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Multiresponse optimization of the properties of albendazole–chitosan microparticles

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ABSTRACT

The loading of albendazole into biodegradable polymeric microparticles provides an attractive alternative to improve the drug dissolution rate. Experimental design and optimization techniques were implemented for the development of albendazole–chitosan microparticles using the ionic interaction method. The effect of seven different factors (chitosan concentration, pH of chitosan solution, stirring rate, stirring time, temperature, ionic agent and pH of ionic solutions) were studied on six responses: the yield, pH, morphology, size, dissolution rate and encapsulation efficiency of the microparticles. During the screening phase, the factors were evaluated at three levels each, in order to identify those which exert a significant effect. Multiple response simultaneous optimization by using the desirability function was then used to find experimental conditions where the system shows the most adequate results. The optimal conditions were found to be: NaOH as ionic agent at a pH value of 13.0, chitosan concentration, 0.50% (w/v) at a pH value of 1.0 and stirring rate, 1000 rpm.

1. Introduction

Albendazole (ABZ), methyl [5-(propyl-thio)-1-*H*-benzimidazole-2yl] carbamate [1], is a benzimidazole derivative with a broad antihelmintic spectrum [2]. ABZ is useful against several gastrointestinal parasites, as well as those producing hydatidosis. The latter disease, caused by *Echinococcus granulosus*, produces hydatidic cysts in kidney, liver and lung. Hydatidosis treatment in humans may be medical or surgical, depending on the extent and lesions accessibility, therefore a systemic action is necessary to decrease the cysts size as a pre-chirurgic treatment. Although ABZ is poorly soluble in water (0.2 μg/mL at 25 °C), it is the most commonly used drug in the medical treatment of echinococcosis [3]. However, ABZ is considered as an *orphan drug*. This term refers to a product treating a rare disease, affecting less than 200,000 individuals in the United States, or less than 5 per 10,000 individuals in the European Union. In Argentina, both medical research and drug development to treat such diseases is financially disadvantageous, and pharmaceutical companies may not be interested in the development of this type of medication. Therefore, it is of considerable importance to design and develop a simple, safe and economic formulation of ABZ, in order to treat patients with echinococcosis.

Following an oral administration, a drug must dissolve in the gastric fluids in order to be absorbed into the systemic circulation. This dissolution process determines the rate and degree of absorption [4]. Different studies have been carried out to improve the aqueous solubility and dissolution rate of ABZ, such as the preparation of solid dispersions with polyvinylpyrrolidone (PVP) [5], inclusion complexes with cyclodextrins [6], and incorporation into an hydrophobic micellar core using surfactants [7]. Another tool used to increase the dissolution rate of poorly water soluble drugs is the microencapsulation with different polymers [8]. The obtained microparticles show different physicochemical properties as compared to the drug itself, and therefore its dissolution rate will be modified.

Chitosan (CH) is a biodegradable and biocompatible cationic polymer [9], frequently used for the development of controlled drug delivery systems [10]. Previous works have developed

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ABZ–chitosan hydrocloride or ABZ-Eudragit microspheres, in order to deliver ABZ specifically into the colon [11,12], and to produce an effective and safe therapy for helminthiasis.

The achievement of certain predictable quality with desired and predetermined specifications is referred with the broad term "Quality by design" (QBD) [13]. This term includes predefined combinations of product design, manufacturing process parameters and raw materials quality providing assurance of suitable quality and performance of desirable products. The understanding of factors and their interaction effects by a designed set of experiments is a very useful component of QBD. Many statistical experimental designs have been recognized as useful techniques to understand the variables and their interactions.

Following these QBD criteria, the purpose of this study is to develop ABZ–chitosan microspheres with high dissolution rate and encapsulation efficiency, in order to obtain a systemic action for the treatment of different hydatidic cysts. To carry out this study, experimental design is a valuable tool, specifically response surface analysis [14]. The effect of several factors in the particle formulation were evaluated, in order to distinguish those which have a significant effect on six responses the yield, pH, morphology, size, dissolution rate and encapsulation efficiency of the microparticles. The final aim was to apply multiple response optimization to improve the properties of the formulation.

2. Materials and methods

2.1. Materials

ABZ was supplied by Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and chitosan by Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals were of analytical grade.

2.2. Methods

2.2.1. Preparation of ABZ–chitosan microparticles

Microparticles were prepared by an ionic interaction method, performed according to the following procedure: ABZ (100 mg) was dissolved at room temperature in acetic acid (25 mL), 25 mL of water were added to the solution and a given amount of chitosan was dispersed in the resulting solution. The suspension was stirred to allow the complete chitosan dissolution in the acetic acid (50%, v/v) and its pH was varied from 1.0 to 3.0 by adding HCl (0.1 N). The pH determinations were carried out in a pH meter Metrohm 744 (Herisau, Switzerland). At the same time, solutions of two different ionic agents (IA) (sodium hydroxide or sodium lauryl sulphate) were prepared at 5.00% (w/v) and their pH were varied from 10.0 to 13.0 by adding NaOH.

The drug–polymer solution was sprayed over the ionic solution and the temperature was kept constant during the process, while the stirring time was maintained to complete the ionic interaction. The stirring rate was stable during this procedure. The samples were washed and centrifuged twice, and finally collected in a drying chamber at 40 °C.

Particle size distribution and mean diameters were determined using scanning electron microscopy (SEM) in a Leitz SEM AMR 1600 T. Samples were previously sputter-coated with a gold layer in order to make them conductive.

2.2.2. Yield determination

The yield was calculated as the ratio between the experimental weight of product and the sum of the weights of all components:

$$\text{yield}\,(\%) = 100 \times \left[\frac{W_{\text{product}}}{W_{\text{ABZ}} + W_{\text{CH}} + W_{\text{IA}}}\right] \quad (1)$$

where W_{product} is the weight of the obtained microparticles and W_{ABZ}, W_{CH} and W_{IA} are the weights of ABZ, chitosan, and ionic agent, respectively.

2.2.3. Determination of ABZ content in microparticles

The encapsulation efficiency (EE) is defined as the percentage of the actual mass of drug encapsulated in the polymeric carrier, relative to the initial amount of loaded drug. For the EE determination, microparticles were dissolved in HCl 0.1 N for 24 h. The amount of loaded drug was determined by spectrophotometric measurements at 291 nm using a LKB-Pharmacia UV spectrophotometer, according to:

$$\text{encapsulation efficiency}\,(\%) = 100 \times \left(\frac{W_{\text{ABZ}}}{W_{\text{t}}}\right) \quad (2)$$

where W_{ABZ} is the actual ABZ content and W_{t} is theoretical ABZ content in the microparticles.

2.2.4. Dissolution studies

All of the ABZ–chitosan microparticles were subjected to dissolution assays in an USP Standard Dissolution Apparatus (Hanson Research SR8 Plus, Chatsworth, USA), equipped with a rotational paddle (50 rpm). The dissolution medium (900 mL of HCl 0.1 N) was maintained at 37 °C. A dispersion powder of microparticles containing ABZ (100 mg) was introduced into the flasks, and the time counter was set to zero. At different time intervals, samples of 5 mL were taken through a filter, and the amount of ABZ released was determined. It was found that chitosan did not interfere with the assay at the working wavelength (291 nm).

3. Results and discussion

3.1. Screening phase

A satisfactory microparticle formulation depends on many factors, and therefore an expanded Plackett–Burman design was built for estimating the main factors affecting its properties. The analysed factors were: chitosan concentration, chitosan solution pH, stirring rate, stirring time, temperature, type of ionic agent and pH of ionic solutions. Each of these factors were evaluated at three levels (a triplicate central point was added to the Plackett–Burman design in order to provide a higher information content for the analysis see Table 1). The factor ranges were selected based on prior knowledge about the system under study. The evaluation consisted in analysing the responses in all the conditions quoted in Table 1. It should be noticed that an excess of IA is necessary to secure the microparticles formation, thus its concentration was set at 5.00% (w/v).

The six analysed responses were: pH of the resulting solution, yield, morphology, size, encapsulation efficiency and microparticle dissolution rate (based on the Q_{30} value, which is defined as the drug concentration solubilized after 30 min). Morphology is a categorical response, and hence values of 1 or 0 were assigned to analyse the morphology: a value of 0 indicates the tendency to form microspheres, while a value of 1 implies a tendency to form microparticles having different non-spherical forms.

An ANOVA test was applied to the experimental data corresponding to the design of Table 1, using the effect of the dummy variables to obtain an estimate of standard errors in the coefficients. As a conclusion of this analysis (Table 2), IA, PC and SR were found to be the most important factors (values of $p < 0.0001$ as quoted in boldface in these tables). Although other factors such as IT, IApH

Table 1
Plackett–Burman design built for factor selection

Experiments	Factors[a]							Responses[b]					
	PC (%, w/v)	SR (rpm)	It (h)	IT (°C)	PpH	IApH	IA	*M*	*S* (μm)	EE (%)	pHS	D_r (Q_{30}) (%)	*Y* (%)
1	0.30	600.00	12.00	37.00	3.00	13.00	N	0	300	70.28	6.4	24.12	70
2	3.00	900.00	12.00	37.00	1.00	10.00	N	0	360	68.15	6.8	20.53	73
3	0.30	900.00	48.00	0.00	3.00	10.00	N	0	60	67.22	6.9	49.82	65
4	3.00	600.00	48.00	37.00	1.00	13.00	N	0	500	71.44	6.5	22.14	90
5	3.00	600.00	48.00	0.00	1.00	10.00	L	1	480	70.12	7.2	23.81	75
6	0.30	900.00	12.00	0.00	1.00	13.00	L	1	75	68.93	6.9	51.12	93
7	3.00	900.00	48.00	0.00	3.00	13.00	N	0	300	69.13	6.7	21.11	72
8	3.00	600.00	12.00	0.00	3.00	13.00	L	1	510	70.12	7.1	22.78	75
9	0.30	600.00	48.00	37.00	3.00	10.00	L	1	415	71.10	6.5	23.33	67
10	0.30	900.00	48.00	37.00	1.00	13.00	L	1	50	72.18	6.7	48.15	93
11	3.00	900.00	12.00	37.00	3.00	10.00	L	1	370	69.65	6.9	23.33	65
12	0.30	600.00	12.00	0.00	1.00	10.00	N	0	330	67.33	7.1	23.67	72
13	1.65	750.00	30.00	18.50	2.00	11.50	L	1	290	70.27	6.7	31.33	73
14	1.65	750.00	30.00	18.50	2.00	11.50	L	1	330	68.15	7.2	32.12	75
15	1.65	750.00	30.00	18.50	2.00	11.50	L	1	260	69.67	6.8	31.19	70
16	1.65	750.00	30.00	18.50	2.00	11.50	N	0	340	65.21	6.4	33.61	64
17	1.65	750.00	30.00	18.50	2.00	11.50	N	0	390	63.12	6.8	32.38	69
18	1.65	750.00	30.00	18.50	2.00	11.50	N	0	280	67.84	7.1	31.37	67

[a] PC: polymer concentration, SR: stirring rate, It: interaction time, IT: interaction temperature, PpH: polymer solution pH, IApH: ionic agent solution pH, IA: ionic agent, N: sodium hydroxide and L: sodium lauryl sulphate.
[b] *M*: morphology, *S*: mean size, EE: encapsulation efficiency, pHS: pH solution, D_r (Q_{30}): dissolution rate and *Y*: yield.

Table 2
Values of *p* obtained for the different factors on the six responses

	S	*M*	EE	pHS	D_r (Q_{30})	*Y*
Model	**<0.0001**	**<0.0001**	0.2606	0.1341	**0.0063**	**0.0022**
PC	**<0.0001**		0.8270	0.3680	**0.0014**	0.5504
SR	**<0.0001**		0.4803	0.8955	**0.0038**	0.4754
It	0.3876		0.3588	0.3680	0.8270	0.4072
IT	0.1526		0.1855	0.0179	0.9573	0.7185
PpH	0.3263		0.9278	0.3680	0.4538	**0.0005**
IApH	0.1010		0.2518	0.1693	0.4585	**0.0008**
IA	0.6824	**<0.0001**	0.0380	0.1833	0.2345	0.0507

Table 3
Significant terms ($p < 0.05$) for each response, coefficient values and ratios between coefficient and maximum response values

Response	Factors	Coefficients	Coefficient/maximum response	*p*
M	IA	0.50	**0.50**	**<0.0001**
S	PC	79.63	**0.16**	**<0.0001**
	SR	−110.0	**0.22**	**<0.0001**
EE	IA	1.14	0.02	0.0380
pHS	IT	−0.18	0.03	0.0179
D_r (Q_{30})	PC	−5.34	**0.10**	**0.0014**
	SR	6.18	**0.12**	**0.0038**
Y	IApH	6.33	0.07	0.0008
	PpH	−3.42	0.04	0.0005

and PpH are also significant at 95% level (i.e., $p < 0.05$), they are considerably less influencing than IA, PC and SR. This is also revealed in Table 3, where the ratios between the coefficients associated with each effect and the maximum response value are collected (higher values are obtained for IA, PC and SR, indicating a correspondingly larger influence on the responses).

The results also revealed a direct relationship between the factor IA and the categorical morphology response *M*: spherical microparticles are desired, and hence NaOH was selected as the ionic agent (see Table 1). The yield *Y* presents some dependence with the factors IApH and PpH (Tables 2 and 3), with signs of the IApH and PpH coefficients being positive and negative, respectively. For the subsequent optimization phase, this implies that these factors should be fixed at their maximum (13.0) and minimum (1.0) values, respectively. Finally, the responses pHS and EE were not influenced by any of the IA, PC and SR factors. Half-normal probability plots for the analysed responses were built which allowed us to reach an analogous conclusion to that obtained from consideration of the values collected in Tables 2 and 3.

Table 4
Central composite design used for the optimization of the responses

Experiments	Factors		Responses			
	Polymer concentration (%, w/v)	Stirring rate (rpm)	Size (μm)	Encapsulation efficiency (%)	Disolution rate Q_{30} (%)	Yield (%)
1	0.50	650.00	330	70.20	24.14	89.30
2	2.50	650.00	500	71.55	25.86	87.11
3	1.50	577.51	700	69.99	18.01	88.16
4	0.09	825.00	60	30.00	43.16	55.77
5	1.50	1072.49	150	71.11	41.88	88.34
6	1.50	825.00	260	72.31	33.93	89.32
7	0.50	1000.00	50	70.00	48.15	85.67
8	2.91	825.00	380	70.14	21.84	89.23
9	1.50	825.00	200	69.98	39.12	87.13
10	2.50	1000.00	250	71.12	36.23	88.97

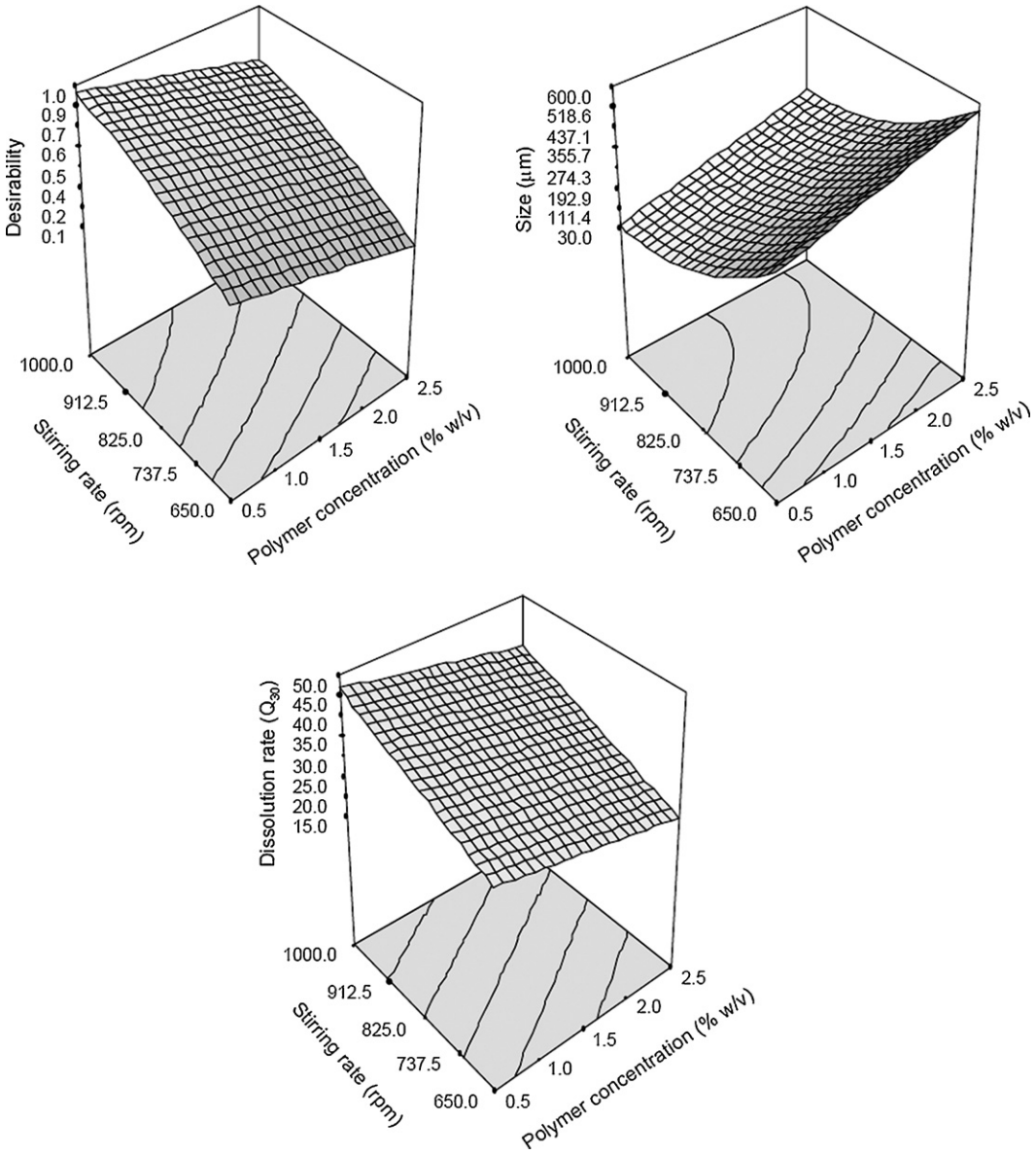

Fig. 1. Response surface plots for the global desirability function, the morphology (M) and dissolution rate (D_r), as indicated.

3.2. Response surface design

A systematic optimization procedure was carried out using response surface methods (RSM), in order to estimate the values of the most important factors leading to the best compromise between maximum dissolution rate and minimum size, the two responses which appear to be most influenced by the studied factors. A central composite design was used for applying the RSM, consisting of 10 experiments (9 experiments and a replicate of the central point), which are combinations of the selected factors in the following ranges: polymer concentration, 0.05–3.00% (w/v) and stirring rate, 500–1000 rpm (Table 4). On the other hand, from results of the screening phase (see above), the pH of both the polymer solution and the IA solution were set at 1.0 and 13.0, respectively. NaOH was selected as IA, due its tendency to form the desired microspheres, and its concentration was set at 5.00% (w/v). The temperature was fixed at 25 °C. All experiments were performed in random order to minimize the effects of uncontrolled factors that may introduce a bias on the measurements.

Table 4 shows the results of the optimized responses (S and D_r), along with the yield and encapsulation efficiency. The latter two responses are included for a final check of the consistency of the results, although they were shown to be less influenced by the studied factors SR and PC. The responses for all the 10 experiments were fitted to polynomial models, using backward elimination to estimate the best models. These results indicated that a quadratic model better explains the behaviour of the response size (S), while a linear model is appropriate for the dissolution rate (D_r). Partial ANOVA results for this optimization design show good statistical indicators (i.e., non-significant lack of fit, and adequate R^2 and model and coefficient standard deviations).

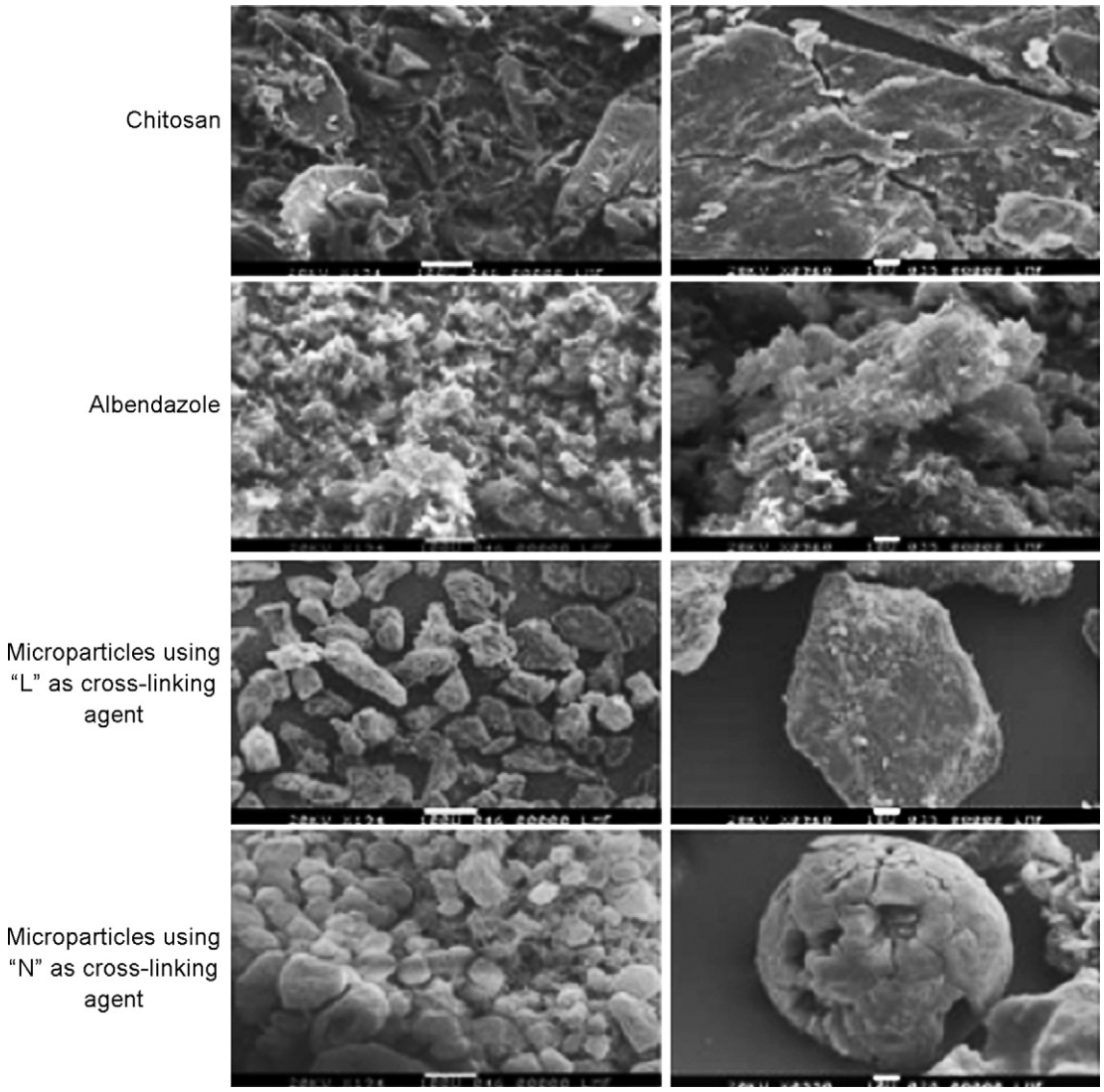

Fig. 2. Photomicrographs of drug, polymer and microparticles obtained using "L" and "N" as ionic agent, as indicated.

As expected, adequate values of the remaining responses morphology, encapsulation efficiency and yield, except for the experiment using an extremely low polymer concentration (0.09%, w/v in Table 4), which yielded both an undesired morphology (i.e., heterogeneous particle shapes) and low encapsulation efficiency. Neither EE nor *Y* did show a significative dependence with the factors PC and SR (as could be anticipated from Plackett–Burman results).

When a simple response is being analysed, the model analysis indicates areas in the design region where the system is likely to give desirable results. However, when several responses are needed to be simultaneously optimized, the desirability function can be employed, which is a function of more than one response. The desirability function includes the priorities of researchers and desires on building the optimization procedure. The procedure involves creating a function for each individual response (d_i) and finally obtaining a global function *D* that should be maximized choosing the best conditions of the designed variables. The function *D* ranges from 0 (value totally undesirable) to 1 (all responses are in a desirable range simultaneously) and is defined by Eq. (3), where $d_1, d_2, \ldots, d_N$ correspond to the individual desirability function for each response being optimized:

$$D = \left[\prod_{n=1}^{N} (d_n)^{w_n}\right]^{1/\sum_{n=1}^{N} w_n} \quad (3)$$

where w_n is a weight which controls the relative importance of each of the analysed factors. In the present work, all weights were set to unity, and hence a simplified version of Eq. (3) was employed:

$$D = \left[\prod_{n=1}^{N} d_n\right]^{1/N} \quad (4)$$

Two responses, as suggested by the analysis of the effect discussed above, were simultaneously optimized: minimum sizes and maximum dissolution rates are desirable. After the optimization procedure was carried out, and adequate models were found for each of these responses, a response surface for the global desirability function was built as a function of the influencing factors *S* and D_r (Fig. 1). As can be seen, higher stirring rates and a lower polymer concentrations produced faster dissolution rates and smaller sizes.

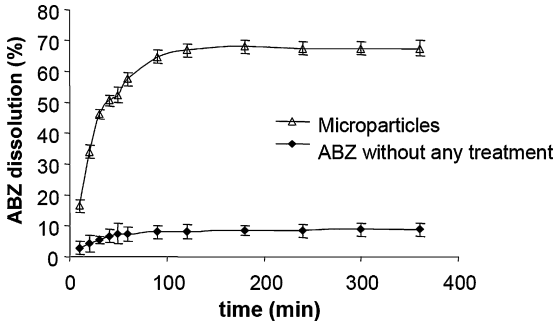

Fig. 3. Dissolution profiles of albendazole without any treatment and microparticles in the optimal conditions.

These results seem to imply that a combination of low polymer concentration and large stirring rate are the best conditions. However, they correspond to a region which might be outside the range of design values. A decrease of drug release with increasing polymer concentrations can be attributed to the increase in the size of the polymer matrix. Initially, higher release rates are observed due to the dissolution of surface-adhered drug. At longer times, drug release is due to the diffusion process, which is much slower when compared to the initial release.

Moreover, the results for extremely low polymer concentrations indicated inappropriate particle morphology and encapsulation efficiency. Therefore, we chose as the best conditions those corresponding to the design point which is closer to the region suggested by the desirability plot, i.e., 0.50% (w/v) for the polymer concentration and 1000 rpm for the stirring rate. The desirability function at this point yields a value of $D = 0.95$ which we consider to be adequate for our purposes.

The morphologic study of polymer, drug, and microparticles, obtained using L and N as ionic agents, is shown in Fig. 2. In the SEM analysis, typical micrographs for ABZ and chitosan are presented at two different magnifications. The polymer is formed by blocks of different forms and sizes. On the other hand, microparticles appear with a relatively uniform size, and a clear difference among particles obtained using L (irregular shape) and N (spherical shape) is observed.

The dissolution profiles for a formulation obtained in the selected conditions were contrasted against ABZ without any treatment (Fig. 3). As can be seen, the microparticles formulation showed an enhanced dissolution rate for ABZ, in comparison with the drug alone.

4. Conclusions

This work demonstrates the properties of ABZ–chitosan microparticles can be improved by rationally analysing the influence of different parameters in the formulation. The procedure is composed of the following four phases: (1) screening the influential factors with a Plackett–Burman design, (2) building a response surface model and (3) finding the optimal conditions. This methodology has been proved to be very efficient in decreasing the particle size and increasing the dissolution of ABZ. In a previous work [12] microspheres of ALB were developed with chitosan hydrochloride, in order to deliver albendazole specifically into the colon. The emulsion method was employed to obtain the microparticles, and glutaraldehyde in toluene was used as the cross-linking agent. In the present work, the microparticles were obtained by the coacervation method, employing chitosan and non-toxic solvents during the procedure. The optimal combination of the microencapsulating materials was found to be 0.50% (w/v) polymer concentration and 1000 rpm stirring rate. On the other hand, the screening phase suggested that polymer and ionic agent pH values should be fixed at 1.0 and 13.0, respectively, in order to obtain maximum yield. Also, NaOH was selected as ionic agent due to its tendency to form microspheres. Temperature and stirring time were fixed at 25 °C and 24 h, respectively.

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