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Effect of chitosan crosslinking on bitterness of artemether using response surface methodology

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Abstract

This work examines the influence of various process parameters on artemether entrapped in crosslinked chitosan microparticles for masking bitterness. A central composite design was used to optimize the experimental conditions for bitterness masking. Critical parameters such as the amounts of artemether, chitosan and crosslinking agent have been studied to evaluate how they affect responses such as incorporation efficiency, particle size and drug release at pH 6.8. The desirability function approach has been used to find the best compromise between the experimental results. The optimized microparticles were characterized by Fourier transform infrared spectroscopy and differential scanning calorimetry. Bitterness score was evaluated by human gustatory sensation test. Multiple linear regression analysis revealed that the crosslinking of chitosan significantly affects incorporation efficiency, particle size and drug release at pH 6.8. The bitterness score of microparticles was decreased to 0, compared with 3+ for pure artemether. The proposed method completed masked the bitter taste of artemether.

Introduction

β-Artemether is one of the artemisinine derivatives that have proved efficient against acute uncomplicated and severe falciparum malaria (Hien & White 1993; Hien 1994) and can clear the parasite even in multiple-drug-resistant falciparum malaria (Bunnag et al 1992).

Artemether has an extremely unpleasant bitter taste. The exact mechanism of this bitterness is not known but it has been reported that drugs like artemether bind to a membrane receptor on the apical taste cells and thus produces bitterness (Yamamoto et al 1998).

Artemether is available as capsules and uncoated tablets. However, these formulations are not appropriate for children because the bitter taste can cause non-compliance and thus hinder therapeutic management (Nahata 1999; Uchida et al 2003).

The development of a palatable formulation has proved difficult, although various taste-masking techniques have been reported, such as the addition of sweeteners and flavours (Barra et al 1999), coating with polymers (Chopra et al 2002), adsorption to ion-exchange resin (Jaskari et al 2001; Vuorio et al 2003), and chemical modifications such as the use of insoluble prodrugs (Borodkin & Yunker 1970; Vyas et al 1973). Each technique has its disadvantages. Addition of sweeteners and flavours is not particularly successful for masking the taste of extremely bitter drugs. Ion-exchange resins are specific for amino groups and sometimes delay drug release. Coating with polymer requires sophisticated instruments. Chemical modification may alter the therapeutic activity of the drug. In such cases, microencapsulation offers advantages in masking bitterness (Sjoqvist et al 1993; Gao et al 2006).

Chitosan [poly (1,4-β-D-glucopyranosamine)] is a polysaccharide derived from naturally occurring chitin by alkaline deacetylation. This polymer has been investigated extensively for applications in various drug-delivery systems. It has appealing intrinsic characteristics that include biodegradability, biocompatibility and lack of toxicity (Sinha et al 2004; Souza et al 2005; Ubaidulla et al 2007). Sodium hydroxide was used as crosslinking agent (Eroglu et al 2007). No reference to any work on the use of chitosan microparticles to mask the bitterness of artemether could be found in the literature. This was therefore the objective of the present study.

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Materials and Methods

Materials

Artemether was a gift from Ajanta Pharma Ltd (Mumbai, India). Chito Clear chitosan (low molecular weight with 86% deacetylation) was a gift from Primax Biopolymers (Reykjavik, Iceland). Methanol was purchased from Qualigens Fine Chemicals (Mumbai, India). Sodium hydroxide, potassium dihydrogen phosphate and acetic acid were purchased from S. D. Fine-Chem Ltd (Mumbai, India). These reagents were used as received. Qualitative Grade 1 filter papers were purchased from Qualigens Fine Chemicals. All water used in the study was deionized double-distilled water.

Preparation of microparticles

Concentrated chitosan solution (1% w/v) in 1% v/v acetic acid was prepared well before required to get a clear solution, free from bubbles. The required quantity of artemether (56 mg) was dispersed and mixed with concentrated chitosan solution (5 mL). The microparticles were prepared by dropping the chitosan solution containing artemether from a glass syringe with an 18G × 0.5 inch flat-cut hypodermic needle, at a flow rate of 2.5 mL min^{-1} into a magnetically stirred (10% w/v) sodium hydroxide solution. The resulting microparticles were allowed to harden for 60 min with gentle stirring using a magnetic flea. Microparticles were prepared in this way using different amounts of artemether, chitosan and sodium hydroxide, as shown in Table 1. The microparticles were collected on qualitative filter paper, washed with deionized double-distilled water, and dried to a constant weight in a vacuum desiccator (Tarsons Products Pvt. Ltd, Baroda, India) for 48 h at room temperature.

Response surface methodology (RSM)

RSM permits the definition of empirical models (usually quadratic polynomials) that accurately describe responses at all values of the studied variables in the experimental region (Ficarra et al 2002). The aim of RSM is to determine conditions that improve a process.

To calculate quadratic regression model coefficients, each variable has to be studied at three separate levels. The central composite design (CCD) is therefore often used to provide an estimate of a second-order equation. The experimental matrices for the three factors (CCD) consist of 14 experiments, expressed in coded variables, which give values for dependent variables, as shown in Table 2. The central point was repeated six times to estimate the experimental error variance. The experiments were performed in a random order to minimize the effects of uncontrolled factors that may introduce a bias to the measurements.

Three experimental responses were studied: Y1 = incorporation efficiency; Y2 = particle size; Y3 = drug release at pH 6.8. A classic second-degree model was postulated for each experimental response, *Yi*, as follows:

$$Yi = b0 + b1A + b2B + b3C + b11A^2 + b22B^2 + b33C^2 + b12AB + b23BC + B13AC \quad (1)$$

where *b*0 is the arithmetic mean of the 20 runs; *bi* is the estimated coefficient for the factors *A, B* and *C*. All experimental results were computed by statistical software (DOE v6.0.5 Stat-Ease, Inc. Minneapolis, MN, USA).

Table 1 Process variables and their levels for central composite design; A and B are amounts of artemether and chitosan, respectively (in g); C is the amount of sodium hydroxide (in mL 10% w/v solution)

Coded values	Actual values		
	A	B	C
−1.63	0.011	0.011	3.15
−1	0.03	0.03	5
0	0.05	0.05	10
1	0.07	0.07	15
1.63	0.114	0.114	24.45

Optimization of responses using desirability function

This technique provides a way to overcome the difficulty of multiple, sometimes opposing, responses (Lewis et al 1999). Each response is associated with its own partial desirability function. If the value of the response is optimum, its desirability equals 1; if it is totally unacceptable, its value is 0. Thus, the desirability for each response can be calculated at a given point in the experimental domain. The optimum is the point with the highest value for the desirability (Rane et al 2007).

We wanted to maximize percentage incorporation efficiency, in order to reduce the total weight of microparticles equivalent to 50 mg artemether. The desirability function of this parameter was calculated using Equation 2:

$$d_i = \left(\frac{Y_i - Y_{\min}}{Y_{\max} - Y_{\min}} \right)^s \quad (2)$$

where d_i is individual desirability and Y_i is the experimental value for percentage incorporation efficiency and *s* is used to change the shape of the desirability goal by weight field. The values of $Y_{\min}$ and $Y_{\max}$ for incorporation efficiency were 52.5 and 91.4%, respectively.

To avoid grittiness of microparticles after oral ingestion, a minimum particle size was also desired. The observed $Y_{\min}$ and $Y_{\max}$ values for particle size were 104.39 and 337.48 μm, respectively.

The bitter taste of the drug generally occurs because of dissolution of the active component in the mouth. The microparticles remain in the mouth for a maximum of 5 min. To avoid dissolution in the mouth, the minimum percentage drug release at 5 min was desired. The values for $Y_{\min}$ and $Y_{\max}$ for

Table 2 Central composite design with the measured responses; A and B are amounts of artemether and chitosan, respectively (in g); C is the amount of sodium hydroxide (in mL 10% w/v solution)

ES	Factors levels			Incorporation efficiency (%)	Particle size (μm)	Dissolution*	Bitterness score
	A	B	C				
1	1	1	−1	67.98	282.33	6.98	3
2	−1	1	1	64.15	322.89	3.92	0
3	0	0	0	82.32	233.49	3.74	1
4	0	0	0	82.83	231.62	3.87	1
5	−1	−1	−1	67.27	264.43	4.67	2
6	1	−1	1	90.65	104.39	4.33	2.5
7	−1	1	−1	52.50	337.48	4.23	1
8	0	0	0	85.32	242.61	3.69	1
9	1	1	1	82.38	253.96	6.76	2.5
10	−1	−1	1	81.81	169.57	3.54	0
11	0	0	0	85.32	239.62	3.92	1
12	1	−1	−1	74.17	217.28	5.97	3
13	−1.63	0	0	54.22	303.49	3.79	0.5
14	0	0	0	80.87	239.18	3.91	1
15	0	0	−1.63	67.59	307,71	5.83	3
16	1.63	0	0	81.34	219.02	6.81	3+
17	0	−1.63	0	85.85	148.47	3.87	2
18	0	1.63	0	66.21	296.22	5.59	1
19	0	0	1.63	91.40	192.26	3.82	0.5
20	0	0	0	82.32	233.41	3.69	1

*Dissolution at pH 6.8: % drug dissolved in 5 min (t5). ES, experimental sequence.

drug release at pH 6.8 in 5 min (t5) were 3.54 and 6.98%, respectively.

The desirability function for particle size and drug release at pH 6.8 was calculated using Equation 3:

$$d_i = \left(\frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}} \right)^s \quad (3)$$

where d_i is the individual desirability and Y_i is the experimental result. The experimental values were acceptable in all the experiments performed; hence, $s = 1$ was chosen in Equations 4–6.

$$d_i = 1 \qquad \text{if } Y_i < Y_{\min} \quad (4)$$

$$d_i = \left(\frac{Y_{\max} - Y_i}{Y_{\max} - Y_{\min}} \right)^s \quad \text{if } Y_{\min} \le Y_i \le Y_{\max} \quad (5)$$

$$d_i = 0 \qquad \text{if } Y_i > Y_{\max} \quad (6)$$

The overall desirability value was calculated from the individual values using Equation 7:

$$D = (d_1 \times d_2 \times d_3 \times d_4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \quad (7)$$

Characterization of microparticles

Incorporation efficiency

The incorporation efficiency was determined by dissolving the artemether microparticles in magnetically stirred (500 rev min^{-1}) mixed phosphate buffer (India Pharmacopoeia III; 5.04 g disodium hydrogen phosphate plus 3.01 g potassium dihydrogen phosphate made up to 1000 mL with water, pH adjusted to about 4) at room temperature for about 90 min. An aliquot of 2 mL was mixed with methanol. The resulting solution was centrifuged at 2500 rev min^{-1} for 10 min (Remi Instruments Ltd, Mumbai, India) and the concentration of artemether in the supernatant determined using UV spectrophotometry at 256 nm. A calibration curve was based on standard solutions in methanol. The incorporation efficiency was determined as (actual drug content / theoretical drug content) × 100.

Particle size

The average particle diameter and size distribution of microparticles were determined using a Malvern Mastersizer 2000 (Malvern Instruments, UK). Approximately 10 mg microparticles were dispersed in 2–3 mL filtered and degassed distilled water containing 0.1% Tween 80 for 1 min using an ultrasonic bath. An aliquot of the microparticle suspension was then added into the small volume recirculation unit and circulated 3500 times per min. Each sample was measured in triplicate in the analysis. Particle size was expressed as the weighted mean of the volume distribution.

In-vitro drug release

The in-vitro release profile of microparticles was determined according to the paddle method described in the US

Pharmacopeia (XXIV). The in-vitro drug release study was carried out in phosphate buffer pH 6.8, as the pH of saliva is in the range 6.3–7.2. An appropriate amount of microparticles containing 50 mg artemether (based on actual drug content) were suspended in 900 mL buffer, and 5 mL samples were withdrawn at 1, 5, 10, 15, 30 and 60 min and analysed using UV spectrophotometry at 256 nm. Each sample was replaced with fresh buffer solution at the same temperature.

Gustatory sensation test

Gustatory sensation tests were carried out according to the method described by Mou-ying et al (1991). This study was approved by the institutional human ethical committee (IHEC) Twenty healthy human volunteers of either sex, aged 23–27 years, were selected on the basis of aquinine taste sensitivity test – 'non-tasters' and 'super tasters' were excluded. Microparticles equivalent to 1 g artemether were dispersed in 100 mL water for 15 s. Immediately after preparation, each volunteer held about 1 mL of the dispersion in the mouth for 30 s and then spat it out. They then rinsed their mouth three times with distilled water. Volunteers waited for 15 min before tasting the next sample. Bitterness level was recorded using the following values: 0 = tasteless; 0.5 = very slightly bitter; 1 = slightly bitter; 1.5 = slightly to moderately bitter; 2 = moderately bitter; 2.5 = moderately to strongly bitter; 3 = strongly bitter; 3+ = very strongly bitter. The bitterness for each sample of microparticles was determined as the mode score (the score assigned by the greatest number of volunteers).

Fourier transform infrared spectroscopy

Spectra for pure artemether, chitosan, blank microparticles and optimized microparticles were obtained using a Fourier transform infrared spectrophotometer (Avatar™ 360 E.S.P™ FTIR spectrometer, Thermo Nicolet Corp., Madison, WI, USA). A total of 2% (w/w) of sample was mixed with dry potassium bromide (KBr; S. D. Fine Chem Ltd., Mumbai, India). The mixture was ground into a fine powder using an agate mortar before compressing into a KBr disc under a hydraulic press at 10 000 psi. Each KBr disc was scanned 16 times at 4 mm s^{-1} at a resolution of 2 cm^{-1} over a wavenumber region of 500–4000 cm^{-1}. The characteristic peaks were recorded.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) of pure artemether, chitosan, blank microparticles and optimized microparticles was performed using a differential scanning calorimeter (DSC 822e, Mettler Toledo, Columbus, OH, USA). Samples were weighed accurately (5–8 mg), sealed in an aluminium pan with a lid and heated (30–390°C) at a scanning rate of 5°C min^{-1}. The purge gas was nitrogen, at a flow rate of 40 mL min^{-1}. An empty aluminium pan was used as reference.

Results and Discussion

This study described a microencapsulation method to reduce the bitterness of artemether.

Experimental design

Preliminary investigations of the process parameters revealed that factors A, B and C (amounts of artemether, chitosan and sodium hydroxide, respectively) highly influenced the incorporation efficiency, particle size, and drug release at pH 6.8, and were therefore used for further systematic studies. The dependent and independent variables were related using mathematical relationships. The fitted polynomial equations relating the response to the transformed factors are shown in Table 3. The polynomial equations can be used to draw conclusions by considering the magnitude of the coefficient and whether it is positive or negative. High values of correlation coefficient (R^2) for all measured variables indicate a good fit. The prediction profiler of interactions between independent variables is shown in Figure 1.

Incorporation efficiency

As shown in Table 3, the amount of chitosan had a negative coefficient, while the amounts of artemether and sodium hydroxide had positive coefficients. This indicates that the incorporation efficiency decreases as the amount of chitosan is increased. Incorporation efficiency was found to be in the range 52–91% and was dependent on the extent of crosslinking and drug loading. The formulations loaded with larger amounts of drug had higher values for incorporation efficiency, reflecting accumulation of more drug molecules. The extent of crosslinking had a significant effect on incorporation efficiency, which increased as the amount of crosslinking agent increased. This is because more extensive crosslinking leads to formation of a more rigid network structure that retains more drug molecules during microsphere preparation (Rokhade et al 2007). The concentrations of entrapped and total artemether content in microparticles

Table 3 Results of regression analysis; A and B are amounts of artemether and chitosan, respectively (in g); C is the amount of sodium hydroxide (in mL 10% w/v solution)

Terms	Incorporation efficiency (%)		Particle size (μm)		Dissolution†	
	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	83.21	NA	236.75	NA	3.81	NA
A	7.03	<0.0001*	−28.08	<0.0001*	0.95	<0.0001*
B	−5.92	<0.0001*	51.17	<0.0001*	0.46	<0.0001*
C	7.20	<0.0001*	−32.94	<0.0001*	−0.49	<0.0001*
A^2	−5.94	<0.0001*	8.89	0.0011	0.55	<0.0001*
B^2	−2.84	0.0002	−5.71	0.0160	0.34	<0.0001*
C^2	−1.54	0.0109	4.66	0.0397	0.37	<0.0001*
AB	2.25	0.0054	−1.47	0.5747	0.44	<0.0001*
AC	0.59	0.3781	−3.98	0.1474	−0.05	0.3962
BC	−0.62	0.3514	20.60	<0.0001*	0.28	0.0008
R^2	0.98	–	0.99	–	0.98	–

Prob > F is the probability of seeing the observed F value if the null hypothesis is true (there is no factor effect). If the Prob > F value is very small (less than 0.05) then the terms in the model have a significant effect on the response, marked with an asterisk. †Dissolution at pH 6.8: % drug dissolved in 5 min (t5). EC, estimated coefficient; NA, not applicable.

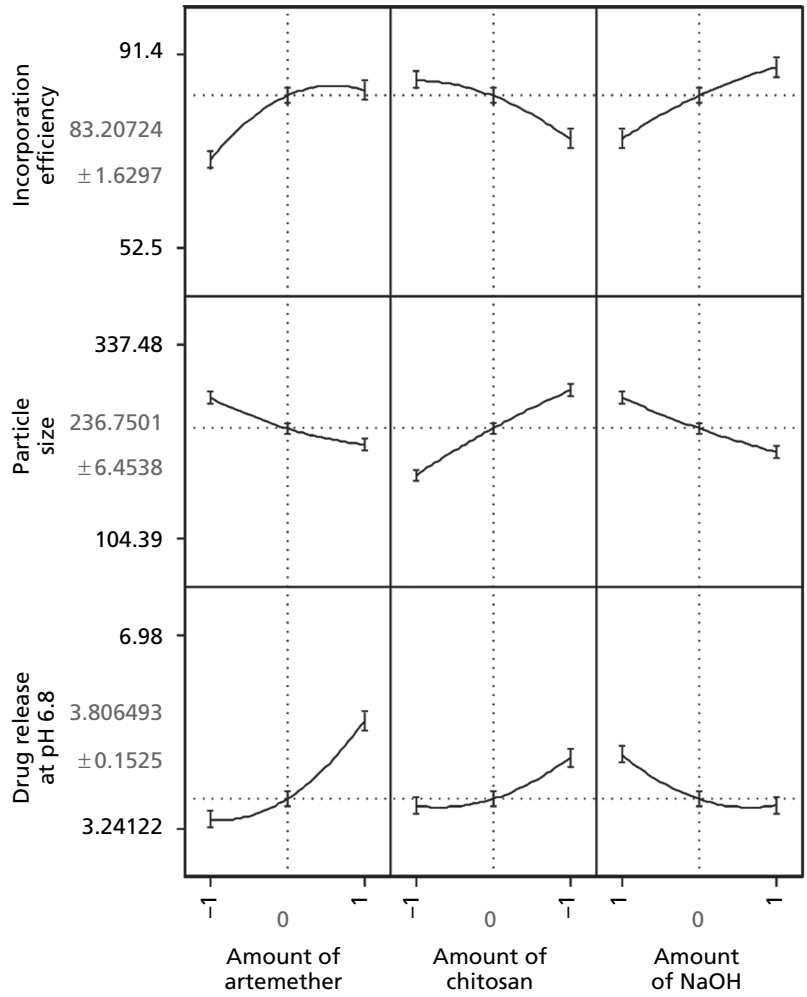

Figure 1 Prediction profiler for interactions between independent variables in microparticles.

and in the filtrate (un-entrapped) were determined spectrophotometrically.

Particle size

As shown in Table 3, the amount of chitosan had a positive coefficient, while the amounts of artemether and sodium hydroxide had negative coefficients. This indicates that particle size increases as the amount of chitosan increases. The particle size is influenced by the calibre of the needle and the viscosity of the chitosan solution. Increased viscosity with higher amounts of chitosan resulted in larger particles. A large amount of sodium hydroxide resulted in smaller particle size because of a high degree of crosslinking. Similar results have been observed for glutaraldehyde (Kumbar et al 2002; Rokhade et al 2007).

In-vitro drug release

As shown in Table 3, the amounts of artemether and chitosan had positive coefficients, whilst the amount of sodium hydroxide had a negative coefficient. This indicates that drug release from the microparticles at pH 6.8 decreases as the amount of sodium hydroxide increases. The amount of chitosan had little effect on the release of drug. Drug release after 5 min decreased as the amount of sodium hydroxide increased. The microspheres produced with larger amounts of sodium hydroxide have more pronounced crosslinking between polymer chains, which retards the release of drug (Ko et al 2002; Remunan-Lopez & Bodmeier 1997). Similar results have been observed with glutaraldehyde (Chourasia & Jain 2004). In addition, drug release from chitosan microparticles increases with increase in drug content (Bayomi 2004). Figure 2 shows the dissolution profiles of pure artemether and optimized microparticles.

Gustatory sensation test

Bitter drugs such as artemether are thought to bind to G-protein-coupled receptors present on the apical taste cell membrane (Yamamoto et al 1998). Chitosan is expected to be insoluble at pH 6.8 and to inhibit drug release. Chitosan is known for its swelling characteristic. Addition of sodium hydroxide increases crosslinking and reduces the swelling

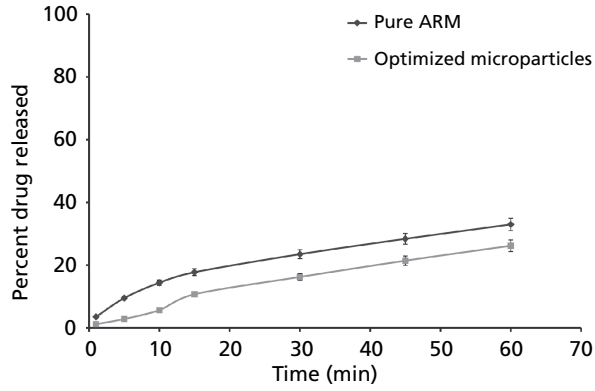

Figure 2 Dissolution profiles of pure artemether and optimized microparticles.

ability of chitosan, which may decrease drug release at pH 6.8. Thus, chitosan forms a physical barrier between artemether and the receptors on the taste cell membranes and thus reduces the bitterness score of artemether in microparticles. The results are in agreement with in-vitro drug release studies.

The bitterness score of optimized microparticles was found to be 0, compared with 3+ for pure artemether.

Optimization using desirability function

Any process can only be authenticated when the variables that affect the process have been optimized to produce a product with good-quality characteristics. Desirability function is an excellent tool for identifying the optimum levels of variables. In this procedure, all the measured responses for independent variables that are thought to affect the quality of the product are taken into consideration. Particle size and drug release had to be minimized and incorporation efficiency maximized for the product to have the desired characteristics. Using the desirability function, all the measured responses were combined to get an overall response (i.e. the overall desirability), which was calculated from the individual desirability of each of the responses using statistical software. The optimum permutation identified had a desirability value of 0.97. The optimum values for the amounts of artemether, chitosan and sodium hydroxide were 0.056 g, 0.03 g and 15 mL, respectively.

Fourier transform infra-red spectroscopy

The physical characteristics of the optimized batch defined from the desirability functions was evaluated further using FTIR and DSC and compared with pure artemether and chitosan. Blank chitosan microparticles were analysed for crosslinking. The samples used for the study were prepared 48 h beforehand and stored in a desiccator before use. Blank chitosan microparticles showed a shift of the absorption peak for –OH from 3430 to 3461 cm^{-1} compared with pure chitosan. In addition, the absorption peak of the amide band, a C–O stretching mode together with an N–H deformation mode, located at 1658 cm^{-1}, shifted to 1666 cm^{-1} compared with chitosan. The absorption bands of blank microparticles were shifted to 3448 cm^{-1} and 1654 cm^{-1} for -OH stretching and the amide band, respectively. This finding confirms the crosslinking of chitosan in the presence of sodium hydroxide. The characteristic peaks of artemether at 2873 cm^{-1} are assigned to C–H stretching vibration in CH_3,CH_2. In addition, the absorption peak at 2844 cm^{-1} can be assigned to C–H stretching vibration in C–O–CH_3. The peak at 1137 cm^{-1} can be assigned to C–O stretching vibration in C–O–C. The peaks at 2953 and 2916 cm^{-1} are assigned to C–H stretching in -CH_3. The spectra for pure artemether, chitosan, blank microparticles and optimized microparticles are shown in Figure 3. The spectrum of optimized microparticles showed all the major peaks of artemether, confirming entrapment of artemether molecules in the chitosan microparticles.

Differential scanning calorimetry (DSC)

Figure 4 shows the DSC profiles for pure artemether, chitosan, blank microparticles and optimized microparticles. The profile for pure artemether showed an endothermic peak at 87.94°C, followed by an exothermic peak at 180.28°C. The endothermic peak corresponding to the melting peak of artemether was shifted towards lower temperatures and with

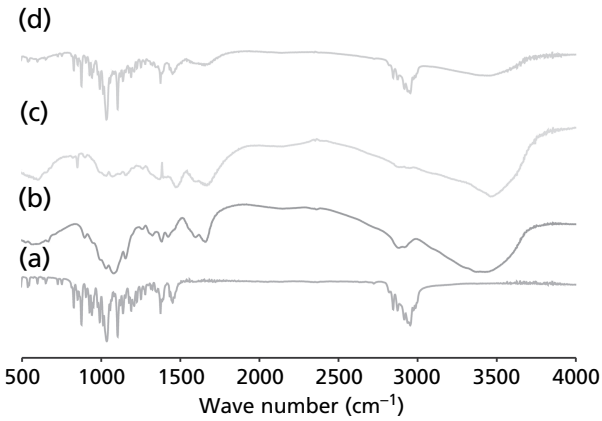

Figure 3 Fourier transform infrared spectra of (a) artemether (b) chitosan (c) blank microparticles and (d) optimized microparticles.

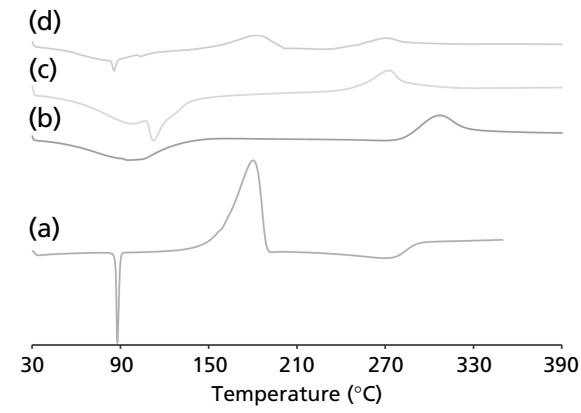

Figure 4 Differential scanning calorimetry profiles of (a) artemether, (b) chitosan, (c) blank microparticles and (d) optimized microparticles.

reduced intensity in the microparticles. This could be attributed to uniform distribution of drug in the polymer crust, resulting in complete miscibility of molten drug in the polymer.

Conclusion

The study demonstrates a significant effect of chitosan crosslinking on the bitterness of artemether. Interactions between artemether, chitosan and sodium hydroxide were found to be statistically significant. The findings suggest that each of these variables has its own significant complementary role in enhancing the process, rather than having an exclusive effect.

Application of experimental design together with desirability function analysis is an ideal tool for optimizing various parameters that have significant effects on the desired properties of the microparticles, such as amount of artemether, chitosan and sodium hydroxide. FTIR and DSC indicated uniform dispersion of artemether molecules in the chitosan microparticles.

The findings reported here may be of value in the pharmaceutical industry for overcoming the problems associated with bitter drugs, which would improve patient compliance and thus effective pharmacotherapy.

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