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Original Research Article

Formulation and Characterization of Lake Color Obtained From Red Cabbage

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ABSTRACT

Colorants are mainly used to impart a distinctive appearance to the pharmaceutical dosage forms. The present study was carried out to develop aluminium lake color of a dye obtained from red cabbage 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 hydroxide 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 a higher temperature (50°C) and a slight acidic pH (6) favor the adsorption. The lake 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.

Introduction

Colorants are characterized by their ability to absorb visible part of the electromagnetic spectrum [380-780 nm]. For good colouring property it has to have high enough absorption coefficient (10 000 to 40 000 l.mol⁻¹.cm⁻¹). Natural food colorings are undergoing a revival within the food industry, due to concerns about synthetic food dyes. The synthetic dyes have been prohibited in foodstuffs because of health concerns. Recent health scares have centered on the presence of banned synthetic dyes in foods. So, in recent years, there has been not only a tendency to limit the use of synthetic colorants but also to replace it by natural pigments, because of natural colorants’ safety and health benefits, strong consumer demand for more natural products. This is particularly true for red colors, and therefore, it become necessary to seek alternative natural sources that could be used by the pharmaceutical and food industries [1].

Further as with natural colors, stability concern is there because they are in dissolved form. It is better to covert the dye into an insoluble form i.e. lake of the dye. Lakes have been defined by the FDA as the "Aluminum salts of FD&C water soluble dyes extended on a substratum of alumina". They are also certified by FDA. Lakes are formed by the precipitation and absorption of a dye on an insoluble base or substrate. The method of preparation of the alumina hydrate and the conditions under which the dye is added or absorbed determines the shade, particle size, dispersability as well as tintorial strength. Other important variables are the temperature, concentration of reactants, final pH, and the speed and type of agitation. The shade or hue of a lake varies with the pure dye content. Aluminium lakes are prepared under aqueous conditions by reacting Aluminium oxide with coloring matter complying with purity criteria set out in the CODEN (USA): IJPB07

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appropriate specification monograph. Following lake formulation, the product is filtered, washed with water and dried. [2]

Material 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 (red cabbage) was purchased from Reliance Fresh, Udaipur.

$\lambda_{\text{max}}$ at various pH are as:

<table>
<thead>
<tr>
<th>Black Carrot</th>
<th>pH 3</th>
<th>pH 6</th>
<th>pH 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$</td>
<td>530</td>
<td>530</td>
<td>608</td>
</tr>
</tbody>
</table>

Batch Mode Studies [4]

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 $\lambda_{\text{max}}$ 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 analyzed to get UV absorbance at its respective $\lambda_{\text{max}}$ while distilled water was used as blank.

Experimental Design[5]

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.

Table 1: Factorial Design Factors and their levels for Adsorbents

<table>
<thead>
<tr>
<th>Aluminium Oxide</th>
<th>Aluminium hydroxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium (0)</td>
<td>High (+1)</td>
</tr>
<tr>
<td>Low (-1)</td>
<td>Medium (0)</td>
</tr>
<tr>
<td>20%</td>
<td>30%</td>
</tr>
<tr>
<td>20%</td>
<td>30%</td>
</tr>
</tbody>
</table>
Table 2: Factorial Design Factors and their levels for pH and temperature

<table>
<thead>
<tr>
<th>Medium (0)</th>
<th>High (+1)</th>
<th>Low (-1)</th>
<th>Medium (0)</th>
<th>High (+1)</th>
<th>Low (-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>8</td>
<td>6</td>
<td>30</td>
<td>50</td>
<td>40</td>
</tr>
</tbody>
</table>

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.

### Kinetics Study[6]

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

1. **Pseudo-first-order rate equation of Lagergren**
   \[
   \log(q_e - q_t) = \log q_e - k_1 t / 2.303
   \]

2. **Pseudo-second-order rate equation**
   \[
   t/q_t = 1/k_2 q_e^2 + 1/q_e t
   \]

The intraparticle diffusion model
\[
q_t = k_i t^{1/2} + C
\]

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 min$^{-1}$) for 1 st order kinetic, $k_2$ (g mg$^{-1}$ min$^{-1}$) is the rate constant of pseudo-second-order adsorption. $k_i$ (mg g$^{-1}$ min$^{-1/2}$) is the intraparticle diffusion rate constant.

### Langmuir and Freundlich Isotherm

These are applied to study the adsorption capacity of the adsorbent.

### Physicochemical Characterization:

The optimized lake was characterized for various physicochemical properties like angle of repose[7], loss on drying[3], carr’s index[8], hausner’s ratio[8], organoleptic properties and limit test for heavy metals[9].

### 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

The standard curve obtained resulted $y$ equation and $r^2$ value as follows.

#### Table 3: Straight Line Equation and $r^2$ values at different pH

<table>
<thead>
<tr>
<th>pH value</th>
<th>$r^2$</th>
<th>Equation of straight Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.9788</td>
<td>$y=0.0052x-0.1175$</td>
</tr>
<tr>
<td>6</td>
<td>0.971</td>
<td>$Y=0.005x-0.113$</td>
</tr>
<tr>
<td>8</td>
<td>0.9949</td>
<td>$Y=0.0005x=0.0139$</td>
</tr>
</tbody>
</table>

Red Cabbage color extracted from black carrot and lake color were formulated using aluminium oxide or aluminium hydroxide or in combination of these two adsorbents at various concentrations. According to factorial design eight 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.
Table 4: Adsorption Profile of Red cabbage Dye by Various Adsorbents

<table>
<thead>
<tr>
<th>Batches</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>59.72±0.47</td>
<td>63.08±0.00</td>
<td>65.51±0.1</td>
<td>72.63±0.17</td>
<td>73.44±0.057</td>
<td>73.61±0.1</td>
</tr>
<tr>
<td>F2</td>
<td>62.68±0.16</td>
<td>63.14±0.06</td>
<td>66.44±0.057</td>
<td>73.21±0.153</td>
<td>75.29±0.057</td>
<td>75.35±0.06</td>
</tr>
<tr>
<td>F3</td>
<td>76.69±0.071</td>
<td>83.39±0.19</td>
<td>88.97±0.07</td>
<td>91.58±0.0</td>
<td>95.25±0.06</td>
<td>95.53±0.07</td>
</tr>
<tr>
<td>F4</td>
<td>60.31±0.43</td>
<td>63.08±0.0</td>
<td>65.63±0.21</td>
<td>72.62±0.17</td>
<td>73.72±0.057</td>
<td>73.89±0.12</td>
</tr>
<tr>
<td>F5</td>
<td>72.88±0.57</td>
<td>76.97±0.0</td>
<td>79.93±0.122</td>
<td>83.46±0.071</td>
<td>86.71±0.12</td>
<td>88.19±0.13</td>
</tr>
<tr>
<td>F6</td>
<td>73.79±0.122</td>
<td>77.04±0.071</td>
<td>79.93±0.12</td>
<td>83.46±0.07</td>
<td>89.88±0.1</td>
<td>90.17±0.071</td>
</tr>
<tr>
<td>F7</td>
<td>72.17±0.19</td>
<td>76.90±0.071</td>
<td>80.99±0.122</td>
<td>85.09±0.141</td>
<td>89.32±0.07</td>
<td>89.39±0.071</td>
</tr>
<tr>
<td>F8</td>
<td>68.92±0.122</td>
<td>71.96±0.07</td>
<td>75.55±0.0</td>
<td>78.45±0.13</td>
<td>80.57±0.0</td>
<td>80.78±0.1</td>
</tr>
</tbody>
</table>

*Values showed % adsorbed ± SEM (n=3)

The batch F3 (adsorbent aluminium hydroxide at 30% w/v concentration) was found to be the best batch as it showed maximum adsorption of the dye. Further this batch was studied for effect of pH and temperature.

Effect of Temperature and pH

Once the suitable adsorbent for black carrot 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. The batch F18, the lake formulated at 50°C and pH 6 showed maximum adsorption of dye and % yield as in the Table 5.

Table 5: Adsorption Profile of Red Cabbage Dye at various pH and temperature

<table>
<thead>
<tr>
<th>Batches</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>F11</td>
<td>57.53±0.1</td>
<td>62.97±0.12</td>
<td>64.99±0.100</td>
<td>68.52±0.115</td>
<td>71.1±0.057</td>
<td>71.12±0.058</td>
</tr>
<tr>
<td>F12</td>
<td>73.79±0.122</td>
<td>76.62±0.071</td>
<td>79.31±0.12</td>
<td>82.76±0.14</td>
<td>91.09±0.01</td>
<td>91.23±0.07</td>
</tr>
<tr>
<td>F13</td>
<td>74.5±0.071</td>
<td>76.28±0.141</td>
<td>78.59±0.186</td>
<td>83.11±0.12</td>
<td>85.65±0.122</td>
<td>85.87±0.1</td>
</tr>
<tr>
<td>F14</td>
<td>59.67±0.231</td>
<td>62.91±0.1</td>
<td>65.39±0.057</td>
<td>72.97±0.11</td>
<td>73.96±0.06</td>
<td>74.07±0.057</td>
</tr>
<tr>
<td>F15</td>
<td>73.30±0.35</td>
<td>76.62±0.07</td>
<td>82.97±0.31</td>
<td>86.85±0.141</td>
<td>93.56±0.07</td>
<td>93.91±0.122</td>
</tr>
<tr>
<td>F16</td>
<td>72.17±0.245</td>
<td>78.03±0</td>
<td>79.79±0.186</td>
<td>79.79±0.122</td>
<td>87.48±0.07</td>
<td>87.98±0.1</td>
</tr>
<tr>
<td>F17</td>
<td>68.93±0.122</td>
<td>71.61±0.071</td>
<td>76.76±0.12</td>
<td>78.59±0.18</td>
<td>80.43±0.19</td>
<td>80.50±0.07</td>
</tr>
<tr>
<td>F18</td>
<td>76.26±0.141</td>
<td>83.61±0.185</td>
<td>88.97±0.075</td>
<td>92.99±0.25</td>
<td>96.59±0.01</td>
<td>97.01±0.07</td>
</tr>
<tr>
<td>F19</td>
<td>71.68±0.244</td>
<td>77.32±0.19</td>
<td>81.91±0.18</td>
<td>85.09±0.14</td>
<td>89.88±0.12</td>
<td>90.17±0.07</td>
</tr>
</tbody>
</table>

*Values showed % adsorbed ± SEM (n=3)
Kinetics Study

The kinetics study of lake formulated from batch F18. The data obtained was fitted into three models pseudo-first-order (Lagrgren), pseudo-second-order (Ho and McKaY) and the intraparticle diffusion model to find the type of kinetics of adsorption.

![Lagrgren pseudo first order model](image1)

**Figure 1:** (A, B, C) Kinetics study according to three models

The kinetic parameters for three kinetic models and correlation coefficients were calculated from the plots (Figure 1). 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 equilibirium[21].

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

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 (Figure 2), which is made between $C_e/Q_e$ and $C_e$ and the values are 322.6 mg/g and 0.00052 l/mg respectively.

Freundlich adsorption isotherm model, used to explain the present adsorption phenomenon, is represented by equation[22, 23]

$$\log Q_e = \log K_f + \frac{1}{n} \log C_e$$

$K_f$ 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 (Figure 3), which are made between log $C_e$ and log $Q_e$ and the values are 2.617 and 1.78 respectively. The n values are between 1 and 10 representing beneficial adsorption.

![Langmuir Plot](image2)

**Figure 2:** Red Cabbage: Langmuir Plot

![Freundlich Plot](image3)

**Figure 3:** Red Cabbage: Freundlich Plot
Physicochemical Characterization

<table>
<thead>
<tr>
<th>Batch</th>
<th>Angle of Repose(θ)*</th>
<th>Tapped Density (gm/cm³)*</th>
<th>Bulk Density (gm/cm³)*</th>
<th>Hausner' ratio*</th>
<th>Carr's Index* %</th>
</tr>
</thead>
<tbody>
<tr>
<td>F17</td>
<td>20.18±0.17</td>
<td>0.45±0.01</td>
<td>0.38±0.00</td>
<td>1.18±0.122</td>
<td>18.42±0.142</td>
</tr>
</tbody>
</table>

*Values showed average±SEM(n=3)

Limit test for heavy metals was found to be in limit. Loss on drying was 0.057± 0.02 to 0.1552 ±0.041 i.e. in the limit of loss on drying according to IP. The lake colors obtained from black carrot dye were of light purple to purple in color and having a vegetable odor with a smooth texture. Particle size ranges from 12.78 µm to 2.84 µm having a particle size of 7.44 µm.

Summary and conclusion

The existence and preparation of blue complexes with aluminium and magnesium from anthocyanins derived from petals and sepals from the flowers of hydrangea and Chinese bellflower is known from the prior art [19-21]. So the present study was aimed to formulate Aluminium Lake of Red cabbage dye and its characterization. It was found that this lake is best formulated when aluminium hydroxide alone is used as an adsorbent rather than using aluminum oxide 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. If both of the adsorbents are used in combination there is a competition of dye of metal surface resulted in decreased adsorption. Temperature and pH also the major factors since temperature is related with the activation energy and pH with the ionic state of the pigment. Itwas concluded that a higher temperature and a slight acidic pH value favors the red cabbage dye uptake. Lake optimized was found to be fine in appearance, good in flow properties and is retain its color and purity when tested for stabilities studies after 3 months. It has also been passed the limit test for heavy metals. The kinetics that followed by adsorption process was pseudo second order and Langmuir and Freundlich confirm that the adsorption process is favorable[18].

Conflict of interest statement

We declare that we have no conflict of interest.

References


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