Theoretical Analysis for Separation of Coagulant Proteins from *Moringa Oleifera* using Nano Structured Alumina

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Abstract

Coagulant protein was separated from extract of *Moringa oleifera* seeds using nano alumina as a bio-adsorbent material by conducting batch and continuous adsorption based on the Isoelectric Point (pI) of nano alumina and *Moringa oleifera*. Characterization of alumina was carried out by SEM and XRD. Adsorption of coagulant protein was observed experimentally by batch adsorption at different pH of adsorbate medium and adsorbent concentration. The results obtained followed the Freundlich adsorption isotherm model. Continuous column test results were performed to determine the breakthrough curves, mass transfer coefficient and number of mass transfer units at different bed height. The overall results indicated that the nano alumina can be used has a adsorbent to separate coagulant proteins based on pI effectively.

Keywords: *nano alumina, fixed bed, coagulant proteins, SEM, XRD, Isoelectric point*

1.0 Introduction

In search of natural coagulants in waste water treatment in place of chemical coagulants is not new, because the natural coagulants originated from plants are not harmful and also results in biodegradable waste which can be easily disposed into the nature without any harmful effects. Knowledge of water treatment using natural coagulants was known to mankind is centuries old [1]. Most investigated plant for extract of natural coagulants is *Moringa oleifera* whose seeds were used for water treatment in the rural areas of Sudan [2] because, *Moringa* seed powder can decrease around 90% of the bacterial load from raw water samples. This coagulant present in the form of protein in the seeds of *Moringa oleifera* is water soluble cationic peptide [14] with Isoelectric Point (pI) above 10 and molecular mass 6.5 Kda [3].

Various protein purification methods have been used to isolate proteins from *Moringa oleifera* seeds depending upon the protein size, shape, charge, hydrophobicity, solubility and biological activity to get good quality products. In general, the initial step involves preparation of crude in salty or buffer solution [4]. Since from the crude, the protein can be separated by various methods. Usually the protein from seeds can be separated by ion exchange [5],

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gel filtration [6] and affinity chromatography techniques, in all these techniques ion exchange (IEX) chromatography stands in the front for purification. In IEX the separation will be based on the adsorption of proteins to immobilized functional groups. These functional groups can be either positively or negatively charged. The most commonly used functional groups are DEAE and CM respectively. In the present purification studies, we have used nano alumina in behalf of functional groups and charged with positive based on the pH of the aqueous solution and pI of the nano alumina [7]. All the oxides can be characterized by their (pI) surface charge, if the pH of aqueous solution in contact with oxide material is below the pI of oxide material the oxide will acquire positive surface charge, if the pH of aqueous solution is above the IEP of oxide material the surface of material will acquire negative charge. The pI of silica, titanium, zirconia and alumina are near to 2, 5, 7 and 9. Alumina particles at neutral pH (around 6) will be meagerly positive charge in aqueous solutions, if the pH is lower than 6.0 the alumina powder is strongly positive charge. This result proposes that alumina materials will undergo DE- flocculation in basic and acidic medium.

In this context, nano alumina can be employed as a bio adsorbent material to separate coagulant proteins from *Moringa oleifera* seeds. The experimental parameters such as, adsorbent material, adsorbent concentration and pH for adsorption using batch adsorption studies were optimized initially, for the optimized batch adsorption studies Freundlich adsorption mathematical model [8] was used to validate the results.

2.0 Experimental Details

2.1 Materials and methods

Nano alumina used as adsorbent, was synthesized by spray pyrolysis method using a precursor. *Moringa oleifera* procured from local market in Bengaluru, was dried in oven, de shelled and powdered and used for experimentation.

The synthesized alumina powder was heat treated at 600^0C for a period of two hours to remove the moisture and other trapped gases etc., and was characterized using XRD and SEM respectively.

2.2 Crude protein extraction

The crude protein was extracted according to Ghebremichael et al. [12], dried *Moringa oleifera* seeds were De-shelled and the core obtained was crushed using a crusher. Oil obtained during crushing was removed by adding the 90% of acetone to the crushed material and centrifuged for 30 min at 10000 rpm at 10^0 C. The solids obtained was dried at 26^0C, after drying the solids were suspended in 5% (w/v) acetate buffer of ammonia (10 mM, pH 7.0) and then vacuum filtered, the filtrate attained was named as crude extract.

2.3 Theoretical Analysis

To study the adsorption behavior and effective adsorption, theoretical analysis is carried out for experimental values obtained from batch adsorption and continuous adsorption. The studies on the adsorption isotherms was to determine the effective rate of adsorption for the batch adsorption studies and mass transfer co-efficient is determined for continuous adsorption studies.

2.4 Batch adsorption

Nano alumina powder was taken in different conical flasks varying from 0.2 to 1.2 g separately, in each conical flask a 15 ml of crude is taken. Each conical flasks were incubated at 26^0C for 45 min with continuous shaking and filtered using vacuum filtration (250 mm Hg) unit. The filtrate obtained was analyzed for protein content using standard Lowry's method.

The results obtained were used to fit the Freundlich adsorption isotherm model for all the systems investigated.

Freundlich Isotherm

ln C^* vs ln v($C_0 - C^*$)

Where, C^* represents concentration of crude extract after adsorption,

C_0 initial concentration of crude extract,

v is the ratio of volume of crude extract to amount of adsorbent,

To determine Freundlich isotherms [8] lnC* v/s lnv*($C_0 - C^*$) is graphically represented by considering the lnC* in Y-axis and ln v*(C_0 - C*) in X-axis, the slope n and intercept k is obtained from the graph, where k and n are Freundlich adsorption parameters.

For the determination of mass transfer coefficient the method described by Vukojevic M [9] et al., is used based on the result from the breakthrough curve. The overall mass transfer coefficient, Ka, for continuous adsorption for the known bed height were evaluated using the following equation

$K_a = (NG_w / H)$ ----------(1)

Where: G_w is the mass flux of solution, kg/min mm^2

K_a is the overall mass transfer coefficient, kg/min mm^3

N is the number of mass transfer units

H is the bed height, m

The Mass flux of solution in the packed column can be evaluated by the following relation:

$G_w = (Q\rho) / (A\epsilon)$ ---------- (2)

Where Q is the flow rate of solution through the column, m^3/min

ρ is the density of the liquid solution passing through the column, kg/m^3

Є is the porosity of the bed

A is the area of the column, m^2

Number of transfer units (NTU) was determined using a graphical representation between ln C^*/C_0 vs 1+ ln C^*/C_0 which was explained by Vukojevic M [9] et al. The value of 1+ ln C^*/C_0 at 0.05 ln C^*/C_0 is evaluated (Fig 8) and used in the following equation to evaluate N

$1 + C^* / C_0 = N (T -1)$ ----------(3)

Where T can be evaluated by knowing the break point and exhaust point in the breakthrough curve.

2.5 Column test

The column test was carried out at an ambient temperature in a glass column (Inner diameter ID=18 mm, length l = 100 mm). The nano alumina with known amount was randomly packed in the column for a bed depth of known arbitrary height. The tap density and the void fraction were found to be 74kg/m^3 and 0.467 respectively and were maintained constant. *Moringa Oleifera* crude of concentration 2.12 mg/ml at normal pH was constantly fed from top of the adsorption column at 60 ml/hr until exhaust point of the adsorption was reached. From the bottom of the column for every fraction of 5 min the effluent samples were collected and analyzed for protein concentration. The conditions maintained in the column for different experimental trials are given in Table 1.

Table 1 Column conditions for different trials

Experimental conditions	Run 1	Run 2
Porosity nano alumina	0.467	0.467
Density of nano alumina kg/m^3	74	74
Bed depth mm	15	25
Flow rate ml/hr	60.0	60.0
Dia of the column mm	18.0	18.0
Initial concentration of protein mg/ml	2.12	2.12

3.0 Results and Discussion

3.1 SEM

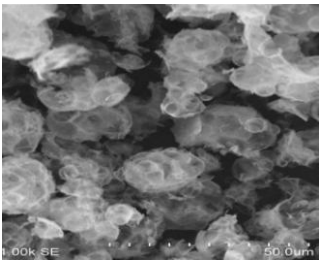

Fig 1 SEM of nano alumina powder

Fig 1 displays the nano-structured alumina particles with hollow, porous morphology, agglomerated with spherical in shape. The spherical shape indicates the salts are completely evaporated and nucleated to the perfect shape, this is in line with the, SEM images of the produced composites reported by Kim [11] which displays spherical, loosely aggregated particles which is due to the low evaporation the solid particles are nucleated.

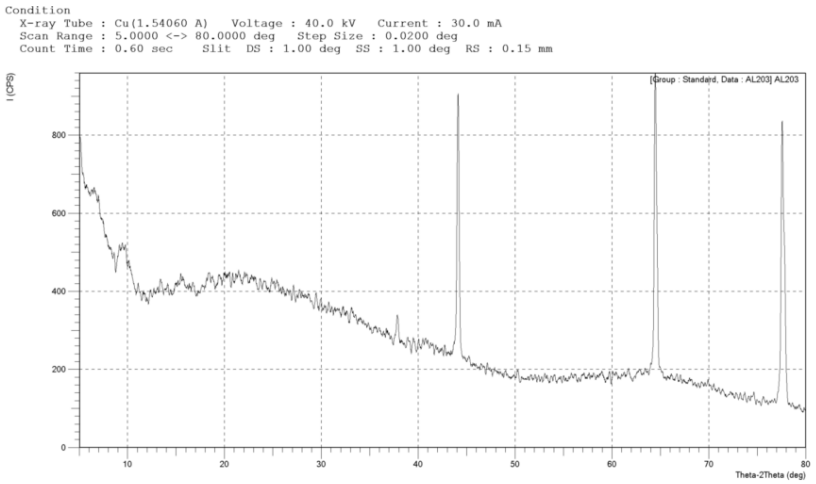

Fig 2 XRD (two theta) plot of nano alumina powder

3.2 XRD

The XRD image of nano alumina (Fig 2) displays 3 peaks at 44°, 64 ° and 77 °, which is as per ICDD (Standard ICDD 29-0063) this is in line with the Martin et al., [10] reported the XRD results for thermally treated nano alumina with well-defined peaks at 45°, 67° and 77.61° and XRD patterns reported by Shighehiro et al., [13] showed 2 peaks at 47° and 65°.

3.3 Study on Adsorption Isotherms

For the results of batch adsorption studies, Freundlich adsorption isotherm mathematical model were used. From the nature of the graph it is confirmed the cationic proteins were adsorbed on nano alumina with a slope of 1.23.

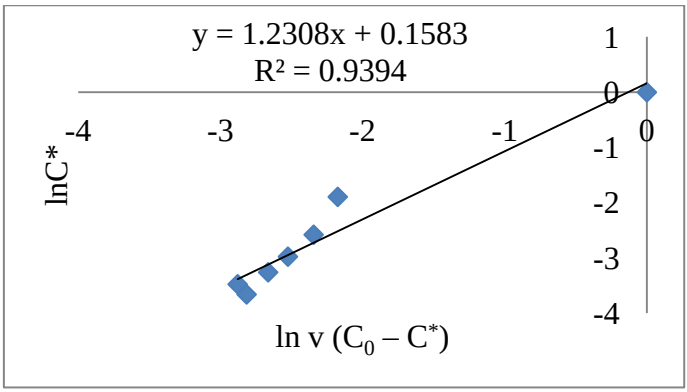

Fig 3 Freundlich isotherm model for adsorption of protein on nanostructured alumina

The Freundlich isotherm constant for alumina is 1.23 (Slope value), which indicates that the alumina is good adsorbents to adsorb the desired proteins.

3.4 Effect of adsorbent

Nano alumina powder were taken in different conical flasks varying from 0.02 to 0.12 g separately, in each conical flask a 15 ml of crude is taken. Each conical flasks were shaken well for 45 min at pH 7.0 and centrifuged for 25 min at 10,000 rpm, the supernatant obtained was subjected for total protein content at 660 nm.

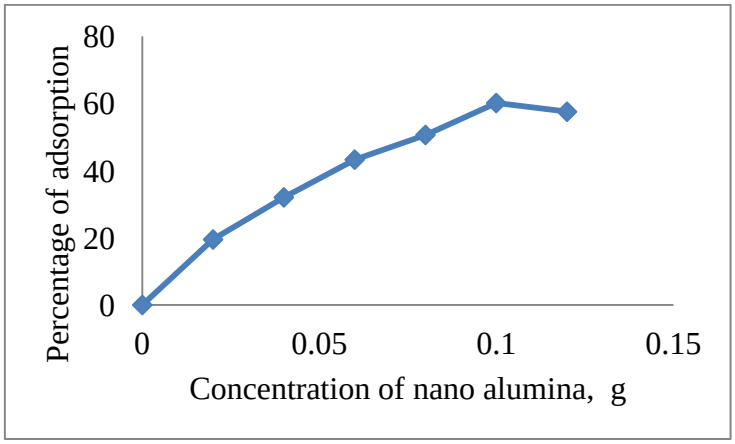

Fig 4 Effect of adsorbent concentration on adsorption of proteins

The graphical representation of effect of adsorbent (Fig 4) represents the increase in adsorption for increased in adsorbent. The adsorption was maximum (60%) when the concentration of the adsorbent was 0.1 g, still further increase in the adsorbent concentration the adsorption of protein was dropped to 56%. This may due to the binding the active site of the adsorbent.

3.5 Effect of solution pH

0.1 g of nano alumina was taken in each of the 4 conical flasks and equal amount of crude proteins were taken in each of the flasks. The pH in each of the conical flask was varied from 2 to 9 (2, 4, 6 and 9). The mixture was mixed well for 45 min and filtered the filtrate obtained was analyzed for adsorption of protein. The results is graphically represented in Fig 5.

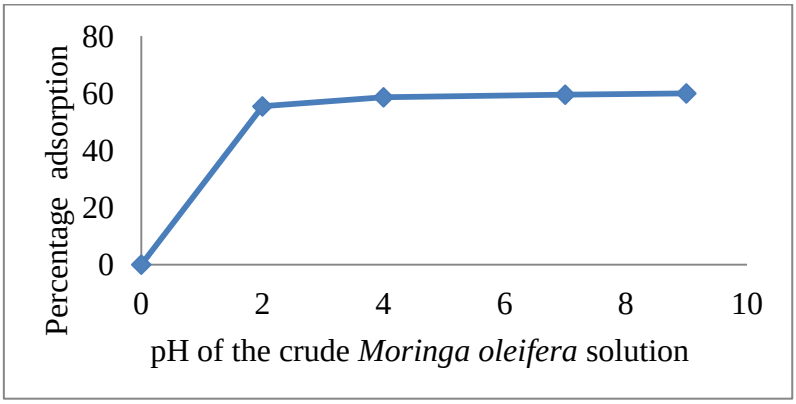

Fig 5 Effect of pH on adsorption

From the above Fig 5 the adsorption of protein was 56% at pH 4.0 and there was a nominal increase in adsorption from 56% - 59% of protein with increase of pH from 4.0 to 9.0 in case of alumina. This was due to the surface charge on nano alumina becomes positive charge (pI 9.0) at pH 7.0 and coagulant protein also gets positive charge and there was a adsorption of anionic charge proteins on nanostructured alumina. The coagulant protein fundamentally a cationic charge at pI 9.6 will not be attracted by the surface of the nano alumina, gets separated due to non-affinity toward nanostructured alumina.

3.6 Break through curves for 15 and 25 mm bed height

Graphical representation of C^*/C_0 V/s time obtained (Fig 6) from the results of continuous adsorption of M*oringa oleifera* proteins over the nano alumina particles at a rate of 60 ml /h for different bed heights, shows the similar pattern of S shaped curve with increase in bed height with increase in break thorough curve. The results of the graph is presented in Table 2.

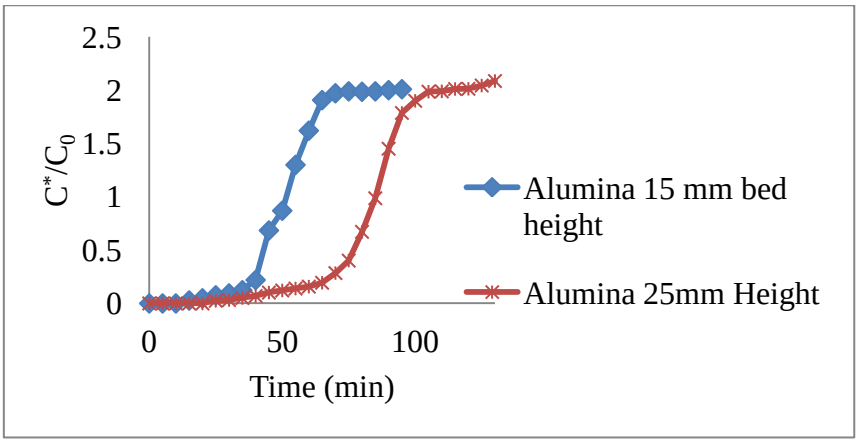

Fig 6: Break through curves for 15 and 25 mm bed height

Fig 6 indicates the break point (Value of X axis for the 0.05 value of Y axis, which refers to 95% of adsorption) for adsorption of protein on nano alumina is 43 min (15 mm Bed), 78 min (25 mm Bed height) and exhaust point (Value of X-axis when the curve in the graph becomes constant) is 65 min (15 mm Bed height), 105 min (25 Bed height), this was due to the rise in bed height and the contact time between nano alumina and crude protein, break time rises by 44% and exhaust time rises by 40%, which indicates rise in height rises percentage of adsorption.

3.7 Evaluation of Mass transfer coefficient through break through curves

Evaluation of mass transfer coefficient the graph of C^* / C_0 vs $1+\ln C^*/ C_0$ (Fig 7 & 8) is plotted, the value of X-axis is evaluated for the 0.05 value of Y-axis. The results obtained from the graphs is used to evaluate the mass transfer coefficient (Table 3)

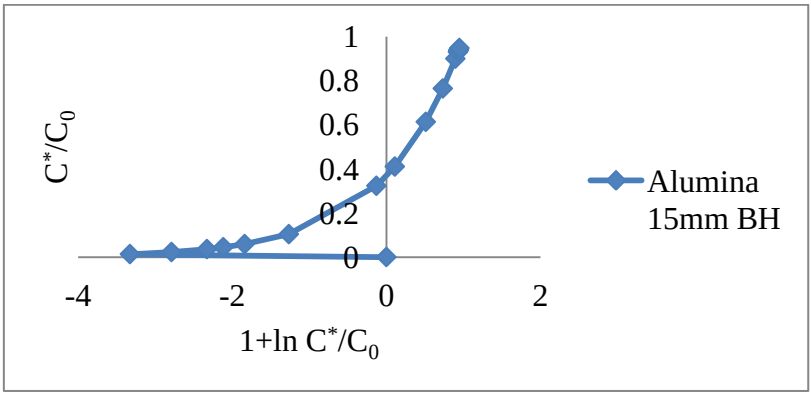

Fig 7 C^*/C_0 vs. $1+\ln C^*/C_0$ to determine the mass transfer coefficient for 15mm BH

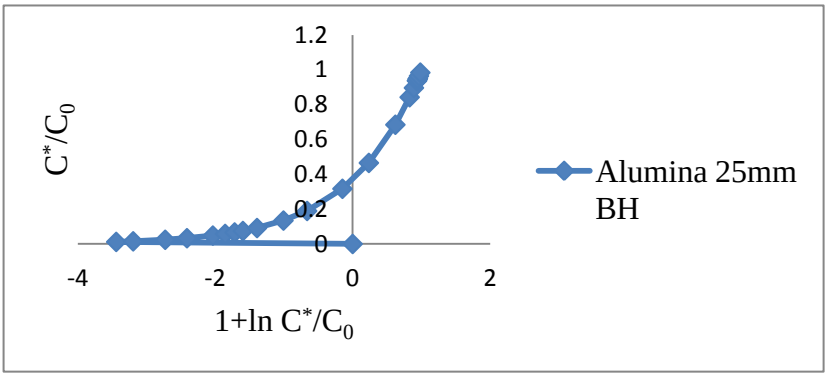

Fig 8 C^*/C_0 vs $1+\ln C^*/C_0$ to determine the mass transfer coefficient for 25 mm BH

Table 2.0: Results of break through curve and determination of T, from the graph Fig 6

Mate-rial	Bed height H meter	Break point T_B min	Exhaust point T_E min	$\tau = T_B/T_E$	Q m^3/min	Diameter of the column Meter	Area m^2	Porosity	Density of crude protein kg/m^3
Nano Alumina	0.015	43	65	0.661	$1x10^{-6}$	0.018	$2.5x10^{-4}$	0.466	970
	0.025	78	105	0.742	$1x10^{-6}$	0.018	$2.5x10^{-4}$	0.466	970

Table 3.0: Determination of Mass transfer coefficient from the graphs Fig 7 & 8

Material	Value of x-axis when the y-axis value = 0.05 (Fig 7 & 8)	NTU in adsorption column N	Mass transfer flux $G_W = (Q*\rho)/A*C$ kg/ m^2 min	Mass transfer coefficient $K_{La} = (N*G_W)/H$ kg/min m^3
Nano Alumina	-2.0	3.7	8.326	2053
	-1.8	6.9	8.326	2297

The results presented in Table 3, indicates the rise in mass transfer coefficient with rise in bed height of nano alumina. Mass transfer coefficient for nano alumina is 34 and 38 kg/s m^3 for 15 mm and 25 mm bed height. From the results the mass transfer coefficient rises with rise in bed height, this may be due to the NTU which rises the adsorption of proteins.

4.0 Conclusion

For adsorption process in which nano alumina, with low density, hollow morphology and spherical in shape can be used as an adsorbent material to separate the proteins. The slope value obtained (1.23) from the Freundlich equation indicates the adsorption process is feasible and the process can be scaled up for commercial purification of the proteins. The results of the continuous adsorption studies predicts the breakthrough curves were in good arrangement and the mass transfer coefficient were independent of bed depth and rises with rise in NTU.

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