

Kinetic and thermodynamic azadirachtin extraction from whole neem fine powder formulation

Sonali Tajane¹, Praful Dadhe¹, Sachin Mandavgane*¹
& Sayaji Mehetre²

¹Department of Chemical Engineering, VNIT, South Ambazari
Road Nagpur, India

²Nuclear Agriculture and Biotechnology Division, BARC,
Mumbai, India

E-mail: mandavgane@gmail.com, sam@che.vnit.ac.in

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Azadirachtin (Aza) has been extracted from powder neem formulation (PNF) obtained by grinding whole neem fruits with an inert material (dolomite) in a hammer mill. Initial study has been carried out to select the appropriate solvent for extraction of Aza from PNF and it is found that methanol is most suitable compare to ethanol, acetonitrile, and water. Three different particles sizes of PNF (HM0.4, HM0.8, and HM1.2) are prepared by changing the hammer mill screen and Aza has been extracted from each sample with methanol at different temperature (35°C, 40°C, 45°C and 50°C). Kinetic and thermodynamic studies have been performed and it is found that with decrease in PNF particle size, the extraction of Aza in methanol increases due to its increase in mass-transfer constant and equilibrium constant values. The extraction process follows first-order kinetics and the values of thermodynamic parameters (ΔG , ΔH , ΔS) indicate that the process is favourable, endothermic, and irreversible.

Keywords: Azadirachtin, Kinetics, PNF, Extraction, Thermodynamics

The use of synthetic fertilizers and pesticides in agricultural practices causes serious environmental pollution. Therefore, to reduce the effect of agricultural practices on the environment, suitable natural materials can be used as insecticides and pesticides. In this regard, neem (*Azadirachta indica*) is considered a potential alternative, as it exhibits sufficient insecticidal property¹. Azadirachtin (Aza) is the key ingredient in neem-based biopesticide, which inhibits the growth of pests. It is a powerful insect antifeedant and growth regulator apart from being a nitrification inhibitor and nematode growth retardant^{2,3}.

Normally, oil seeds cannot be converted into fine powder because they contain oil. Thus, neem fruit powder is coarser, and is widely available in cake form (neem cakes), which have comparatively smaller

surface area. However, this problem can be overcome by grinding whole dry neem fruits with an inert material that can absorb and adsorb the released oil, making it possible to obtain free-flowing fine powder of dried whole neem fruit (PNF).

In this study, whole neem fruits were cogrinded with an inert material (dolomite) to obtain free-flowing fine powder. Dolomite is an anhydrous carbonate mineral composed of calcium magnesium carbonate [$\text{CaMg}(\text{CO}_3)_2$]. Consequently, the obtained powder materials also act as a source of essential nutrients (i.e., calcium and magnesium), which promote plant growth^{4,5}. Dolomite is cheap and easily available, and also helps to neutralize the pH of acidic soil. A hammer mill is the most suitable equipment for grinding dry neem fruits. The powder obtained by grinding has large surface area and shows better biological activity owing to the presence of limonoids (e.g., salannin and nimbolide), which also helps to increase the shelf life of the products⁶.

It is well-known that Aza is the key component in PNF and thus, a thorough analysis of this key component can help in understanding the properties of PNF, which can then be utilized for identifying its suitable applications. In most cases, solvent extraction is used for analyzing Aza. Ambrosino *et al.*⁷ extracted Aza from neem seed kernels using a supercritical extraction method, with supercritical and liquid carbon dioxide (CO_2) as extractive agents in a three-separation-stage supercritical pilot plant. Jadeja *et al.*⁸ used pressurized hot-solvent-extraction method to obtain Aza-rich extracts from defatted neem kernels. In another study, microwave-assisted extraction of Aza from neem at atmospheric condition was studied by Dai *et al.*⁹.

In this work, optimization of solvent for extraction of Aza from PNF is presented. The effects of temperature and particle size on the kinetics of Aza extraction from PNF are studied. Thermodynamic parameters such as change in Gibbs free energy, enthalpy, and entropy were determined to understand the mode of extraction.

Experimental Section

Dry neem fruit and dolomite powder were purchased from a local market in Nagpur (Maharashtra, India). Aza (95% pure) was purchased

from Sigma Chemical Co., Methanol, water, acetonitrile (all HPLC grade) were from Fisher Scientific India.

Formulation of PNF

Whole dry neem fruits were subjected to a pretreatment method that included washing, cleaning, and drying. The cleaned dry fruit was then pulverized along with dolomite in a hammer mill (RPM-9000, HP-2 Shubh baby micro pulverizer, India). The experimental setup is described elsewhere¹⁰; however, in this study, three different classifying screens, namely, HM1.2, HM0.8, HM0.4, were used to obtain three different size samples of PNF. The average diameter of PNF obtain from above mention hammer mill screen are 555,495 and 390 respectively. The moisture content was determined according to the Bureau of Indian Standards (IS 3579-1966) and was found to be $6\% \pm 5\%$ (w/w).

Optimization of solvent for extraction

Azadirachtin is one of the limonoids present in whole neem fruits. These limonoids are soluble in organic solvents. To identify the best solvent, Aza was extracted using four different solvents, namely, methanol, ethanol, acetonitril, and water. In brief, the extraction procedure is as follows: 10 g of PNF was dissolved in 100 mL of the respective solvent and extracted using a sonicator for 30 min. All extract samples were centrifuged, syringe filtered, and loaded onto an HPLC column for analysis. Figure 1 shows the amount of Aza extracted from PNF using the different solvents.

HPLC analysis

The Aza content in PNF was determined by HPLC (Water Make, Model no-515) using a C-18 analytical

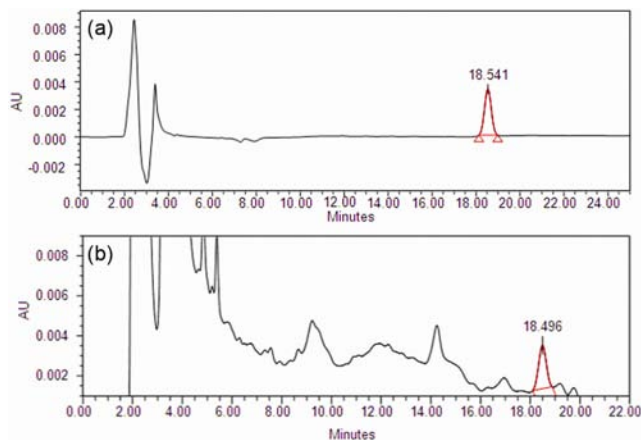


Fig. 1 — HPLC peak of Aza with retention time of 18.496 min: (a) standard Aza sample in methanol; (b) crude extract of the PNF sample in methanol.

column with water-acetonitril mixture (65:35) as the mobile phase (flow rate 1 mL/min and a UV detector operated at 214 nm). About 10 μ L of the sample loaded onto the column. The retention time of Aza was 18.49 min⁸. The HPLC peaks at this retention time for standard Aza extract in methanol and the PNF extract in methanol are shown in Figs 1a and b, respectively. The calibration curve was plotting using a known concentration of Aza solution.

Aza extraction with methanol at different temperatures

Analysis showed that Aza is effectively extracted from PNF using methanol as the solvent, compared with the other three solvents studied. Thus, PNF samples of different particle sizes were extracted using methanol for 3 h with PNF (weight in g) to solvent volume (mL) ratio of 1:5. The extraction study was carried out at four different temperature (35, 40, 45, and 50°C) using water bath shakers (REMI Mumbai). Samples were obtained after every 30 min, which were centrifuged to remove solids, syringe filtered, and then finally loaded onto HPLC columns for analysis. This procedure was repeated for each particle size.

Extraction kinetics

To design and analyze any extraction process on a large scale, it is necessary to carry out relevant kinetic studies. During the extraction of Aza from PNF, no chemical reaction is involved, and thus, the mass-transfer kinetic model was applied to represent the experimental data. Extraction of Aza from PNF is mass-transfer controlled and the rate-controlling step is the transfer of Aza from PNF (solid) to bulk of methanol (liquid). Therefore, the mass-transfer rate from PNF to the solvent can be written as follows:

$$\frac{dW_A}{dt} = k \cdot A(C_{Ai} - C_A) \quad \dots (1)$$

where dW_A/dt is the mass-transfer rate of Aza (g/s); C_A is the concentration of Aza in bulk (organic solvent; g/L) at any time t ; C_{Ai} is the concentration of Aza at equilibrium (g/L); k is the mass transfer coefficient (m/s); and A is the mass transfer area (m^2).

Because the extraction was performed in a batch, the volume of solvent can be considered “constant” during the process. Thus, equation (1) can be rewritten as follows:

$$\frac{dW_A}{dt} = k \frac{A}{V}(W_{Ai} - W_A) \quad \dots (2)$$

Let $A/V = a$ (m^2/m^3)

$$\frac{dW_A}{dt} = ka(W_{Ai} - W_A) \quad \dots (3)$$

where, ka is the volumetric mass-transfer coefficient (m^3/s). Equation (3) is solved for the following condition: at $t = 0$, the mass of Aza in the bulk of liquid is 0 (i.e., $W_A = 0$), where W_A is the mass of Aza in the bulk of liquid at time t . Integration of equation (3) by applying this condition gives following result:

$$W_A = W_{Ai}[1 - \exp(-k.a.t)] \quad \dots (4)$$

Further, equation (4) can be expressed in yield per mass of PNF by rearranging the terms

$$Y_A = Y_{Ai}[1 - \exp(-k.a.t)] \quad \dots (5)$$

$$\ln\left[1 - \frac{Y_A}{Y_{Ai}}\right] = -k.a.t \quad \dots (6)$$

The mass-transfer constant (ka) for each system was determined from the slope of the plot of $\ln[1 - (Y_A/Y_{Ai})]$ versus t . The coefficient of determination was also obtained for each linear equation.

Thermodynamic parameters

The thermodynamics parameters ΔG , ΔH , and ΔS for the extraction process were determined using following equations:

$$\ln K = \frac{\Delta G}{R T} = -\frac{\Delta H}{R T} + \frac{\Delta S}{R} \quad \dots (7)$$

$$K = \frac{Y_T}{Y_u} = \frac{M_L}{M_S} \quad \dots (8)$$

where K is the equilibrium constant; Y_T is the yield in % of Aza at temperature T ; Y_u is % of Aza remaining in PNF; M_L is the amount of Aza in liquid at equilibrium temperature T ; M_S is the amount of Aza in PNF at equilibrium temperature T ; R is the universal gas constant ($8.314 \text{ J/mol}\cdot\text{K}$); ΔG is the Gibbs free energy (J/mol); ΔH is enthalpy (J/mol); and ΔS is entropy ($\text{kJ/mol}\cdot\text{K}$).

Results and Discussion

Optimization of solvent for Aza extraction from PNF

The amount of Aza extracted from PNF was better in methanol than in other solvents, and the order of amount extracted is as follows: methanol > ethanol > acetonitrile > hot water > cold water. These results are in line with those by Kaushik 11. Therefore, methanol was used for extracting Aza from PNF and in-depth kinetic and thermodynamic studies were carried out.

Extraction kinetics

Results of the kinetic study showed that the extraction of Aza increases with temperature (Fig. 2)

Besides, the mass-transfer constant increases as the particle size of PNF decreases (Table 1), because with the decrease in PNF particle size, the mass-transfer coefficient increases, which offers less resistance to

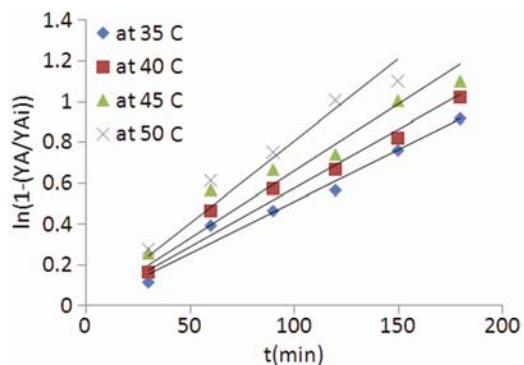


Fig. 2 — (a) Extraction kinetics of Aza from PNF (HM1.2) at different temperatures using methanol as the solvent.

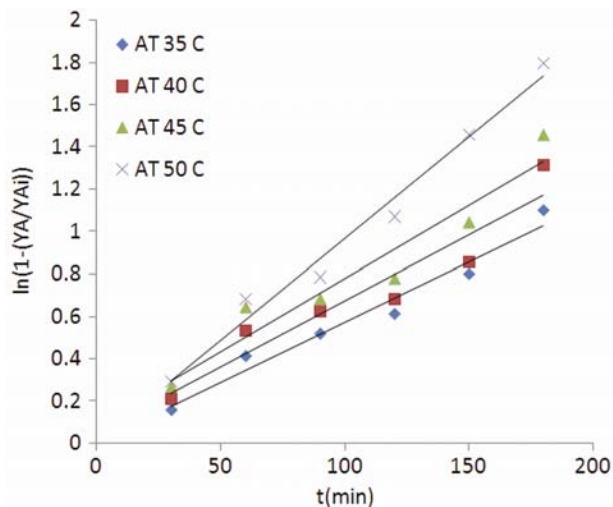


Fig. 2 — (b) Extraction kinetics of Aza from PNF (HM0.8) at different temperatures using methanol as the solvent.

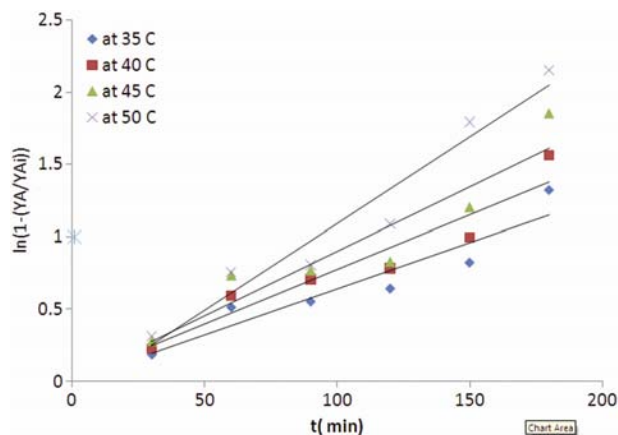


Fig. 2 — (c) Extraction kinetics of Aza from PNF (HM0.4) at different temperatures using methanol as the solvent.

Table 1 — Mass transfer constant and coefficient of determination for extraction of Aza in methanol at different temperatures for the three samples of PNF.

T(K)	HM1.2 (555um)		HM0.8 (495um)		HM0.4 (390um)	
	Ka (m/s) × (m ² /m ³)	R ²	Ka (m/s) × (m ² /m ³)	R ²	Ka (m/s) × (m ² /m ³)	R ²
308	5 × 10 ⁻³	0.97	5 × 10 ⁻³	0.964	6 × 10 ⁻³	0.888
313	5 × 10 ⁻³	0.955	6 × 10 ⁻³	0.904	7 × 10 ⁻³	0.906
318	6 × 10 ⁻³	0.894	7 × 10 ⁻³	0.912	9 × 10 ⁻³	0.874
323	8 × 10 ⁻³	0.921	9 × 10 ⁻³	0.981	11 × 10 ⁻³	0.939

The value in parenthesis is the average diameter of PNF obtained through corresponding hammer mill screen.

Table 2 — Thermodynamic equilibrium constants for Aza extraction at different temperatures for the three samples of PNF

T (K)	1/T	K (J/mol)		
		HM1.2	HM0.8	HM0.4
308	0.003247	1.5	2	3.28
313	0.003195	1.77	2.70	3.76
318	0.003145	2	3.61	5.38
323	0.003096	2.75	5	7.57

the transfer of Aza from the smaller PNF particles to the solvent (methanol); additionally, as the particle size decreases, the surface area per unit volume increases, which subsequently improves the contact between the PNF and solvent. This promotes a higher Aza transfer rate.

Thermodynamic parameters

The value of equilibrium constant K in Table 2 indicates that with the increasing temperature of the extraction process, the value of K increases. The effect of particle size on K was studied and it was observed that with the decrease in particle size, the value of K increases.

From Table 3 (Free energy of Aza extraction), it can be seen that for all three PNF samples, the value of ΔG (J/mole) is negative and the magnitude increases with temperature and decreases with increase in particle size of PNF. Compared with smaller particles, larger particles achieve equilibrium faster. The negative sign indicates that the extraction of Aza is consequently feasible.

A graph between $\ln K$ versus $1/T$ was plotted, the values of ΔH and ΔS were determined, and these are presented in Table 4. It can be seen that all values of ΔH are positive and increase with decrease in PNF particle size. This result indicates that the Aza extraction is endothermic in nature. The positive value of ΔS indicates that the Aza extraction process is irreversible.

Table 3 — Gibbs free energy for Aza extraction at different temperatures for the three samples of PNF

T (K)	ΔG (J/mol)		
	HM1.2	HM0.8	HM0.4
308	-1024.28	-1774.57	-2588.88
313	-1483.3	-2576.26	-3435.01
318	-1824.26	-3119.75	-4441.67
323	-2714.96	-4320.84	-5424.55

Table 4 — Enthalpy and entropy values for Aza extraction for the three samples of PNF

Hammer mill size (mm)	ΔH (J/mol)	ΔS (J/mol)
HM1.2	32216.75	1.557614
HM0.8	48520.5	2.359875
HM0.4	56003.1	2.749579

Conclusion

The maximum yield of Aza from PNF was achieved using methanol as the solvent ($t = 50^\circ\text{C}$; particles size, HM0.4). The extraction process follows the first-order kinetics. It was found that with decrease in PNF particle size, the extraction of Aza in methanol increases due to the increase in mass-transfer constant and equilibrium constant values. It was found that ΔG is negative, ΔH is positive, and ΔS are positive, which indicate that the extraction process is feasible, endothermic, and irreversible.

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