Original Research Article

Optimization of Green Tea Catechin Loaded Niosomes by Thin Film Hydration Technique using Food Grade Surfactants

ABSTRACT

Aims: Catechins, belonging to polyphenols, have received a great attention because of their health benefits. But they are met with limited efficacy in food applications due to several reasons such as poor aqueous solubility, poor stability, and low bioavailability in GI conditions.

Study design: Nanoencapsulation in the form of niosomes is expected to provide efficient delivery of these bioactive components.

Duration of study: The study was conducted in Dairy Chemistry Division, ICAR-National Dairy Research Institute underNational Agricultural Science Fund (NASF) project between June 2018 and March 2021.

Methodology: Theprocessing parameters for preparation of catechin loaded niosomes were optimized. Different parameters such as type and concentration of non-ionic surfactant (NIS), stabilizer and ratio of NIS:stabilizerwere optimized to prepare catechin loaded niosomes by Thin Film Hydration Technique using Food Grade Surfactants.

Results: Among all the optimized formulations, the ones prepared with Tween 60 and lauryl alcohol (1.5:1) and Tween 80 and lauryl alcohol (1:0.5) at 250 µM concentration by thin film hydration and high shear homogenization at a speed of 15000 rpm for 15 min, showed desired particle size of 58.48 and 60.69 nm and entrapment efficiency of 85.82 and 85.69%, respectively. They also exhibited uniform size distribution and stability as measured by polydispersity index and zeta potential.SEM micrographs also confirmed the formation of catechin loaded niosomes.

Conclusion: It is concluded that 250 µM concentration of NIS, T60 or T80 and lauryl alcohol as stabilizer at a ratio of 1.5:1 and 1:0.5, respectively by thin film hydration and high shear homogenization at 15,000 rpm for 15 min was the optimized formulation for preparation of catechin loaded niosomes.

Keywords: Catechins; Entrapment efficiency; Thin film hydration; Non ionic surfactants, stabilizers

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1. INTRODUCTION

Green tea has attracted the consumer's interest due to its therapeutic benefits against a wide variety of disorders, ranging from antiobesity to anticancer. Several scientific reports showed that green tea exhibits antiobesity, antioxidant,anticancer and other biological and pharmacological functions, hence making them an excellent source for food and nutraceutical applications. The health promoting properties of green tea are mainly due to their polyphenol content; about 60–80% of polyphenols are catechins [1].However, their food application is limited due to their low bioavailability, poor water solubility and stability under gastrointestinal conditions. Therefore, to alleviate these limitations, catechins could be nanoencapsulated in the form of niosomes to improve their bioavailability.

Niosomes are closed bilayer vesicles formed by self assembly of non ionic surfactants and stabilizers in aqueous media. The most common factors that affect the physico-chemical properties of niosomes include method of preparation, type and concentration of non ionic surfactants, stabilizers, hydration medium and nature of the bioactive compound being encapsulated[2].Catechins are slightly soluble in water; among the catechins Epigallocatechin gallate is highly hydrophilic in nature[3]. Among the various non ionic surfactants used for the preparation of niosomes, Tweens in combination with cholesterol as stabilizer at 1:1 ratio were reported to show higher encapsulation efficiency for water- soluble compounds[4]. Hence, Tween 60 and Tween 80 were choosen as non ionic surfactants as they were reported to provide high encapsulation efficiency for hydrophilic compounds. Cholesterol is a common additive used to prevent leakiness and stabilize bilayer vesicles. It was reported that the stable niosomes can be prepared using fatty alcohols instead of cholesterol to avoid gel-liquid phase transition of niosomes[5]. Use of cholesterol in functional foods may not be preferred because of its reported adverse health effects such as increased risk of cardiovascular diseases and cancer[6]. Therefore, in this study cholesterol was substituted with fatty alcohols such as lauryl and cetyl alcohol and the niosomes thus obtained were compared with the ones stabilized using cholesterol. Based on the preliminary studies and literature reports, the various parameters such as the speed of homogenization, stabilizers, type and concentration of NIS and ratio of NIS and stabilizers were optimized for the preparation of catechin loaded niosomes.

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2. Materials and Methods

Green tea catechins (>97% HPLC), Tween 60 (T60), Tween 80 (T80), lauryl alcohol, cetyl alcohol and cholesterol,Dihexadecyl phosphate (DCP), ethanol, methanol and phosphate buffered saline(PBS) were procured from Sigma-Aldrich Chemicals Co, (St. Louis, MO, USA). All the reagents were freshly prepared before analysis. Milli-Q water and double distilled water were used wherever necessary.

For the optimization of preparation of niosomes, the following parameters were studied:

1. Speed (5000, 10000 and 15000 rpm) of high shear homogenization
2. Type of stabilizer (lauryl alcohol andcetyl alcohol)
3. Type of non-ionic surfactants (Tween 60 and Tween 80)
4. Concentration of non-ionic surfactant (150, 200 and 250 µM) and
5. Ratio of non-ionic surfactant and stabilizer (1:0.5, 1:1 and 1.5:1)

2.1 Preparation of catechin loaded niosomes by thin film hydration and high shear homogenization method

Niosomes were prepared by thin film hydration andhigh shear homogenization method, the flow diagram of catechin loaded niosomes wasshown in Fig. 1.The amount of catechin used for formulation was kept constant 25 mg/100 mL. Amount of Dihexadecyl phosphate (DCP) was added at 5 mg to 100 mL of ethanol. The blank niosomes were also prepared using the same method without incorporation of catechin. During the whole preparation process, working solutions were protected from exposure to the light.

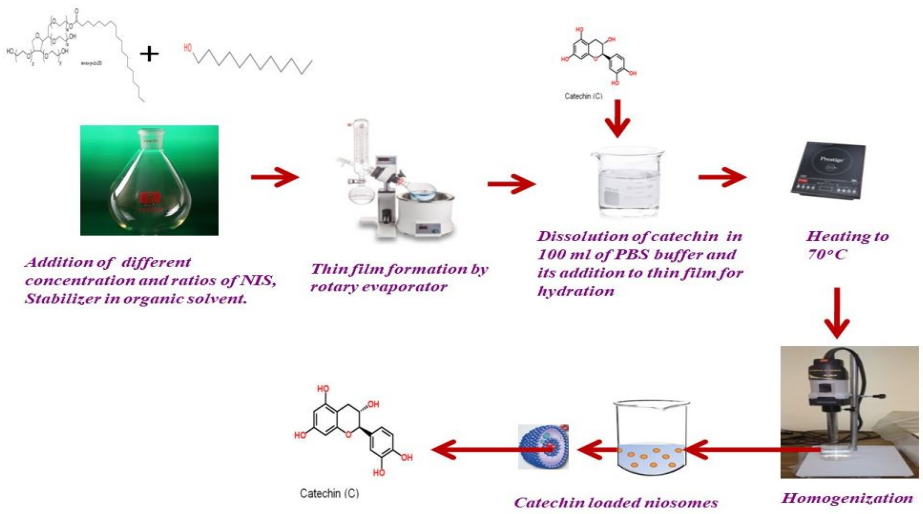

Fig.1. Preparation of catechin loaded niosomes by thin film hydration and high shear homogenization method.

The processing parameters were optimized based upon the vesicle size and encapsulation efficiency (%) of the niosomes.

2.1.1 Mean hydrodynamic diameter and polydispersity index

Dynamic light scattering (DLS) is the most common measurement technique to analyze size of the nanoparticles. The hydrodynamic diameter can be determined by measuring the random changes in the intensity of light scattered from a suspension or solution under the Brownian motion to obtain broadness of the hydrodynamic size distribution. The mean hydrodynamic diameter (particle size) and polydispersity index (PDI) of the catechin loaded niosomes weredetermined by dynamic light scattering (DLS) on a Litesizer™ 500 (Anton paar, GmbH, Pvt. Ltd., Austria). The niosome sample solution (1 mL) was diluted to 10 mL with Milli Q-water and homogenized for 1 min to get homogenous suspension. The samples were transferred to the cuvette and measured thrice at 25°C and at a back scattering angle of 173°.

2.1.2 Zeta potential

Zeta potential of particles is typically measured by phase analysis light scattering using Litesizer™ 500 (Anton paar, GmbH, Pvt. Ltd., Austria). The niosome samples were analyzed for zeta potential value to determine the stability of the formulation. The analysis was carried out by diluting the sample (1 mL) with Milli Q-water (10 mL) as dispersion medium and measured thrice at a temperature of 25°C.

2.1.3 Encapsulation efficiency

The encapsulated catechin was separated from free catechin by dialysis method reported by Sravani *et al*. 2018 with minor modifications. Three mL of catechin niosomal suspension was loaded into 12 kDa Mw cutoff dialysis membrane bag (HiMedia, Pvt. Ltd., Mumbai, India), clipped at both ends and dialyzed for 2 h using dialysate medium with constant stirring. Later, the permeate was collected as free catechin and determined the concentration by measuring the absorbance at 273 nm using Hitachi UV-VIS

spectrophotometer (UH5300, Hitachi High Technologies Corporation, Japan). The concentration of free catechin present in the samples was calculated using standard linear curve prepared at different concentration of standard catechin (0-100 µg/mL). The encapsulation efficiency (EE) was calculated by the following formula:

$$Entrapment\ efficiency\ (\%) = \frac{C_T - C_F}{C_T} X100$$

Where C_T is total amount of catechin taken in the dialysis bag and C_F is free or unentrapped catechin in the permeate.

2.1.4 Scanning electron microscopy

The surface morphology of the niosomes was studied by field emission scanning electron microscope (FESEM) (Zeiss Ultra 55 with patented GEMINI column technology, Carl Zeiss AG, GmbH, Oberkochen, Germany).

3. Statistical analysis

All experiments were performed at least in triplicate, and the results were summarized as mean values ± standard deviation. The experimental data on particle size, PDI values, zeta potential and encapsulation efficiency were subjected to one-way ANOVA or two-way ANOVA using SPSS 23.0 software.

4. Results and Discussion

4.1 Optimization of speed of high shear homogenization and stabilizers

High shear homogenization (HSH) is an important step in the preparation of niosomes, initially the speed of the homogenization, using three speeds viz., 5000, 10000 and 15000 rpm for 15 min was optimized. It is a process of reducing the particle size by applying the shear stress. Decreasing the particle size increases the solubility of bioactive and hence, improves its bioavailability [7]. T60 at 150 µM concentration and lauryl alcohol (LA) or cetyl alcohol (CA) at a ratio of 1:1, DCP @ 5 mg to induce charge on the niosomes and catechin @ 25 mg/100 mL were taken in a round bottom flask and the contents were subjected to rotary evaporation and followed by HSH at different speeds. The niosomal formulations were characterized for particle size, polydispersity index, zeta potential and encapsulation efficiency and the data are presented in Table 1. Particle size is a very critical attribute, which affects the stability, encapsulation efficiency and release profile of bioactive. The results have shown that the particle size decreased significantly ($p<0.05$) from 125.32to 87.86 nm, 502.83 to 449.86 nm, respectively when lauryl and cetyl alcohol were used as stabilizers with increasing speed from 5000 to 15000 rpm for 15 min. The reduction of particle size with increasing homogenization speed might be due to the applied shear stress, turbulence and ripple effects, which broke up the vesicles effectively and thus, produced lower particle size. Therefore, the homogenization speed of 15,000 rpm was choosen for further studies. Similar results of reduction in mean vesicle size of resveratrol loaded niosomes using stearic acid as stabilizer with increasing speed of homogenization [8].

Polydispersity index (PDI) is a parameter to describe the size distribution of nanoparticles. The narrow size distribution of niosomal vesicles exhibits high stability due to Brownian motion of nanoparticles[9]. Lower PDI of 22 to 26%, obtained for the niosomes prepred with lauryl alcohol which

indicates theuniform distribution of particles and better stability. The speed of homogenization had no significant ($p<0.05$) effect on PDI.

Zeta potential (ZP) is another important parameter to reflect the physico-chemical property and storage stability of nanoparticles in aqueous media. The surface charge of nanoparticles and binding type between additives used for formulation determines the rate of release and encapsulation efficiency of bioactives[10].Zeta potential of lauryl and cetyl alcohol catechin loaded niosomes increased from -21.0 to -25.33 mVand -16.23 to -24.53mV, respectively with increasing speed from 5000 to 15000 rpm. But no significant ($p<0.05$) difference was observed between 5000 and 10000 rpm, whereas significant difference was observed with 15000 rpm when compared with the other two speeds.

Encapsulation efficiency (EE) also increased with increasing speed from 67.33 to 79.66% for lauryl alcohol from 5000 to 15000 rpm. A good correlation was observed between homogenization speed and vesicular size and EE; higher the speed, smaller the particle size and higher the encapsulation efficiency for catechin loaded niosomes. At higher homogenization speed due to more turbulence and ripple effects,the particle size was reduced.

Table 1. Optimization of homogenization time on catechin loaded niosomes prepared with lauryl alcohol or cetyl alcohol

HSH (15000 rpm) / min	Parameter	Blank-LA*	Catechin-LA*	Blank-CA*	Catechin-CA*
5000	Mean hydrodynamic diameter (nm)	125.32±2.58	113.13±2.43^{aA}	510.34±5.21	502.83±6.67^{aB}
10000		107.07±3.23	98.66±2.94^{bA}	491.89±4.16	460.66±3.10^{bB}
15000		87.86±6.30	73.15±2.61^{cA}	382.69±7.25	449.86±3.76^{cB}
5000	PDI (%)	26.54±1.94	22.53±1.05^{aA}	28.47±1.34	43.30±3.40^{aB}
10000		24.27±1.41	23.30±0.79^{aA}	27.67±1.54	41.80±2.81^{bB}
15000		22.56±2.53	26.23±2.21^{aA}	24.98±1.35	30.20±1.99^{cB}
5000	Zeta potential (mV)	-19.65±1.85	-21.0±1.60^{aA}	-17.94±1.94	-16.23±1.32^{aB}
10000		-20.70±1.55	-23.21±1.36^{aA}	-18.28±1.05	-18.30±1.10^{bB}
15000		-25.00±0.26	-25.33±1.72^{aA}	-24.92±1.89	-24.53±1.30^{cB}
5000	Encapsulation efficiency (%)	-	67.33±2.08^{aA}	-	Non homogeneous
10000		-	75.66±2.51^{bA}	-	Non homogeneous
15000		-	79.66±4.04^{bA}	-	70.00±2.00^{cB}

*****Results are expressed as mean± SD, η=4. All the formulations were prepared by using Tween 60 as NIS, LA or CA as stabilizer - 150 µM concentration in 1:1 ratio. Values within a column with different superscripts (a, b, c) and row (A, B) differ significantly ($p<0.05$).

Further, use of lauryl alcohol showed lower particle size and appeared to be a better stabilizer than cetyl alcohol. This could be due to the fact that longer alkyl chain fatty alcohols have more hydrogen bonds and charges, which may be responsible for bigger vesicle size. Catechin niosomes prepared with cetyl alcohol showed more viscous and non homogeneous suspension at lower rpm as shown in Fig. 2, whereas at 15000 rpm, the EE was 70%. The type of stabilizer and additives used for preparation of niosomes would

also affect the vesicle size and encapsulation efficiency [11]. This study showed that homogenization speed of 15,000 rpm and lauryl alcohol are preferable to prepare catechin loaded niosomes with lower particle size.

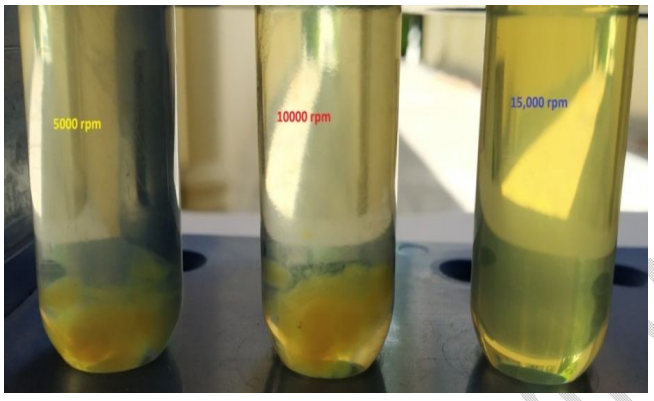

Fig.2. Effect of homogenization speed on catechin loaded niosomes prepared with cetyl alcohol

4.1.1Scanning electron microscopy and Transmission electron microscopy

Morphological characterization of blank and catechin loaded niosomes prepared using T60 using lauryl alcohol ancetyl alcohol was carried out using scanning electron microscope and the images are shown in Fig. 3 The SEM images showed that the vesicles were monodispersed exhibiting spherical structure and smooth shape, and narrow particle size distribution; the surface was apparently free from visible pores and cracks. Mean particle size as obtained in SEM analysis of catechin niosomes prepared using T60 using lauryl alcohol showed smaller size of 58 nm when compared with niosomes prepared with T60 using cetyl alcohol (450 nm), which could be due to relatively high viscosity of cetyl alcohol than lauryl alcohol. These results correlated well with the results obtained by DLS measurements.

4. 2Optimization of type and concentration of non ionic surfactants (T60/ T80)

In another trial, two non-ionic surfactants namely T60 or T80 @ 150, 200 and 250 µM concentrations were used to prepare niosomes. Zidovudine niosomes with various concentrations of 30, 50, 60, 70, 90, and 120 µM of Tween 60 or Tween 80, and cholesterol using thin film hydration method and concluded that stable vesicles were not formed with these concentrations and crystal like structure was observed [12]. Therefore, 150 to 250 µM of T60 and T80 concentration was chosen for this study. In this trial, non ionic surfactant and lauryl alcohol were kept at a ratio of 1:1.

From the data in Table2, it may be observed that the mean particle size of catechin niosomes reduced significantly ($p<0.05$) with increase in concentration of non-ionic surfactants from 150 to 250 µM; 74.15 to 60.67 and 74.73 to 64.71 nm for T60 and T80, respectively. The increase in concentration of NIS reduced the particle size; this could be attributed to increased adsorption of non ionic surfactant molecules to lipid and aqueous interface, which leads to decrease in interfacial tension, and thus forms lower particle size or it might be due to higher level of surfactant molecules, which diffused from lipid to aqueous phase, thereby produced the smaller particle size[13].The particle size of vitamin E loaded nanoemulsion decreased with increase in concentration from 2.5 to 10% of Tween 20, 40, 60 and 80, whereas increasing the concentration further increased the particle size due to highly viscous nature of Tweens and it is more difficult to

spontaneous breakup of oil to water interface[14]. EGCG niosomes prepared using 200 µM of Tween 60 and cholesterol at 1:0.25 molar ratio by thin film hydration and ethanol injection method showed the particle size was ~100 and 60 nm, respectively[15].

Table 2. Optimization of concentration on catechin loaded niosomes prepared with Tween 60/ Tween 80 and lauryl alcohol as stabilizer

Concentration (µM)	Parameter	Blank-T60*	Catechin-T60*	Blank-T80*	Catechin-T80*
150	Mean	91.86±5.23	$74.15±1.58^{aA}$	80.13±5.81	$74.73±1.49^{aA}$
200	hydrodynamic	82.39±6.49	$71.14±2.96^{bA}$	85.39±3.40	$71.38±3.69^{bA}$
250	diameter (nm)	71.86±3.44	$60.67±5.02^{cA}$	88.61±1.38	$64.71±4.74^{cB}$
150	PDI (%)	23.19±3.23	$28.26±0.72^{aA}$	23.16±2.65	$25.11±1.88^{aB}$
200		21.56±2.04	$17.0±1.49^{bA}$	23.25±0.75	$18.11±2.00^{bB}$
250		18.59±2.42	$14.3±4.48^{cA}$	19.41±1.15	$15.10±2.10^{cA}$
150	Zeta potential	-24.99±0.26	$-23.7±1.82^{aA}$	-22±3.00	$-21.08±2.28^{aB}$
200	(mV)	-24.27±1.25	$-23.53±0.58^{aA}$	-24.63±1.65	$-21.70±2.30^{aB}$
250		-28.23±2.92	$-26.40±2.00^{aA}$	-26.53±2.38	$-22.63±3.15^{aB}$
150	Encapsulation	-	$80.31±5.14^{aA}$	-	$75.33±3.51^{aB}$
200	efficiency (%)	-	$86.00±3.60^{bA}$	-	$85.40±3.66^{bA}$
250		-	$86.33±1.52^{bA}$	-	$85.99±1.00^{bA}$

*****Results are expressed as mean± SD, η=3.All the formulations were prepared with NIS, stabilizer in 1:1 ratio. Values within a column with different superscripts (a, b, c) and row (A, B) differ significantly ($p<0.05$).

Niosomes prepared using T60 showed significantly lower particle size than T80 with respect to all the concentrations used for formulation. Particle size and zeta potential of catechin loaded niosomes prepared with T60 and lauryl alcohol analyzed by DLS are depicted in Fig. 3. Both T60 and T80 have similar polar head groups and alkyl chain length (C18), which accounts for almost the same HLB value. Size of the vesicles depends upon length of the alkyl chain of non ionic surfactants[16]. The formation of bilayer vesicles depends highly upon the hydrophilic–lipophilic balance of the surfactant, the chemical structure of the components and the critical packing parameter. The possible reason for lower particle size of catechin niosomes with T60 is due to its high hydrophilic head moiety with a HLB of 14.9. T80 with HLB of 15 and one double bond, which is equivalent to removal of $-CH_2$ group from the saturated alkyl chain has an effect on critical micelle concentration.

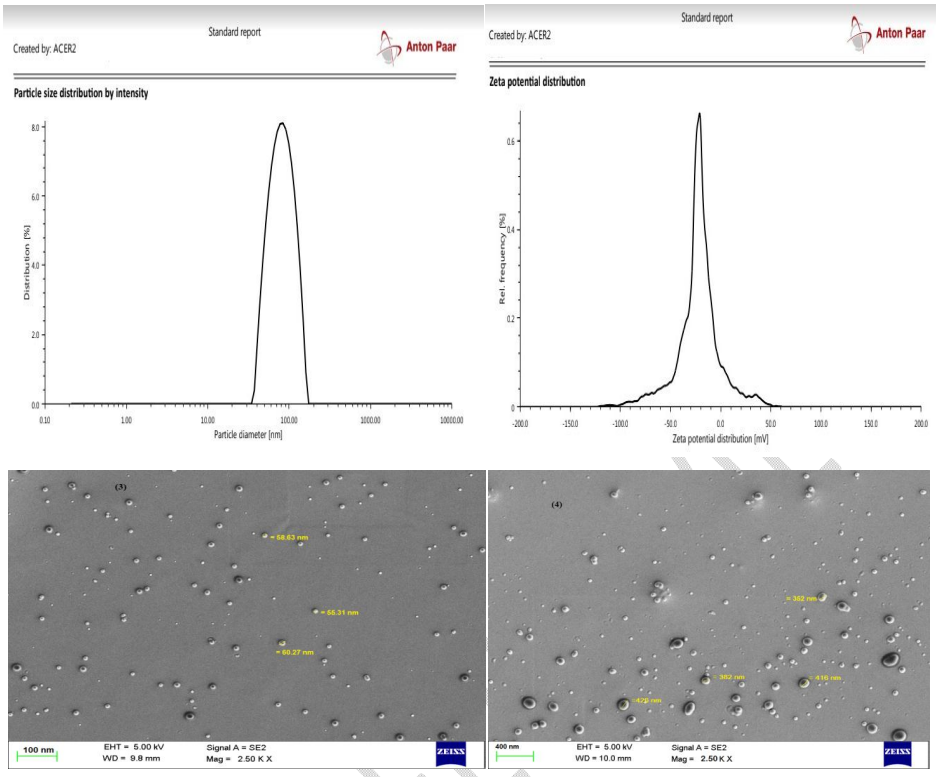

Fig. 3. Images of (1) particle size and (2) zeta potential by DLS (3) T60 and lauryl alcohol and (4) T60 and cetyl alcohol by SEM of catechin loaded niosomes.

Interestingly, there is no strong correlation observed between particle size and HLB value. Almost the same HLB value is a rough guide often used for selecting surfactants. The molecular geometry of non ionic surfactants plays an important role for determining the functional performance. The non polar tails in T60 are saturated, whereas T80 are unsaturated and hence more kinked[17]. This suggests that vesicle size increases with increase in hydrophilicity. T60 can create hydrophilic environment between niosomal surfaces and hydrating medium. Hence, the hydrated niosomes require low energy for uniform dispersion throughout aqueous medium, which is an important requirement for efficient homogenization. Due to its large hydrophilicity, it slightly reduces critical packing parameter (geometry of vesicle formed by non ionic surfactant), which may produce more spherical vesicles and consequently decrease the vesicle size to minimal and decreases the curvature of bilayer vesicles and consequently, the size of vesicles[18].The zidovudine niosomes was optimized with different non-ionic surfactants namely, Tween 20, 40, 60 and 80, and cholesterol as stabilizer with addition of DCP using thin film hydration method and concluded that vesicle size formulated with T80 (2.66 µM) was slightly larger than that using T60 (2.48 µM)[12].

T60 or T80 had no effect on PDI values and narrow size distribution was observed with both the surfactants, which indicates that the size of vesicles was relatively homogeneous. However, the PDI value decreased significantly ($p<0.05$) ranging about 25 to 15% with increase in concentration of NIS. The results

showed that zeta potential of all the catechin niosome samples was negative. Inclusion of dihexadecyl phosphate, an anionic species, also had effect on vesicle size and zeta potential and it imparted negative potential on the catechin niosomes. The negative charge on the catechin niosomes may be due to the interaction among T60 or T80, lauryl alcohol, DCP and catechins. Niosomes prepared with T60 showed higher zeta potential (-26.40 mV) than those prepared with T80 (-22.63 mV), whereas the concentration had no significant ($p<0.05$) effect. Higher zeta potential possesses greater stability to nanoparticles by decreasing the vander Wall forces, which are responsible for aggregation of particles. The inclusion of DCP reduced the vesicle size and increased the zeta potential of liposomal encapsulation of (+)-catechin. Zeta potential of starch based nanovesicles of catechins prepared using horse chestnut catechin, water chestnut catechin and lotus stem catechin showed −18.05, −21.5 and −18.05 mV, respectively[19].

It is evident from the Table 2,when the concentration of T60 or T80 increased from 150 to 250 µM, the EE increased from 80 to 86% and 75 to 85%, respectively. Catechin or EGCG niosomes prepared with Span 60 using cholesterol by thin film hydration and showed an encapsulation efficiency of 49.48 and 53.05%, respectively[20]. These findings are consistent with the previous studies of Ruckmani and Sankar (2010), who reported that higher EE was observed with lower HLB value of non ionic surfactant T60 (82.4%) than T80 (79.5 %). EE also depends upon the properties of bioactive compound, NIS and also its interaction between bilayers. T60 and T80 are hydrophilic non ionic surfactants, which can easily interact with hydroxyl group and gallolyl moiety of hydrophilic catechin, mainly EGCG and thus favours high encapsulationefficiency[15].

Among the different concentrations, viz.,150, 200 and 250 µM of T60 or T80, used for formulation of catechin niosomes, it was observed that 250 µM concentration of T60 or T80 had shown lowest particle size and highest EE than other concentrations. Therefore, 250 µM concentration of T60 or T80 was used for further studies.

4.3Effect of ratio of NIS: stabilizer on catechin loaded niosomes

In order to establish the ratio of NIS and stabilizer the preliminary studies were conducted using different ratios of non ionic surfactant T60 or T80, at 250 µM concentration and and stabilizer lauryl alcohol were selected in 1:0.5, 1:1 and 1.5:1 to prepare catechin loaded niosomes by thin film hydration and high shear homogenization. It may be observed from the data given in Table 3 that with respect to T60, a surfactant : stabilizer ratio of 1:0.5 produced niosomes with higher size (67.12 nm) than the ratio of 1:1 (66.60 nm) and1.5:1 (58.48 nm), the differences being statistically significant. Increase in T60 beyond certain concentration with a low amount of stabilizer may increase particle size. Whereas, in case of T80, no significant difference ($p<0.05$) was observed in the sizes, which were 60.69 and 60.59 nm, when NIS:stabilizer ratio was 1:0.5 and 1:1, respectively. Increase in ratio to 1.5:1 increased the vesicle size to 66.75 nm, which might be attributed to higher viscosity of T80 at this concentration when compared to the other ratios. Similar results were found by preparation of nanoencapsulated green tea catechins using zein with different core to wall ratios 1:0.5, 1:10. 1:50 by electrospraying technique reported that lower ratio 1:0.5 nanoencapsulates possessed lower particle size due to relatively low viscosity values[21]. PDI% and zeta potential showed no significant ($p<0.05$) difference in catechin niosomes prepared with different ratios of T60 and lauryl alcohol. However, the significant difference was observed with 1:0.5 ratio of T80 and lauryl alcohol than other ratios.

The encapsulation efficiency of catechins prepared used T60 was 87.32, 86.28 and 85.82%, and for Tween 80, it was 85.69, 85.59 and 85.63% for 1:0.5, 1:1 and 1.5: 1 ratios, respectively. Changes in the ratio of Tween 60 and lauryl alcohol have brought a marginal change in encapsulation efficiency. Increasing the ratio had no significant ($p<0.05$) effect on EE in niosomes prepared with T80, whereas in case of T60 it decreased. This might be due to the level of surfactant to lipid ratio to prepare niosome is generally 10-30 mM.Increasing the ratio of NIS increases the amount of encapsulation, but for highly viscous NIS, the level of NIS: lipid ratio is too high and therefore, increasing the ratio may decrease the % of EE[21]. Nanoencapsulation of catechins with zein as wall material using electrospraying method showed significantly ($p<0.05$) higher encapsulation efficiency for 1:50 (95.27%) core to wall ratio followed by 1:10 (92.75%) and 1:05 (89.96%) samples [22]. Hence, it was observed that increased ratio of T60 and lauryl alcohol decreased the particle size and EE, whereas using T80 showed no difference for 1:0.5 and 1:1 but increased particle size at 1.5:1.

Table 3. Optimization of ratio of NIS and stabilizer of catechin loaded niosomes prepared with Tween 60/ Tween 80 and lauryl alcohol

Ratio of NIS : stabilizer	Parameter	Blank-T60*	Catechin-T60*	Blank-T80*	Catechin-T80*
1:0.5	Mean	98.86±2.41	67.12±7.1^{aA}	77.55±5.26	60.69±0.08^{aB}
1:1	hydrodynamic	78.11±5.49	66.60±8.24^{aA}	88.62±1.38	60.59±4.76^{aB}
1.5:1	diameter (nm)	77.98±4.31	58.48±8.39^{bA}	102.29±4.21	66.75±3.23^{bB}
1:0.5	PDI (%)	21.96±2.19	24.29±3.15^{aA}	19.38±1.04	23.08±3.75^{abA}
1:1		19.66±1.51	24.03±4.48^{aA}	19.41±1.16	19.06±2.10^{aB}
1.5:1		17.05±2.12	13.83±6.43^{bA}	23.62±1.80	18.10±2.26^{bB}
1:0.5	Zeta potential	-20.89±2.12	-26.17±1.45^{aA}	-24.98±1.93	-24.15±3.2^{aB}
1:1	(mV)	-27.50±3.71	-26.39±2.86^{aA}	-25.31±3.55	-22.48±3.15^{bB}
1.5:1		-26.49±0.75	-29.66±3.05^{aA}	-18.83±1.48	-22.09±1.95^{bB}
1:0.5	Encapsulation	-	87.32±1.35^{bA}	-	85.69±1.1^{aB}
1:1	efficiency (%)	-	86.28±1.7^{aA}	-	85.59±1.0^{aA}
1.5:1		-	85.82±1.85^{aA}	-	85.63±0.41^{aA}

*****Results are expressed as mean± SD, η=3.All the formulations were prepared with NIS @ 250 µM concentration. Values within a column with different superscripts (a, b, c) and row (A, B) differ significantly ($p<0.05$)

5. Conclusion

The particle size and EE strongly depends upon the type of surfactants and NIS to stabilizer ratio. Among the different parameters such as homogenization speed viz; 5000, 10000 and 15,000, stabilizers lauryl alcohol and cetyl alcohol, two non ionic surfactants such as T60 or T80, with different concentrations such as 150, 200 and 250 µM NIS, NIS: stabilizer ratios such as 1:0.5, 1:1 and 1.5:1, for preparation of catechin loaded niosomes. It is concluded that 250 µM concentration of NIS, T60 or T80 and lauryl alcohol as stabilizer at a ratio of 1.5:1 and 1:0.5, respectively by thin film hydration and high shear homogenization at 15,000 rpm for 15 min was the optimized formulation for preparation of catechin loaded niosomes. The optimized formulation can be utilized for fortification of foods.

CONSENT

The manuscript does contain any individual person's data in any form (including any individual details, images or videos).

ETHICAL APPROVAL

Manuscript does not involve any biological studies, the use of any animal or human data or tissue "Not applicable".

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Comment [AM5]:

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