Optimization of Batch Extraction Parameters for Aloin from Aloe Vera Gel

N.R. Jawade, S. Chattopadhyay

Abstract—Extraction of aloin from Aloe Vera was carried out in a series of solvents methanol, ethanol, isopropyl alcohol (IPA) and water respectively. Maximum yield of extraction of aloin was estimated in methanol on the basis of solubility and therefore was used to investigate influence of dry gel particle size, speed of agitation, dry gel loading and temperature etc; on the recovery in a stirred batch extractor. The analysis of aloin in extracted sample was done by liquid chromatography. Effective intraparticle diffusivity of aloin in methanol was estimated using unsteady state mass diffusion model, and activation energy of diffusion using Arhenius equation. Particle size 0.042-0.841 mm, agitation speed of 700 rpm, dry gel loading 7.5 % (w/w), temperature 65 °C were found to be the optimum parameters from the experimental work.

Keywords: Aloe Vera, Aloin, Batch extraction, Diffusivity, Optimization

1. INTRODUCTION

Aloe Vera (Aloe Barbadensis Miller) is a tropical succulent plant, of liliaceous family (Fig 1). It contains biologically active substances grouped as vitamin, mineral, amino acid, salicylic acid, anthraquinone, mono and polysaccharide, sugar, enzyme, lignin, and fatty acid [13]. Gel is a thin, clear, jellylike substance that can scrap from the leaf. [Fig.2]. The parenchyma tissue produces the gel [10]. Gel contains 99% water and rest occupies most of the part by polysaccharides and active compounds [1]. Aloein, yellow-brown compound at levels from 0.1 to 0.66 % of leaf dry present in cells adjacent to the rind of the leaf in gel. It is used as laxative agent to maintain digestion system treating constipation by inducing bowel movements [3] [7].

Aloin is an anthraquinone glycoside and its chemical structure is as shown in fig. 3. It’s molecular weight is 418 and has molecular formula C_{21}H_{22}O_{9}. Its IUPAC name is 8-Dihydroxy-10-(β-D-glucopyranosyl)-3- hydroxymethyl)-9(10H)-anthracenone [1]. Once ingested, aloin increases peristaltic contractions in the colon, and induces bowel movements. It also prevents the colon from absorbing water from gastrointestinal tract, and leads to softer stools [5] [7].

In this work, we look into engineering aspects like extraction rate, diffusion coefficient, and activation energy of diffusion of aloin from dry gel particles in batch extraction process. A series of solvents e.g. methanol, ethanol, water and IPA were investigated along with factors such as dry gel particle size, speed of agitation, dry gel loading, and temperature influencing (limiting by boiling point of solvent), and recovery of the aloin by extraction. An unsteady state diffusion model is used to estimate diffusion coefficients [9] [11] [12] [14].

II. EXPERIMENTAL WORK

2.1) Materials & method

Aloe Vera leaves were collected from local nursery. The leaves were washed with water and rinds were removed. The inner gel scapped and cut into pieces, solar dried (30-45 °C for 3 weeks) and dry gel particles were collected. The dry gel particles were screened using sieves in the range of 0.42-0.841, 0.841 to 1.68, 1.68- 3.36 & 3.36 – 6.73 mm respectively. Standard aloin sample was obtained from Fluka, USA for calibration. Solvents used for the extraction and high performance liquid chromatography (HPLC) analysis were of AR and HPLC grade from S. D. Fine Chemicals, India.

2.2) Soxhelt extraction

The maximum recoverable aloin was estimated by Soxhelt extraction using methanol. 5 % (w/w) dry gel particles of size 0.42 – 0.841 mm were taken in Soxhelt with 200 ml methanol. Extraction was carried out for 24 hours. Samples free from dry gel were collected at the end, stored in a freezer, and analyzed using HPLC to determine the concentration of aloin in each extract.

2.3) Batch extraction experiment

Batch extraction was conducted in a fully baffled 250 ml stirred borosilicate cylindrical glass vessel (7 cm ID, and 9 cm height) to estimate extraction kinetics and to analyze influence of the operating parameters. In a typical experiment, the vessel was charged with the dry gel along with 200 ml solvent. A volume of solvent in all experiment was taken 200 ml and dry gel loading changed accordingly. A four pitch bladed 45° turbine agitator of 3.5 cm diameter was
used to stir the mass at a predetermined rpm. All experiments were conducted in a thermostatic bath. The temperature of system was measured with accuracy of ±1 °C. Samples were collected at different time intervals for entire duration of the extraction and analyzed by HPLC to estimate the concentration of aloin. At the end of each experiment, the solution was filtered and volume was adjusted at its initial value to avoid errors in the aloin concentration due to vaporization loss. The effect of solvent of varying polarity (e.g. methanol, ethanol, water, and IPA), speed of agitation (300, 600, 900, and 1200 rpm), particle size (0.42-0.841, 0.841-1.68, 1.68-3.36, 3.36-6.73 mm), solid loading (2.5 %, 5 %, 7.5 %, and 10 %) and temperature (30 °C, 40 °C, 50 °C, 60°C and 65 °C) were investigated to select optimum conditions for the batch extraction.

2.4) Chromatographic conditions

The HPLC analysis conducted using a Symmetry® C-18 (4.6 x 250 mm, 5 μm) column equipped with with 5 μl sample loop, online uv detector. The gradient method was used for mobile phase. The detection was done at 290 nm. Fig. 4 shows the spectra of methanolic extract of aloin

![Chromatogram of MeOH extracted aloin](image)

Fig. 4: Chromatogram of MeOH extracted aloin (0.42 -0.841 mm, 5 %, 700 rpm & 30 °C)

III. RESULTS AND DISCUSSION

3.1) Selection of solvent

Fig. 5 shows the concentration of extracted aloin in different solvents after one hour of batch extraction at 30 °C. Aloin showed the maximum extraction in methanol and it decreased gradually with ethanol to water and comparatively low with IPA. The results of extraction in the same solvents at their respective boiling points are shown in fig 6. About 88% aloin was extracted within 30 minutes using methanol at its boiling point.

![Selection of solvent for extraction of aloin](image)

Fig. 5: Selection of solvent for extraction of aloin

The initial rates of extraction was, however, higher in the case of methanol (0.0761 mg/ml min) at its boiling point, compared to using ethanol (0.0504 mg/ml.min), water (0.0085 mg/ml.min) and IPA (0.0034 mg/ml.min) respectively.

![Effect of solvent at its boiling point](image)

Fig. 6: Effect of solvent at its boiling point

Methanol at boiling point shown 88 % extraction of aloin within 30 min, which is 74 % with ethanol, 22 % with water and 7 % with IPA respectively. The differential rates of extraction in different solvents can be due to varying solubility of aloin in these solvents.

The size of solvent molecules plays a crucial role in the extraction as diffusion is the only mechanism by which the solvent can penetrate into the solid matrix of natural material. It may be possibly due to increase in polarity of solvent and decrease in molecule size of solvent, except water. The increase may be attributed to higher solubility and diffusivity of the species at higher temperatures of extraction. A smaller size of methanol helps it to penetrate better inside the matrix of dry gel which may gradually become difficult for larger size alcohols like ethanol and IPA. However it is observed that extraction of aloin using water is less even water molecule is small than methanol and ethanol. It was observed that degradation of aloin took place while using water as solvent for extraction. It may be due to hydrolysis of aloin at higher temperature. Also solubility of aloin in water is reported 8300 mg/l of water [2]. Since methanol is the best solvent for the extraction of aloin, it was used for the subsequent experimentation, unless stated otherwise.

3.2) Soxhlet extraction

The maximum amount of recoverable aloin content in the raw material was determined by Soxhlet extraction using methanol. A 24 hour of extraction showed the aloin content in the dry gel to be 3.433 %. It is 0.0515 % on the basis of fresh Aloe Vera leaf. This was considered as a basis for calculation of percentage extraction of aloin.

3.1 Effect of speed of agitation

The extraction of aloin was studied with methanol at 30 °C at speed of agitation 300 to 1200 rpm respectively.
Fig. 7: Effect of speed of agitation

Fig. 7 shows that percentage extraction increases with the increase in agitation speed. The rise was considerable from 600 rpm to 900 rpm, but above 900 rpm, the rise was not appreciable. Increased turbulence in the suspension reduces the interfacial solid-liquid mass transfer resistance and attains a limiting value at and beyond 700 rpm, indicating thereafter intraparticle diffusion control.

The critical speed of agitation (critical suspension velocity) of solid–liquid systems in agitated vessel is calculated by Zwietering correlation [6] [7].

\[ N_{js} = \frac{\rho g (x - \rho_l)}{\rho_l} 0.45 x^{0.13} d_p^{0.2} D^{0.85} \]  

where, \( N_{js} \) is the critical speed of agitation (RPS), \( S \) is the constant for given geometry and depends on the tank diameter to agitator diameter ratio, is the kinematic viscosity of liquid (m²/s), \( \rho_s \) is the solid density (kg/m³), \( x \) is the solid to liquid ration in percentage by weight, \( d_p \) is the particle diameter (m), \( D \) is the agitator diameter (m). The speed of agitation should be below the critical speed for better suspension in vessel at the experimental conditions. Critical speed of agitation found 721-848 rpm for 0.42 mm-6.73 mm & temperature between 30 °C - 50 °C. Therefore, speed of agitation was selected at 700 rpm for all subsequent studies.

3.4 Effect of dry gel particle size

Since at higher speed of agitation intraparticle diffusion process is the rate controlling step, the particle size would have a significant effect on the extraction rates.

Fig. 8: Effect of dry gel particle size

Fig. 8 shows that, at 700 rpm speed and 30 °C temperature, using different particle sizes in the fraction 0.42 - 0.841, 0.841 - 1.68, 1.68 - 3.36, and 3.36 - 6.73 mm respectively. With reduced size, the rate of extraction of aloin was expectedly increased. For smaller particles, diffusion path decreases, and specific surface area increases, both of these helping in an increased recovery of aloin.

3.5 Effect of dry gel loading

The influence of percentage solid loading (w/w) on the extraction rate of aloin was investigated in methanol at 700 rpm speed; with 0.42-0.841 mm particle size at 30 °C. Fig. 9 shows that, rate of extracted aloin increased with increased solid loading. The higher extraction rate of aloin in methanol suggests that solubility of aloin is higher in methanol than other used solvents in experiments. Theoretically, large quantity of solvent makes the solution dilute. This causes larger concentration difference to develop between the interior of plant cells and the external solvent, thus higher rate is expected for higher solid loadings in a fixed amount (volume) of a solvent to enrich it.

Fig. 9: Effect of dry gel loading

For the initial 30 minutes, there was rapid increase in percentage extraction in all dry gel suspension loadings. Since the solvent is free of any solute, thus solute experiences large driving force to get dissolved in the solvent, but at later stage, the concentration gradient decreases, affecting the rate of extraction. The extracted equilibrium concentration of aloin when compared with the maximum amount of aloin present in the dry gel had shown increasing at higher loading. At higher loading, the nature of suspension is also different than that of lower dry gel loading due to the critical speed of suspension increases with increase dry gel loading. After 50 min of extraction, differences in percentage release of solute aloin became imperceptible. At higher dry gel loading, the concentration gradient at the solid-liquid interface increases with time as the amount of solvent remains unchanged. The maximum extraction of aloin was achieved at 7.5% of dry gel loading.

3.6 Effect of temperature

The effect of temperature on aloin extraction was investigated at 30 °C to boiling point of methanol by maintaining the speed of agitation at 700 rpm, 5% dry gel loading and 0.42-0.841 mm particle size.
Fig. 10: Effect of temperature

Fig. 10 shows that with increase in temperature, the percentage recovery of aloin is increased. This increase can easily be attributed to higher solubility of aloin at higher temperature. The kinetic energy as well as diffusivity of solvent increased with temperature, and thus solvent penetrated better inside the cellular matrix leading thereby faster release of the aloin molecules took place.

IV. MATHEMATICAL MODELING OF THE ALOIN EXTRACTION

4.1) Unsteady state diffusion model

A general diffusion model of solid-liquid extraction (Simeonov,1999;Seikova,2004,Wongkittipong,2004[11][12][14]. It is used for the characterization of the experimental data of aloin from the dry gel derived form Aloe Vera leaves. The model equation is based on Fick’s second law.

Over a flat surface, unsteady state mass balance

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \tag{2}
\]

Where, C is the concentration of aloin at any time t, D is the diffusivity of solute in solid matrix; x is radial distance in the direction of mass transfer. The initial and boundary conditions are given below

Initial conditions, at t = 0, for the continuous phase

\( C_s = 0 \) (Concentration of solute in the solution, i) For the dispersed phase

Boundary conditions

\[ \frac{\partial C(t,x)}{\partial t} \bigg|_{x=0} = 0 \tag{3} \]

At the interface (x = e), equality of flux of solute (in mass): The coverall flux of the solute obtained by integrating over the entire dry gel solid particle) is

\[ F = -D \cdot A \left( \frac{\partial C(t,x)}{\partial x} \right) \bigg|_{x=e} \tag{4} \]

The incoming flux in the liquid is

\[ F = V_L \frac{dC_L(t)}{dt} \tag{5} \]

where, \( V_L \) is the solvent volume and A the specific area. This model was used to estimate diffusion coefficients (D) of aloin and values are tabulated in table I.

![Image 47x577 to 291x729](image1)

![Image 310x339 to 554x492](image2)

![Image 310x595 to 554x711](image3)

![Image 310x712 to 554x866](image4)

**Table I**

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Experimental Aloe content</th>
<th>Extraction of aloin %</th>
<th>Diffusivity m^2/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>0.00268</td>
<td>55.331</td>
<td>4.3765 x 10^{-11}</td>
</tr>
<tr>
<td>40</td>
<td>0.00351</td>
<td>68.155</td>
<td>5.9775 x 10^{-11}</td>
</tr>
<tr>
<td>50</td>
<td>0.00407</td>
<td>79.029</td>
<td>8.145 x 10^{-11}</td>
</tr>
<tr>
<td>60</td>
<td>0.00444</td>
<td>86.213</td>
<td>10.750 x 10^{-11}</td>
</tr>
<tr>
<td>65</td>
<td>0.00454</td>
<td>89.126</td>
<td>12.576 x 10^{-11}</td>
</tr>
</tbody>
</table>

A good fit between experiments and concentration profiles was obtained as shown in fig.7-10 Initial rate of extraction of aloin is very rapid till 30 min which is quite obvious due to large concentration gradient. The coefficient of diffusion for aloin in methanol varies from 4.3765 x 10^{-11} to 12.576 x 10^{-11} m^2/s for the temperature range 30 to 65 °C. The diffusivity of aloin found increased with increased temperature.

4.3) Activation energy of aloin diffusion

Activation energy of the different process was calculated from the diffusion coefficient at different temperatures using Arrhenius law.

\[ D = D_0 e^{-E / RT} \tag{6} \]

where, D = diffusion coefficient, \( D_0 \) = constant, E = activation energy, R = gas constant.

Activation energy of the diffusion process of aloin is calculated from the slope from = 6/eq.6 by plotting ln D vs. 1/T as shown in fig. 11. It is found 25.673 KJ/mol.

V. CONCLUSION

In this study, extraction of aloin from dry gel of Aloe Vera was carried out in a batch extractor. Influence of operating parameter such as solvent, speed of agitation, dry gel particle size, dry gel loading, and temperature were investigated. Methanol has higher solvation power, and it shows greatest release on extraction of aloin than ethanol, water and IPA. Agitation speed of 700 rpm, particle size 0.042-0.841 mm and temperature 65 °C , and time of extraction 30 minute were found to be the optimum parameters from the experimental work. The unsteady state diffusion model was used to estimate diffusion coefficient 4.3765 x 10^{-11} to 12.576 x 10^{-11} m^2/s for the temperature range 30 to 65 °C. The diffusivity of aloin found increased with
increased temperature. The activation energy for diffusion of aloin in methanol is 25.673 KJ/mole using Arhenius equation.

VI. ACKNOWLEDGEMENT

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VII. REFERENCES

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