Aqueous Two-Phase Separation (ATPS) Methods for Oleic acid extraction from Neem leaves

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Abstract. The aqueous two-phase separation system (ATPS) signifies an environmentally responsible approach for the extraction of bioactive compounds from a plants basis, as it is a liquid-liquid fractionation technique centred on the inconsistency of two aqueous solutions. In this investigation, various experimental parameters are optimized as the speed of agitation (200, 300 400 and 500 rpm) and solvent ratio (1:1, 2:3 and 3:2) with 20 % (w/w) of Ammonium Sulphate (AMS) salt composition and 30 % (w/w) of Polyethelyene Glycol (PEG). The obtained extract contains alkaloids, flavonoids, tannins, glycosides, acids and total phenolic compounds (TPC). The extracted Oleic acid by the ATPS method was measured with gallic acid equivalent (GAE) of TPC extracted from neem leaves powder. The determined concentration of oleic acid in the practice of TPC is 8.033 mg of GAE/g from the optimized experimental parameter. The optimized results can be cast off for a commercial process on an industrialized scale. Also, the mathematical modelling investigation was done to intent the critical impeller speed (Njs) with the Zwittering model. The identified model calculates the essential speed of agitation (rpm) for maximum extraction yield.

Keywords: Oleic acid, Total phenolic compounds (TPC), Aqueous Two-Phase Separation System (ATPS), Gallic Acid Equivalent (GAE), Critical Impeller Speed (Njs).

1. Introduction

Herbs are used for flavouring, food, medicine, or perfume from ancient times. Culinary use naturally differentiates herbs are implying the leafy green portions of spice also a product from a different part of the plant containing seeds, roots, bark, and fruits. Furthermore, medicinal contents available in the plant's parts are used for the production of some pharmaceutical products such as aspirin, colchicine, ephedrine, morphine, physostigmine, pilocarpine, quinidine, reserpine and vincristine, etc [1-2]. The approach of isolating the bioactive components from the medicinal plants and used for the manufacturing of some pharmaceutical products are becoming prominent. Generally, organic solvents such as methanol, ethanol and diethyl ether are usually helped for extracting the bioactive compounds as of plant basis by the outmoded extraction arrangements. These solvents are relatively expensive,

needs distinctive processing conditions and most importantly disposal of the solvents is a major concern as they are not environmental responsive [3].

Conventional extraction processes are time-consuming and need more solvent for carrying out an operation, also after extraction, the added cost of purification and solvents recovery makes the process uneconomical.

Whereas, ATPS two-stage extraction is developing as a successful and flexible green system for the downstream handling of biomolecules. Fluid two-stage frameworks are low unpredictability frameworks with high adaptability [4]. That is, an expansive assortment might be acquired utilizing substances that pursue the Green Chemistry guideline on ecotoxicity, biodegradability, bioaccumulation and constancy, limiting waste and amplifying yields. Furthermore, they conform to the guideline of changeover of naturally safe structures to permit work under air weight. Since the 1970s numerous classified and out examinations have announced the filtration of proteins and other biologic materials utilizing ATPS, and numerous specialists have considered different operations of ATPS for the extraction and cleaning of organic products [5]. However, the utilization of such frameworks for the recuperation of phenolic mixes from plant materials is extremely constrained. In addition, there is broad writing about the thermodynamic properties of ATPS be that as it may, to the best of our insight, their application to crude unpurified examples has been very constrained [6].

Subsequently, the late nineteenth-century fluid two-phase extraction has been identified to the entire world. Aqueous two-phase can be framed through an extensive diversity of characteristics or else engineered water-solvent polymers& salt blends [7]. Watery two-phase extraction is developed for protected, sparing partition and cleaning of biomolecules, for example, proteins and catalyst extraction. Fluid extraction has numerous favourable circumstances; it is biocompatible, has low interfacial surface pressure among stages and it has high water content, the procedure can incorporate and the ability for strengthening [8-9].

Likewise, the level of corruption for biomolecules is low. In any case, two-polymer and polymer-based salt frameworks have developed quickly and a considerable measure of effort has been placed keen on concentrate this strategy utilizing these sorts of aqueous two-phase separation systems (ATPS). Aqueous two-phase extraction is known as an operative, adaptable and significant developing green method for the subsequential treating of biomolecules. This strategy has points of interest completed traditional extraction systems similar to, simplicity of scaling-up, condition benevolent, minimal effort, fit for nonstop activity and is effective for some sorts of trials exceptionally for the fixation and refinement of biomolecules. The utilization of partiality in ATPS can affect the developed recuperation earnings and developed refinement bends of bio consistent items such as it is an essential phase recuperation strategy [10]. Water as the foremost constituent of together stages in ATPS practices a moderate setting for bioactive molecules to distinct and polymers steady to the assembly and biotic doings through further liquid-liquid extraction approaches could impairment natural goods since of the development circumstances and biological solvents such method reduces the purity of active ingredient present in the extract.

There are two fundamental sorts of ATPS: polymer-polymer and polymer–salt frameworks. The mind-boggling expense of some shaping stage polymers (e.g dextran) limits the use of these frameworks, just legitimized when the expense of the result of intrigue is extensive. Consequently, the choice of the more temperate polymer–salt frameworks is profoundly suggested [10-11].

The novelty of the proposed work is that during the extraction itself two different layers of aqueous solution and salt is obtained, which can help further to reduce the cost of separating components. Also, the systems can be designed by partying a diversity of components in water and two-polymer and polymer-salt systems have developed quickly. The said work majorly focuses on the extraction of oleic acid from neem leaves to powder using ATPS (water + polymer + salt) based on PEG and ammonium sulphate. The aim is to optimize various experimental parameters (time, ATPS composition, particle size) for the removal of Oleic acid from neem leaves powder and its additional practices as natural antioxidants.

2. Material and Methods

The Neem powder was obtained from Hari Parshuram Aushdhalya, Pune., Polyethelyene Glycol (PEG) was procured from SRL Chemicals Pvt Ltd, Mumbai. Folin Ciocalteu's reagent was procured from Qualigens Fine Chemicals, Mumbai. Ammonium Sulphate (AMS) was procured from S.D. Fine Chemicals Pvt Ltd, Mumbai.

2.1. Batch extraction

Batch extractions are a modest method for the extraction of bioactive compounds. The stages in this method are equipped with a 50 ml glass reactor with a four-bladed glass turbine impeller and the combination to be divided is supplementary. Subsequently collaborating, phase parting is proficient each by resolving below gravity. The stages are disconnected and investigated to improve the alienated constituents of the preliminary mixture. The object product would be focused at any of the stages and the pollutants in the additional form. In various cases, reclamation and attentiveness of the product that produces beyond 90% can be attained with a particular extraction stage.

One particular phase removal does not give adequate retrieval, recurrent extractions can be supported obtainable in a sequence of communicating and parting components [12]. The fluid dividers into two stages, each covering added 80% liquid. When basic biomolecules are supplementary to these combinations, biomolecules and cell wreckages are dividers among the stages; by choosing suitable circumstances, cell remains can be limited to one stage as the object bioactive molecule barriers. The segregating of biomolecules among segments mostly be contingent on the equilibrium connection of the arrangement. The partition coefficient is demarcated as [12-13].

$$K = \frac{C_{AT}}{C_{AB}}$$

Where C_{AT} is the equilibrium attentiveness of constituent A in the upper phase and C_{AB} is the equilibrium adsorption of A in the lesser phase. If constituent A helps the greater stage the worth of K will be better. In numerous aqueous arrangements, K is continual finished with an extensive collection of deliberations, as long as the molecular possessions of the stages are not transformed. The theoretic yield in the topmost stage, Y_T, can be premeditated relative to the capacity ratio of the stages, R (up to volume / below volume), and the partition coefficient *K* of the object molecule as follows [12-13]:

$$Y_T = \frac{V_T C_{AT}}{V_o C_o} = \frac{V_t C_{AT}}{V_t C_{AT} + V_B C_{AB}} = \frac{1}{1+[\frac{1}{KR}]}$$

Similarly, the theoretic yield in the bottommost stage, Y_B is known by,

$$Y_B = \frac{1}{1+[\frac{1}{KR}]}$$

Consequently, by changing anyone like K or R we can effortlessly upsurge or reduction the profit of the object particle [14]. Additional constraint recycled to describe two-phase partitioning is the concentration factor, δc, distinct as the ratio of produce attentiveness in the favoured stage to the original product attentiveness.

$$\delta_{C,T} = \frac{C_{AT}}{C_{Ao}} \quad \text{(Product partitions to the higher phase)}$$

$$\delta_{C,B} = \frac{C_{AB}}{C_{Ao}} \quad \text{(Product partitions to the lower phase)}$$

2.2. Determination of the binodal

By settlement, the constituent mainly in the lower layer is represented as abscissa and the predominant element in the upper stage is represented as ordered. The three systems are explained realistically.

2.2.1 Turbidometric titration

In the tubing, with suitable backup solutions, formulate systems through different configurations of recognized weight. Note the added size due to titration, for example, if 5 g methods are organized, use 10 ml tubes. As an example, shows the systems for different systems that can use PEG-phosphate and PEG-dextran, and the essential designs. This can be replicated in a worksheet to permit easy intention. Note down the mass of the tube and titration drop by drop, with suitable dilution till the scheme is zeroed, i.e, a stage is formed.

This can be done through the scheme is continuously mixing or accumulation a droplet, collaborating, adding a second drop and continues the same process. To confirm that it has formed a single-stage system, the schemes must be centrifuged (for example 1000-2000 g, 5 min). Record the concluding mass of the tube and estimate the mass of the additional dilution just before the formation of a phase. Since the number of graduate systems is relative to the total of points in the binodal, superior precision is obtained with a superior number of schemes [15].

2.2.2 Cloud point method

Balance 5 g of a standard solution of constituent X into a 25 ml narrowed flask. Then add drop by drop, a reserve solution of the Y component up to the principal indication of turbidity, which is the cloud point. Note the weight of the Y component necessary for the mixture to become cloudy. This provides the first point in the binodal. Also, add a known weight of diluent lower the cloud point and duplication as indicated [15-16].

2.2.3 Determination of the Tie line

Measuring the connection line for polymeric schemes comprising an optically active composite, for example, PEG-dextran, PEG-Ficoll and ethylene oxide-propylene as well as oxide-Reppal PES 100.

2.2.3.1 Polymeric Methods Comprehending One Optically Active Polymer

Formulate a standard curve for the lively constituent, in the variety of 0 to 10% (p / v), i.e. inside the linear series, through the identical sections arrange a second standard curve for the refractive measure of the index. If the scheme is arranged in a shield, the average curves for the clean constituents must be completed with a similar safeguard, since the salts similarly subsidize the refractive index. Get ready the phased scheme for investigation, assembly sure that the phase components mix well; let the phases separate.

To ensure complete separation, centrifuge at low speed (for example 1000-2000 g, 5 min). The system proportions should be appropriate to permit deduction of at least 5 g upper and lower stage for phase concentration investigation and an additional quantity for density extents. Distinct the higher and subordinate stages building certain not to origin stage relations. Make the suitable dilutions, for example, watery 5 g of phase with the suitable solvent to 25 ml in a volumetric container. Extent the visual revolution for every stage and estimate the individual concentrations. The concentration of another constituent is resolute by determining the refractive index of the developed and subordinate stage and deducting the influence of the refractive index acquired from the optically lively constituent [17].

2.2.3.2 Polymer-Salt Systems

Formulate a standard curve aimed at salt conductivity within the linear series (in% w / v). Arrange the stage schemes as indicated above and eliminate 5 g of samples of the higher too subordinate phases and

diluted with liquid and lyophilized, then note the dehydrated weight. Eliminate an additional section from the higher and subordinate stage, diluted with water and extent the conductivity of every stage. Estimate the salt absorption and deduct the mass involvement of the dehydrated mass of the section [16-18].

3. Results and Discussion

In this investigation, various experimental parameters were optimized such as speed of agitation (200 rpm, 300 rpm, 400 rpm & 500 rpm) and solvent ratio (1:1, 2:3 and 3:2). Extract samples were pipette out at specific time intervals like every 15 min and further standard Folin–Ciocalteu's method was used for analysis purposes. The obtained outcomes indicated that the TPC concentration of TPC in the extraction stage at a specific time. By changing the parameters speed of agitation and solvent ratio in the batch reactor at different times the results were optimized and used for further study.

3.1 Speed of agitation

About 5 g of powder of neem leaves was weighted and fed to 50 ml of batch reactor along with AMS salt (20 %w/w) and 50 ml of PEG (30 %w/w) at a temperature of 30°C till the extraction rate was a steady-state. The maximum speed of agitation produces high turbulence in the batch reactor and increases the rate of mass transfer [11]. The results of various experiments were performed for multiple agitation speeds is shown in figure 3.1 The experimental results shows that the concentration of TPC at 200 rpm significantly low as compared to 500 rpm, but there could be a marginal difference of TPC concentration of 400 and 500 rpm, speed of agitation., therefore for the further study, speed of agitation (400 rpm) was used. The circulation of TPC compounds from the neem leaves powder in the solvents could expand with the accumulative agitation speed. An added rise in the agitation speed has no substantial effect on final extraction yield; it clues that external mass transfer fighting is inconsequential at 400 rpm

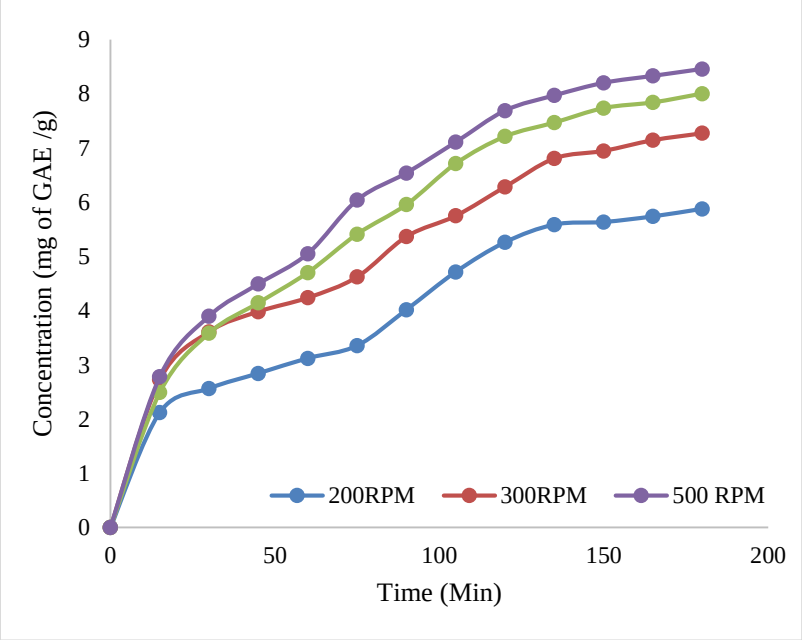

Fig. 3.1 Concentration of TPC obtained from Batch at temp 30^0C speed of agitation 200, 300, 400 & 500 rpm)

3.2 Effect of Solvent Ratio (AMS: PEG)

The TPC concentration values in the extract were considered for changed extraction times at different solvent ratios (1:1, 2:3 & 3:2) and the same is shown in Figure 3.2. About 5 g of powder of neem leaves

was weighted and fed to 50 ml of batch reactor along with AMS salt (20 %w/w) and 50 ml of PEG (30 %w/w) at a temperature of 30°C till the extraction rate was a steady-state. There was an increase in TPC concentration and experiential for solvent ratio 01:01. A substantial quantity of solvent favours an additional concentration gradient and cuts diffusional resistance that rises the rate of extraction rate.
There was a rise in TPC concentration, for the solvent ratio of 1:1 to 3:2. A substantial quantity of solvent tends to the added concentration gradient and drops diffusional resistance that rises the rate of extraction [11].

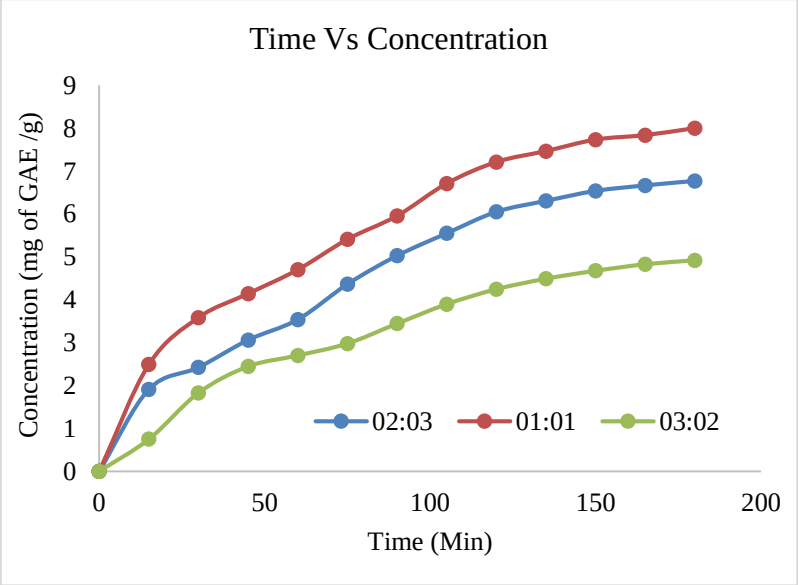

Fig.3.2 Effect of Solvent Ratio (AMS: PEG)

4. Conclusion

The investigation of the ATPS method was beneficial for the sub sequential treating of biomolecules. Also, the experimental parameters were optimized such as speed of agitation and solvent ration and the optimized parameters as 400 rpm and 01:02 solvent ration respectively with AMS salt (20 % w/w) & PEG (30 % w/w). The obtained extract also contains alkaloids, flavonoids, tannins, glycosides, acids and total phenolic compounds (TPC). The extracted Oleic acid by ATPS technique was measured with gallic acid equivalent (GAE) of TPC extracted from neem leaves powder. The determined concentration of oleic acid in the practice of TPC is 8.033 mg of GAE/g from the optimized experimental parameter. The optimized results can be used for a commercial process on an industrial scale.

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