

Development and optimization of the activated charcoal suspension composition based on a mixture design approach

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In this study, a new drug product containing activated charcoal was designed and developed. The excipient levels in the pharmaceutical formulation were optimized using a mixture design approach. The adsorption power of the activated charcoal suspension was selected as the critical quality attribute influencing the efficacy of medical treatment. Significant prognostic models ($p < 0.05$) were obtained to describe in detail the interrelations between excipient levels and the adsorption power of the formulation. Liquid flavour had a critical impact on the adsorption power of the suspension. Formulations containing the largest amount of liquid flavour showed the lowest adsorption power. Sorbitol was not adsorbed onto activated charcoal so strongly as liquid flavour. A slight increase in the content of carboxymethylcellulose sodium led to a marked decrease in adsorption power. The obtained mathematical models and response surface allowed selection of the optimal composition of excipients in a final drug product.

Keywords: activated charcoal, adsorption power, phenazone, experimental design, *Quality by Design* concept

The Quality by Design concept is an essential part of the modern approach to pharmaceutical quality. It involves designing and developing formulations and manufacturing processes to ensure predefined drug product quality (1). Due to the current trend of being Quality by Design compliant, the use of experimental designs in pharmaceutical sciences is increasing (2, 3). This strategy can shorten a drug development phase and reduce the overall experimental work and costs (4, 5). Furthermore, the use of experimental design techniques enables better understanding of the effect of material attributes and process factors on the final drug product quality (5).

This paper focuses on the application of experimental design in the development and optimization of a new drug product containing activated charcoal. The composition of excipients in the formulation was optimized using a mixture design approach according to the ICH Q8 guidelines (6). The adsorption power of the activated charcoal suspension was considered as the critical quality attribute to be studied in order to establish the optimal

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excipient levels in a final drug product. Mixture design was applied to investigate the effect of varying the ratios of excipients and their interactions on the adsorption power of the pharmaceutical dosage form. Due to the fact that the response variable (adsorption power) is a function of the properties of different components present in the suspension, a mathematical model was constructed to better understand and predict the output response with respect to input variables.

EXPERIMENTAL

All chemicals and solvents were of analytical reagent grade. Phenazone (99%) and carboxymethylcellulose sodium salt (high viscosity) were obtained from Sigma-Aldrich (USA). Activated charcoal was provided by Pharma Cosmetic (Poland). Sorbitol and HPLC-grade acetonitrile were purchased from POC (Poland). Liquid flavour (cola aromatic base dissolved in triacetin) was purchased from Hoffmann (Poland). Ultrapure water was obtained from a Milli-Q water purification system from Millipore (USA).

Preparation of the activated charcoal suspension

In the first stage of the study, the suspension included the following excipients: sorbitol (as a sweetener), liquid flavour and purified water.

The required amount of sorbitol (according to Table I) was dissolved in purified water. The obtained solution was mixed with the activated charcoal in a porcelain mortar and then liquid flavour was added.

In the second stage, a carboxymethylcellulose sodium salt (CMC) was added as a suspending agent to the formulation (Table II). Sorbitol was dissolved in purified water. The obtained solution was heated in a beaker to a temperature of 35 °C and agitated, the required amount of the polymer was dispersed in sorbitol solution. Agitation was continued without heating to obtain the appropriate consistency of the formulation and then 0.01 g of liquid flavour was added. The obtained polymer dispersion was poured onto the charcoal powder in a mortar and mixed thoroughly. In this way, two grams of activated charcoal was incorporated into 10 g of the prepared gel formulation.

Adsorption power of activated charcoal suspension

The un-adsorbed phenazone concentration in the filtrate was measured using the RP-HPLC method. Adsorption power was measured as follows: 1.8 g of activated charcoal suspension was weighed into a 100-mL ground-glass stoppered conical flask, then 25-mL of a freshly prepared phenazone solution (10 mg mL⁻¹) was added. This was shaken thoroughly for 15 min at room temperature and filtered. One (1.0) mL of the filtrate was transferred into a 25-mL volumetric flask and made up to the mark with ultrapure water. The obtained phenazone solution (1.0 mL) was transferred into a 25-mL volumetric flask and diluted with ultrapure water to the mark, mixed and filtered through a 0.22- μ m membrane filter prior to being injected into a chromatographic column. Each suspension sample was prepared in triplicate. The concentration of phenazone in the investigated filtrate was calculated using the calibration curve prepared at nine different concentration levels (in the range of 2.54–20.36 mg L⁻¹).

HPLC method

A reversed phase high performance liquid chromatography method for the determination of phenazone in filtrate was developed with the aid of a HPLC system (Shimadzu, Japan) equipped with two LC-20AD solvent delivery pumps, a DGU-20A degasser and a diode array detector (SPD-M20A). Chromatographic experiments were performed on a GraceSmart™ RP 18 column (150 mm × 4.6 mm, 5 μm, WITKO, USA), combined with a SecurityGuard™ precolumn Gemini C18 (length 4 mm, internal diameter 3 mm, Phenomenex, USA). The mobile phase consisted of acetonitrile and water in a volume ratio of 25:75. All chromatographic experiments were performed in the isocratic mode. The flow rate was set to 0.5 mL min⁻¹ and the injection volume was 20 μL. Chromatograms were recorded at 242 nm and were analyzed by means of the LCSolution software.

Experimental design

Mixture design with three factors was used for the optimization of excipient levels in the activated charcoal suspension. Mixture design is a special type of response surface methodology. In these experiments, the factors are the ingredients of a mixture and, consequently, their levels are not independent (5). The adsorption power of activated charcoal suspension was selected as the response variable. In the mixture design approach, the response is a function of the proportions of excipients present in a drug product (5, 7). The advantage of this methodology is the reduction of the number of experiments needed to optimize the response while maintaining the representativeness. The STATISTICA® 10.0 software (StatSoft, Tulsa, OK, USA) was used to construct the experimental design as well as to analyze the data. Experimental designs for the developed pharmaceutical dosage

Table I. Design matrix for formulations without carboxymethylcellulose sodium salt

Formulation	Sorbitol (%) ^a	Liquid flavour (%)	Purified water (%)	Adsorption power ^{b,c}
1	15.0	5.0	80.0	35.7 ± 0.5
2	0.0	5.0	95.0	37.9 ± 0.2
3	7.5	2.5	90.0	39.3 ± 0.9
4	7.5	0.0	92.5	43.9 ± 0.3
5	0.0	2.5	97.5	40.9 ± 0.8
6	15.0	0.0	85.0	43.1 ± 0.1
7	15.0	2.5	82.5	40.4 ± 0.3
8	15.0	0.0	85.0	42.0 ± 0.1
9	15.0	5.0	80.0	35.6 ± 0.7
10	0.0	5.0	95.0	37.3 ± 0.6
11	7.5	2.5	90.0	39.4 ± 0.4
12	0.0	0.0	100.0	45.6 ± 0.3
13	0.0	0.0	100.0	46.1 ± 0.2
14	7.5	5.0	87.5	35.6 ± 0.2

^a The solution was mixed with the activated charcoal (2 g) in a porcelain mortar and then liquid flavour was added.

^b Phenazone (g) per 100 g activated charcoal.

^c Mean ± SD, *n* = 3.

form are given in Tables I and II. The proportion for each excipient is expressed as a fraction of the mixture for each treatment combination; the sum of excipient proportions is equal to 100 %. The design resulted in fourteen suspension mixtures.

Table II. Design matrix for formulations containing carboxymethylcellulose sodium salt (CMC)

Formulation	Sorbitol (%)	CMC (%)	Purified water (%)	Adsorption power ^{a,b}
1	15.0	1.0	84.0	33.9 ± 0.1
2	0.0	0.0	100.0	45.2 ± 0.3
3	0.0	0.5	99.5	43.6 ± 0.4
4	7.5	1.0	91.5	34.9 ± 0.4
5	0.0	1.0	99.0	36.0 ± 0.4
6	15.0	0.5	84.5	41.0 ± 0.8
7	7.5	0.0	92.5	44.0 ± 0.1
8	15.0	1.0	84.0	33.6 ± 0.4
9	0.0	0.0	100.0	45.4 ± 0.1
10	7.5	0.5	92.0	42.1 ± 0.3
11	15.0	0.0	85.0	43.3 ± 0.7
12	7.5	0.5	92.0	42.3 ± 0.3
13	0.0	1.0	99.0	36.4 ± 0.5
14	15.0	0.0	85.0	44.0 ± 0.1

^a Phenazone (g) per 100 g activated charcoal.

^b Mean ± SD, $n = 3$.

RESULTS AND DISCUSSION

In the first stage of the study, three suspension excipients: sorbitol, liquid flavour and purified water were considered to be input variables with adsorption power as the response. Liquid flavour was used to improve the taste and smell of the suspension. Sorbitol was included in the formulation as a sweetener. Due to the cathartic action of sorbitol, the combination of activated charcoal and sorbitol counteracted the constipation produced by charcoal alone. As a result, the elimination of toxic substances from the body was much easier. The upper limits of the two were found to be 15 % for sorbitol and 5 % for liquid flavour. The imposed restrictions for sorbitol content resulted from the dose recommended by the *European Pharmacopoeia* (8). Higher content of sorbitol in a dosage form may lead to gastric irritation and diuretic effect.

Fourteen different pharmaceutical formulations were prepared according to the design matrix described in Table I. The adsorption power of each suspension was determined by HPLC. Analysis of variance was carried out. Model significance, lack of fit and determination coefficient, which indicate the model fitness, were evaluated. The multiple regression analysis indicated that the polynomial model was significant ($p < 0.05$) in predicting the adsorption power of the suspension (Table III). It could explain almost 98 % of all variances in the data ($R^2 = 0.9803$) and an insignificant lack of fit was obtained.

Table III. Analysis of variance (ANOVA)

	Sum of squares	Degrees of freedom	Mean square	Test <i>F</i>	<i>p</i> value ^a
Predictive model for formulations without CMC					
Model	165.23	5	33.05	79.47	< 0.01
Total error	3.33	8	0.42		
Lack of fit	2.39	3	0.80	4.25	0.08
Pure error	0.94	5	0.19		
Total adjusted	168.55	13	12.97		
Predictive model for formulations containing CMC					
Model	251.17	5	50.23	799.06	< 0.01
Total error	0.50	8	0.06		
Lack of fit	0.15	3	0.05	0.73	0.58
Pure error	0.35	5	0.07		
Total adjusted	251.67	13	19.36		

^a*p* value less than 0.05 is considered significant.

Residual analysis was also carried out in order to verify the assumptions used in statistical analysis. Residuals are the difference between the observed and predicted values (calculated from the mathematical model). Normal distribution of the residual values was confirmed using the normal probability plot and the Shapiro-Wilk test.

The adsorption power of activated charcoal suspension is a function of the levels of excipients present in the pharmaceutical formulation. The obtained predictive model (for formulations without CMC) can be described by the following equation:

$$v = 43.60 x + 13.04 y + 45.75 z - 0.76 xy - 6.46 xz - 1.16 yz$$

where *v* is the predicted response (adsorption power), *x* is the fraction of sorbitol in the mixture, *y* is the fraction of liquid flavour in the mixture, *z* is the fraction of purified water in the mixture.

The results of this study can be also graphically represented in the so-called triangular graph. Response surface describes the functional relationship between the adsorption power and excipient levels. As can be seen in Fig. 1a, liquid flavour has a critical impact on the adsorption power of the activated charcoal suspension. Formulations containing the largest amount of liquid flavour showed the lowest adsorption power values.

All suspensions, prepared in accordance with the design matrix from Table I, were characterized by unsatisfactory consistency. Thus, in the next stage of the study, carboxymethylcellulose sodium salt was included as a suspending agent in the pharmaceutical formulation in order to achieve appropriate consistency. This polymer increased the viscosity of the formulation and thereby reduced the sedimentation rate of suspended charcoal particles. Upper limits of the two excipients in the suspension were determined to be 15 % sorbitol and 1 % carboxymethylcellulose sodium salt. These excipient levels in the pharmaceutical formulation were optimized by the mixture design approach based on adsorption power measurements. A total of fourteen experiments were performed accord-

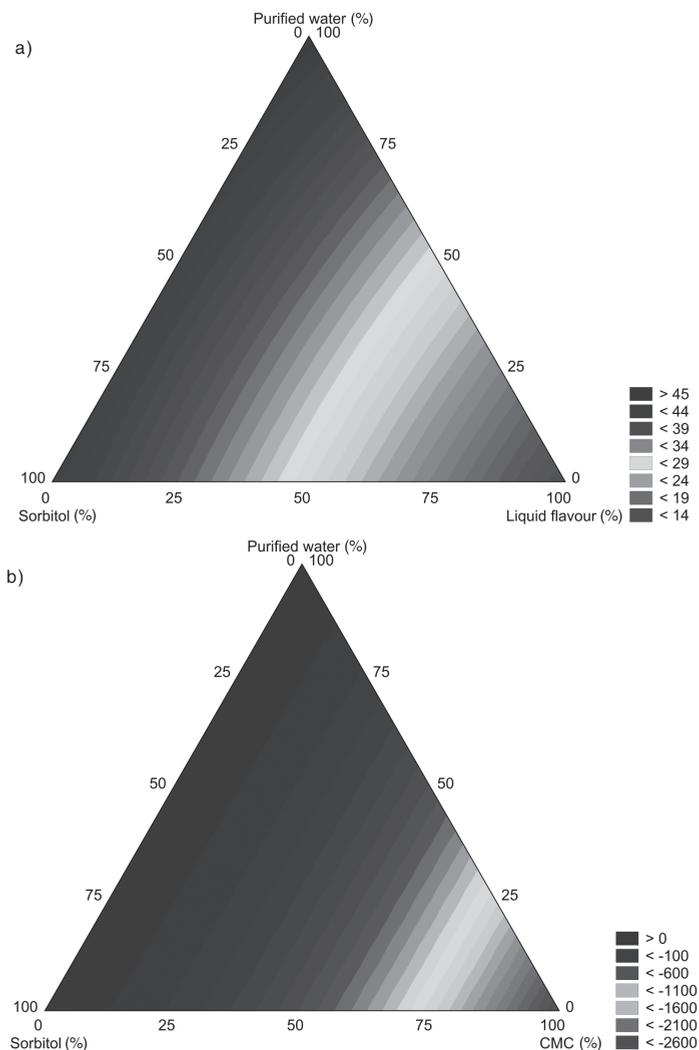


Fig. 1. Mixture response surface contour plot: a) for formulations without carboxymethylcellulose sodium salt (CMC), b) containing CMC.

ing to the design matrix given in Table II and the average adsorption power values were used in data analysis. A polynomial model was generated for the response value using multi-linear regression analysis. Adequacy of the developed model and the statistical significance of the regression coefficients were tested using the analysis of variance. The results indicated that the model was significant ($p < 0.05$). It could explain almost 99 % of all variances in the data ($R^2 = 0.9980$) and insignificant lack of fit was obtained (Table III). Moreover, the assumption of normal distribution of the residuals was also confirmed by the normal probability plot and the Shapiro-Wilk test.

The adsorption power of activated charcoal suspension (for formulations containing CMC) can be described by means of the obtained mathematical model, as follows:

$$v = 43.49 x - 2620.55 y + 45.27 z + 2675.36 xy - 1.04 xz - 2690 yz$$

where v is the predicted response (adsorption power), x is the fraction of sorbitol in the mixture, y is the fraction of carboxymethylcellulose sodium salt in the mixture, z is the fraction of purified water in the mixture.

Graphical interpretation of the effects of excipients on the drug product quality and thus searching for the optimal formulation is possible by means of the so-called mixture response surface contour plot. As can be seen from Fig. 1b, carboxymethylcellulose sodium salt influences the adsorption power. A slight increase in the content of this polymer leads to a marked decrease of adsorption capacity. However, the use of 0.5 % carboxymethylcellulose sodium salt gives a suspension with satisfactory consistency and adsorption power. Due to the adsorption of liquid flavour onto activated charcoal, it was excluded from the formulation. Finally, sorbitol, in an amount of about 12 %, was used as a sweetener to improve the taste of the charcoal suspension.

According to the results of the present study, it can be stated that 0.5 % carboxymethylcellulose sodium salt and 12–14 % sorbitol might be used in the formulation in order to achieve the desirable adsorption power of the final suspension.

The application of this experimental methodology allowed a better understanding of the influence of formulation excipients on the critical physicochemical quality attribute (adsorption power) of the activated charcoal suspension. In contrast to the traditional experiment-based and trial-and-error approach, the applied methodology enabled to minimize the number of experiments to be conducted, without a significant loss of information. Prognostic mathematical models were generated to describe in detail the interrelations between excipients and the critical quality attribute of the final suspension.

CONCLUSIONS

In this study, a new drug product containing activated charcoal was designed and developed using the mixture design approach. It allowed the modeling and analysis of the adsorption problem and thus selection of the optimal qualitative and quantitative composition of excipients, which guarantees the efficacy of medical treatment. The results indicated that the mixture design is a useful tool for the activated charcoal drug product design and development, in accordance with the *Quality by Design* strategy.

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