Experimental and modeling studies on extraction of amyrins from latex of mandar

(*Calotropis gigantea*)

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The effect of various process parameters, such as solvent, temperature, speed of agitation, and solid loading, on the percentage extraction of α-amyrin and β-amyrin from the latex of *Calotropis gigantea* has been studied. Extraction of α-amyrin and β-amyrin is performed with different solvents such as methanol, ethanol, propanol, and chloroform. Methanol is found to be the best solvent for both α-amyrin and β-amyrin. As the temperature of the extraction increases, easy release of amyrin takes place, because of destruction of cellular structure of the matrix. The percentage extraction increases with the increase in speed of agitation. Assuming the flat geometry of the particles, the process has been modeled and the results are compared with experimental data at different experimental conditions. The values of energy of activation are obtained as 40.64 kJ/mol for alpha amyrin and 26.96 kJ/mol for beta amyrin.

**Keywords:** α-amyrin, Activation energy, β-amyrin, *Calotropis gigantea*, Latex, Mandar

Amyrins belong to the class of pentacyclic triterpenoids and have been isolated from the oleoresin *Manila elemi* and numerous other resins and saps. The two amyrins, (α and β) are found to be similar in their behavior in solution and thus represent the stereoisomers which differ only in the position of the methyl group. Both the compounds have same molecular weight but they differ in position of CH₃ group. These compounds are stereoisomer of each other.

The latex of *Calotropis gigantea* is applied to soften the outer skin portion of human while removing thorns. The latex and related species are commonly used on fresh cuts to stop bleeding. It is also used as an anti-inflammatory agent in folk medicine1. Tribal people use this latex for easy delivery abortion and for other ailments2.

The mixture of α-amyrin and β-amyrin triterpenes acts as an antinociceptive agent (reducing sensitivity to painful stimuli). The mixture also promotes anti-inflammatory, antiulcer, antitumor and hepatoprotective actions. Anti-inflammatory effect of alpha and beta amyrin from *Protium heptaphyllum* was reported by Holanda3. The medical uses of this class of compounds suggest their great potential in drugs formulation. Investigations of mixtures of alpha and beta amyrins established their gastro protective, contraceptive, antipruritic4 and hepatoprotective (ability to prevent damage to the liver) behavior against acetaminophen-induced hepatotoxicity. Alpha amyrin has topical (In medicine, a topical medication is applied to body surfaces such as the skin or mucous membranes) anti-inflammatory properties also5. Thakur et al6 have extensively studied the extraction of different terpinoids like 3' methyl butanoates of α-amyrin and ψ-taraxasterol, besides the known 3' methyl butanoates of three triterpine alcohols. These compounds were identified as isorhamnetin-3-O-rutinidine and isorhamnetin-3-O-β-D-glucoside from its C- NMR spectra7.

The clot dissolving property of the latex is also reported to be much stronger than the trypsin and papain enzymes and the dissolution of blood clot is a pre-requisite in the process of wound healing. In vivo plasmin enzymes in the presence of matrix metalloproteases are responsible for such processes8. The *Saussurea lappa* plant is rich in sesquiterpenoid lactones and terpenoids. Several reports are available on their isolation9-11.

The rate of discovery of new terpenoids has increased over the last ten years largely as a result of

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the increase in the sophistication of separation and analytical techniques. The goal of the present research work is to find a better solvent through a systematic experimental study to understand the influence of the main operating parameters on the extraction. The diffusion model proposed by Wongkittipong\textsuperscript{12} is fitted with experimental data and activation energy required for diffusion of both the compounds is estimated. The coefficient of effective diffusion for both amyrins has been estimated at different temperatures with methanol as solvent. The order of magnitude of effective diffusivity estimated in the present work agrees well with that for the extraction of andrographolide from the leaves of \textit{Andrographis paniculata}\textsuperscript{12} extraction of thymol from seeds of \textit{Trachyspermum ammi}\textsuperscript{13} and extraction of catechin hydrate and epicatechin from Indian green tea leaves\textsuperscript{14}.

**Experimental Procedure**

**Soxhlet extraction**

The objective of the soxhlet extraction is to determine the maximum recoverable content of the solute in the raw material. A mass of 13.33 g of the fresh latex of \textit{Calotropis gigantea} was introduced in the soxhlet apparatus with 400 cm\textsuperscript{3} of methanol. The extraction was performed for 24 h with methanol.

**Batch extraction**

The objective of the batch experiments is to measure the global kinetics of extraction and to investigate the influences of the operating parameters. A mass of 5 g fresh latex was introduced into the extraction vessel, equipped with a mechanical stirrer and containing 150 cm\textsuperscript{3} solvent. The samples (0.5 mL) without particles were collected at different time intervals and cooled quickly; stored in a refrigerator until HPLC analysis was performed. The \(\alpha\)-amyrin and \(\beta\)-amyrin concentrations in the liquid phase were then measured by HPLC.

**Methods of analysis**

The analysis was performed with high performance liquid chromatography (HPLC) with a system from Waters, USA. The HPLC consisted of Waters 600 pump, Waters 2487 UV detector, and Rheodyne injection valve (5 µL sample loop). The analytical HPLC column was a Waters X-Terra \textsuperscript{\textregistered} MS C\textsubscript{18} 5 µm (3.9 x 150 mm, column Waters Co., USA). The gradient system of acetonitrile and water both containing 0.01% phosphoric acid were used as the mobile phase\textsuperscript{15}. The retention time for alpha amyrin and beta amyrin were 60.05 min and 58.14 min respectively. The injection volume was 5 µL and the UV wavelength was set at 210 nm. Empower (ver.1.0) was used as the data acquisition system. The column temperature was maintained at 31 °C during analysis. Calibration of alpha amyrin and beta amyrin has been conducted separately by using various standard solutions procured from Sigma Aldrich (USA). All solvents (water, acetonitrile) used for high performance liquid chromatography (HPLC) analysis were of HPLC grade (E-Merk) and obtained from Abhishek Scientific Mumbai. The concentrations of \(\alpha\)-amyrin and \(\beta\)-amyrin versus corresponding peak area were plotted separately. The plot showed a perfect straight line with \(R^2>0.99\). Figure 1 shows HPLC chromatograms of standard alpha amyrin, beta amyrin and solvent extracted sample with methanol as a solvent respectively.

**Results and Discussion**

**Effect of solvent**

\(\alpha\)-amyrin and \(\beta\)-amyrin were extracted from latex of \textit{Calotropis gigantea} in four different solvents, namely methanol, ethanol, propanol, and chloroform at a constant temperature of 30 °C. Figures 2a and b show the percentage extraction of \(\alpha\)-amyrin and \(\beta\)-amyrin in different solvents. It is observed that in one hour 84% of \(\alpha\)-amyrin and 78% of \(\beta\)-amyrin get extracted in methanol whereas, chloroform could extract only 66% of \(\alpha\)-amyrin and 53% of \(\beta\)-amyrin respectively. Chloroform being most non-polar of all selected solvents gives minimum extraction as compared to other solvents. Amongst the chosen alcohols the polarity of methanol and ethanol is comparable, but higher % extraction is observed with methanol as compared to ethanol. This could be due to higher values of diffusion coefficient of amyrin with methanol as compared to ethanol. In addition, amyrins are highly soluble in methanol; hence, the % extraction of amyrin is highest in methanol than in other solvents. The concentrations obtained at this time are considered to be equilibrium concentrations attained during the extraction. The trend lines in the figures are the fitted lines with the steady-state diffusion model, discussed hereunder.

**Effect of agitation**

Mechanical agitation by a rotating device is a suitable method for dispersing solids into liquids to decrease the mass transfer resistance. High turbulence produced during agitation gives better mass transfer
coefficients which are necessary for effective dispersion of solid into liquids. To determine the optimum speed and to ensure that the external mass transfer does not interface, the extraction was studied by varying the speed of agitation from 400 rpm to 1000 rpm. The percentage extraction of $\alpha$-amyrin and $\beta$-amyrin increases with increase in the speed of agitation up to 800 rpm and remained constant thereafter (Figures 3a and b). Hence, to study the effects of other parameters (solvent, temperature and solid loading) all other experiments were carried out at the optimized speed of agitation unless otherwise mentioned. The % extraction of amyrin increases with increase in speed of agitation up to 800 rpm and thereafter remains constant, probably because of solubility limitations at that speed of agitation.

Effect of temperature

The effect of temperature on the rate of extraction of amyrins from latex of *Calotropis gigantea* with methanol was investigated in an agitated vessel at 1000 rpm in the temperature range 30-55 °C. The percentage of extraction of both $\alpha$-amyrin and $\beta$-amyrin is found to increase with increase in temperature. The initial rate of extraction of alpha amyrin is very rapid up to 20 min and thereafter it attains a steady state value. Also, the steady state values of percentage extractions of amyrins increases with increase in temperature (Figures 4a and b). This is because higher the temperature of solvent, the faster is its penetration ability and lesser time is required to extract the same amount of amyrins at higher temperature, and also the final percentage of amyrin

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Fig. 1—Chromatogram of standard alpha amyrin (A), beta amyrin (B) and solvent extracted sample (C) at 1000 rpm using methanol as a solvent.
extracted is higher at higher temperature. A higher value of effective diffusion coefficient signifies that more amyrins can be extracted into the solution from the latex of Calotropis gigantea in a short time.

**Effect of solid loading**

The effect of solid loading on the extraction of α-amyрин and β-amyрин was studied with methanol at 1000 rpm, and at room temperature (30°C). (Figures 5a and b) show that the percentage extraction of α-amyrin and β-amyrin decreases as solid loading of latex increases. The suspension of latex is observed to be viscous in the solvent during extraction which leads to decrease the surface area available for extraction with the increase in solid loading. Since the suspension becomes viscous it is difficult for the solvent to diffuse inside the latex and dissolve the amyrins and hence, the percentage extraction decreases with increase in loading.

**Modeling section**

**Mass transfer model**

In the present study an attempt has also been made to estimate the diffusion coefficient using a steady state diffusion model proposed by Wongkittipong et al. for the extraction of andrographolide from
plants. The general diffusion model developed by Wongkittipong et al.\textsuperscript{12} and Seikova et al.\textsuperscript{16} for solid liquid extraction is described by the following equations:

\[
\frac{\partial c(t, x)}{\partial t} = D \frac{1}{x^{\phi-1}} \frac{\partial}{\partial x} \left( x^{\phi-1} \frac{\partial c(t, x)}{\partial t} \right) \tag{1}
\]

where \(D\) is the effective diffusivity (m\(^2\)/s).

For flat shape the following Fick’s second law of diffusion is used:

\[
\frac{\partial c}{\partial t} = D \left( \frac{\partial^2 c(t, x)}{\partial x^2} \right) \tag{2}
\]

Diffusivity of \(\alpha\)-amyrin and \(\beta\)-amyrin was determined by fitting experimental data with different conditions. The diffusivity values were used to estimate the energy of activation using the following Arrhenius equation:

\[
D = D_0 \left( e^{ \frac{-E_D}{RT} } \right) \tag{3}
\]

where \(D_0\) is the frequency factor; and \(E_D\), the energy of activation.
The particle diameter was discreetized in space with second-order finite differences. For the boundary equations, a same order finite difference was used. The Crack–Nicolson method was used to solve these equations. The coefficient of diffusion was then calculated from the experimental data points using a simple method based on a quadratic criterion. Percentage extraction was plotted against time from the experimental data at different temperatures as well as that computed from the model and the plot shows good agreement between the two.

### Numerical treatment

The particle diameter was discreetized in space with second-order finite differences. For the boundary equations, a same order finite difference was used. The Crack–Nicolson method was used to solve these equations. The coefficient of diffusion was then calculated from the experimental data points using a simple method based on a quadratic criterion. Percentage extraction was plotted against time from the experimental data at different temperatures as well as that computed from the model and the plot shows good agreement between the two.

### Comparison between experimental results and model predictions

The diffusion coefficient ($D$) was determined by fitting the experimental data in the model for different experimental conditions. The $\chi$-square test analysis between % extraction values obtained from the model and experiments at different conditions was performed. The diffusion coefficient obtained in each case corresponding to the minimum value of $\chi^2$ squared is reported in Table 1 ($\chi^2 = \sum [X_{\text{exp}} - X_{\text{model}}]^2$, where $X$ is the fractional extraction of amyrin in solvent). The coefficient of effective diffusion for alpha amyrin varies from $6.31 \times 10^{-13}$ to $22.32 \times 10^{-13}$ m$^2$/s and that for beta amyrin varies from $2.91 \times 10^{-13}$ m$^2$/s to $7.21 \times 10^{-13}$ m$^2$/s with methanol at different temperatures. As expected, $D$ values increase with temperature of extraction and so does the rate of extraction. The higher the temperature of solvent, the faster is its penetration ability and lesser time is required to extract the same amount of amyrins at higher temperature, and also the final percentage of amyrin extracted is higher at higher temperature. A higher $D$ value signifies that more amyrins is extracted into the solution from the latex of *Calotropis gigantea* in a short time. The energy of activation can be calculated from Arrhenius equation [Eq (3)]. The plot of $\ln D$ with $1/T$ for both the amyrins is shown in Fig 6. The values of energy of activation are estimated as 40.64 kJ/mol for alpha amyrin and 26.96 kJ/mol for beta amyrin. Similarly, it is observed that the coefficient of effective diffusivity is higher for methanol and the effective diffusivity decreases with decrease in polarity of the solvent. The order of magnitude of effective diffusivity estimated in the present work agrees well with that for the extraction of andrographolide from the leaves of *Andrographis paniculata*\(^{12}\) the extraction of thymol from seeds of *Trachyspermum ammi*\(^{13}\) and extraction of catechin hydrate and epicatechin from Indian green tea leaves.\(^{14}\)

### Table 1—Effective diffusion coefficients for alpha amyrin and beta amyrin at different experimental conditions as estimated by the model

<table>
<thead>
<tr>
<th>Exp. conditions</th>
<th>Diffusion coefficient $\times 10^{13}$, m$^2$/s</th>
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<tbody>
<tr>
<td></td>
<td>$\alpha$ - amyrin</td>
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<tr>
<td>Solvent</td>
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<tr>
<td>Methanol</td>
<td>6.31</td>
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<td>Ethanol</td>
<td>4.07</td>
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<tr>
<td>Propanol</td>
<td>3.29</td>
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<tr>
<td>Chloroform</td>
<td>2.29</td>
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<td>Speed of agitation, rpm</td>
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<tr>
<td>400</td>
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<tr>
<td>600</td>
<td>6.15</td>
</tr>
<tr>
<td>800</td>
<td>6.20</td>
</tr>
<tr>
<td>1000</td>
<td>6.31</td>
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<tr>
<td>Temperature, °C</td>
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<td>55</td>
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<td>Solid loading(^a), %</td>
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<tr>
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<td>6.22</td>
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<td>2.50</td>
<td>6.31</td>
</tr>
<tr>
<td>5.00</td>
<td>6.44</td>
</tr>
</tbody>
</table>

\(^a\)Gram of raw material/cm$^3$ of solvent.

**Fig. 6**—Plot of $\ln D$ versus $1/T$ for estimation of activation energy

**Conclusion**

Extraction of alpha and beta amyrin was performed with methanol, ethanol, propanol and nonpolar solvent like chloroform. The effect of different
parameters such as speed of agitation, temperature, solid loading has been extensively studied. As the temperature of the extraction increases, easy release of amyrin takes place, because of destruction of cellular structure of the matrix. The % extraction of amyrin increases with increase in speed of agitation up to 800 rpm and thereafter it remains constant, probably because of solubility limitations at that speed of agitation. The experimental results are used to estimate the effective diffusion coefficients at different experimental conditions. It is observed that coefficient of effective diffusion increases with increase in temperature. The coefficient of effective diffusion \( (D) \) for alpha amyrin varies from \( 6.31 \times 10^{-13} \) m\(^2\)/s and for beta amyrin it varies from \( 2.91 \times 10^{-13} \) m\(^2\)/s to \( 7.21 \times 10^{-13} \) m\(^2\)/s with methanol at different temperatures. Similarly it is observed that coefficient of effective diffusivity is higher for methanol and \( D \) value decreases with decrease in polarity of the solvent. Two factors govern the percentage extraction of amyrins, namely the penetration of solvents and the capacity of solvent to solubilise the product. The percentage extraction in non polar solvent is less than that in polar solvent. The values of energy of activation are obtained as 40.64 kJ/mol for alpha amyrin and 26.96 kJ/mol for beta amyrin.

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