



Lake Color Formulation of Beetroot Extract and its Optimization

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ABSTRACT

Red Beetroot having a lot of medicinal properties also used for its coloring properties from ancient time for food products. The present study was carried out to develop aluminium lake color of a dye obtained from beet root using different adsorbent (s) (Aluminium oxide or aluminium hydroxide or combination of both) in different ratio by simply mixing them to get dye adsorbed onto the surface of adsorbent. Batch Mode studies were carried out for 60 minutes. Aluminium oxide at a concentration level of 30% w/v found to be the choice of adsorbent after optimization because maximum adsorption of dye from reaction mixture after 60 minutes and maximum % yield. After optimization of adsorbent the effect of pH and temperature also studied by formulating lake by varying these two parameters. It was observed that red color was degraded at higher temperatures and higher pH. The lake was formulated only from a few batches which are processed at low temperature and a lower pH value. Still the lake obtained from F11 (Temperature 30°C and pH 3) was found to be showing maximum adsorption and % yield which was then characterized for various physicochemical properties like angle of repose, Carr's index, hausner's ratio, loss on drying, particle size and limit test for heavy metals. The lake was found to follow pseudo second order kinetics.

Keywords: Beetroot, lake color, Adsorption, Aluminium Lake, pseudo second order.

INTRODUCTION

Beetroot (*Beta vulgaris*) is the main source of natural red dye, known as "beetroot red". Betanine is the main component of the red colorant extracted from *Beta vulgaris*. Immediately after extraction, betanine is exposed to degradation.¹

Beet (*Beta vulgaris* subsp. *vulgaris*) has been bred to produce a number of varieties, some of which are used as fodder and some of which are used for human food consumption: chard (var. *cicla*), sugar beet (var. *altissima*), and beetroot (var. *rubra*). Beetroot is a variety with a strongly colored root.

The purple root owes its color to the presence of betalains. Betalains are found in other plants than beetroot, but beetroot is the only allowed source of betalain colorant in the EU and the USA. Betalains are actually comprised of two groups of pigments: the red-purple betacyanidins and the yellow betaxanthins, both of which are water-soluble.

Betacyanidins are conjugates of cyclo-DOPA and betalamic acid, and betaxanthins are conjugates of amino acids or amines and betalamic acid. Just as with anthocyanins, the betacyanidins (aglycones) are most often glycosylated, in which case they are called beta-cyanins, and the sugar residue may also be acylated. The red-purple betacyanins comprise the major part of pigments in beetroot, and of these a single one, betanin, comprises 75–95 %. The pigments are not extracted, instead the beetroots are pressed and the water partially removed to give a product containing typically 0.5 % pigments. The

major disadvantage of beet-root color is its low heat stability.²

Foods have been coloured red using beetroot juice in the home for many years. In the eighteenth century, for example, Mrs. Raffald used beetroot colouring, in a recipe for pink pancakes, in *The Experienced English Housekeeper* (1769).

Today, it is used to provide colour in a wide variety of dishes. It is not just for its red colour, however, that *Beta vulgaris* has been utilized. For many years, extracts of leaf beet and spinach were used to colour confectionary green.

Laura Mason, in her book about the prehistory of sweets, lists beets among the plants used to colour sweets, up until the Victorian era in Britain.

Beet leaf green was a benign addition compared to some of the things that were added to colour sweets in former times, such as dye from quinces cooked in pewter (lead), acid fruits cooked in untinned copper vessels (copper), and Prussian blue (cyanide).

Following a scandal, when fatalities occurred after lozenges were consumed in 1850, a major reappraisal of the colourings and ingredients used in foodstuffs was undertaken.

This eventually led to modern food-safety legislation. In the 1860s, however, new synthetic pigments replaced many of the old colourings (including beet green), and these gave an intensity and consistency of colour not previously seen in confectionary. Betalains are important



natural colourings within the food industry. The main source of betalains is beetroot. During commercial extraction, beet roots are first crushed, and the coloured juice is collected and concentrated.

Betalain pigments are sold to the food industry either as juice concentrates or powders. Juice is concentrated under vacuum until it comprises around 60-65% of total solids.

Freeze drying techniques are used to produce a powder, typically containing 0.3-1.0% pigment.³

Due to its stability problems it may be beneficial to convert it into a more stable form that is lake color.

Lakes have been defined by the FDA as the "Aluminum salts of FD&C water soluble dyes extended on a substratum of alumina".

Lakes also must be certified by the FDA. Lakes, unlike dyes, are insoluble and color by dispersion.

Consequently, the particle size of lakes is very critical to their coloring capacity or tinctorial strength.

Generally, the smaller the particle size, the higher the tinctorial strength of lakes due to increased surface area for reflected light.

Lakes are formed by the precipitation and absorption of a dye on an insoluble base or substrate.

The base for the FD&C lakes is alumina hydrate.⁴

Here in the present study we have tried to prepare an aluminum lake of beet color, the adsorbent and the adsorption process is optimized and the experiment design is fully factorial.

MATERIALS AND METHODS

Aluminium oxide (Thomas Baker- Mumbai), Aluminium hydroxide gel dried (Thomas Baker- Mumbai), Sodium hydroxide pellets (Sisco Research laboratories Pvt. Ltd - Mumbai), Potassium-di-hydrogen ortho-phosphate (Sisco Research laboratories pvt.ltd- Mumbai), Hydrochloric acid (Thomas Baker- Mumbai) and Buffer tablets (pH 7) (Himedia laboratory, Mumbai) were of laboratory grade, provided by pharmaceuticals laboratory, BN PG College, Udaipur.

The vegetable for color extraction (Beet Root) was purchased from Reliance Fresh, Udaipur.

Reagent Preparation

0.1 N NaOH, 0.1 N HCl, 0.1 N Phosphate buffer pH 7 was prepared.⁵

Extraction of Dye

All the 1 kg beets were washed well; removing any dried dirt or mud from their surface.

Then peeled and chopped the beets and the tops into manageable pieces. Added the beets to the blender and blended well.

Extract was strained through a piece of cheese cloth to get the pulp and any large pieces of vegetable out. Filtrate of red beet color obtained was 500 ml.

Preparation of Standard Curves

Beet root dye was estimated at the λ_{\max} at various pH using water as media obeyed Beers law.

λ_{\max} at various pH are as:

Table 1: Standard Curve Data

	Beet Root		
λ_{\max}	pH 3	pH 6	pH 8
	482	482	484

Batch Mode Studies

Adsorption experiments were carried out by agitating adsorbents (aluminium oxide or aluminium hydroxide or aluminium oxide + aluminium hydroxide) with dye solution of desired concentration and pH in a 100 ml beaker at desired temperature. A good contact has been made between adsorbent and dye by agitating on hot plate magnetic stirrer of cosolab at low rpm in a 100 ml round bottom flask.

Dye concentration remained in the solution was determined spectrophotometrically by monitoring the absorbance at desired λ_{\max} using single beam UV-VIS Spectrophotometer.

Experiment was continued to 1 hr and 2 ml of each sample was withdrawn at 10 minutes time interval (In sample tube) replacing with the same amount of distilled water. All the samples were then centrifuged for 20 minutes at medium rpm.

One ml of the supernatant solution was diluted to 5ml and analysed to get UV absorbance at its respective λ_{\max} while distilled water was used as blank.⁶

Experimental Design

A 3^2 full factorial design was employed to systematically study the combined influence of the effect of independent variables (Adsorbent type- Aluminium oxide and Aluminium hydroxide) on the dependent variables i.e. % adsorbed.

In this design 2 factors are evaluated, each at 3 levels, and experimental trials are performed at all 9 possible combinations.⁷

After optimizing adsorbent type and its level again a 3^2 full factorial design was employed to systematically study the combined influence of the effect of independent variables of Batches (pH and temperature) on the dependent variables % adsorbed. In this design 2 factors are evaluated, each at 3 levels, and experimental trials are performed at all 9 possible combinations.

A statistical model incorporating interactive and polynomial terms is used to evaluate the response.



Table 2: Factorial Design Factors and their Levels for Adsorbents

Aluminium Oxide			Aluminium Hydroxide		
Medium (0)	High (+1)	Low (-1)	Medium (0)	High (+1)	Low (-1)
20%	30%	0%	20%	30%	0%

Table 3: Factorial Design Factors and their Levels for pH and Temperature

pH			Temperature (°C)		
Medium (0)	High (+1)	Low (-1)	Medium (0)	High (+1)	Low (-1)
3	8	6	30	50	40

Table 4: Beet Root: % Adsorbed at various time intervals (Minutes) for Batch

Batches	% Adsorbed at various time interval (Minutes) *					
	10	20	30	40	50	60
F1	74.55 ± 0.12	79.34 ± 0.3	82.34 ± 0.17	85.54 ± 0.00	85.60 ± 0.17	87.12 ± 0.01
F2	75.24 ± 0.13	80.88 ± 0.12	83.33 ± 0.172	86.11 ± 0.00	86.57 ± 0.12	87.42 ± 0.298
F3	78.66 ± 0.28	85.61 ± 0.27	91.24 ± 0.45	92.79 ± 0.02	93.16 ± 0.12	93.99 ± 0.12
F4	79.41 ± 0.22	82.72 ± 0.11	85.76 ± 0.02	90.22 ± 0.09	91.35 ± 0.12	91.61 ± 0.07
F5	78.20 ± 0.07	84.49 ± 0.12	90.11 ± 0.12	91.66 ± 0.12	92.34 ± 0.27	93.14 ± 0.10
F6	78.17 ± 0.12	81.62 ± 0.00	84.43 ± 0.17	89.17 ± 0.11	90.23 ± 0.19	90.57 ± 0.02
F7	79.65 ± 0.12	86.44 ± 0.27	91.98 ± 0.29	93.55 ± 0.298	96.11 ± 0.19	97.47 ± 0.172
F8	78.19 ± 0.112	84.99 ± 0.22	90.13 ± 0.11	93.22 ± 0.17	93.98 ± 0.27	94.55 ± 0.21

Table 5: Beet Root: % Adsorbed at various time intervals (Minutes) for Batch F11 to F19

Batches	% Adsorbed at various time interval (Minutes) *					
	10	20	30	40	50	60
F11	79.12 ± 0.01	87.44 ± 0.27	92.65 ± 0.19	94.15 ± 0.17	97.11 ± 0.09	98.47 ± 0.27
F12	78.2 ± 0.00	84.49 ± 0.11	90.11 ± 0.12	91.66 ± 0.09	92.34 ± 0.17	93.14 ± 0.3
F13	Color degraded					
F14	78.17 ± 0.12	81.62 ± 0.11	84.43 ± 0.09	89.17 ± 0.00	90.23 ± 0.27	90.57 ± 0.00
F15	74.55 ± 0.12	79.34 ± 0.12	82.34 ± 0.19	85.54 ± 0.298	85.6 ± 0.00	87.12 ± 0.17
F16	Color degraded					
F17	75.24 ± 0.17	80.88 ± 0.17	83.33 ± 0.33	84.12 ± 0.17	85.09 ± 0.23	86.77 ± 0.12
F18	Color degraded					
F19	Color degraded					

*Values showed % adsorbed ± SEM (n=3)



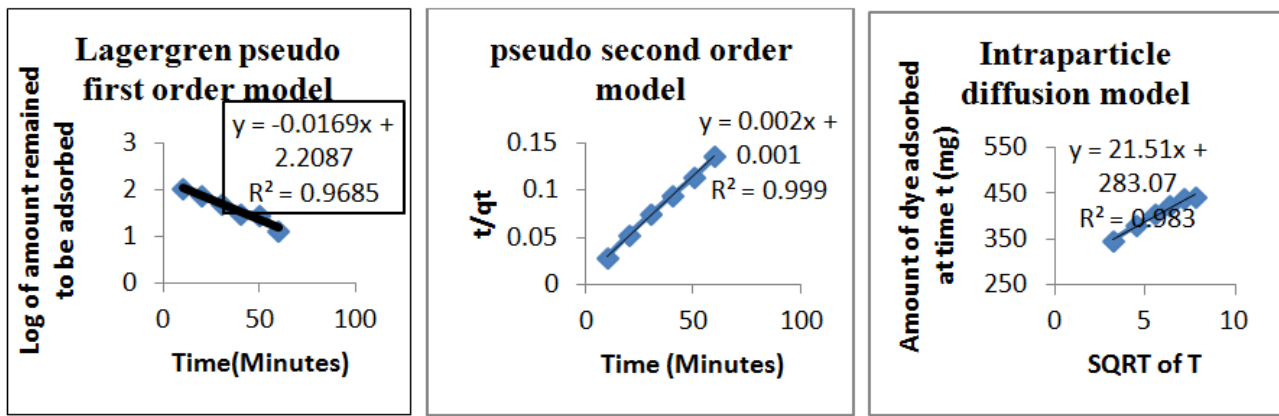


Figure 1: Kinetic Models For Batch F7

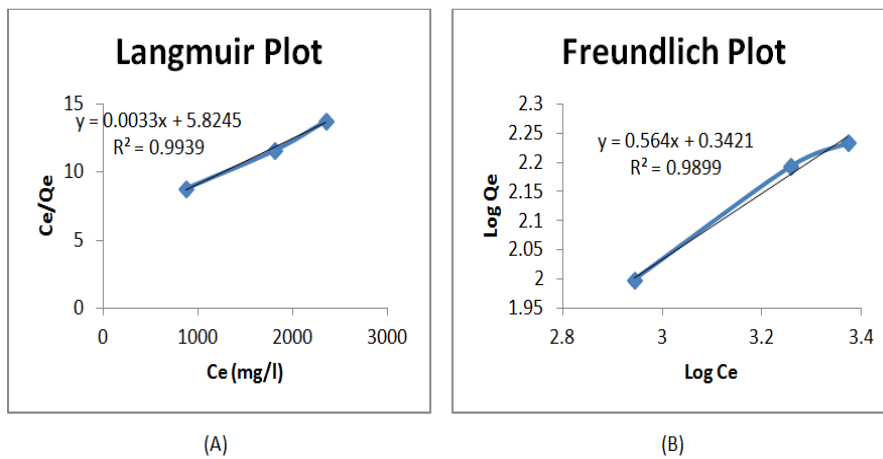


Figure 2: (A) Langmuir Plot (B) Freundlich Plot

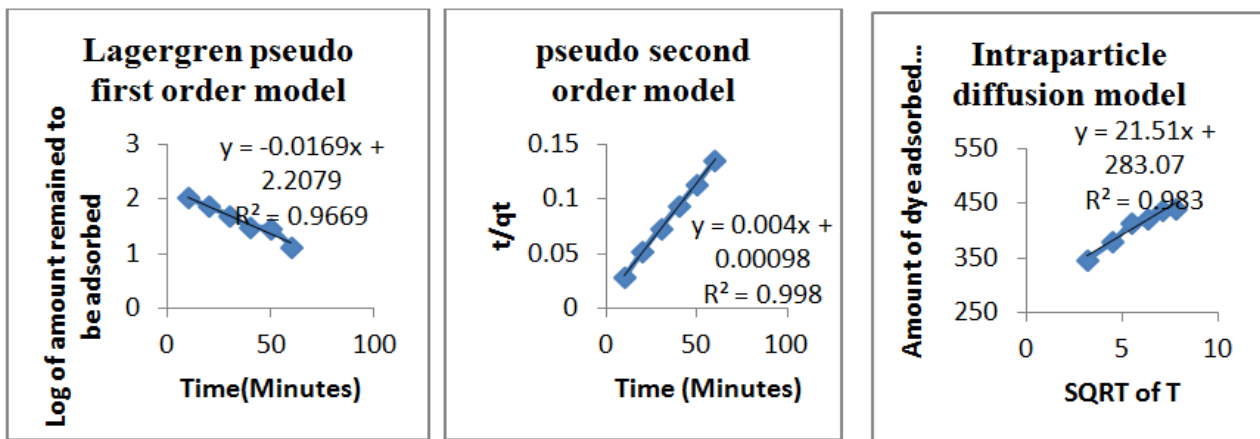


Figure 3: Various Kinetic Models for F11

Kinetics Study

The data from one hour adsorption study were fitted into three kinetics models:

Pseudo-first-order rate equation of Lagergren

$$\log(q_e - q_t) = \log q_e - k_1 / 2.303 * t$$

Pseudo-second-order rate equation

$$t/q_t = 1/k_2 q_e^2 + 1/q_e * t$$

The intra particle diffusion model

$$q_t = k_t t^{1/2} + C$$

Where q_e and q_t are the amounts of the dye adsorbed (mg) at equilibrium and at time t (min), respectively.

k_1 is the adsorption rate constant ($L \text{ min}^{-1}$) for 1st order kinetic, k_2 ($g \text{ mg}^{-1} \text{ min}^{-1}$) is the rate constant of pseudo-second-order adsorption.

k_t ($mgg^{-1} \text{ min}^{-1/2}$) is the intra particle diffusion rate constant.⁸

Langmuir and Freundlich Isotherm

These are applied to study the adsorption capacity of the adsorbent.⁹⁻¹¹



Physicochemical Characterization

The optimized lake was characterized for various physicochemical properties like angle of repose¹², loss on drying⁵, carr's index¹³, hausner's ratio¹³, organoleptic properties and limit test for heavy metals.¹⁴

Stability Studies

The optimized formulation after characterization were subjected to stability studies under normal conditions temperature and humidity in desiccators for three months in packed in aluminium foil to protect it from any sunlight. After three months the lake were assayed and compared from the initial product for its specific color.

RESULTS AND DISCUSSION

Batches from F1 to F8 were formulated and % dye adsorbed (By UV spectrophotometry) and % yield were calculated at every 10 minutes till one hour. The Adsorption data are shown in Table 4. The batch F7 (adsorbent aluminium hydroxide at 30% w/v concentration) was found to be the best batch as it showed maximum adsorption of the dye. For confirmation of above said results, % yield of the product was also calculated at the end of each experiment. These also supports that maximum yield was observed when aluminium oxide was used as adsorbent at 30 % w/v concentration.

Kinetics Study

The kinetics study of lake formulated from batch F7 was done to get adsorption rate constant and to know the adsorption dynamics.

The data obtained was fitted into three models pseudo-first-order (Langergren), pseudo-second-order (Ho and Mckay) and the intraparticle diffusion model (Figure 1) to find the type of kinetics of adsorption. From this data it was observed that the adsorption followed pseudo second order reaction kinetics having a greatest r^2 value = 0.999.

Adsorption Isotherm

Lagmuir isotherm has been applied for adsorption equilibrium.¹⁵

$$C_e/Q_e = 1 / (K_L b) + (1/K_L) C_e$$

Where C_e is equilibrium concentration (mg/l), Q_e is amount of dye adsorbed at equilibrium (mg/g) and K_L and b is Langmuir constants related to adsorption capacity (mg/g) and energy of adsorption (l/mg) respectively. K_L and b are determined from the slope and intercept of Langmuir plot, which is made between C_e/Q_e and C_e and the values are 303.03 mg/g and 0.00057 l/mg respectively. (Figure 2 (A))

Freundlich adsorption isotherm model, used to explain the present adsorption phenomenon, is represented by equation¹⁶

$$\log Q_e = \log K_f + (1/n) \log C_e$$

K_f and n are constant incorporating all factors affecting the adsorption process (adsorption capacity and intensity). The values K_f and n were calculated from the intercept and slope of the plot, which is made between $\log C_e$ and $\log Q_e$ and the values are 2.1948 and 1.773 respectively. The n values are between 1 and 10 representing beneficial adsorption. (Figure 2 (B))

Effect of Temperature and pH

Once the suitable adsorbent for beet root dye and its concentration was optimized for lake formulation, a series of experiments of lake formulation were conducted at different temperature (30, 40 and 50°C) and various pH (3, 6 and 8) while keeping the adsorbate and adsorbent concentration, contact time, agitation speed constant. Here the batches kept at higher temperature shows degradation in their color due to stability issue of beet color.²

The batch F11, the lake formulated at 30°C and pH 3 showed maximum adsorption of dye and % yield as in the Table 5.

Kinetics Study

Data of batch F11 was fitted into three models to find kinetics (Figure 3).

Again correlation coefficients supports the adsorption to be follow pseudo second order reaction kinetics having a greatest r^2 value = 0.998.

To confirm these evidences at the end of the experiment % yield was calculated. It also confirms the above said results.

Physicochemical Characterization

The optimized batch was tested for limit test of heavy metals was found to be in limit. (NMT 5mg/kg) loss on drying, the values lies from 0.057± 0.17 to 0.1549 ±0.04 i.e. in the limit of loss on drying according to IP.

The lake colors obtained from Beet Root dye were of pink purple to purple in color and having a vegetable odor with a smooth texture. Particle size ranges from 10.71 µm to 1.59 µm having a particle size of 6.37 µm.

SUMMARY AND CONCLUSION

The present study was aimed to formulate Aluminium Lake of beet root dye and its characterization. It is found that this lake is best formulated when aluminium oxide alone is used as an adsorbent rather than using aluminum hydroxide or a combination of aluminium oxide and aluminum hydroxide.

Adsorption is a surface phenomenon so depends upon concentration of reactant. If there is a concentration of aluminium oxide is high as upto 30% w/v there is a better adsorption than lower ranges.

Temperature and pH also the major factors since temperature is related with the activation energy and pH with the ionic state of the pigment.



It was concluded that a lower temperature and a lower pH value favor the adsorption process.

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Conflict of Interests

The authors report no conflicts of interest in this work.

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