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Research Article

The Influence of Sodium Hyaluronate, L-Leucine and Sodium Taurocholate on the Nebulization of Aqueous Betamethasone-17-Valerate Suspensions

Mina I. Tadros[1,2]

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Abstract. The purpose of this research was to evaluate the variables that are suggested to influence the adsorption of the hydrophilic hyaluronic acid (HA) onto the surface of the hydrophobic betamethasone-17-valerate (BV) particles in order to formulate a nebulizable suspension. The adsorption of HA from aqueous solutions (0.04% to 0.16%, *w/v*) to a fixed BV concentration (0.04%, *w/v*) under different experimental conditions, was investigated. The method of preparation of HA-BV suspensions involved suspending BV particles either in the hydrated HA solution (method 1) or in water followed by addition of solid HA (method 2). Other variables like the time required for the adsorption to complete and temperature at which adsorption is carried out were studied. The nebulization of the suspensions was tested via an air jet nebulizer connected to a twin stage impinger. In order to improve the nebulization behavior of the optimized suspension, L-leucine or sodium taurocholate was incorporated in increasing concentrations (0.01–0.04%, *w/v*). The optimized suspension, having a nebulization efficiency of 33.75%, was achieved following the adsorption of HA (0.1%, *w/v*) onto BV particles adopting method 2 of preparation and extending for three days at 4 °C. Incorporation of either l-leucine or sodium taurocholate significantly decreased the aggregate size of the optimized suspension and consequently caused significant increases in the nebulization efficiency to reach 46.87% and 56.25%, respectively.

KEY WORDS: adsorption; betamethasone-17-valerate; hyaluronic acid; L-leucine; nebulization; sodium taurocholate.

INTRODUCTION

The use of aerosols for the treatment of diseases has increased dramatically in the last few years. For locally active drugs, a significant advantage of the aerosol delivery is its ability to give much higher local drug levels than those achieved by systemic administration (1).

Inhaled corticosteroids are usually recommended first-line therapy for persistent asthma of all severities (2). Recently, Derendorf *et al.* (3), suggested that the important pharmacokinetic and pharmacodynamic characteristics that can enhance the efficacy or prolong the anti-inflammatory effects of inhaled corticosteroids include small particle size, high glucocorticoid-receptor-binding affinity, long pulmonary residence time and rapid conjugation.

Betamethasone-17-valerate (BV) is a model corticosteroid that is commonly investigated in the control of asthma (4–6). The usual adult dose of BV is 200 μg four times daily. Indeed, the formulation of a uniform aqueous suspension of BV that is capable of providing constant drug levels at the site of action for a prolonged time is a challenging issue due to the highly hydrophobic nature of BV (log P=4.2). In this study, the adsorption of the hydrophilic polymer hyaluronan (hyaluronic acid, HA), under different experimental conditions, onto the surface of BV particles was investigated to obtain uniform aqueous drug suspensions that could be inhaled via nebulization. Due to the bioadhesive properties of HA (7), these suspensions are suggested to be capable of prolonging the drug activity in the lung by avoiding rapid clearance by the mucociliary escalator.

HA is a naturally occurring polymeric hydrogel based on a linear polysaccharide comprised of repeating units of D-glucuronic acid and *N*-acetyl-D-glucosamine, linked by ß-1, 4 and ß-1, three glycosidic bonds (8).

Several properties support the unique utility and choice of HA in the pulmonary delivery system. HA is naturally present in the lung where its role includes protection of lung elastin from the damage associated with inflammatory lung disease and in the repair of lung injury (9).

In addition, aerosolized HA has been shown to significantly reduces the bronchial hyper-reactivity to muscular exercise in asthmatics (10). Animals treated with HA prior to the induction of experimental emphysema develop significantly less disease than untreated controls. Although clinical trials involving nebulized HA are not expected to yield a measurable treatment effect for at least several years, it is proposed that the special ability of this polysaccharide to retain water may increase the elasticity of lung elastic fibers, producing a relatively rapid improvement in pulmonary

[1] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Kasr El-Aini, Cairo, Egypt.
[2] To whom correspondence should be addressed. (e-mail: mina_ebrahim@yahoo.com)

mechanics (11–12). HA turnover in the lung has not been investigated, however, it was anticipated that the use of small quantities of HA in a pulmonary delivery system is unlikely to lead to undesirable accumulation (13).

Finally, it has been shown that molecules of hyaluronan in the pulmonary alveolus are flexible coils which self-aggregate. Hyaluronan solutions are quite viscous and bind to cell surface receptors and to proteins in the extracellular matrix. The networks formed with self-aggregated hyaluronan with or without proteins create gels whose properties depend largely upon the molecular weight of the hyaluronan and its concentration (14). High molecular weight HA was thus investigated in this work, in an attempt to anchor the BV particles long enough in the lung for a prolonged action. Similar interactions between the hydrophilic HA and hydrophobic regions of drugs as fluticasone propionate (13), molecules like lecithin (15) and surfaces like graphite (16) were reported.

The influence of certain natural surfactants, L-leucine and sodium taurocholate, on the properties of aqueous HA–BV suspensions was investigated aiming at improving their suspending properties and consequently their nebulization behaviour. Among the common amino acids, L-leucine was found to be the most surface active (17). L-leucine is a natural anti-adherent amino acid that is commonly employed in dry powder inhaler (DPI) formulations to improve the dispersibility of drug particles like disodium cromoglycate (18), ß-estradiol (19) and tobramycin sulfate (20). Indeed, the deposition of spray dried powders intended for pulmonary gene therapy was significantly increased by the incorporation of L-leucine (21).

Sodium taurocholate is a natural bile salt that is commonly employed in pharmaceutical preparations as an absorption enhancer (22–23). Recently, it was reported that the bioavailability of inhaled insulin powder (24) and nebulized solution (25) was significantly improved with *in vitro* and *in vivo* upon incorporation of sodium taurocholate.

In the current work, the possible adsorption of HA, from different solution concentrations, on BV particles under different experimental conditions was studied. The nature of the interaction between HA and BV was investigated after freeze drying the resulting suspensions. The nebulization behaviour of HA–BV suspensions before and after incorporation of L-leucine or sodium taurocholate, in different concentrations, was also reported.

MATERIALS AND METHODS

Materials

Micronized betamethasone-17-valerate was donated by Egyptian Int. Pharmaceutical Industries Co.(10th of Ramadan city, Egypt). Particle size analysis in 0.1% (*w*/*v*) Tween 80 using Malvern Mastersizer-S (Malvern Instruments, Worcs, UK) gave a volume mean particle diameter ($d_{50\%}$) of 1.5 µm, with a d_{10}–$d_{90\%}$ range of 0.84–4.73 µm. High molecular weight hyaluronic acid sodium salt from *Streptococcus equi* ($1.5–1.8\times10^6$ Dalton) was purchased from Fluka BioChemika (St. Louis, Mo, USA). L-Leucine was obtained from E. Merck (Darmstadt, Germany). Sodium taurocholate and methanol were from Sigma Chemicals (St. Louis, Mo, USA).

Methods

Optimization of the Factors Influencing the Adsorption of HA from Aqueous Solution on BV Particles Suspended in Water

Initial experiments were carried out using different HA aqueous solution concentrations and a fixed BV concentration of 0.04% (*w*/*v*) to investigate the influence of the following experimental variables on adsorption including: (1) HA aqueous solution concentrations, (2) the method of addition of HA and BV, (3) The time period required for the adsorption process to complete and (4) the temperature at which the adsorption process occur.

Five HA aqueous solution concentrations were tried 0.04%, 0.08%, 0.10%, 0.12% and 0.16%, *w*/*v*. Two methods were used to investigate the influence of addition of HA and BV. In method 1, HA was added to 10 ml of distilled water and allowed to hydrate for 72 h at 4 °C. Four milligrams of BV was then added to the hydrated HA solution and sonicated for 30 min. For method 2, a crude suspension of BV in water was prepared by adding 10 ml of distilled water in aliquots to 4 mg of BV. The dispersion was sonicated and then the dry HA was added to the sonicated dispersion. The prepared BV suspensions were left after sonication for different periods (24, 72 or 120 h) at different temperatures (4 or 25 °C) to adsorption to take place whilst stirring continuously using magnetic stirrers adjusted at 75 rpm.

Characterization of HA–BV Particles

1. Suspension formation

Dispersions of BV particles in water (0.04%, *w*/*v*) were obtained by vortexing for 30 s and compared to those suspensions prepared after the adsorption of HA on BV particles has taken place from different HA solution concentrations. All suspensions were monitored visually for sedimentation over a 1 week.

2. Investigation of the possible chemical interactions between HA and BV

The HA–BV suspension obtained after optimization of the previous factors was freeze dried (Savant Novolyphe-NL500, Holbrook, NY, USA). The freeze dried suspension was subjected to the following investigations.

(a) Fourier transform infrared (FT–IR) spectroscopy

FT–IR spectra were obtained on a FT–IR spectrophotometer (Genesis II, Mattson, USA). Samples of BV, HA and the freeze dried suspension were prepared in KBr discs (2 mg sample in 200 mg KBr). The scanning range was 400 to 4,000 cm^{-1} and the resolution was 1 cm^{-1}.

(b) Differential scanning calorimetry (DSC)

The DSC thermograms were recorded on a differential scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan). Two-milligram samples of BV, HA and the freeze dried suspension were heated in hermetically sealed aluminium pans over a temperature range of 30 to 300 °C at a constant rate of 10 °C /min under nitrogen purge (30 ml/min).

Incorporation of L-Leucine or Sodium Taurocholate into the Optimized HA–BV Suspensions

Two series of the optimized HA–BV suspension were prepared. Each containing one of the following surfactants; L-leucine or sodium taurocholate, in four different concentrations (0.01%, 0.02%, 0.03% and 0.04%, *w/v*). The particle size of these suspensions was determined in triplicates at 25 °C using a laser diffraction analyzer (Malvern Mastersizer-S, Malvern Instruments, Worcs, UK). The resulting data, in addition to those obtained with the control optimized HA–BV suspension prepared without surfactants, were analyzed by SPSS statistics program (SPSS Inc., Release 14.0 for Windows, Chicago) applying one-way ANOVA followed by post hoc multiple comparisons using the least square difference (LSD) test. Differences between series were considered to be significant at $P<0.05$.

Nebulization of the Prepared Suspensions

The nebulization of prepared HA–BV suspensions, before and after incorporation of L-leucine or sodium taurocholate, was carried out, in triplicates, using a compressor nebulizer system (Medel® AS3, Class II b, Italy), supplied with an air jet nebulizer (Medejet® Basic, Italy). The compressor air flow was 10 l/min and the average nebulization rate was 0.38 ml/min. The aerodynamic behavior of the generated aerosols was evaluated using the twin-stage impinger (TSI) (Copley Instruments, Nottingham, UK) following the stated procedure in the British Pharmacopoeia (2004) (26).

A volume of 2 ml of each suspension (containing the equivalent to 800 μg of BV) was filled into the nebulizer. The mouthpiece of the nebulizer was attached to the TSI by means of a suitable adaptor. Seven and 30 ml of methanol was used as the collecting solvent (4) in the upper and lower impingement chambers, respectively. The vacuum pump of the impinger was switched on and after 10 seconds the nebulizer was switched on. After complete nebulization of the suspensions, the nebulizer was switched off. After 5 s the pump of the impinger was switched off. The nebulizer was disconnected and the impinger was dismantled. The inner surfaces of the impingement chambers were washed separately with methanol and the washings were collected in graduated flasks. The drug fractions collected in stage 1 and 2 of the TSI as well as the remaining drug fraction in the nebulizer after aerosolization (the dead volume), were determined spectrophotometrically (Shimadzu UV-1601 PC Double Beam, Kyoto, Japan) at λ_{max} 240 nm. The nebulization efficiency percentage was calculated as follows (1):

$$\text{Nebulization efficiency (\%)} = \left[\frac{\text{Drug fraction collected in stage 2 of the TSI}}{\text{Total drug dose submitted to nebulization}(800\mu g)}\right] \text{X } 100 \quad (1)$$

Statistical analysis of the results was carried out as mentioned previously using SPSS statistics program (SPSS Inc., Release 14.0 for Windows, Chicago) applying one way ANOVA followed by Post Hoc multiple comparisons using LSD test.

RESULTS AND DISCUSSION

1. Appearance of suspensions and its significance

Fig. 1 illustrates the appearance of dispersions prepared by just vortexing BV particles in water and those suspensions obtained by the adsorption of HA on BV particles at 25 °C over 3 days using method 2. It is clear that the particles prepared from a HA solution concentration of 0.1% (*w/v*) gave a smooth suspension that showed little sedimentation after 1 week storage (Fig. 1d). In contrast, the suspensions prepared from lower HA solution concentrations [0.04% and 0.08% (*w/v*); Fig. 1b and c, respectively] and higher HA solution concentrations [0.12% and 0.16% (*w/v*); Fig. 1e and f, respectively] formed cruder suspensions with many particles either at the surface or settled at the bottom of the vial. The control BV particles (Fig. 1a) did not form a suspension as the hydrophobic particles floated on the surface. HA–BV particles prepared by method 1 were poorer suspensions (not shown).

2. Lack of chemical interaction between BV and HA

Figs. 2 and 3 show the FT–IR spectra and DSC thermograms of BV, HA and the optimized freeze dried suspension, respectively. It is observed from both figures that FT–IR spectrum and the DSC thermogram of the freeze dried suspension seemed to be only a summation of BV and HA FT–IR spectra and thermograms, respectively. This could indicate that there is no chemical interaction between BV and HA and therefore, the adsorption of HA onto the hydrophobic BV surface was assumed to be governed only by physical hydrophobic interactions.

3. Influence of natural surfactants on the particle size of the optimized HA–BV suspension

As shown in Table I, the addition of increasing amounts of L-leucine to the optimized HA–BV suspension caused a monotonic reduction in the aggregate size of the suspension over the entire range of concentrations studied. The aggregate size were reduced from 4.55 to 2.79 μm upon the addition of 0.04% (*w/v*) L-leucine. In contrast, the aggregate size of the optimized HA–BV suspension was significantly decreased (ANOVA, $P<0.05$) upon the addition of increasing concentrations of sodium taurocholate. Post hoc multiple

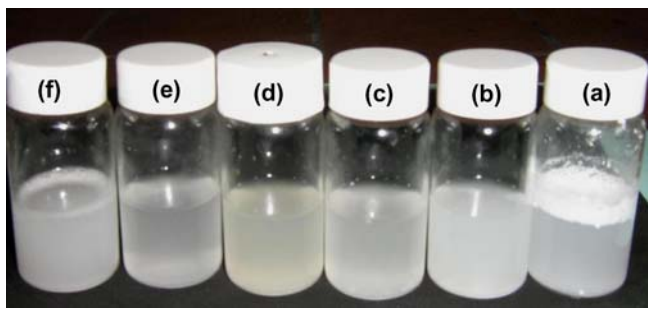

Fig. 1. A photo showing an aqueous BV dispersion (0.04%, *w/v*; *a*) and BV suspensions prepared by adsorption of HA solution concentrations of 0.04% (*b*), 0.08% (*c*), 0.10% (*d*), 0.12% (*e*) and 0.16% (*w/v*; *f*) using method 2 at 25 °C for 3 days

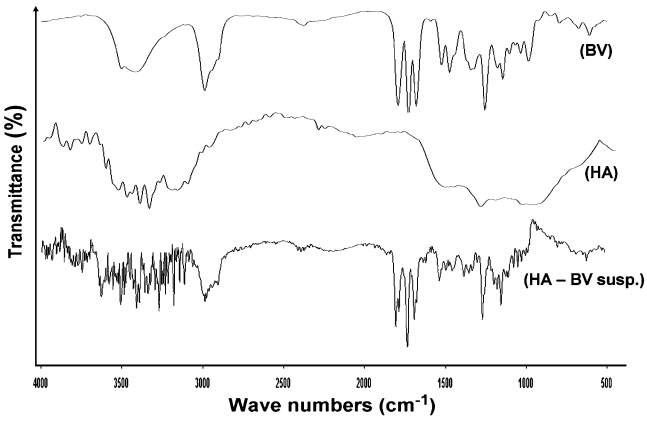

Fig. 2. FT–IR spectra of BV, HA and the optimized HA–BV suspension

comparisons adopting LSD test revealed that there is no significant difference ($P<0.05$) between the aggregate size of HA–BV suspension (1.67 μm) upon the addition of 0.04% (*w*/*v*) sodium taurocholate and the individual particle size (1.5 μm).

As more surfactant is added, the attractive forces between individual particles decreases, effectively decreasing the sticking probability between colloiding particles; aggregate sizes are therefore reduced (27). Upon studying the cohesive forces of salbutamol sulphate in a model non-pressurized fluorinated liquid containing poly ethylene glycol 400, Traini *et al.* (28) concluded that in the absence of any stabilizing agents, strong attractive forces were present between particles. The addition of a low concentration of poly vinyl pyrrolidone (PVP) to this system had the most significant influence on drug particle cohesion (ANOVA, $P<0.05$).

L-leucine is considered as a very weak surfactant. Its hydrophobic tail resembles very much that of n-butyl alcohol while the hydrophilic head contains the polar $-NH_2$ and –COOH groups (29). When compared to other amino acids, L-leucine possesses a high surface activity due to its strong hydrophobic characters. Therefore, the inability of L-leucine to cause marked reductions in the aggregate size of the optimized HA–BV suspension could be related to its hydrophobicity.

On the other hand, the hydrophilic nature of sodium taurocholate could provide a suitable explanation to the abrupt decrease in the aggregate size of the optimized HA–BV suspension upon addition of increasing amounts of this natural bile salt.

Nebulization Behavior of the Suspensions

Figs. 4 and 5 show the drug deposition percentages of different HA–BV suspensions obtained by the adsorption of HA from different solution concentrations on BV particles at 25 °C over a 3-day period using method 1 and 2, respectively. It is clear from both figures that HA solution concentration is directly proportional to the drug nebulization efficiency percentages and inversely proportional to the dead volume percentages. These relations were held true till a certain HA concentration (0.1% *w*/*v*) above which the above relationships are reversed; further increases in HA concentration lead to significant ($P<0.05$) reductions in the drug nebulization efficiency percentages and significant ($P<0.05$) increments in the dead volume percentages.

Interestingly, significantly ($P<0.05$) higher drug nebulization efficiency percentages were achieved with all HA solution concentrations when the adsorption process was carried out using method 2 of preparation.

HA is a network forming polymer which takes up preferred conformations forming primary, secondary and tertiary structures in water at different polymer concentrations (30). The primary structure of HA in solution has not been isolated, but is probably a simple sequence of sugars at very low concentrations of HA acting independently in solution. At higher concentrations the secondary structure forms. In this structure HA molecules aggregate in pairs in solution to form ordered twofold helical structures adopting extended random coil conformations (31). HA in the twofold helix has extensive hydrophobic patches of about eight C–H units sequenced along its chain on the interior faces of the helix which stabilize the structure, whilst the exterior is stabilized by H bonds (32). At higher concentrations, secondary structure aggregates become entangled to form an extended three-dimensional network, the tertiary structure (33). The concentration of HA for conversion to tertiary network structure for HA of similar molecular weights to that used in this work has been reported to be 0.1% (*w*/*v*) (34–35).

As suggested from visual observations, the ability of the particles to suspend in water was related to the quantity of HA adsorbed. the amount of HA adsorbed to a 0.04% (*w*/*v*) BV dispersion from different concentrations of HA solution increased to a maximum as the concentration of HA increased and then decreased. The concentration of the HA solution that is suggested to give the maximum adsorption, in this study, is similar to what is mentioned in literatures (0.1%, *w*/*v*). When higher solution concentrations were used, adsorption decreased with increasing HA concentration. This could be related to the aggregation of HA molecules in the secondary conformation to form tertiary network structures. This may be responsible for the non-equilibrium isotherms.

At equilibrium, the meshwork of secondary structure HA solution is held together by a large number of fairly weak interactions that can be broken apart by mechanical stresses such as stirring thereby exposing the hydrophobic patches along the HA chains. Therefore, it was suggested that HA

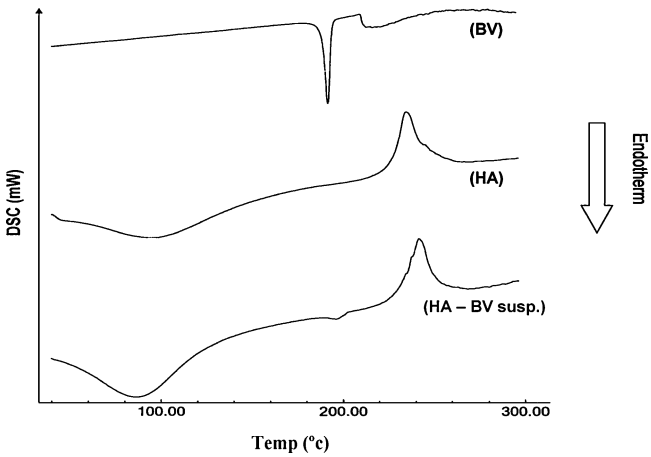

Fig. 3. DSC thermograms of BV, HA and the optimized HA–BV suspension

Table I. The Influence of Incorporating L-Leucine or Sodium Taurocholate, in Increasing Concentrations, on the Aggregate Size of the Optimized HA–BV Suspension

Formula	Type of Surfactant	Surfactant Concentration (*w/v*)	Aggregate Size (μm)
Optimized suspension	–		4.55±0.25
L1	L-Leucine	0.01	4.03±0.14
L2	L-Leucine	0.02	3.51±0.27
L3	L-Leucine	0.03	3.18±0.16
L4	L-Leucine	0.04	2.79±0.21
S1	Sodium taurocholate	0.01	3.28±0.31
S2	Sodium taurocholate	0.02	2.65±0.13
S3	Sodium taurocholate	0.03	2.12±0.23
S4	Sodium taurocholate	0.04	1.67±0.11

anchors onto the hydrophobic BV surface via physical hydrophobic interactions with these patches. Similar mechanisms are believed to be responsible for the interaction of HA with other hydrophobic molecules. For example, it has been shown that fluticasone propionate (FP) also form complexes with HA in the secondary structure. FP opens out the HA molecules by preventing intra molecular associations that occur in HA solution and bind to HA hydrophobic patches (13). In this study, as the concentration of secondary structure HA in solution increases, there are more chains in solution available for adsorption, and adsorption increases with increase in concentration. The adsorption maxima (0.1%, *w/v*) correspond to a conversion of HA solution conformation from secondary to tertiary network structures. Reduction in adsorption at higher solution concentrations of HA implies that adsorption of HA onto BV from a tertiary network structure is not as effective as from secondary extended structures. The strong HA–HA interactions favored in the tertiary network structures at high concentrations appear to limit the availability of hydrophobic patches for interaction with BV and adsorption reduces.

The availability of hydrophobic patches for adsorption may explain the influence of preparation variables on the adsorption process. Method 1 (using previously hydrated HA) always gave reduced adsorption than method 2, involving the addition of non-hydrated HA to FP suspension. FP particles at maximum adsorption [0.1% (*w/v*) formed a smooth suspension after preparation by method 2, indicating that the surface of the drug particle had been made more hydrophilic by the presence of adsorbed HA. In contrast, the FP/HA particles [0.07% (*w/v*)] generally formed a crude suspension after preparation by method 1 with many particles either at the surface or settled at the bottom of the vial, implying little adsorption of HA onto the surface of the particles. A possible explanation for reduced adsorption with method 1 is that fully hydrated HA is less likely to be adsorbed onto the surface of BV because its solution structure, stabilized by the hydrophobic patches, has already formed, whereas when hydration and adsorption occur simultaneously, as in method 2, BV particles can compete with the HA molecules for the hydrophobic patches so that some of the HA will be adsorbed onto BV rather than onto another HA molecule to form secondary structure.

It could be concluded that the highest drug nebulization efficiency percentage (27.18%) was achieved when the HA–BV suspension was prepared by the adsorption of 0.1% (*w/v*) HA solution onto BV particles at 25 °C, over 3 days, using method 2. This suspension was chosen for further optimization studies.

Fig. 6 shows the influence of the time required for the adsorption process to complete on the drug deposition percentages of the HA–BV suspension obtained by the adsorption of 0.1% (*w/v*) HA solution on BV particles at 25 °C over 3 days using method 2. It is clear that significantly ($P<0.05$) higher drug deposition percentages were obtained when the adsorption process was carried for 3 or 5 days. As supported by visual examination, a crude suspension was obtained when the adsorption process was carried out over 1 day only. This could indicate that the a longer period is needed for the adsorption process to take place completely. Indeed, extending the adsorption period to continue for 3 or 5 days favored the production of more uniform suspensions. Post hoc multiple comparisons adopting LSD test revealed that there was no significant difference ($P>0.01$) in the drug nebulization efficiency percentages when the adsorption process was carried out over a 3- or 5-day period. Therefore, it was assumed that a 3-day period is sufficient for completion of the adsorption process.

Significantly ($P<0.01$) higher drug nebulization efficiency percentage (33.75%) was achieved when the adsorption process was carried out at 4 °C. The higher viscosity of HA solution at 4 °C (8.0 mPas; Brookfield DV-II + PRO, Brookfield Engineering Labs., Inc., Middleboro, MA, USA) compared to the viscosity of the same solution at 25 °C (6.0 mPas) was suggested to have marked effects on the solution conformation of HA from which the adsorption occurred.

Cowman and Matsuoka (36) investigated the structure of hyaluronan and found that the molecular domain of a high molecular weight HA chain occupies a sphere. Because the molecular domains are quite large, HA chains interfere with each other at even low concentrations. With increasing concentration, molecular weight or decreasing temperature, the polymer chains begin to approach each other and interact hydrodynamically. On the other hand, over the temperature range of 25–60 °C, the persistent length decreases leading to a decrease in the intrinsic viscosity. This reflects the increased

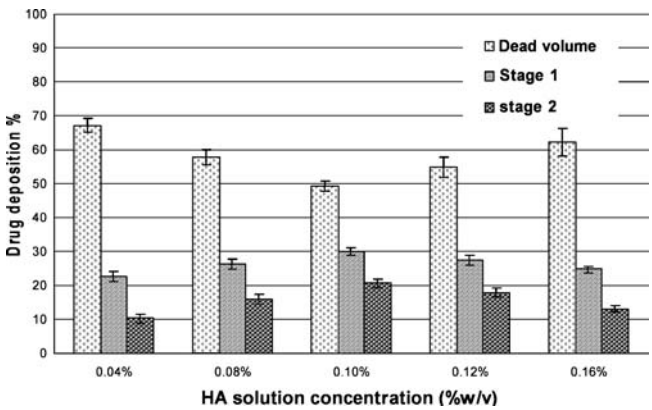

Fig. 4. The influence of HA solution concentration on the drug deposition percentages of the HA–BV suspensions prepared at 25 °C over three days using method 1

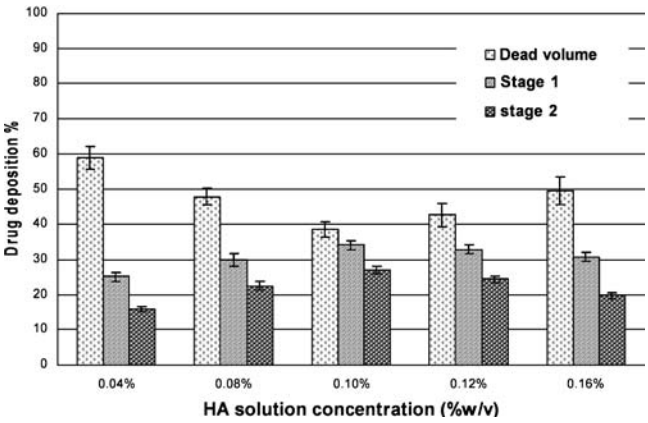

Fig. 5. The influence of HA solution concentration on the drug deposition percentages of the HA–BV suspensions prepared at 25 °C over three days using method 2

population of high energy conformers at high temperature. The bulk density was confirmed to decrease as a result.

Indeed, more uniform suspensions with higher drug nebulization efficiencies were obtained when the adsorption process was carried out at 4 °C.

Conclusively, the highest drug nebulization efficiency percentage (33.75%) was achieved with the optimized HA–BV suspension prepared by the adsorption of 0.1% HA (*w/v*) on BV particles at 4 °C over a 3-day period and adopting method 2 of preparation.

Figs. 7 and 8 show the influence of increasing L-leucine and sodium taurocholate concentration on the drug nebulization efficiency percentage of the optimized HA–BV suspension, respectively. It is clear that, there is a direct relationship between the surfactant concentration and the drug nebulization efficiency percentages. Statistical analysis of the data revealed that significantly ($P<0.01$) higher drug nebulization efficiency percentages are obtained with the suspensions prepared using sodium taurocholate.

Indeed, the incorporation of 0.04% (*w/v*) of sodium taurocholate (formula S4) significantly ($P<0.001$) increased the drug nebulization efficiency percentage from 33.75%, with the optimized HA–BV suspension, to 56.25%. On the other hand, the dead volume percentage was significantly

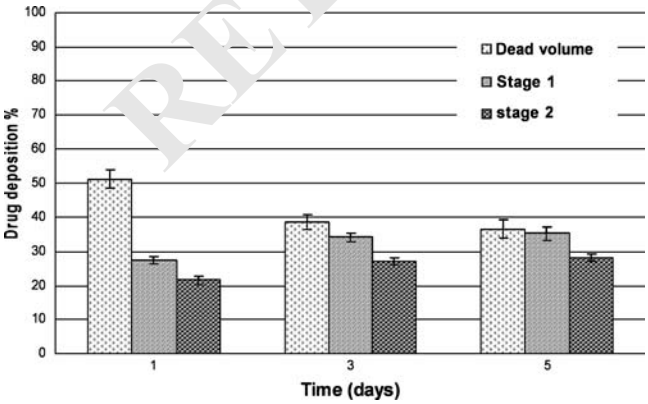

Fig. 6. The influence of the time required for the adsorption process to complete on the drug deposition percentages of the HA–BV suspensions prepared with 0.1% (*w/v*) HA solution concentration at 25 °C using method 2

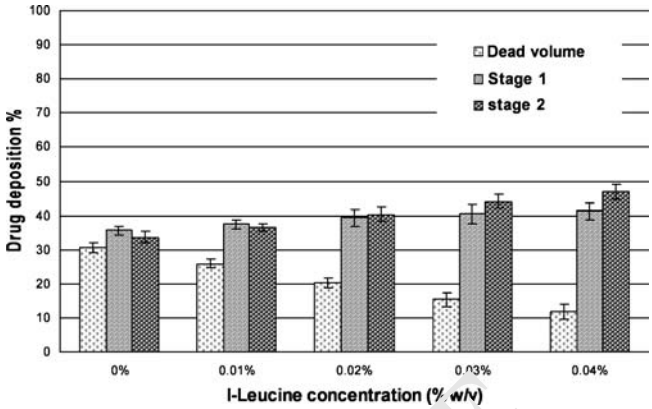

Fig. 7. The influence of L-leucine concentration on the drug deposition percentages of the optimized HA-BV suspensions

($P<0.001$) reduced from 30.62%, with the optimized HA–BV suspension, to 0.62%. These results could be related to the ability of sodium taurocholate to cause significant reductions in the aggregate size of the optimized HA–BV suspension from 4.55 to 1.67 μm, which is closer to the individual particle size (1.5 μm). The lower the particle size, the higher drug nebulization efficiency percentage (37). As suggested by Johansson *et al.* (23), sodium taurocholate further increases the bioavailability of the nebulized drugs by opening of tight junctions between the adjacent airway epithelial cells.

CONCLUSIONS

The hydrophilic HA molecules can adsorb, under specific conditions, onto the surface of hydrophobic BV particles due to physical interactions between the surface of BV particles and hydrophobic patches along the HA chains. The extent of adsorption is influenced by the conformation of the HA molecules in the solution from which adsorption occurred. Optimized HA–BV suspensions are achieved following the adsorption of HA (0.1%, *w/v*) on BV particles at 4 °C, over a 3-day period and adopting method 2 of preparation. Incorporation of L-leucine or sodium taurocholate significantly decreased the aggregate size of the optimized suspension and consequently caused significant increases in the drug nebulization efficiency percentages.

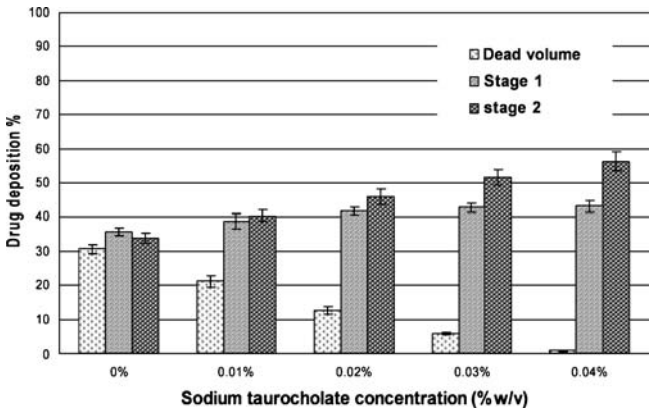

Fig. 8. The influence of sodium taurocholate concentration on the drug deposition percentages of the optimized HA–BV suspensions

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