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Modelling and optimization of extraction yield of the natural red madder dye

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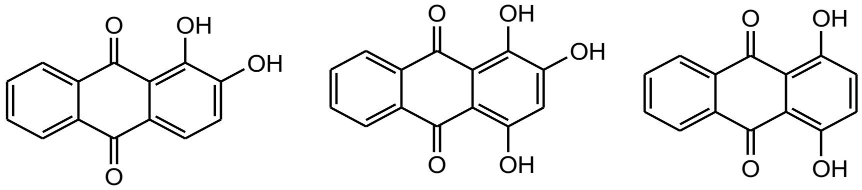
Abstract

Alizarin and purpurin are the main colored compounds of madder. The optimization of extraction (Rdt%) of red dye madder has been carried out using Central Composite Design (CCD in the following) taking into account 3 factors (concentration of the acid, concentration of the alkali, and extraction time).The choice of the solvent, acid and alkali was initially determined by a simple screening. The Central composite experimental design and Response Surface Methodology (RSM) were used to determine the best operating conditions for a maximum yield of extraction. Experimental results of the CCD showed that the best operating conditions to obtain the maximum extraction yield (91.8%) were the following: HCl2.95%, NaOH2.95% and 1,25 hours extraction time.

1. Introduction

Rubia tinctorum L, is a plant of the Rubiacees family [1]. The different dyes of Rubiacees belong to the anthraquinonoid organic chemicals [2-6]: they all have the same basic skeleton, with hydroxyl, carboxyl, glycoside groups that modulate properties and colors. The two most known are alizarin and purpurin [3,4]. Madder can be used against kidney and bladder diseases and as antibacterial and antifungal [7]. It would have the ability to dissolve kidney stones [8]. It has also been used in the dyeing of wool [9,10].



Alizarine Purpurin Quinizarin

Figure 1: The main dyes of madder components

The aim of this study is to model and optimize the extraction of madder. The influence of the nature of the solvent, the alkali and the acid on the extraction yield, will be predicted by the analysis of the screening design. Using a Composite Central Design (CCD) [11], and response surface methodology (RSM) [12] a modelling of the effects of these parameters on the performance will be performed.

Our approach can be summarized in three main steps. At first, the best parameters were selected by means of a screening design model. A second step based on the effects of the factors and their interaction on the yield in the form of an equation model. In the final step, optimization will be carried out using contour graphs.

2. Material and Methods

2.1. Preparation of samples for extraction of color components

Air-dried samples containing moisture were ground in a Wiley mill and stored in plastic bags at room temperature (25-27 °C) [13] in the dark.

The extraction yield in this paper is the ratio between the obtained absorbance for a given trial and the maximum absorbance as mentioned in 2.2 below.

2.2. Anthraquinone determination

0.3 g of dried matter was extracted with 2 ml 80% ethanol at 80°C during one hour. The dye solution was separated. Samples from the extracts were measured using a spectrophotometer UV (UNICAM UV/Vis) at 434 nm. Alizarin was used as a standard [14].

2.3. Chemicals

All reagents used in this study are of analytical quality: methanol (CH_3OH), ethanol (C_2H_5OH), methylene chloride (CH_2Cl_2), hydrochloric acid (HCl), sodium hydroxide (NaOH) and sulphuric acid (H_2SO_4).

2.4. Extraction

A sample of 10 g root of the madder was extracted. These extractions were carried out with 25 ml of respectively MeOH, CH_2Cl_2 and EtOH at their boiling points according to the following shedules: 1hr 15min, 2hrs, 5hrs, 10 hrs 30min, 16 hrs and 19hrs 46 min. After vacuum filtration, each extraction sample was evaporated and the residue was treated with 70 ml of ethyl acetate and 70 ml of distilled water (three times). After washing the organic layer with various alkali reagents (NaOH, $NaHCO_3$ and KOH) at different concentrations (3%, 5%, 8%, 11% and 13%), the aqueous layer obtained was acidified with HCl or H_2SO_4 (3%, 5%, 8%, 11% and 13%). The aqueous layer was treated with ethyl acetate and the final extract was evaporated to yield the colored compound. Note that the experience does not last long to consider the molecules sensitivity to the light.

3. Statistical analysis

3.1. Screening designs

The extraction of the red dyes from the madder depends on several parameters such as the solvent (CH_2Cl_2, MeOH or EtOH), the acid (HCl or H_2SO_4 at 5%) and the alkali (NaOH, KOH or 5% $NaHCO_3$) And finally the extraction time (2 hours to 16 hours) (Table 1). The experimental design method is known as a well suited strategy for optimizing any possible system [15]; it is time and resource saving as it prevents from investigating individually all relevant factors by conducting a huge number of trials. Each of the twenty four extractions was performed using the conditions generated by the Plackett-Burman design as described in (Table 2).

Table 1: Experimental conditions for screening design

Solvent	CH_2Cl_2	MeOH	EtOH
Acid (5%)	HCl	-	H_2SO_4
Alkali (5%)	NaOH	KOH	$NaHCO_3$
Time of extraction	2 h	-	16 h

3.2. Response surface design

Statistical analysis is frequently performed in agriculture, biology and chemistry to study the empirical relationships between one or more measured response surface and a number of variables (parameters). The most widely used surface response for three to six factors are Box–Behnken [16-19].and central composite designs.

This part of the paper treats the construction and analysis of a central composite design in which the response is the yield of extraction of the red dye madder (Rdt %), and the variables x_i are: concentration of the acid (% HCl), concentration of the alkali (% NaOH), and time of extraction (t [hours]): x_1, x_2, and x_3 respectively (Table 3).

The values of the transformed variable x_i and the values of the real variables are summarized in (Table 4). The 18 trials to be run are of orthogonal design (which means that the coefficients do not change when the model's parameters change). They are given in (Table 4).

The first 8 experiments are generated by a 23 factorial design; the ± 1 coded values x_i were obtained by means of the following relation:

$$X_j = (x_j - x_{j0})/\Delta x_j$$

Where X_j is the coded value of the j^{th} independent variable, xj the natural value of the jth independent variable, Xj_0 the natural value of the j^{th} independent variable at the center point, Xj_o the natural value of the j^{th} independent variable at the center point, and Δx_j is the step change value.

Table 2: Plackett–Burman experimental screening design

Trial	Acid (5%)	Time (h)	Alkali (5%)	Solvent	Rdt%
1	H_2SO_4	2	$NaHCO_3$	MeOH	5.4
2	H_2SO_4	2	NaOH	MeOH	9.6
3	HCl	2	KOH	MeOH	9.3
4	HCl	2	NaOH	MeOH	11.3
5	HCl	2	$NaHCO_3$	MeOH	3
6	H_2SO_4	2	KOH	MeOH	23.4
7	HCl	16	$NaHCO_3$	CH_2Cl_2	18
8	HCl	16	NaOH	CH_2Cl_2	60
9	H_2SO_4	16	NaOH	EtOH	33.1
10	H_2SO_4	16	$NaHCO_3$	EtOH	21.1
11	H_2SO_4	16	KOH	EtOH	42.5
12	HCl	2	KOH	CH_2Cl_2	51.1
13	HCl	2	NaOH	EtOH	66.3
14	H_2SO_4	2	NaOH	EtOH	21.3
15	H_2SO_4	2	$NaHCO_3$	EtOH	5.8
16	HCl	2	KOH	CH_2Cl_2	40.1
17	HCl	2	NaOH	CH_2Cl_2	54
18	H_2SO_4	16	KOH	MeOH	11.6
19	H_2SO_4	16	$NaHCO_3$	MeOH	5.7
20	H_2SO_4	16	NaOH	MeOH	11.9
21	HCl	16	KOH	MeOH	30.1
22	HCl	16	$NaHCO_3$	MeOH	7.4

The experiments are 6 points on six axes, at a distance of ± α from the center. The last four experiments in (Table 4) have been performed in the center. The distance calculated such as the square of Xj^2 and the variables are orthogonal and rotatable. In the current design space, we generated 14 experiments and four others in the center. The parameter α is close to 1.682 for each factor.

The values of the factors coding the concentration of the acid, concentration of the alkali, and time of extraction, used in this design space and the response (Rdt%), are reported in (Table 4). In the experimental design, the relation to estimate responses (ŷ) is:

$$\hat{y} = b_0 + \sum_{j=1}^{3} b_j X_j + \sum_{j=1}^{3} \sum_{\substack{j'=1 \\ j \neq j'}}^{3} b_{jj'} X_j X_{j'} + \sum_{j=1}^{3} b_{jj} X_j^2$$

Let $b_u X_u$ be the general term of; the 10 terms (1constant + 3 variables j+3 interactions jj'+ 3 squared variables jj = 10) generally used for the construction of the model, and the normal equation gives the b_u coefficients with the least-squares method:

$$b_u = Y_u / \sum_{i=1}^{n} X_{iu}^2 \quad \text{where} \qquad Y_u = \sum_{i=1}^{n} X_{iu} y_i$$

X_{iu} and y_i being the X_u and y_i values for the i^{th} experiment; Y_u is named contrast.

Table 3: Experimental range and levels of the independent test variable

	Coded variables X_1, X_2, X_3^b				
Natural variables (x_j)	-1.682	-1	0	1	1.682
Concentration of the acid (% HCl)	2.954	5	8	11	13.046
Concentration of the alkali (% NaOH)	2.954	5	8	11	13.046
Time of extraction (t[h])	1.249	5	10.5	16	19.751

b. $X_1 = (x_1 - 8)/3$; $X_2 = (x_2 - 8)/3$; $X_3 = (x_3 - 10.5)/5.5$

Table 4: Central composite design matrix used for three independent variables

Run	X_1	X_2	X_3	(Rdt %) exp y_i	(Rdt %) cal $\hat{y}_i$	Residue e_i
1	-	-	-	54	51.95	2.049
2	+	-	-	77	74.68	2.312
3	-	+	-	24	19.52	4.472
4	+	+	-	82	80.26	1.735
5	-	-	+	60	59.60	0.397
6	+	-	+	62	64.34	-2.340
7	-	+	+	41	41.17	-0.179
8	+	+	+	84	83.91	0.084
9	-1.682	0	0	22	24.97	-2.979
10	1.682	0	0	80	80.03	-0.037
11	0	-1.682	0	71	71.41	-0.410
12	0	1.682	0	58	60.60	-2.606
13	0	0	-1.682	45	50.25	-5.256
14	0	0	1.682	62	59.76	2.240
15	0	0	0	31.56	31.59	-0.031
16	0	0	0	32	31.59	0.409
17	0	0	0	31.62	31.59	0.029
18	0	0	0	31.70	31.59	0.109

4. Results and discussion

4.1. Screening design

The extraction yield of the red dye madder (Rdt%) was calculated for each series (Table 2). The extraction yields were then analyzed using multiple linear regression by means of Statgraphics software. The calculated regression coefficients have been standardized to facilitate the assessment of the relative efficiency (Eq1).

Eq1

$$\text{Rdt }\% = 25.35\ S_1 - 19\ S_2 - 6\ S_3 + 2.62\ A_1 - 2.62\ A_2 + 11.9\ B_1 + 2.99\ B_2 - 14.93 B_3 + 8.09\ T$$

With:

S_1: Solvent CH_2Cl_2 ; S_2: Solvent MeOH; S_3: Solvent EtOH; A_1: Acid HCl; A_2: Acid H_2SO_4; B_1: Alkali NaOH; B_2: Alkali KOH; B_3: Alkali $NaHCO_3$; T: Time of extraction.

The extraction data (Rdt %) were analyzed to identify the effects of the primary factors. All parameters and their relative weight in the screen design are those in the previous equation (Eq. 1). CH_2Cl_2, NaOH, HCl and extraction times have a very significant effect on yield (Rdt%). MeOH, EtOH, KOH and $NaHCO_3$ and H_2SO_4 have a weak effect. EtOH is the favorable solvent according to our screening (Table 2) and other authors [20-21], although CH_2Cl_2 has an important effect, it is discarded because suspected of being carcinogenic, damages the environment. The extraction time seems to be non-significant in the range of 2-16 hours. Therefore we were able to retain NaOH and HCl and a reduced time of 2 hrs.

4.2. *Response surface design*

The parameters were selected on the basis of the results from the screening design and also from an existing level of understanding of the CCD processes. (Table 4) shows the experimental data for extraction yield of the red dye madder (Rdt%). The ten terms are easily calculated by substituting data values in the expressions for the least squares estimates of the coefficients (Table 5). The fitted response surface expressed in real variables is:

Eq 2

$$(\text{Rdt}\%) = 31{,}5958 + 16{,}3678\ X_1 - 3{,}21169\ X_2 + 2{,}82569\ X_3 + 7{,}39413\ X_1^2 + 12{,}1649\ X_2^2 + 8{,}27679\ X_3^2 + 9{,}5\ X_1X_2 - 4{,}5\ X_1X_3 + 3{,}5\ X_2X_3$$

From this equation, it is possible to compute estimated values ($\hat{y}_i$) and the corresponding residuals $e_i = y_i - \hat{y}_i$ (Table 4). An estimate of the variance of the experimental error (s_r^2) was obtained by dividing the residual sum of squares $\sum e_i^2$ (Table 4), by ν(number of degrees of freedom (df) =18–10= 8), (Table 5).

$$S_r^2 = \sum e_i^2 / \nu = 148.783 / 8 = 18.5978$$

The experimental value of the F distribution is obtained by dividing the mean square of the coefficient b_u (MS_u) by the variance of the experimental error (s_r^2).

$$F_{exp} = MS_u / s_r^2$$

The mean square estimate of the coefficients (MS_u) is obtained by dividing the sum of squares estimates of the coefficients (SS_u) by their degree of freedom ($\nu_u = 1$).

$$MS_u = SS_u / \nu_u$$

The effects of the process factor were statistically sorted using the Variance Analysis (ANOVA) which subdivides the total variation of a data set into components associated with the specified variation sources.

Table 5 shows ANOVA results for the extraction yield of the red dye. The ratio F used to determine the statistical significance of the effects of this is a ratio of two estimates independent of the experimental error. Associated with this ratio is a value P which quantifies the probability of committing an error by associating an effect with a given factor. The P value also provides the exact level of significance of a hypothesis test. The R-square values indicate the percentage change in response that is explained by the variation of factors in the experiment. The significance of the effects can be estimated by comparing the values of the b_u^2 / s_{bu}^2 ratio with a critical value, $F_{0.95}$ (1, 8) = 5.317 of the F distribution, at 95% confidence level with 1 and 8 degrees Freedom, according to the results presented in (Table 5). In this case, 9 effects have P values less than 0.05, indicating that they are significantly different from zero at the 95% confidence level. The best fitting response function is then conveniently written as follows by (Eq3). The quantities in brackets are the coefficient corresponding to standard deviation.

Eq3

$$\begin{aligned}(\%\ Rdt) = {} & 31{,}5958 \pm (0.097) + 16{,}3678\ (\pm 0.053)X_1 - 3{,}21169\ (\pm 0.053)X_2 + 2{,}82569\ (\pm 0.053)X_3 \\ & + 7{,}39313 \pm 0.055)X_1^2 + (12{,}1649 \pm 0.055)\ X_2^2 + (8{,}27679 \pm 0.055)\ X_3^2 \\ & + (9{,}5\ \pm 0.069)\ X_1X_2 - (4{,}5\ \pm 0.069)\ X_1X_3 + (3{,}5\ \pm 0.069)\ X_2X_3\end{aligned}$$

Table 5: ANOVA for response surface model of extraction yield of the red dye madder

Parameter	**b_u**	**SS**	**df**	**MS**	**$F_{exp.}$ value**	**P-Value**	**significance**
b_0	31.5958	-	-	-	-	-	-
b_1	16.3678	3659.13	1	3659.13	336.56	0	**
b_2	3.21169	140.884	1	140.884	12.96	0.007	**
b_3	-2.82569	109.055	1	109.055	10.03	0.0133	*
b_{12}	9.5	722	1	722	66.41	0	**
b_{13}	-4.5	162	1	162	14.9	0.0048	**
b_{23}	3.5	98	1	98	9.01	0.017	*
b_{11}	7.39313	691.618	1	691.618	63.61	0	**
b_{22}	12.1649	1872.35	1	1872.35	172.23	0	**
b_{33}	8.27679	866.83	1	866.83	79.73	0	**
Residual	-	86.9763	8	10.872	-	-	-
Total Corr	-	7487.17	17	-	-	-	-

**: significant at level of 1% ($F_{(0.01)}$(1,8) = 11.258, *: significant at level of 5%($F_{0.05}$(1,8) = 5.317

The optimum conditions obtained by the model are given in (Table 6) below.

Table6: Factor levels and their optimum

Factor	**Low**	**High**	**Optimum**
Concentration of the acid (%HCl)	-1.682	1.682	-1.682
Concentration of the base (% NaOH)	-1.682	1.682	-1.682
Time of extraction (t[h])	-1.682	1.682	-1.682

4.3. Validation of the model

Considering the elaborated model and the optimum parameters, shows that the optimum yield is 95%. In order to validate such results, the extraction of the dye was carried out in the optimum conditions (t = 1.249 hrs, (% HCl): 2.954, (%NaOH): 2.954). The experimental yield-test was close to 92%. This experimental finding shows that the proposed model is valid either statistically or on the experimental point of view.

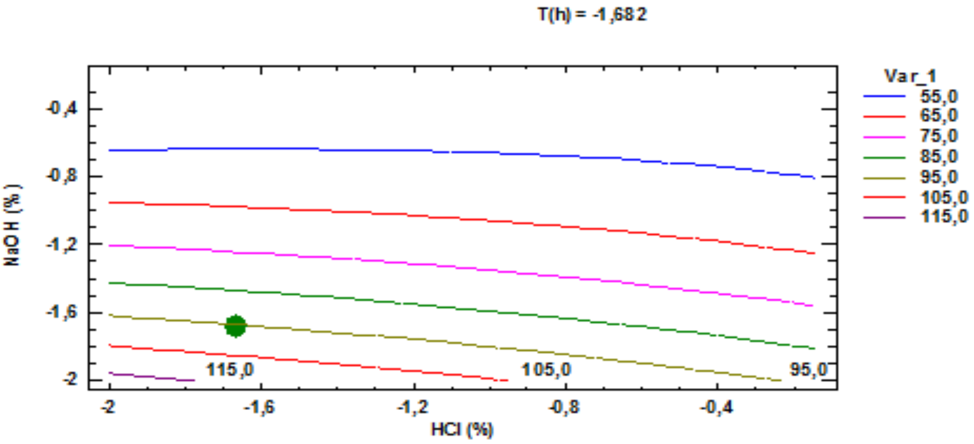


Figure 2: Contours of estimated response surface (Rdt%)

Conclusion

Using the Composite Central Design (CCD) analysis and the Response Surface Methodology (RSM), it was possible to determine the optimal conditions for high yield extraction (Fig.2). The model was tested by performing an experimental extraction under these optimal conditions (Table 6). The optimum yield obtained was (91.8%). The realization of the experimental extraction and the determination of the optimum extraction are tested successfully, validating the proposed model.

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