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Preparation of large porous biodegradable microspheres by using a simple double-emulsion method for capreomycin sulfate pulmonary delivery

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Abstract

The aim of this work was to evaluate if a simple double-emulsion method could be used for developing a new formulation of large porous microspheres (MS) potentially useful for capreomycin sulfate (CS) pulmonary delivery. Poly(DL-lactide-*co*-glycolide) was used for MS preparation. A simple W/O/W double-emulsion/solvent evaporation preparation method was employed and MS were characterized by UV spectrophotometry, particle size, and scanning electron microscopy. A computer-generated response surface method (RSM) was employed to evaluate % drug content, volume mean diameter (VMD), and span upon variation of two numeric and two categorical factors.

MS size distribution was found to be strongly affected by the homogenization method and the type of emulsifier employed. Mean diameters ranged from 1 to 20 μm. The MS presented a proper morphology, with a highly porous interior and a rough surface. Peptide content ranged between 1 and 20%. The region of optimality was referred to as a low VMD and span values, and a high drug content. The best results were found when using a 20% loading, 19.8–3.2 dichloromethane/acetone ratio, ultraturrax mixing, and HPMC as emulsifier.

The double-emulsion method allowed the preparation of CS loaded large porous MS having suitable characteristics to match respirability requirements. The use of RSM helped to establish the conditions to obtain formulations potentially useful for a possible CS pulmonary delivery, by using a simple preparation method with a consistent time, cost, and material saving.

Keywords: Capreomycin sulfate; Large porous microspheres; Tuberculosis; RSM

1. Introduction

Recently, tuberculosis (TB) infections have been found to worryingly spread all over the world. Several factors, such as HIV epidemics, immigration from developing countries and failure of DOTS (directly observed treatment short course) therapies, contributed to increase the TB incidence (Maher et al., 2002; Vigorita et al., 1994; Ferrarini et al., 1999).

Moreover, improper use of antibiotics in chemotherapy easily produces multiple drug-resistance (MDR) strains. MDR TB requires extensive, expensive, and very toxic chemotherapy to patients. In addition, most antibiotics are relatively ineffective for the treatment of intracellular infections, such as TB, due to their poor penetration into cells or decreased intracellular activity. The improvement of the anti-microbial agent efficacy against microorganisms located inside cells has been achieved by drug entrapment within liposomes (Gursoy, 2000; Deol and Khuller, 1997; Pinto-Alphandary et al., 2000; Le Conte et al., 1994). Moreover, potential liposomal capreomycin sulfate (CS) formulation have been developed for inhalation use (Giovagnoli et al., 2003; Ricci et al., 2006). CS is a water-soluble peptide used, intramuscularly (15–20 mg/kg/day), in combination with other effective drugs, in the treatment of TB that failed to respond to first-line agents (Martindale, 1997). Recently, it has been demonstrated that CS can be useful in the treatment of MDR TB (Fattorini et al., 1999).

Liposomes, however, show some drawbacks related to their instability and low loading capacity. In this regard, biodegradable polymeric microspheres (MS) may represent a valid alternative owing to their well know properties. In particular, poly(lactide-*co*-glycolide) (PLGA) polymers have been employed for producing respirable MS for anti-tubercular use (Suarez et al., 2001; Zhou et al., 2005; Sharma et al., 2001). To date, the alveolar CS dose needed to achieve a therapeutic

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intracellular effect is not known, but it could be theorized that low concentrations may be sufficient since the very peculiar target site of action. Moreover, low CS amounts may be preferable in order to avoid drug absorption and the harmful side effects associated with a systemic exposure. This also justifies the use of polymeric particles in place of other well known targeting systems, such as liposomes, which, are known to retard or partially avoid macrophage uptake when delivered to the alveoli (Evora et al., 1998). Polymeric MS, in turn, being prone to macrophage uptake by virtue of a longer residence time and the non-self characteristics of the polymeric material, can enhance intracellular targeting. However, fabrication of a suitable inhalable powder for pulmonary delivery is indeed complex. In this regard, a proper drug loading, size, and morphology characteristics represent the key factors affecting the applicability of such formulations. Therefore, the well established characteristics for obtaining a respirable powder have to be accounted when choosing a preparation method. Such characteristics can be summarized by the following equation:

$$d_{ae} \cong d_g \sqrt{\frac{\rho_p 1}{\rho_0 \chi}} \quad (1)$$

where d_{ae} is the mass median aerodynamic diameter (MMAD) (Schlesinger, 1985), d_g the mean geometric diameter, ρ_p the particle density, ρ_0 is 1 g/cm^3 and χ is the shape factor.

It is reported a d_{ae} upper limit of 4.7 μm in order to fulfill the respirability characteristics (Byron and Patton, 1994; Brain and Valberg, 1979; Zainudin, 1993). Of course, this result can be achieved by changing either particle size or density and shape.

Although, spray-drying is a method of choice to fabricate MS for inhalation purposes, in this paper an alternative approach was investigated by employing a simple double-emulsion method. Although there are methods exploiting mild conditions for the production of spray-dried PLGA MS (Wang and Wang, 2002; Nguyen et al., 2004; Gavini et al., 2003, 2005), there are some issues related to the use of solvents and the thermal behavior of PLGA polymers, which are in some cases sensitive to the temperatures used for the production of spray-dried powders (e.g. polymer annealing may occur). On the other hand, although possessing many scaling up problems, the double-emulsion method is simple and low cost and ensures the preservation of polymer characteristics, moreover, when properly modified, it can be useful for the entrapment of hydrophilic molecules (Giovagnoli et al., 2004; Kang and Singh, 2001; Schrier and DeLuca, 2001). Unfortunately, this technology often produces particles with a too large MMAD and size distributions to be suitable for inhalation. These adverse features may, however, be compensated by proper particle morphological characteristics, such as shape and porosity. In fact, large porous MS can be used for inhalation (Abdellaziz et al., 1999; Edwards et al., 1997, 1998). It was found that, although having large geometric diameters, their irregular shape and high porosity provide adequate aerodynamic characteristics, thus allowing their delivery as an aerosol powder. In this paper, parameters as kind of emulsifier, type of stirring, loading, and dichloromethane/acetone ratio were modified to investigate the possibility of producing potentially inhalable CS loaded large porous PLGA MS. To achieve this goal, a computer generated response surface method (RSM) was employed in order to assess preparation variable effects on particle characteristics. The MS suitability was regarded as drug content, volume mean diameter (VMD), and dimensional dispersity, which was referred to as span. MS were characterized by spectrophotometry, scanning electron microscopy (SEM) and particle size analysis.

Prior to effect investigation, model suitability and prediction efficiency were investigated by check-point analysis, while the preparation variable effects were assessed by response surface evaluation. Finally, best CS loaded MS batches were evaluated to disclose the real possibility of using such a simple method for fabricating MS having a potential therapeutic usefulness. In this regard, their characteristics in terms of VMD and span were considered fundamental to embed the MS obtained with possible respirability properties.

2. Materials and methods

2.1. Materials

CS from *Streptomyces Capreolus*, polyvinylalcohol (PVA, 30–70 kd) and hydroxypropylmethylcellulose (HPMC) were purchased from Sigma–Aldrich Chemical (Milan, Italy). Poly(DL-lactide-*co*-glycolide) Resomer RG502H (Mw ∼10 kd) was purchased from Bidachem s.p.a. (Florence, Italy). Acetone was provided by Farmitalia Carlo Erba (Milan, Italy) and dichloromethane by J.T. Baker (Milan, Italy). Ultra pure water was obtained by reverse osmosis through a Milli-Q system (Millipore, Rome, Italy). All other reagents and solvents were of the highest purity available.

2.2. Microsphere preparation

CS loaded MS were prepared according to a W/O/W double-emulsion method (Giovagnoli et al., 2004). From 270 to 285 mg of RG502H PLGA polymer was dissolved in about 1 mL dichloromethane or dichloromethane/acetone solution, while CS amounts corresponding to 5–20% (w/w) with respect to batch size (300 mg) were dissolved in 100 μL water solution. Then, the CS solution was mixed to the organic phase containing the polymer to obtain a primary W/O emulsion. This primary emulsion was vortexed for 2 min and then slowly injected into 40 mL of 6% PVA or 3% HPMC water solution under mechanical stirring (1500 rpm, at 25 °C) to form the final W/O/W double emulsion. After 1 min, the final emulsion was diluted in 500 mL of deionized water maintained at 25 °C. In order to evaporate the organic solvent, the emulsion was kept under stirring for about 2 h. The resulting MS were filtered by a Millipore 5 μm nitrocellulose filter (Millipore, Milan, Italy), washed with deionized water and freeze-dried overnight.

2.3. Evaluation of CS content

The amount of CS encapsulated was evaluated by UV spectrophotometry using a UV/VIS Agilent 8453 spectrophotometer

Table 1
d-Optimal design set up

Factor	Name	Type	Low Actual	High Actual	Low Coded	High Coded	Responses	
A	Loading	Numeric	5%	20%	-1	+1	Y_1	Drug content
B	Dichloromethane/acetone ratio	Numeric	70/30	100/0	-1	+1	Y_2	VMD
C	Stirring system	Categorical	Ultraturrax	Propeller	-1	+1		
D	Emulsifier	Categorical	HPMC	PVA	-1	+1	Y_3	Span

(Agilent, Germany). The CS absorbance was read at 268 nm. CS was extracted from the MS by incubation of a weighed amount of MS in 1 mL of a 1 M NaOH solution overnight at r.t. After complete MS hydrolysis, the clear solution was neutralized with an equal volume of a 1 M HCl solution. Upon proper dilution, the solution was submitted to spectrophotometric analysis. The peptide entrapped in the MS was expressed as % drug content (Eq. (2)) and as encapsulation efficiency (Eq. (3)):

$$\%\text{drug content} = \frac{\text{amount of entrapped drug}}{\text{MS weight}} \quad (2)$$

$$\text{encapsulation efficiency} = \frac{\text{actual loading}}{\text{theoretical loading}} \times 100 \quad (3)$$

All measurements were carried out in triplicate and the error expressed as S.D.

2.4. Scanning electron microscopy

MS surface structure and porosity were investigated by scanning electron microscopy (SEM) using a Philips XL30 microscope (Philips Electron Optics, Heindoven, NL). Samples were prepared by placing an amount of dried MS powder onto an aluminum specimen stub. The samples were dried overnight and were sputter coated with gold prior to imaging (EMITECH K-550X sputter coater Ashford, Kent, UK). Coating was performed at 20 mA for 4 min.

2.5. Particle size distribution

An Accusizer 770 (PSS Inc., Santa Barbara, CA) using the technique "Single Particle Optical Sensing" was used to determine the size distribution of the MS preparations. The lyophilized particles were suspended into deionized water and a small amount of Tween 80® was used as surfactant to prevent MS aggregation. Analysis were performed in triplicate and size was expressed as VMD. The population dispersity was referred as span and calculated as reported in the following equation:

$$\text{span} = \frac{d_{90} - d_{10}}{d_{50}} \times 100 \quad (4)$$

where d_{90}, d_{10}, and d_{50} are the mean diameters at the 90%, 10%, and 50% of the population distribution, respectively.

2.6. Model validation and check-point analysis

A computer generated d-optimal design was employed to evaluate main effects and interactions of the factors chosen on % peptide content, VMD, and span. The factors were selected according to the parameters characterizing the preparation method: % peptide loading, solvent ratio, type of stirring system, and type of emulsifier (Table 1).

A polynomial regression model was employed for the d-optimal design. The dataset used consisted of 59 batches comprising two center point replicates of CS loaded MS. The model was evaluated in term of statistical significance by using ANOVA analysis. The Box-Cox diagnostic test and normal probability plots of residuals were used to check variance and the need of mathematical transformation on dataset and to assess the presence of possible outliers. Check-point analysis was carried out in order to establish the model predictivity and, then, to assess its reliability in describing the MS behavior. The check points were selected according to the response surface plots thereby obtained (Table 2), at the lower and higher level of each of the two categorical factors, and all measurements were performed in triplicate. The response actual values were compared with the predicted values by means of an unpaired-*t* test and bias estimates were quantified.

2.7. Response surface evaluation and interpretation

On the basis of the validated models, response surface plots were generated for each response so as to determine the effects of the preparation variables on the selected MS characteristics. In this regard, desirability was assessed and investigated in order to determine the conditions for best particle features in order to establish whether or not the MS produced could be accounted for the development of respirable formulations for the MDR TB treatment. This evaluation was accomplished by considering MS properties mainly referred as VMD, which was regarded as one of the most important features, along with particle morphology, affecting respirability, especially if considering its fundamental contribution to MMAD.

All statistics and experimental design analyses were performed by using a Design-Expert® v. 6.0.11 software (Stat-Ease, Inc., Minneapolis, MN).

Table 2
Check-point analysis

Factors C and D	Check-point		Predicted %drug content	Predicted VMD	Predicted span	Actual % drug content ± S.D*	Actual VMD ± S.D*	Actual span ± S.D*
	Factor *A*	Factor *B*						
Ultraturrax-HPMC	20	85:15	1.3***	12.1***	1.6***	1.5 ± 0.6***	13 ± 4***	1.1 ± 0.5***
Ultraturrax-PVA	20	85:15	4.8***	19.5**	1.2***	6.2 ± 2.0***	12 ± 7**	1.1 ± 0.3***
Propeller-HPMC	20	85:15	10.2***	45.7***	1.3***	11 ± 4***	54 ± 15***	1.7 ± 0.5***
Propeller-PVA	20	85:15	14.3**	73.8***	1.0**	19.6 ± 3.5**	68 ± 14***	1.5 ± 0.4**

* $n = 3$.
** Not significant difference ($P = 0.100$, $\alpha = 0.05$).
*** Not significant difference ($P = 0.700$, $\alpha = 0.05$).

3. Results and discussion

3.1. Characterization of CS loaded MS

The W/O/W method employed produced MS with a promising morphology as shown in Fig. 1. The method was modified by using a concentrated stabilizer solution to favor a rapid particle formation upon injection of the primary W/O emulsion and to limit size increase as well as CS leakage from the soft matrix. The dilution of the final W/O/W emulsion is a standard procedure needed to decrease the stabilizer concentration in the first place, and to allow a better dispersion of the MS being formed in order to reduce the probability of particle aggregation upon hardening.

The high rate of evaporation adopted for MS hardening provoked the formation of large pores and cavities within the polymeric matrix. This contributes to reduce the density and compactness of the particles, with a supposed decrease in the MMAD value as reported by Eq. (1). Moreover, the high porosity observed increases the unevenness and roughness of the surface with a positive effect on the shape factor χ and, thus, possibly on their aerodynamic properties. As a prove, Fig. 1 Panel C shows a cross-section of a PLGA MS obtained with the W/O/W method. The interior of the particle is clearly full of cavities which are the result of the extraction of the water droplets from within the matrix. This feature is important, as already mentioned, to reduce the density and the MS so as to obtain a favorable MMAD value. These morphological characteristics were the same for all the batches obtained regardless of factor variations.

3.2. Assessment of model predictivity

Particle size in terms of VMD, and span and CS drug content were monitored upon variation of the selected factors. The experimental design applied to the three responses chosen was validated by assessing model adequacy. The polinomial equations

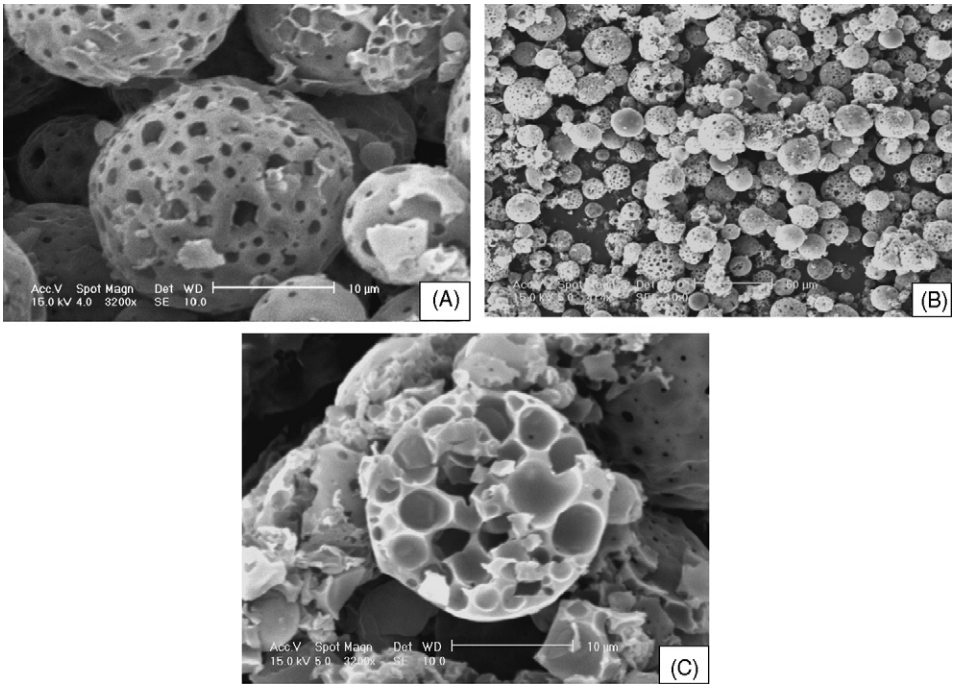

Fig. 1. SEM microphotographs of CS loaded PLGA MS batches used for the experimental design (Panels A and B). MS cross-sectional image is also reported showing the porous internal structure (Panel C).

describing the effects of the four factors on the responses as derived from the d-optimal design are here below reported:

$$Y_1 = 1.94 + 0.57A - 0.34B - 0.93C + 0.29D - 0.17A^2 + 2.59B^2 + 0.99AB - 0.23AC + 0.043BC + 0.35B^2 - 0.23A^2B + 0.30AB^2 + 0.41B^2C - 2.38B^4 - 1.20AB^3 \quad (5)$$

$$Y_2 = 3.71 + 0.082A - 0.077B - 0.076C + 0.24D - 0.39A^2 - 0.083AC + 0.25BD - 0.51A^2C \quad (6)$$

$$Y_3 = 0.66 + 0.15A + 0.053B - 0.083C + 0.098D + 0.21AB \quad (7)$$

where the terms A, B, C, and D represent the four factors as reported in Table 1 and Eqs. (5)–(7) are the models describing the behavior of the drug content, VMD, and span, respectively.

ANOVA and statistical validation of the mathematical models recorded a high significance ($P < 0.0001$, $\alpha = 0.05$) as compelled by large F values. Although the presence of not significant terms as result of the need of preserving model hierarchy, Eqs. (5)–(7) showed satisfactory characteristics in terms of r^2 values, which were 0.8558, 0.4206, and 0.3508, respectively. On the other hand, diagnostics did not point out any issue related to the presence of outliers or lack of normality (data not shown). In addition, the investigation of response curvature showed a not significant lack of fit, which was correlated to a proved non-linearity of the polynomial equations obtained. Although the models were statistically validated, a further confirmation of their ability to predict particle features has yet to be provided. In fact, in order to use Eqs. (5)–(7) for investigating the conditions corresponding to best particle properties, model predictivity needs to be disclosed and assessed exactly. In this regard, predicted and actual values were compared to highlight model performances and effectiveness (Fig. 2A–C). The correspondence between calculated and experimental data was quite higher for drug content (Fig. 2A) for which correlation reached 0.8992, and lower for VMD and span that showed a larger deviation from the ideal trend (Fig. 2B and C) ($r^2 = 0.5025$, 0.4080, respectively). This, may be ascribed to a stronger dependence of VMD and span on categorical factors, which determine, to a larger extent, particle size, size distribution, an inflated variance, and thus variability of the response.

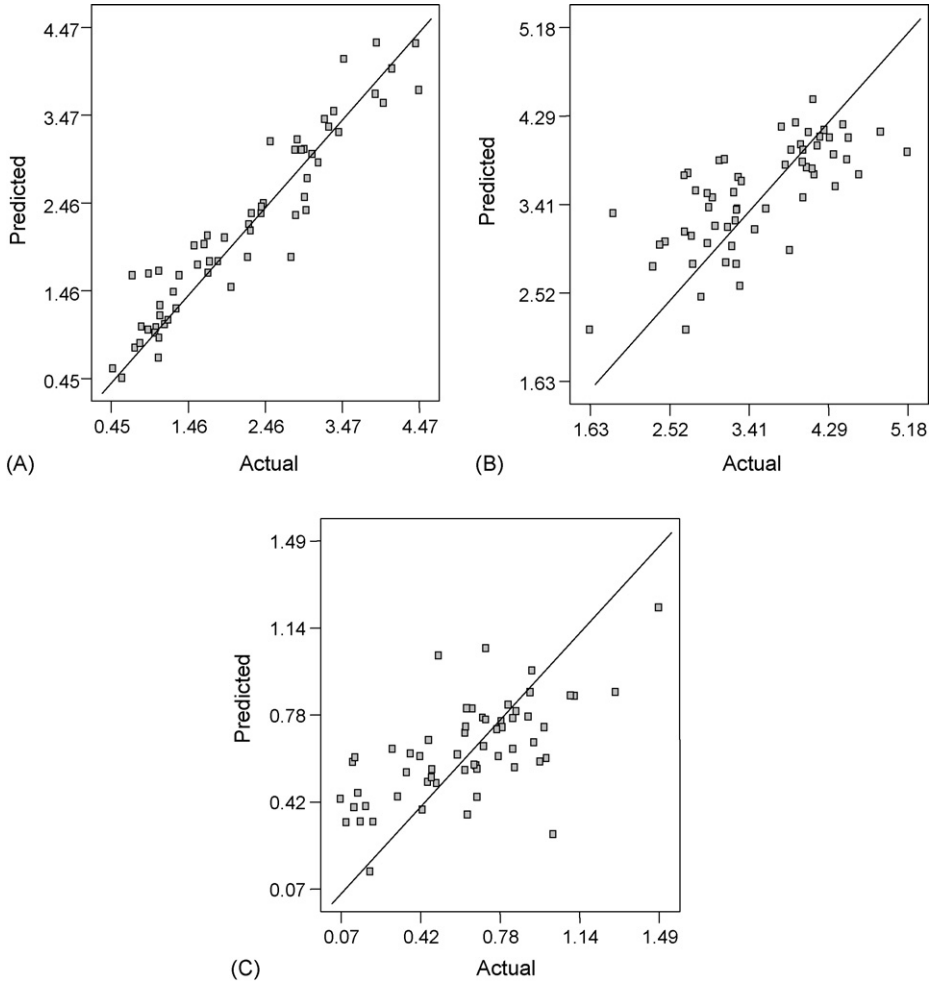

Fig. 2. Plots of predicted and actual values as obtained from the Eqs. (5)–(7) for drug content (Panel A), VMD (Panel B), and span (Panel C).

Table 3
Biases between the predicted and actual response values obtained from the check-point analysis

Factors C and D	Check-point		%Drug content bias	VMD bias (%)	Span bias (%)
	Factor *A*	Factor *B*			
Ultraturrax-HPMC	20	85:15	13.3	6.9	31.3
Ultraturrax-PVA	20	85:15	22.6	38.5	8.3
Propeller-HPMC	20	85:15	7.3	15.4	23.5
Propeller-PVA	20	85:15	27.0	7.9	33.3

Therefore, four check-points were selected within the design space as reported in Table 2. These points corresponded to spots in the optimality area where the model reports improved MS characteristics. The calculated and measured responses were in close agreement as from the values reported in Table 2. The *t*-test performed on the predicted and actual values obtained in triplicate confirmed a not significant difference between the two groups of data ($P = 0.100$, $P = 0.700$) either for drug content and VMD or span, at 95% significance level. Moreover, an additional analysis was performed on bias estimates (Table 3). As reported, the differences between the actual and the model generated responses are mainly below 15%, even though, in some cases, biases as large as 20% or more than 30% were recorded. Although apparently high, these biases have to be considered sufficiently adequate when a very complex system is investigated. In fact, the double-emulsion method is easy to be performed but it is controlled by many parameters, which represent sources of variation able to lower precision and reproducibility. This can be seen also in Table 2 where the S.D.s related to the true values are actually quite large, reflecting reproducibility problems. Such an issue, is also evident from the ANOVA validation of Eqs. (5)–(7), where the coefficient of variation (C.V.) of each response ranged from 15 to 18% to values as high as 40% (data not shown). In light of these observations, the agreement between predicted and actual values was considered satisfactory.

3.3. Response surface evaluation and variable effects

Important considerations are raising from the observation of the contribution of the single factors to the response values. In fact, the model coefficients, which estimate the factor effect on each response, pointed out a slightly higher contribution of the factor *D* to VMD and the factor *A* to span with respect to the other factors. As confirmed by the presence of large higher order coefficients in Eqs. (6) and (7), an even more important effect of interactions was seen. Nevertheless, span (Eq. (7)) was influenced only by the AB term, while the other interaction terms were negligible and omitted for model improvement. On the other hand, Eq. (5) showed a larger contribution of the C coefficient and a remarkable effect of high order interactions. These observations remarked a large non-linear effect of B and a quite important contribution of C on the response, which can be explained by the intrinsic importance of stirring and the solvent diffusion and extraction processes, which strongly affect drug entrapment in particulate systems. In fact, along with an increased acetone content, the fast diffusion-solvent extraction becomes more and more incisive upon emulsification, and that favors dramatically drug leakage from within the forming particle. Moreover, the stirring method weights heavily on drug content, which can be strongly modified whether ultraturrax or propeller mixing are chosen.

From these observations, it is clear that the categorical factors had a relevant influence on size and size distribution as well as drug content, especially in the non-linear terms which possess a predominant effect. Moreover, the D contribution to VMD resulted of a great extent, confirming the importance of the choice of a right emulsifier able to reduce size as well as to control size dispersity.

The validated models, represented by Eqs. (5)–(7), were used to build up the corresponding response surfaces. The best target features were found to be a maximum drug content, a minimum VMD as well as a low span value. According to the results obtained by fitting the models to experimental data, the highest MS suitability was reached when ultraturrax and HPMC were used for the CS loaded MS preparation. This condition corresponds to the low levels of the two categorical factors, therefore the response surface plots were built by keeping factors C and D constant at their low level, and varying the numerical factors A and B (Fig. 3). In this situation, the drug content was seen to reach a maximum of about 5%, when loading was increased and solvent ratio tended to its lowest value (70/30). In turn, VMD was minimum if loading was either at its lower or higher level, and solvent ratio was maximum (100/0), whereas span reached about 1 when both factors A and B were increased. It is clear that not all the conditions assessing best values for the three responses exactly matched. In fact, an opposite effect on drug content and VMD was observed for factor *B*, whereas span and VMD seemed to undergo to the same variation when factor *B* was increased. This effect correlates well with the observations, previously reported, based on the coefficient estimates of Eqs. (5)–(7), where the solvent ratio turned to radically enforce the non-linear terms of the models, confirming the delicate balance existing between the amount of organic solvent and the kind of emulsifier used, which has to be perfectly assessed to ensure suitable MS aerodynamic properties.

The optimal condition for obtaining potentially suitable CS loaded MS was established by using the desirability function when ultraturrax and HPMC were employed. Thanks to this mathematical tool it was possible to assess the best compromise among the three response improvement regions (Fig. 4). According to that, an improvement can be achieved at high loadings (20%) and at low acetone amounts in the organic solution (96.8/3.2). In this conditions, it was found that reasonably low VMD and span values can be achieved, while maintaining theoretically acceptable CS content levels. In fact, a VMD as low as 9–15 μm and a span value of about 1–0.9 were recorded when both loading and solvent ratio were set within this region. These conditions also allowed the achievement of a 5–7% CS content. The batches obtained observing these predicted conditions revealed quite a good size distribution profiles (Fig. 5A). Moreover, increasing the CS loading from 5 to 20% proved to be

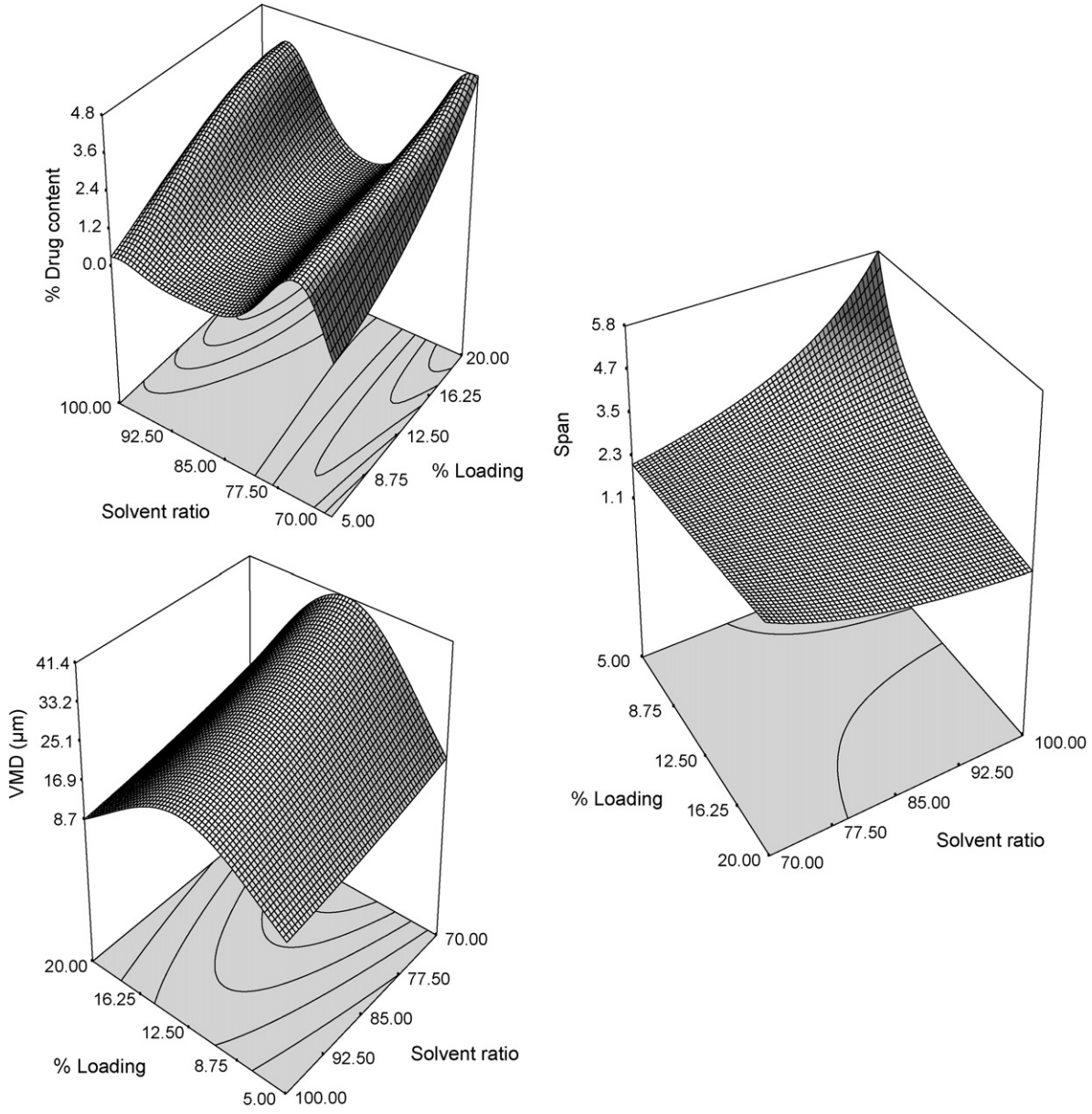

Fig. 3. Response surface plots of the validated models for drug content, VMD, and span. All the plots were built at the lower level of the two categorical factors (Ultraturrax, HPMC).

very useful to highlight the effect of this factor on VMD and span (Fig. 5B). In fact, although a 5% loading decreased VMD very much, however it increased span quite a lot with respect to higher loadings. Moreover, of course a low loading produces a low content, which is not desirable, therefore the compromise adopted for achieving the optimality is justified in order to obtain an acceptable CS content. Furthermore, VMD around 10–15 μm may be considered adequate because of the high MS porosity, which should decrease consistently the MS density and increase the shape factor, with an important reduction of the MMAD value as reported by Eq. (1). Unfortunately, there are some drawbacks related to the use of HPMC. In fact, some aggregates were seen in all cases, but mainly when employing HPMC as emulsifier (Fig. 6). Hence, care has to be paid upon emulsification of the primary emulsion with the HPMC solution in order to avoid strong aggregation and the undesired formation of too large particles. This phenomenon was not always easy to control, although a good dispersion limits the cluster building up, and it increased by increasing acetone content in the solvent mixture as well as at 100% dichloromethane. This is the reason why an exact acetone amount was used (∼3%), although practical limits impair very precise adjustments of the solvent ratio, as consequence of their high volatility. In spite of that, the formation of a large population with a VMD of 8–10 μm was always observed.

Although this phenomenon reflected negatively on reproducibility, as earlier reported, the use of HPMC favored the formation of smaller CS loaded MS with a sufficient content. Therefore, in any case, this emulsifier was of choice over other stabilizers, such as PVA.

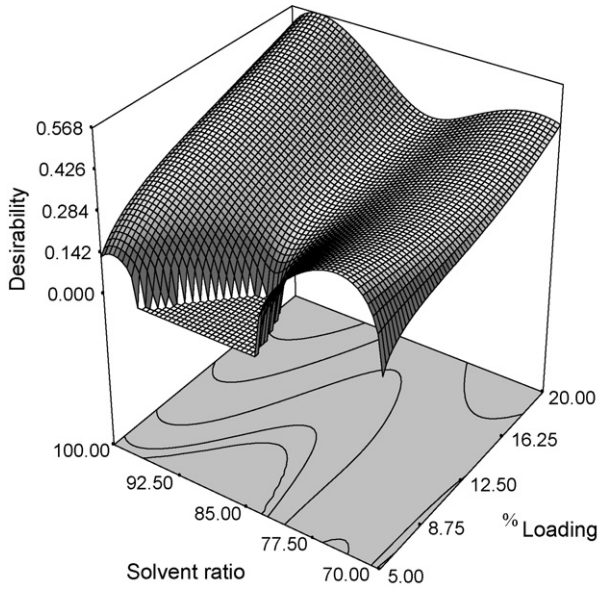

Fig. 4. Desirability response surface profile built at the lower level of the two categorical factors (Ultraturrax, HPMC).

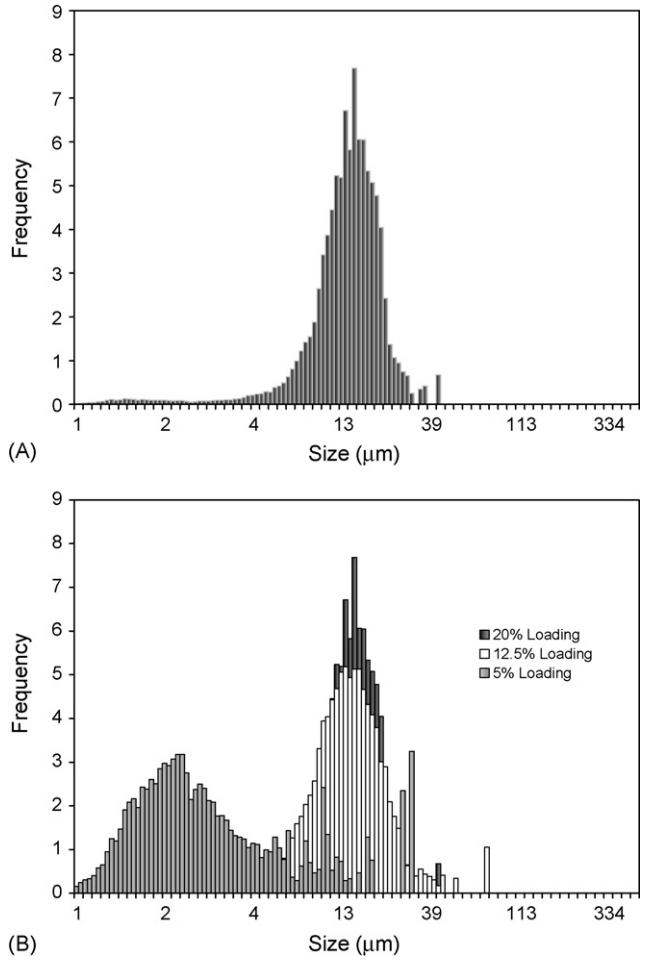

Fig. 5. VMD size distribution profile of the best batch obtained on the basis of the experimental design results (Panel A). The effect of loading in the same conditions is also reported (Panel B). The batches were generated around the optimality region outlined by the desirability function at the lower level of the two categorical factors.

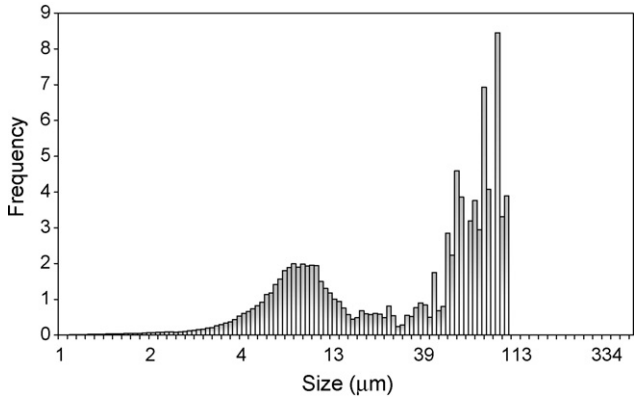

Fig. 6. VMD size distribution profile of one batch, obtained at 15% loading, putting in evidence aggregation phenomena. Note the presence of a suitable population at around 8–10 μm.

Further considerations concern the size of the particle produced. Early studies showed that large porous particles can be used effectively for lung delivery of drugs (Abdellaziz et al., 1999; Edwards et al., 1997, 1998). In fact, although having large geometric diameters with mean geometric diameters as large as 10–20 μm (Edwards et al., 1998) or 8.5 μm (Edwards et al., 1997), the high porosity confers proper aerodynamic characteristics and the MMAD results lower than the 4.7 μm limit set for respirability. As a consequence, large non-porous particles can be inhaled much more efficiently than small non-porous particles, probably because of a much lower fractional surface area available for particle–particle contact, which makes them less prone to aggregation (Abdellaziz et al., 1999). A similar behavior could be thought for the highly porous large CS loaded MS obtained in this work, since a size (8–10 μm) comparable to that reported in the literature was obtained (Abdellaziz et al., 1999; Edwards et al., 1997, 1998). Hence upon these considerations many hopes account the possibility of using CS loaded MS, obtained by a double-emulsion method, for a practical (clinical) application in CS lung delivery.

4. Conclusion

In conclusion, it was possible to fabricate CS loaded PLGA MS with good characteristics by using a simple double-emulsion method. This method, even though poorly applicable to large-scale production, possesses an implicit easiness and the ability to preserve PLGA polymer characteristics.

Although the MS respirability was not proven, the properties in terms of morphology, size and drug content were satisfactory and are supposed to influence positively MS aerodynamic features. On these basis, the CS loaded MS may be considered potentially useful as a tool for improving drug-resistant antitubercular treatments.

The next step, of course, is to formulate a proper inhalable powder in order to allow respirability tests able to provide the actual aerodynamic properties and the respirable fraction of the produced particulate system.

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