherosclerosis[6,14].

One way to prevent the above problems is to block the generation of free radicals. In this regard, recently, there is increased interest in the use of dietary antioxidants, specifically; phenolic compounds[11]. Which have been documented as highly effective protective agents against various chronic degenerative diseases. It has been postulated that the biological activity associated

with the phenolic compounds is associated, at least in part with its antioxidant properties, due to the ability to chelate metals and trap free radicals.

However, one problem with the phenolic compounds is their bioavailability, which, at least for green tea is quite low, less than 5%[9]. Then this compound must be used repeatedly and continuously to maintain an effective level in the human body. These problems can be solved using various protection schemes. Few protection schemes have been associated with phenolic compounds. Maeyama et al.,(2005) developed a delivery system based on biodegradable polyesters and protected(-)-epigallocatechin-3-gallate[8]. Fang et al.,(2005) evaluated a system based on liposomes for controlled release of catechins to the skin[3,4].

Liposomes are microscopic vesicles formed regularly by phospholipids, aqueous medium with a variable size between 50 and 500nm[16]. They offer several advantages as vehicles for the controlled release of active compounds such as polyphenols. Typical scheme is that the active compound to pass through a passive bodies to reach the target tissue. In this regard, liposomes did not release their contents until their membranes are destabilized by an external agent, which can be change in pH, metal ions, temperature, light, enzymes and conformational changes[2].

However in the literature, our knowledge there is no reports on the effect of the concentration of polyphenols of the liposomes. The aim of this study was to evaluate the effect of gallic acid concentration on the physicochemical characteristics, entrapment efficiency and the antioxidant capacity of liposomes.

MATERIALS AND METHODS

Materials

Lecithin (Solae, St Louis, MO., USA), Span 80, gallic acid, methanol, Folin reagent, sodium carbonate, DPPH (Sigma, St. Louis, MO, USA).

Preparation of liposomes

Solutions phosphatidylcholine (5% v/v) were dissolved in a solution of chloroform: methanol (1:1). The organic solvent was evaporated in a rotating evaporator at 39-40°C, traces of solvent were removed by maintaining the lipid film to vacuum for 1 hour (Büchi R-205). The lipid film obtained was then hydrated with deionized water which was included with gallic acid at concentrations of 10, 100 and 1000μg/mL.

To determine the hydration efficiency an experimental run was carried out according to the method of[3,4].

Determination of total phenols

The gallic acid content was determined by Folin-Ciocalteu method using gallic acid as standard, briefly, samples of 125μL were added to 500μL of water and 125μL of Folin-Ciocalteu reagent (2N). The mixture was stirred vigorously and left to stand for 6 min, subsequently were add 1.25mL of a 7% solution of sodium carbonate and 1mL of water and stirred and let stand for 90 min. Absorbance was read at 760 nm using a UV-Vis Cary 50 Bio (Varian, Palo Alto, CA, USA). The total phenol content was expressed as gallic acid equivalent (GAE: gallic acid mg/mL sample)[1]. All tests were performed in duplicate.

Efficiency of entrapment of gallic acid

The encapsulation efficiency of gallic acid in liposomes was determined by the total phenol content. The total phenols were evaluated in gallic acid loaded liposomes, before and after chemical partition with chloroform:methanol solution (1:1). The fractions were separated by size exclusion chromatography. Glass columns were used 15×1 cm, packed with Sephadex G-25 and using a phosphate buffer solution (pH 7.4) as mobile phase. Subsequently the fractions were centrifuged at 4500 rpm for 10 min in a Heraeus centrifuge Labofuge 400R (Thermo Electron Corporation, Germany). The fractions were analyzed by the Folin-Ciocalteu method to determine the percentage of entrapment, which was constructed as follows:

Encapsulation Efficiency (%)=[(Concentration of gallic acid in the sample-the concentration of gallic acid in methanol)/Concentration of gallic acid in the sample]×100[3,4].

Free radical scavenging

To evaluate the antioxidant capacity, we used the following procedure. Samples of 100μL were added to 2.9mL of a solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in methanol (concentration of DPPH was 0.2mM). The mixture was stirred vigorously and read the absorbance of the resulting solution at 515 nm in a

UV-vis spectrophotometer (Cary 50 Bio UV-visible, Varian, Palo Alto, CA, USA).

Chain breaking rate

The determination of the rate of chain breaking(k) was obtained from the methodology proposed by[10], where the k value was calculated from obtaining a third-order kinetic model, as shown in the following formula:

$$1/A^3 - 1/Ao^3 = -3kT(2)$$

where A and absorbance at time t and Ao is the absorbance at the initial time and k is the empirical constant speed of radical breaking chain.

Statistical analysis

All experiments were performed at least twice. The data were analyzed by ANOVA (analysis of variance) and Tukey test, (p=0.05). (Statistica v 4.3, StatSoft Inc., Tulsa, OK, USA).

RESULTS

The conditions used for the development of stable liposomes were 5% lecithin, 20% of aqueous phase and the use of a pH of 2. From the above conditions was performed kinetic analysis of the evolving structure of liposomes, for 20 hours, taking pictures every hour. The results are shown in Figure 1.

The results of total phenol content in liposomes are shown in TABLE 1 for the different fractions collected (name directly in the TABLE).

The results of efficiency are shown in TABLE 2 showed a marked effect of concentration on efficiency.

TABLE 3 presents the results of the percentage of free radical scavenging activity (% RSA) by the method of DPPH*(2,2-diphenil-1-picrylhydrazyl) using for comparison the effect of gallic acid at a concentration of 100μ/mL (81.34±5.52%) (At 60 min).

TABLE 3 presents the results from samples of liposomes called directly (without partition, without chromatographic separation) and the major fraction with chromatographic separation and partition.

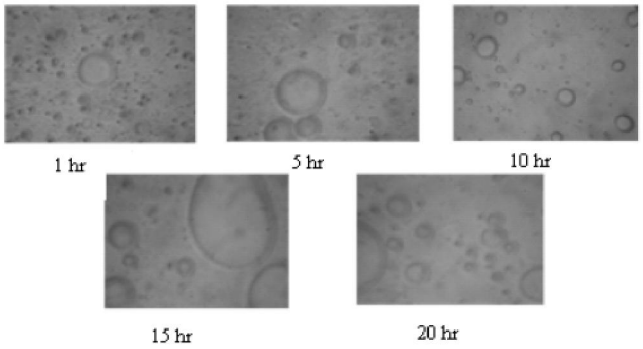

Figure 2 : Microphotographs of liposomes (100x)

TABLE 1 : Total phenols content in direct and with partition fractions from liposomes at 10, 100 and 1000 of μg of gallic acid/mL

	Fraction	Liposomes 10μg of gallic acid/mL	Liposomes 100μg of Gallic acid/mL	Liposomes 1000μg of Gallic acid/mL
Direct	Fraction1	ND	ND	ND
	Fraction2	ND	63.1467±26.7352	38.9881±14.0723
	Fraction3	ND	25.1022±5.6465	35.9539±7.1208
	Fraction4	ND	19.2333±5.7446	14.8110±5.8892
	Fraction5	ND	9.6942±2.1256	5.1525±1.8428
	Fraction6	ND	8.5969±1.7391	15.9450±5.5265
Partition	Fraction1	ND	9.0850±2.1708	ND
	Fraction2	ND	7.7834±3.9362	12.7334±1.3198
	Fraction3	ND	11.1200±0.0000	15.4934±4.1330
	Fraction4	ND	4.6768±2.4844	1.8500±0.0000
	Fraction5	ND	ND	0.1800±0.0282
	Fraction6	ND	4.1334±0.0000	3.1250±0.0000

***data shown are mean±standar deviation**
****ND No detectable**

TABLE 2 : Efficiency of encapsulation of gallic acid (%)

Fraction	Efficiency (%)		
	Liposome (10μ/mL)	Liposome (100μ/mL)	Liposome (1000μ/mL)
Fraction1	ND	ND	ND
Fraction2	ND	55.363	2.625
Fraction3	ND	14.556	1.296
Fraction4	ND	14.556	1.296
Fraction5	ND	9.694	0.497
Fraction6	ND	4.464	1.282

***C No determinated**

TABLE 3 : Radical scavenging activity (RSA) (%) and chain breaking rate (k) for gallic acid encapsulated in liposomes

Sample	% RSA (60 min)		
	Liposome (10μg/mL)	Liposome (100μg/mL)	Liposome (1000μg/mL)
Gallic acid as control(100 ppm)	-	81.344±5.522	-
Direct	42.545±6.744	39.907±2.909	40.378±8.296
Partition	1.659±0.000	10.882±1.523	8.676±0.631
K(O.D.-3/min.mg m.s.)	0.017±0.001	0.052±0.010	0.174±0.000

***Data shown are mean±standard deviation**

DISCUSSION

Results obtained from micrographs showed that the major structural feature liposomes (lipid bilayer thickness, uniformity of size, well defined spherical shape) were obtained between 10 and 12 hours of hydration (Figure 2). This result is agreeing with reports about the hydration time for liposome[12].

It was determinated amount of phenolic compounds trapped in liposomes. From the three concentrations of gallic acid used (10, 100 and 1000μg/mL) were obtained 6 fractions after partition, each one was subjected to a partition with a solution of chloroform-methanol. According to the partition coefficient of gallic acid (0.45)[7], when the partition is done, gallic acid tends to go to the polar portion (methanol) of the solution, site where absorbance readings were obtained.

Where a strong presence of total phenols was seen in fractions 2 and 3 at 100 and 1000μg/mL. The remaining fractions of these concentrations showed very small values of gallic acid. For the concentration of 10μg/mL gave absorbance readings of total phenols values not detectable.

The fractions analyzed after the partition (partition in TABLE 1) showed lower values than those obtained in the direct fraction. The resulting values are interesting, since it is necessary to remember that the liposome solution has concentration of 10, 100 and 1000μg/mL and 10μg/mL but was not possible to obtain readings above partly due to the small amount of gallic acid incorporated in liposomes and the possible interference of lecithin in reading.

The liposome solution has two main components, phosphatidylcholine and watery solution of gallic acid, then the readings should be associated with the presence of gallic acid and/or possible interference of lecithin, it was decided to evaluate the lecithin under the Folin-Ciocalteu protocol, giving an equation $Y=0.005X+8.72$ ($R^2=0.987$) validates a lecithin concentration range 1000 to 5000μg/mL, where Y, is the reading of EAG and Xeq , is the concentration of lecithin in μg/mL, with this data readings were corrected total phenols.

Several authors indicate that the concentration of the aqueous phase in the formulation of a liposome has a great influence on the percentage of entrapment reached[3-5,15] report that an increase in water content can cause an upset in the micellar structure, on the contrary, if the concentration of lecithin increases with respect to the micelle water passes from a spherical shape to a cylindrical shape, but if it continues to increase the concentration of lecithin cylindrical micelles acquire a form similar to the lattices observed in semi-dilute solutions or concentrated polymer[13]. Then it must be a balance between the amount of water and the amount of lecithin development of liposomes and obtaining a good percentage of entrapment. In this regard the liposomes with 10μg/mL of gallic acid showed the highest amount of water that caused a much more disordered system by the amount of water and there was no entrapment of gallic acid. In the stage of hydration, excess water caused the phenolic compound remained in the continuous phase without arriving at the site of liposome entrapment as his presence was not detected.

Liposomes with 100μg/mL of gallic acid showed the best percentages of entrapment in the different fractions collected, adding all fractions as a whole is bumping up to a 98.06% efficiency. Moreover liposomes with 1000μg/mL of gallic acid showed a very low efficiency; together have only a 7.75% of entrapment. At this concentration the amount of water was 10 times lower than that used for the formulation of liposomes to 100μg/mL, which could reduce the mobility of gallic acid.

The results of % RSA for direct samples showed no statistically significant differences between them ($p>0.05$). The partition fraction showed a value of RSA% very low compared to direct samples, which is understandable since the partition fraction is diluted three times with respect to the direct sample.

The greatest value of k was found in the lowest concentration, while the lowest value of k was found associated with the highest concentration (1000μg/mL), showing a linear behavior between the value of k and the concentration of gallic acid used. In its pure form gallic acid has a k value of 2.57±0.10 (D.-3/min.mg ms) to 50.94±5.76μg/mL (IC 50). In any case, the samples reached this value, but there be emphasized that the present concentration, even at 1000μg/mL solutions of liposomes reached only 10μg/mL of gallic acid.

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CONCLUSIONS

Lecithin liposomes with gallic acid showed an efficiency of up to 98% in terms of total phenols entrapment. There is a marked influence of phenol concentration on efficiency, finding the best results at 100μg/mL, with respect to % ARL no statistical differences were observed. K showed a positive linear relationship (R^2>0,987) between the rate of chain breaking and gallic acid concentration used.

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