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Optimization of the colloidal properties of chitosan nanoparticles encapsulating alpha-arbutin

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ABSTRACT

Melasma is a highly prevalent skin disorder in which patients exhibit hyperpigmented patches on the face. This provokes the essentiality to search for effective treatment strategies, among which are the topical nanosystems. The current study aimed to optimize the prepared chitosan nanoparticles (CSNPs) to enhance the topical delivery as well as the therapeutic potential of alpha-arbutin (α-arbutin), being employed as a skin whitener for melasma treatment. Drug-free nanoparticles were prepared using chitosan polymer and the polyionic tripolyphosphate Sodium salt (TPP) employing the ionic gelation technique. The colloidal properties regarding particle size (P.S), polydispersity index (PDI), and zeta potential (ζ-potential) were evaluated either without adjusting the pH of chitosan or TPP solutions and after its adjustment. The optimized nanoparticles were selected for drug loading. Results revealed that only the TPP concentration had a significant effect on the P.S of drug-free nanoparticles, in which upon increasing its concentration from 0.02 to 0.1%, P.S decreased significantly. Also, only chitosan concentration affected the EE% of the loaded nanoparticles, in which the increase in chitosan concentration from 0.10 to 0.20% was coupled with a significant increase in EE%, however further increase in its concentration from 0.20 to 0.30% resulted in a significant decrease in EE%. All formulations exhibited sufficiently positive ζ-potential values ranging from +37.30 to +42.90 mV. The optimization of the nanoparticles revealed that the P.S of CSNPs decreased significantly upon adjusting the pH of both chitosan and TPP solutions. Loading α-arbutin into chitosan solution resulted in significantly higher EE% compared to its loading into TPP solution. Hence, the proper optimization of CSNPs enhanced their colloidal properties and consequently the topical therapeutic potential of α-arbutin.

Keywords: *alpha-arbutin; chitosan nanoparticles; cosmeceuticals; melisma; skin.*

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1. INTRODUCTION

Melasma is defined as a common, acquired, benign chronic hyper melanosis, being attributed to the hyperactivity of melanocytes on different face regions, such as the forehead, malar region, and chin. Consequently, preparing an appropriate formulation for the topical treatment of melasma

presents a promising approach. Nowadays, polymeric-based drug delivery systems are acquiring much consideration in treating several dermatological diseases on the account of their ability to ameliorate drugs' activity, delay and control drugs' release, and augment drugs' deposition or retention inside the skin, thus enhancing their therapeutic efficacy **[1, 2]**. Among the promising topical polymeric systems are the chitosan-TPP nanoparticles. Chitosan is the N-deacetylated chitin derivative, constituted mainly of N-acetyl-D-glucosamine and D-glucosamine units. It is a natural, biocompatible, biodegradable non-toxic, and cationic polymer **[3]** along with reported topical merits on the account of its high positivity, penetration enhancement potential, sun screening, and skin protection effects **[4]**. Tripolyphosphate Sodium salt (TPP) is an inorganic polyionic compound having a chemical structure of **($Na_5P_3O_{10}$)** together with the polyphosphate Penta-anion Sodium salt.

Among several available skin whitening agents, arbutin was reported to be a potent skin depigmenting agent that can interfere with melanin synthesis and accumulation. Arbutin is a naturally occurring beta-D-glucopyranoside hydroquinone derivative, existing in the dried leaves of specific plant species, like bearberry in two different isomeric forms (alpha and beta isomers). Its acts by suppressing the tyrosinase enzyme present in the melanosomes, which boosts melanin production at non-cytotoxic concentrations other than reducing the synthesis and expression of such enzyme **[5]**. It is reported that arbutin's activity comes from its structural homologies with its substrate (tyrosine), which causes the competitive suppression of tyrosinase catalytic activity **[6]**. Many studies have revealed that α-arbutin (or 4-hydroxyphenyl alpha-glucopyranoside) shows more substantial inhibitory potential on human tyrosinase, reaching ten times greater compared to arbutin (beta isomer), promoting its efficacy and stability in attaining the required whitening efficacy on the skin **[7-10]**.

Consequently, the present study aimed to formulate optimized α-arbutin-loaded CSNPs to enhance their therapeutic efficacy when applied topically on the skin. Optimization of the colloidal properties of the nanoparticulate systems (namely; P.S, PDI and ζ-potential) was carried out by preparing drug-free CSNPs and investigating the influence of different formulation parameters on these properties. The effect of α-arbutin loading on the physical properties of the prepared CSNPs was also studied in an attempt to attain optimized α-arbutin-loaded CSNPs with optimum physical properties and maximum entrapment efficiency.

2-Materials and methods

2.1.Materials

Alpha-arbutin was purchased from making cosmetics scientific company, USA. Chitosan [(from shrimp shells) (low molecular weight 50 kDa with a degree of acetylation of 75%)] and tripolyphosphate sodium salt (TPP) was purchased from Alpha Aesar, Germany. Glacial acetic acid was obtained from El-Gomhorea pharmaceutical company, Cairo, Egypt. Nanosep® centrifuge tubes with an ultra-filter of molecular weight cut off 100 kDa were purchased from Pall Life Sciences (USA).

2.2.Methods

2.2.1-Preparation of CSNPs by ionic gelation method

2.2.1.1.Preliminary study for optimization of the preparation conditions of drug-free CSNPs

A preliminary study was performed to determine the optimum conditions for the preparation of drug-free CSNPs with suitable P.S, PDI and ζ-potential intended for topical delivery. As presented in **Table 1**, fifteen drug-

free CSNPs **(P1-P15)** were prepared using the ionic gelation technique between the positively charged chitosan polymer and the polyionic tripolyphosphate sodium salt (TPP) and tested for their P.S, PDI, and ζ-potential. Chitosan polymer was dissolved in 1% acetic aqueous solution (pH= 3) at one of three concentrations (0.10, 0.20 or 0.30 % w/v) under magnetic stirring (Lab Tech LMS -1003, Korea) for one hour **[11-14]**. CSNPs were formed spontaneously following the dropwise addition of 4 ml of sodium TPP aqueous solution at one of five concentrations (0.02, 0.04, 0.06, 0.08 or 0.10 % w/v) to 10 ml of the chitosan solutions under magnetic stirring (Lab Tech LMS -1003, Korea) at 600 r.p.m for two hours at room temperature (Chitosan: TPP, 2.5:1, v/v) **[15]**.

2.2.1.2.In process pH measurement

The pH of both sodium TPP aqueous solutions and chitosan solutions for all formulations was measured using a digital pH meter (JANEWAY 3505, UK) following direct measurement without dilution.

2.2.1.3.Effect of pH alteration on the characteristics of the obtained drug-free CSNPs

To study the effect of pH change on the P.S, PDI, and ζ-potential of CSNPs (**Table 1**), another fifteen blank formulae were prepared **(P16-P30)** based on the previously applied procedure, yet the pH of chitosan and TPP solutions was altered to 5.00± 0.11 and 2.00± 0.16, respectively by adjustment with 0.1 M NaOH and 0.1 M HCl using a digital pH meter (JANEWAY 3505, UK) **[16]**.

Table 1. Effect of composition on the colloidal properties of drug-free CSNPs before and after pH adjustment (mean ± SD, n = 3)

Formula code	Chitosan concentration (% w/v)	TPP concentration (%w/v)	pH of Chitosan solution/ TPP solution	Mean P.S (nm) ± S.D.	Mean PDI ± S.D.	Mean ζ-potential (mV) ± S.D.
P1		0.02		1021.00 ± 18.30	0.74 ± 0.03	+37.60 ± 0.23
P2	0.10	0.04	3.00/ 8.65	940.00 ± 12.21	0.73 ± 0.04	+38.20 ± 0.72
P3		0.06		814.50 ± 10.45	0.71 ± 0.03	+38.90 ± 0.64
P4		0.08		748.90 ± 20.12	0.64 ± 0.06	+39.40 ± 0.29
P5		0.10		668.00± 15.41	0.61 ± 0.05	+40.20 ± 0.35
P6		0.02		1049.00 ± 30.90	0.74 ± 0.11	+41.20 ± 0.11
P7		0.04	3.00/ 8.65	962.20 ± 28.50	0.74 ±0.11	+42.40 ±2.11
P8	0.20	0.06		869.60 ± 10.72	0.71 ±0.02	+42.90 ±1.69
P9		0.08		754.50 ± 15.40	0.65 ±0.04	+40.20 ±1.34
P10		0.10		656.10±11.84	0.64 ±0.01	+40.90 ± 0.53
P11		0.02		1043.30 ± 12.13	0.76 ±0.04	+42.00 ± 0.19
P12	0.30	0.04	3.00/ 8.65	951.90 ± 20.36	0.74 ±0.03	+38.20 ±1.21
P13		0.06		866.20 ± 13.22	0.72 ± 0.02	+41.10 ±0.43
P14		0.08		795.80 ± 10.34	0.65 ± 0.01	+38.80 ±0.27
P15		0.10		701.00 ± 13.61	0.66 ± 0.02	+42.40 ± 0.42
P16		0.02		700.50 ± 10.26	0.58 ± 0.23	+38.00 ± 0.31
P17		0.04		622.00±21.18	0.52 ±0.19	+38.20 ±1.29
P18	0.10	0.06		515.00±10.38	0.49 ±0.34	+39.80 ±0.64
P19		0.08	5.00/ 2.00	435.00±28.01	0.45 ±0.11	+37.30 ±1.26
P20		0.10		324.10 ± 18.31	0.43 ± 0.16	+38.10 ± 0.49
P21		0.02		750.90 ± 12.73	0.59 ± 0.32	+41.90 ± 0.22
P22		0.04		648.00±28.19	0.55 ±0.18	+41.10 ±0.52
P23		0.06		556.30±11.04	0.50 ±0.27	+41.90 ±0.31
P24		0.08	5.00/ 2.00	429.10±33.10	0.42 ±0.16	+42.00 ±0.38
P25	0.20	0.10		319.20 ± 17.60	0.45 ± 0.10	+42.80 ± 0.11
P26		0.02		732.40 ± 15.11	0.59 ± 0.13	+40.20 ± 0.20
P27		0.04		652.90±12.34	0.57 ±0.08	+39.40 ±0.44
P28		0.06	5.00/ 2.00	578.00±14.01	0.53 ±0.23	+41.30 ±0.36
P29	0.30	0.08		485.40±22.15	0.41 ±0.21	+38.60 ±0.27
P30		0.10		365.00 ± 17.10	0.43 ± 0.28	+40.10 ± 0.39

2.2.1.4.Preliminary study for optimization of the preparation conditions of α-arbutin loaded- CSNPs

As shown in **Table 2**, another twelve CSNPs formulations loaded with 10 mg α-arbutin **(P31-P42)** were prepared employing the previously mentioned procedure and tested for their EE%, P.S, PDI and ζ-potential, in which the drug was loaded either in the 10 ml chitosan solutions at concentrations (0.10, 0.20 or 0.30% w/v) or in the 4 mL of TPP solutions at concentrations (0.02 or 0.10 % w/v).

Table 2. Effect of chitosan and TPP concentrations after pH adjustment on the P.S, PDI, ζ-potential, and EE% of loaded CSNPs (the drug was loaded either into the chitosan or TPP solution) (n=3)

Formula code	Chitosan concentration* (%w/v)	TPP concentration** (%w/v)	Mode of drug incorporation	EE % Mean ± S.D.	Mean P.S (nm) ± S.D.	Mean PDI ± S.D.	Mean ζ-potential (mV) ± S.D.
P31	**0.10**	**0.02**	In chitosan solution	70.32±1.69	712.70 ± 25.63	0.59 ± 0.13	+38.80 ± 0.51
P32		**0.10**		71.63±1.03	342.00 ± 36.29	0.45 ± 0.22	+39.90 ± 0.38
P33	**0.20**	**0.02**		81.09±4.04	723.00± 21.96	0.61 ± 0.19	+39.00 ± 0.71
P34		**0.10**		81.78±1.31	361.40 ± 28.34	0.50 ± 0.10	+41.40 ± 0.12
P35	**0.30**	**0.02**		66.65±2.29	745.00 ± 30.11	0.59 ± 0.13	+42.00 ± 0.48
P36		**0.10**		63.68±2.46	372.30 ± 12.89	0.45 ± 0.28	+40.70 ± 0.05
P37	**0.10**	**0.02**	In TPP solution	32.25±9.43	720.00 ± 23.10	0.51 ± 0.26	+39.70 ± 0.28
P38		**0.10**		34.34±7.58	336.00 ± 32.36	0.44 ± 0.15	+38.00 ± 0.56
P39	**0.20**	**0.02**		57.13±6.94	726.30 ± 15.84	0.52 ± 0.39	+40.20 ± 0.19
P40		**0.10**		59.37±10.09	355.00 ± 28.51	0.44 ± 0.16	+39.40 ± 0.45
P41	**0.30**	**0.02**		33.09±6.77	739.20 ± 18.49	0.55 ± 0.27	+41.80 ± 0.25
P42		**0.10**		34.15±5.21	377.00 ± 27.93	0.45 ± 0.14	+41.50 ± 0.61

* pH of chitosan solution = 5.00
** pH of TPP solution = 2.00
10 mg drug was inserted into the chitosan or TPP solution
The average pH of the resultant CSNPs dispersions was 4.91

2.3. Characterization of CSNPs

2.3.1. Determination of the P.S, PDI, and ζ-potential of CSNPs

The P.S, PDI, and ζ-potential of the prepared CSNPs were assessed utilizing a dynamic light scattering particle size analyzer (model ZS3600, Malvern Instruments Ltd., Worcestershire, UK), following appropriate dilution with water **[17, 18]**.

2.3.2. Determination of α-arbutin entrapment efficiency (EE%) in CSNPs

The free (unentrapped) drug was parted from the entrapped drug present in the prepared CSNPs employing Nanosep® centrifugal tubes **[19, 20]**. A volume of the CSNPs formulation equivalent to 0.5 ml was inserted in the Nanosep® centrifugal tubes and centrifuged into a high-Speed cooling centrifuge (SIGMA-3-30KS, Germany) for 30 min at 3000 rpm at -4 ◦C. The free (unentrapped) α-arbutin was quantified in the filtrate after dilution with distilled water utilizing UV-spectrophotometer (Biochrom Libra S60, UK) at 283 nm, using distilled water as blank. No absorbance interference was detected from the CSNPs at the aforementioned wavelength. The entrapment efficiency (EE%) was computed applying the subsequent equation **[20]**:

$$EE\% = \frac{At - Af}{At} \times 100 \qquad \textbf{(Eq.1)}$$

Where A_t is the total amount (entrapped and unentrapped) of α-arbutin loaded into the formulation, and A_f is the amount of the unentrapped (free) α-arbutin.

2.4. Statistical analysis

Data statistical analysis was performed using Graphpad® Instant software applying way ANOVA followed by Tukey Kramer post-test. Data were estimated as mean ± standard deviation (n=3).

3. Results and Discussion

3.1. Preparation of CSNPs by ionic gelation method

CSNPs were efficiently prepared to utilize the ionic gelation technique. This method is the most commonly used owing to the high stability of the produced particles, utilization of water-based solutions without the inclusion of organic solvents, and mild reaction conditions, besides being simple and cost-effective. Low molecular weight chitosan was favored in the current work due to its relatively better solubility as well as colloidal stability **[21]**. In the current method, CSNPs are synthesized owing to the ionic interaction between two oppositely charged species which are the positive amine groups present on the chitosan molecule and the negative polyionic TPP molecule **[21, 22]**. The obtained CSNPs were found to be transparent with a smooth and homogenous appearance without phase separation.

3.2. Characterization of CSNPs

3.2.1. Effect of the formulation parameters on the P.S, PDI, and ζ-potential of drug-free CSNPs

Initially, fifteen drug-free formulae (**P1-P15**) were formulated employing the ionic gelation technique. The pH of chitosan and TPP solutions was measured and was found to be 3.00± 0.07 and 8.65± 0.05, respectively. Afterward, these formulations were evaluated in terms of P.S, PDI, and ζ-potential without adjusting the pH of either chitosan or TPP solutions. As presented in **Table 1**, it was obvious that upon increasing TPP concentration from 0.02 to 0.10% at constant chitosan concentration, the P.S of nanoparticles decreased significantly ($P<0.05$), which could be attributed to the augmented TPP molecules availability which in turn could interact with chitosan free amino groups within the formed nanoparticles. Moreover, it was suggested that additional anion incorporation could further increase the cross-linking occurring between chitosan chains, hence illustrating the decrease in the size of CSNPs along with increasing the concentration of TPP **[23]**. Such increment in the internal cross-linking resulted in more tightly bound chitosan chains within the nanoparticles, thus causing the particles to be condensed, promoting a gradual reduction in particle size. Furthermore, cross-linking inhibits the free primary amino groups availability on chitosan molecule, hereby preventing different nanoparticles to be self-aggregated **[23]**. Accordingly, formulations (**P1**, **P6,** and **P11**) prepared to utilize the lowest concentration of TPP (0.02%, w/v) exhibited a micrometer size range compared to those prepared with higher concentrations. On the other hand, by increasing chitosan concentration from 0.10 to 0.30% at constant TPP concentration, an insignificant increase ($P> 0.05$) in P.S was observed similar to what was reported by **Tilkan and Özdemir, [24]**, showing the insignificance of the increase in chitosan concentration on the obtained particle size during the encapsulation of flurbiprofen into chitosan microspheres.

Regarding PDI values, they varied from 0.61 to 0.76, similar to the studies of **Azevedo et al. [25]**, **Abouelhag et al. [26],** and **Raj et al. [27]**who also formulated CSNPs using the ionic gelation technique. As shown in **Table (1)**, the PDI values insignificantly changed ($P>0.05$) either upon increasing the TPP concentration from 0.02 to 0.10 % w/v at constant chitosan concentration, or upon increasing chitosan

concentration from 0.10 to 0.30% at constant TPP concentration, similar to what was reported by **Yien et al. [28]**. In addition, all formulations were positively charged along with ζ-potential values ranging from +37.60 to +42.90 mV, which is commonly encountered with chitosan-based nanoparticulate systems owing to the high positivity of the chitosan molecule due to the presence of NH_3^+ **[29-32]**. Moreover, the ζ-potential of the prepared CSNPs was not affected significantly (*P>0.05*) by the change in the concentration of chitosan or TPP as shown in **Table (1)** similar to the previous findings of **Zaki et al. [17]** who investigated the cytotoxicity of CSNPs towards mouse hematopoietic stem cells, **Silva et al. [33]** who adopted the formulation of CSNPs for daptomycin delivery in the ocular treatment of bacterial endophthalmitis and **Tamara et al. [34]** who attempted the formulation of chitosan/protamine hybrid nanoparticles.

3.2.2. Effect of pH alteration on the characteristics of the obtained drug-free CSNPs

To study the effect of the pH of both preparation phases on the characteristics of the obtained CSNPs, an additional fifteen drug-free formulae (**P16-P30**) were prepared (**Table 1**), yet the pH of chitosan and TPP solutions was altered to 5.00± 0.11 and 2.00± 0.16, respectively based on previous authors' reports **[20, 35-37]**. The process of ionic gelation was reported to be pH-responsive, thus the physicochemical properties of the prepared nanoparticles could differ with pH change **[38]**. As demonstrated in **Fig. 1**, it can be deduced that upon changing the pH of both chitosan and TPP solutions, the P.S of the current CSNPs decreased significantly (*P<0.05*) when compared to the 1st fifteen formulae (**P1-P15**) prepared without pH adjustment. Chitosan solutions at pH 5.00 promoted the production of smaller-sized particles, as at this pH, chitosan chains became more condensed compared to more acidic solutions, owing to the lower degree of primary amines protonation, hence resulting in the formation of nano-sized particles **[39]**. Meanwhile, chain compaction promotes TPP cross-linking with much denser particles, in contrast to more linear chitosan chains at more acidic pH solutions **[23]**.

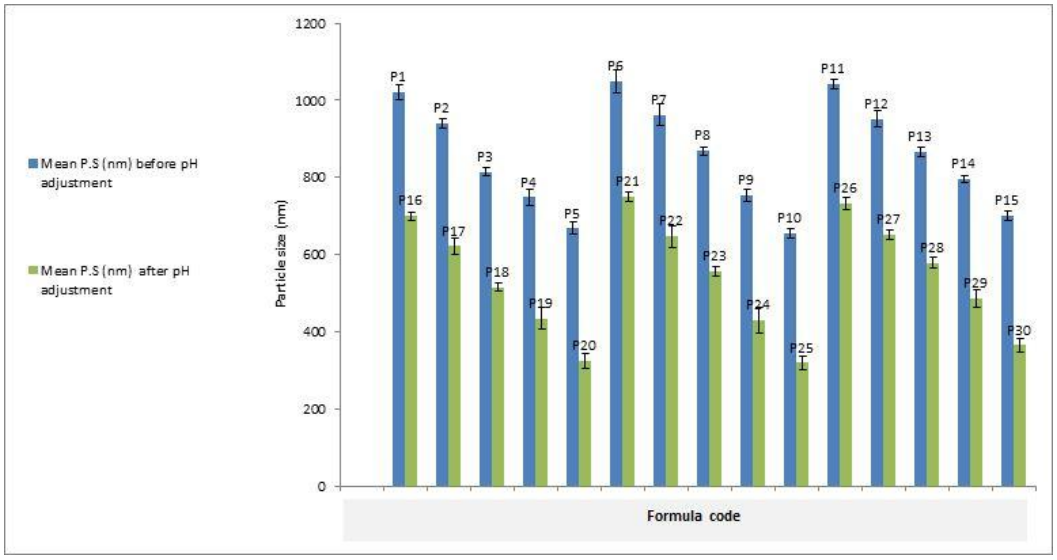

Fig.1. Effect of pH change of chitosan and TPP solutions on the particle size of drug-free CSNPs

It was also reported that the pH of TPP solutions greatly influences the electronegative potential of such molecule when interacting with chitosan free amine groups so that at lower pH values, TPP molecule renders less reactive to react chemically with chitosan owing to its neutralization by more positive ions (H_3O^+ and H^+) present in solution. Therefore, TPP interacts with fewer amino groups (NH_3^+) present on chitosan molecules, resulting in a decrease in the size of nanoparticles besides being more monodispersed. Though, at a more basic pH, TPP becomes more reactive in solution owing to the decrease in the neutralization of positive ions, increasing its affinity to interact not only with chitosan free amine groups but also with the same groups present on the already produced CSNPs resulting in larger particle size **[23]**. It was reported that the degree of ionization of TPP molecule depends on the solution pH value, in which in the original solution of TPP (pH 8.65), it was found that TPP molecule is ionized into OH^- and TPP ions ($HP_3O_{10}^{4-}$ and P_3O_{10} 5^-). Though, at low pH, P_3O_{10} 5^- anions are only present. Chitosan-TPP nanoparticles formulated in the original TPP solutions are subjected to deprotonation and minor ionic-crosslinking, however, the prepared chitosan nanoparticles in acidic TPP solutions are entirely subjected to ionic-crosslinking **[35]**. Moreover, it was reported that the transient exposure of chitosan solution to a high pH of the TPP solution (8.65 in the unadjusted solutions) may promote chitosan molecules aggregation, thus promoting the increase in the available number of nuclei for the maturation of nanoparticles leading to higher P.S **[40]**. Similar to what was previously mentioned with formulations **P1-P15**, on increasing the concentration of TPP from 0.02 to 0.10% at constant chitosan concentration, the P.S decreased significantly (*P<0.05*), whereas, by increasing chitosan concentration from 0.10 to 0.30% at constant TPP concentration, an insignificant increase (*P> 0.05*) in P.S was noted.

Regarding the PDI values, they ranged from 0.41 to 0.59 indicating more monodisperse CSNPs and greater particle stability. As shown in **Fig. 2**, PDI values significantly decreased (*P<0.05*) upon altering the pH of both chitosan and TPP solutions compared to the formulae prepared without pH alteration **(P1-P15)**. This correlates with the P.S. decrease since, at lower pH, TPP is neutralized by more positive ions becoming less reactive with chitosan molecule. At higher pH, the TPP molecule is neutralized by fewer positive ions and hence possesses a higher affinity to react with chitosan molecule by cross-linking not only with chitosan chains but also with the formed CSNPs resulting in aggregation **[23]**. Similar to what was encountered with formulations (**P1-P15**), the PDI values showed insignificant changes (*P>0.05*) upon increasing the TPP concentration from 0.02 to 0.10 % w/v at constant chitosan concentration, or upon increasing chitosan concentration from 0.10 to 0.30% at constant TPP concentration. Also, all formulations exhibited positive charges with ζ-potential values in the range of +37.30 to +42.80 mV which were not affected significantly (*P>0.05*) by the pH alteration of either chitosan or TPP solutions as presented in **Fig. 3**, matching the outcomes of **Masarudin et al. [23]**, which might be attributed to the closeness of the pH values of the final solutions (4.11 without adjustment and 4.91 with adjustment). Similar to what was encountered with formulations (**P1-P15**), the change in the concentration of chitosan and TPP showed insignificant effect on the surface charge of these nanoparticles (*P>0.05*) as previously discussed. Based on the previous results, formulae prepared without alteration of the pH (chitosan solution of pH = 3.00 and TPP solutions of pH 8.65) were excluded from further investigations.

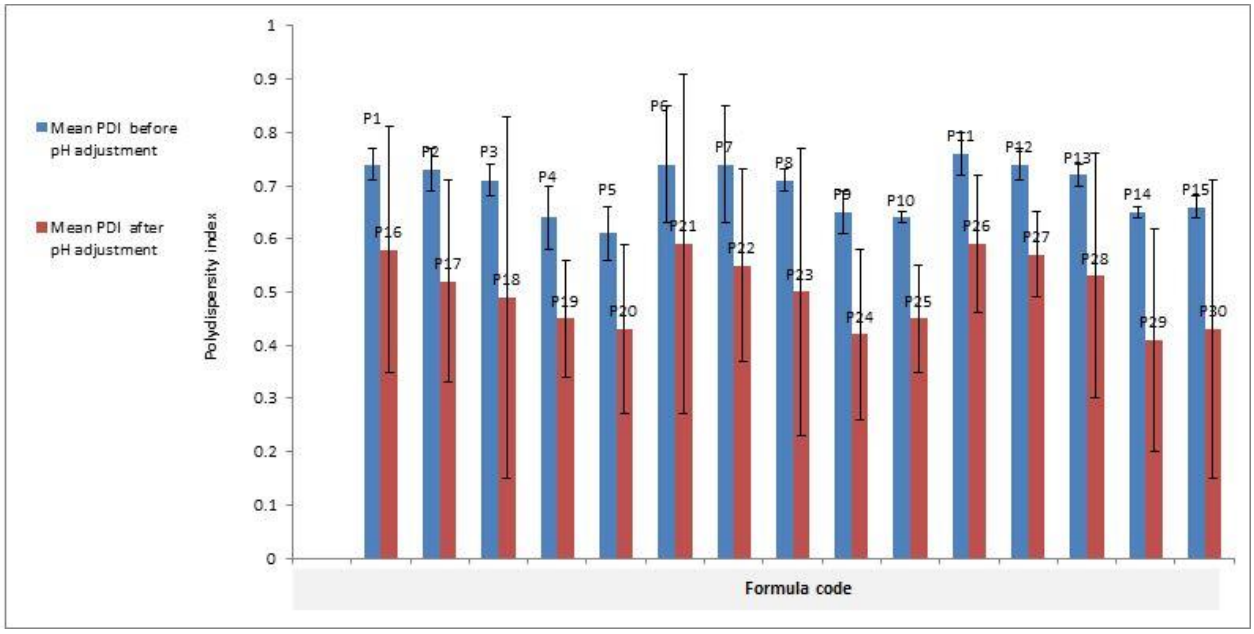

Fig. 2: Effect of pH change of chitosan and TPP solutions on the polydispersity index of drug-free CSNPs

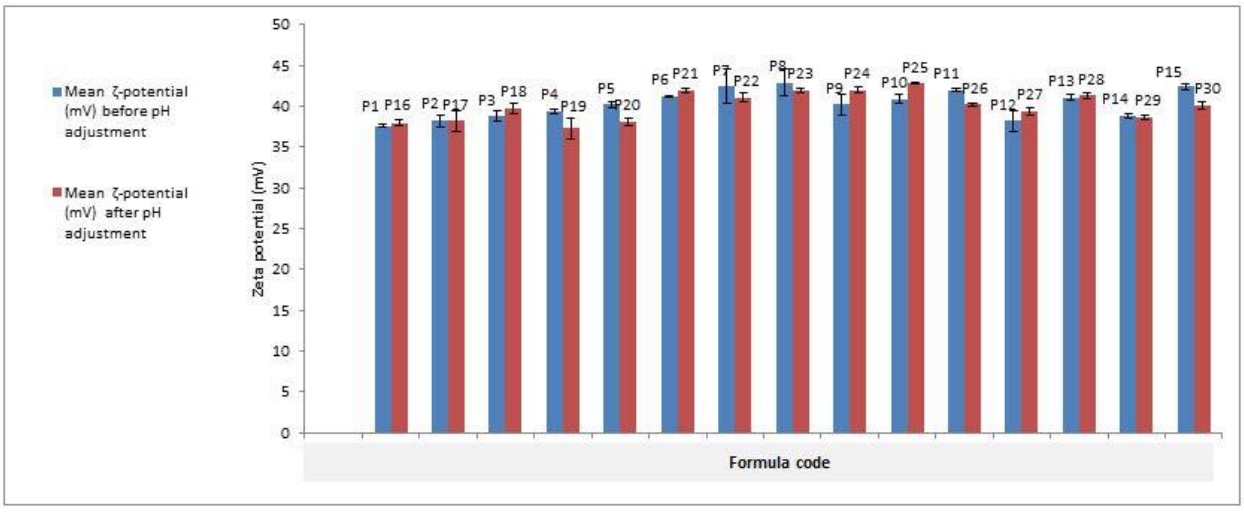

Fig. 3: Effect of pH change of chitosan and TPP solutions on the zeta potential of drug-free CSNPs

3.2.3. Effect of drug loading and mode of drug incorporation on the P.S, PDI, ζ-potential, and EE% of CSNPs

To study the effect of drug loading on the characteristics of the α-arbutin loaded CSNPs formulae, six formulae (**P31-P36**) were prepared to utilize the three chitosan concentrations (0.1, 0.2, and 0.3, % w/v) and the minimum and maximum concentrations of TPP (0.02 and 0.10, % w/v) while loading 10 mg drug into the chitosan solution. Afterward, the pH of both chitosan and TPP solutions was adjusted to 5.00 and 2.00, respectively. As shown in **Table 2**, the increase in chitosan concentration from 0.10 to

0.20% resulted in a significant increase in EE% values (*P<0.05*) due to the formation of more nanoparticles in the medium. Whereas, a further increase in chitosan concentration from 0.20 to 0.30% resulted in a significant decrease in EE% (*P<0.05*) because high chitosan concentrations might cause a dramatic increase in viscosity and gel formation, which hindered the encapsulation of α-arbutin, thereby decreasing the encapsulation efficiency **[38-40]**. Increasing the TPP concentration from 0.02 to 0.10 % w/v at constant chitosan concentration did not have a significant effect (*P>0.05*) on the EE% of the drug which came in parallel with the results of **Wu et al. [12]**.

Similarly, P.S decreased significantly (*P<0.05*) upon changing the pH of both chitosan and TPP solutions with an insignificant change in the PDI values (*P>0.05*), along with comparable ζ-potential values (*P>0.05*), which could be assigned to the uncharged nature of α-arbutin **(Martial Safety Data Sheet-Making cosmetics scientific company, USA)** as displayed in **Table 2**, suggesting that loading the drug into the CSNPs affected neither the P.S nor PDI, which was similar to the findings of **Leelapornpisid et al. [13]** and **Silva et al. [33]**.

For the confirmatory purpose, another six formulae were prepared (**P37-P42**) similar to the previously mentioned procedure while loading the 10 mg of the drug into the TPP solution instead of chitosan solution to investigate the effect of the mode of drug incorporation either in chitosan or TPP solutions on the P.S, PDI, ζ-potential and EE% of the prepared CSNPs formulae. The formulae were compared to the previous six formulae (**P31-P36**) regarding their EE%, P.S, PDI, and ζ potential. Likewise, the three chitosan concentrations (0.1, 0.2 and 0.3, % w/v) and the minimum and maximum concentrations of TPP (0.02 and 0.10, % W/V) were employed. As displayed in **Table 2**, the increase in chitosan concentration from 0.10 to 0.20 % was coupled with a significant increment in the EE% values (*P<0.05*) and then followed by a significant reduction (*P<0.05*) upon further increasing the chitosan concentration from 0.20 to 0.30%, similar to the findings observed with formulations (**P31-P36**). Similarly, increasing the TPP concentration from 0.02 to 0.10 % w/v at constant chitosan concentration did not have a significant effect (*P>0.05*) on EE%. However, results revealed that loading the drug into the TPP solution displayed significantly lower EE% (*P< 0.05*) when compared to its loading into the chitosan solution **(Fig. 4)**, which might be ascribed to the possible covalent bond interaction between the hydroxyl groups present in chitosan and the glucose moiety of α-arbutin **[14]**.

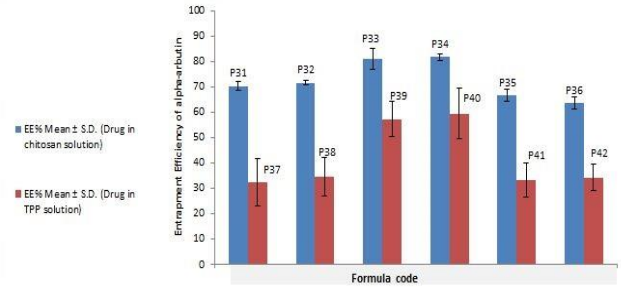

Fig. 4: Effect of mode of drug incorporation in chitosan or TPP solutions on the EE% of loaded CSNPs

Regarding P.S, PDI, and ζ-potential, formulae (**P37-P42**) exhibited comparable results (*P> 0.05*) to formulations (**P31-P36**). Therefore, it can be deduced that loading the drug either into chitosan or TPP solution does not affect the P.S, PDI, or ζ-potential of the prepared CSNPs, following the results of **Silva et al. [33]**.

Conclusion

Chitosan nanoparticles have been successfully prepared and characterized in the present study. The nature and composition of various attributes as well as the conditions applied in CSNPs formulation exhibited major effects on their colloidal properties. Therefore, optimization of formulations' preparation

parameters was essential to produce nanoparticles with acceptable physical properties. It was generally observed that the particle size of the prepared systems decreased upon increasing the concentration of TPP along with changing the pH of both chitosan and TPP solutions. The incorporation of α-arbutin into CSNPs didn't affect the particle size, PDI values and surface charge of the prepared systems, however higher chitosan concentrations resulted in the reduction of the encapsulation efficiency of α-arbutin loaded-CSNPs. Consequently, the obtained results present a guiding map for the effective formulation of α-arbutin-loaded CSNPs.

Declarations

Ethics approval and consent of participation

Not applicable

Consent of publication

Not applicable

Data and materials availability

All data produced or analyzed throughout this study are included in the current manuscript.

Competing interests

No competing interests were found between the authors.

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