



FUNGAL STRAIN OF *ASPERGILLUS ORYZAE* IMMOBILIZED ON SILICA GEL FOR Au(III) SORPTION

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Biomasses have significant impact on the development of new solid phase extraction methods for metal analysis. Adsorbent, prepared by incorporating dried biomass (*Aspergillus oryzae*) immobilized on activated silica, was presently developed for Au(III) enrichment. The main factors affecting the adsorption-desorption process like pH, sample volume, eluent and eluent flow rate were optimized. Under the optimum conditions, good recovery was obtained at about 99.4 ± 0.1 %, performed by Flame Atomic Absorption Spectrometry. At optimized pH 2.0, Au(III) was quantitatively sorbed and recovered with 1:1 mixture of 0.2 M HCl mixed with 0.2 M thiourea. The enrichment factor was found as 125, and the limit of detection is $0.88 \mu\text{g L}^{-1}$ whereas limit of quantification is $2.93 \mu\text{g L}^{-1}$. The proposed method was successfully applied in the analysis of tap water, pharmaceutical formulated product and synthetically prepared dummy sample. Equilibrium data also were fitted by linear regression methods through Langmuir, Freundlich, Temkin, Dubinin and Radushkevich (D-R) and Harkin –Jura isotherm models.

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Introduction

It is well known that Au (III) has received an increasing amount of attention in research due to its wide applications in industries, utilization in Chemistry, biology, medicine, corrosion resistant as well as economic activity and occurrence on the Earth at very low natural content.¹⁻² The concentration of Au(III) in environmental samples is extremely low, e.g. 0.5 ng g^{-1} for 10 g samples (rocks, sediments, soils) and 0.05 ng mL^{-1} for 1 L water samples reported by Medve in 2004.³ Hence determination of Au (III) in environmental and biological samples is difficult. Therefore, it is a challenge for researchers to develop an effective method for analysis of trace level of Au (III).^{4,5}

Commonly used techniques for elemental Au (III) analysis is Flame Atomic Absorption Spectrometry (FAAS) and Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES), however, they suffer from spectral and chemical interferences. Separation and pre-concentration is an essential step for the accurate measurement and analysis of the various metal species.^{6,7} To enhance the accuracy and sensitivity in the determination of Au (III), an enrichment step is necessary.⁸ Nowadays, solid phase extraction (SPE) has gained importance as a separation and pre-concentration method as it is a clean, simple, flexible technique with availability of a large number of sorbents.^{9,10} For the determination of Au(III), many solid phase extraction methods have been developed e.g., polyethylenimine (PEI) ion-exchange polymer which is coated with alumina in the presence of NaNO_3 ,¹¹ rubeanic acid (dithiooxamide) chelate on silica gel,¹² cysteine modified silica gel (SiG-cys),¹³

multiwalled carbon nanotubes (MWNTs),¹⁴ octadecyl silica membrane disks modified by pentathia-15-crown-5,¹⁵ and low density polyhydroxy polyurethane foam (LPPF)¹⁶ as a solid support material. Due to availability, less environmental contamination and cost, biosorbents have gained an advantage in solid phase extraction,^{17,18}

Biosorption involves the use of immobilized biomass for heavy metal removal.¹⁹ Sorbents such as *Fomitopsis carnea*,²⁰ *L-cysteine*,²¹ *tricarbohydrazide modified attapulgite*,²² oat and wheat biomasses,²³ and *sargassum natans*²⁴ have been reported for Au(III) sorption-desorption. Heavy metal decontamination using live cells often causes restriction, as a continuous supply of nutrient is required and there is also the possibility of toxicity to the microorganism. This can be overcome by the use of dead microbial biomass, which involve the use of ion exchange as a mechanism for transport through the cell wall and does not require the significant condition for the maintenance of a viable biomass.

Fungal biomass is advantageous due to low cost and easy availability from fermentation and pharmaceutical industries.²⁵ Dead cells of different varieties of *Aspergillus oryzae* species have been utilized for heavy metal removal. The treatment of biomass with activated silica shows significant changes in the cell wall.²⁶ Alkali treatment of *Tolypocladium* and *Penicillium oxalicum* species improves deacetylation of chitin and causes an increase in the binding sites, thereby enhancing the sorption capacity significantly.²⁷

In the present work, a newly developed sorbent, based on *Aspergillus oryzae* immobilized on activated silica (AOAS) has been investigated for enrichment of Au (III). Sorbet was successfully applied for Au (III) enrichment from the Ayurvedic formulated product, dummy sample and tap water prior to its FAAS determination.

Experimental

The determination of Au (III) was carried out using a flame atomic absorption spectrophotometer *AAAnalyst* 200 (Perkin Elmer, USA) equipped with an air-acetylene burner with deuterium arc background correction. The Au(III) hollow cathode lamp was operated at 10 mA current. The absorbance was measured at 217 nm with a spectral band width of 0.5 nm. The pH values were measured with Elico LI-127 digital pH meter (Elico India Ltd., India) supplied with a combined glass electrode. Double distilled water was obtained from LAB-SIL quartz double distiller (LAB-SIL Instruments Pvt. Ltd., India). A glass column (150 mm x 10 mm, J-sil, India make) was used for column studies. The fungal biomass was separated from liquid media using research centrifuge Model R-24 (REMI, India). Fourier transforms infrared spectrometer, model Spectrum one FTIR spectrometer (Perkin Elmer, USA) was used for characterization of sorbent. Scanning electron microscopy (SEM) [QUANTA-200, FEI Ltd., Netherlands] was used for surface morphological study.

Reagents and solutions

The stock standard solution containing 1000 mg L⁻¹ solution of Au (III) was prepared by dissolving appropriate amount of (HAuCl₄.H₂O) from Sisco Research Laboratories in slightly acidic double distilled water. The solution was standardized volumetrically,²⁸ and working standard solutions were prepared by appropriate dilution. Silica gel 60-120 mesh which was activated with conc. HCl²⁹ was used as solid support for sorbent preparation. For culture, all media were used from Himedia laboratories Pvt. Ltd. All other chemicals used were of Analytical Reagent grade.

Preparation of *Aspergillus oryzae* immobilized activated silica

A laboratory strain of lyophilized *Aspergillus oryzae* obtained from National Collection of Industrial Microorganism (NCIM), Pune, India was used for sterilization. Sterilization of liquid medium and glass wares was done by autoclaving at 121°C for about 20 min. A fungus cultivated in solid media composed of sabouraud dextrose broth 30 g L⁻¹ and agar 20 g L⁻¹ was used for the growth of the fungal biomass which was preserved by refrigerating at 4°C. Starter culture was prepared by loop full inoculation of the cultivated fungus in 100 mL liquid medium (without agar) which was followed by 48 hrs incubation at 25 °C. Experimental culture was prepared using 250 mL of liquid medium inoculated with 5 mL of the starter culture. After incubation the biomass was stored for 25 days at 25°C. The growth media was then centrifuged at 7500 rpm for 30 min for separation of biomass. The residual biomass was then thoroughly washed with double distilled water number of times to ensure removal of residual growth and was followed by oven drying of the fungal biomass at 80 °C for 24 h, which was then grounded and sieved through a 100 mesh sieve. The dead cells of *Aspergillus oryzae* were used for immobilization on activated silica for preparation of the sorbent.

Exactly 100 mg, 150 mg, 200 mg of the biomass was homogenized with 1.0 g of activated silica and wetted with double distilled water. The paste was kept for drying in an

oven at 40, 60 and 80 °C for 1h. The wetting and drying step was repeated till *Aspergillus oryzae* got immobilized on silica. FTIR spectroscopy was used for characterization of this prepared sorbent. This AOAS sorbent was used for all studies.

General procedure

A glass column packed with 350 mg of the AOAS was pre-conditioned to the desired pH by double distilled water adjusted using dilute NH₃ and HCl. A sample solution containing 20 µg of Au(III) was adjusted to the appropriate pH and passed through the column. The sorbed Au(III) was eluted with 10.0 mL of 1:1 mixture (0.2 M HCl + 0.2 M thiourea) and its concentration was determined by FAAS.

Determination of Au(III) in Ayurvedic sample

Initially 10 powdered gold containing tablets were transferred into a platinum crucible and incinerated in a muffle furnace, gently at first, the temperature was then gradually increased to around 600 to 700 °C for three h to remove carbon from sample.³⁰ After cooling, remaining powder was dissolved in aqua regia to extract Au(III) and diluted to 250 mL with double distilled water.

Determination of Au(III) in dummy prepared sample

Separate solutions containing calcium (100 mg L⁻¹), potassium (1000 mg L⁻¹), sodium (1000 mg L⁻¹), chloride (1000 mg L⁻¹) and Au(III) (100 mg L⁻¹) were prepared. From above solutions 38 mL, 16.2 mL, 17.8 mL, 3.6 mL and 4.6 mL of Ca, K, Na, chloride and Au(III) respectively were taken, mixed to equilibrate and diluted up to in 250 mL.

Batch adsorption studies

A batch adsorption study was done using AOAS shaking with 100 µg ml⁻¹ solution on wrist action mechanical shaker. The effect of variables such as solution pH, contact time, initial adsorbate concentration and revolution per minutes (rpm) was studied. Equilibrium studies were conducted with 50 mg of AOAS sorbent, which was added to 50.0 mL aqueous solution of Au(III) (10-480 µg 50 mL⁻¹) in reagent bottles and pH adjusted to 2 at 31 °C. The solutions were agitated on a wrist action mechanical shaker for 15 min at 140 rpm. Desorption of Au(III) from the modified sorbent was done by shaking the sorbed resin after filtration, with 1:1 mixture of 0.2 M HCl + 0.2 M thiourea for 30 min at 180 rpm. After equilibration, the phases were filtered and analysed by FAAS.

Results and discussion

Characterization of AOAS

The immobilization of *Aspergillus oryzae* on activated silica and Au(III) sorbed on sorbent was checked by FTIR spectrum (Figure1). The peak observed at 1069.85 cm⁻¹ was assigned for Si–O–Si stretching. Another peak observed at 974.62 cm⁻¹ indicated the presence of Si–O–H stretching

vibrations of activated silica (Figure 1A),³¹ where as band observed at 947.46 cm^{-1} was unchanged during sorption-desorption. While, the spectrum of AOAS (Figure 1B) showed a band between $3600 - 3200\text{ cm}^{-1}$ due to $-\text{OH}$ groups and bands between $3000 - 2800\text{ cm}^{-1}$ were assigned to stretching vibrations of CH_2 and CH_3 groups. A peak observed at 1652.43 cm^{-1} was attributed to $\text{C}=\text{O}$ stretching in carboxyl or amide groups on the biomass. The spectrum also showed peaks at 1644.63 cm^{-1} and 1059.25 cm^{-1} which corresponds to the $-\text{N}-\text{H}$ deformation mode coupled with $\text{C}-\text{O}$ stretching from the amide groups present on the cell wall of biomass. A large band with several peaks between $1200 - 800\text{ cm}^{-1}$ may be attributed to the polysaccharide ring present on the biomass.³² A comparison of Figure 1A and B with Figure 1C, showed that peaks observed at 3564.11 , 1644.63 and 796.89 cm^{-1} indicated the immobilization of *Aspergillus oryzae* on activated silica. The spectra showed some variations after Au(III) sorbed on AOAS. As shown in Figure 1D, the peaks observed at 3259.06 cm^{-1} and 1644.63 cm^{-1} disappeared, which proved that the amide group of biomass was involved in the binding of Au(III).

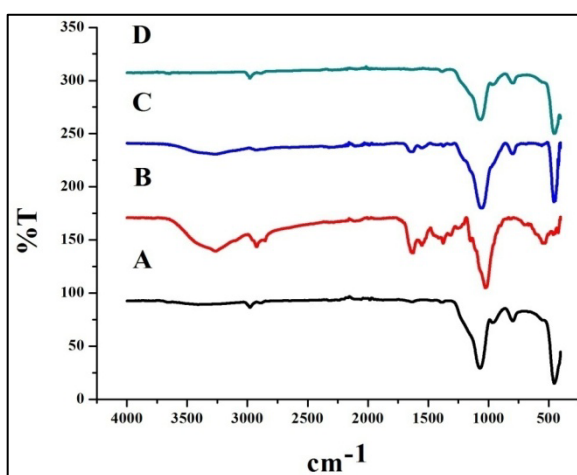


Figure 1. FTIR of (A) activated silica (B) *Aspergillus oryzae* (C) AOAS (D) Au(III) sorbed on AOAS.

Effect of amount of the sorbent and temperature on sorbent recovery

The amount of sorbent is an important parameter that affects the sorption and recovery of the analyte. Quantitative retention is affected when the amount of sorbent varies. For this purpose, different amounts of the sorbent (100–200 mg) were studied. The results showed that quantitative recoveries of the metal ions were obtained when the sorbent quantity was greater than 150 mg. With 100 mg of the sorbent, the lowest recovery was obtained, whereas 150 mg and 200 mg showed quantitative recoveries. Therefore 150 mg was chosen for further experiments.

Temperature is a parameter that affects the physical conditions of biomass. At $40\text{ }^{\circ}\text{C}$ reduction in the recovery of Au(III) was observed, whereas at $80\text{ }^{\circ}\text{C}$ color of the biomass changed which was not useful for further study. At $60\text{ }^{\circ}\text{C}$ temperature quantitative recoveries were obtained, hence this was chosen as the operative temperature.

Characterization of AOAS surface morphology

Scanning electron microscopy (SEM) was used to study the surface morphology of AOAS and for the evaluation of adsorbed Au(III) on AOAS. Figure 2a shows the high-resolution SEM images of AOAS surface while variation in Figure 2b indicated that Au(III) was adsorbed on AOAS. Irregular structure was observed in Figure 2b, indicating the adsorption of Au(III) on AOAS.

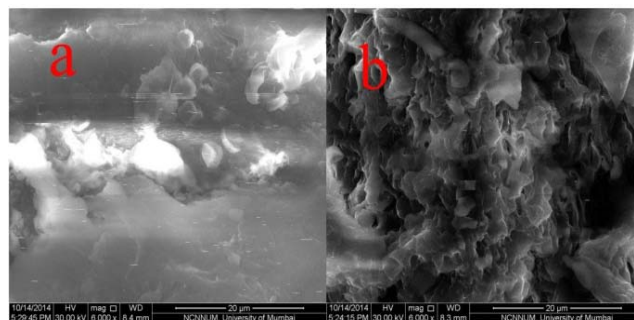


Figure 2. SEM images of (a) AOAS (b) Au (III) adsorbed on AOAS.

Effect of pH

pH is one of the most important parameter, because formation of strength of bond between the metal and microbial biomass varies with pH during the sorption of metal ions. Therefore, sorption of Au(III) on the columns containing AOAS sorbent were studied from pH 1.0 – 6.0 (Figure S1). pH range of 1 to 3 was studied with over ten digits, among which pH 2 showed maximum recovery with more than 99 % adsorption. Au(III) sorption increased with increase in pH on AOAS sorbent and it was quantitative ($99.7 \pm 0.2\%$) at pH 2.0. It was observed that only 45 % of Au(III) was sorbed on activated silica. It clearly indicated that, immobilization of the biomass on silica was necessary for complete sorption of Au(III). Hence, pH 2.0 was considered as the optimum pH used for further studies.

Sample flow rate and sample volume

The flow rate of sample solution has considerable influence on solid phase extraction, as high flow rates diminish the contact between the analyte and sorbent. Au(III) sorption was studied for sample flow rate of $1.0 - 4.0\text{ mL min}^{-1}$. Au(III) was quantitatively sorbed from $1.0 - 3.0\text{ mL min}^{-1}$ sample flow rate. While, flow rate $> 3.0\text{ mL min}^{-1}$ resulted in a decrease of Au(III) sorption. Hence, a sample flow rate of 1.0 mL min^{-1} was maintained in all experiments.

Sample volume is considered as one of the most important parameters studied for real sample analysis in solid phase extraction methods. Sample solution (25 - 1250 mL) containing $20\text{ }\mu\text{g Au(III)}$ at pH 2.0 was passed through the column. The recovery of Au(III) was done quantitatively even up to 1250 mL of sample solution. The eluent volume used was 10 mL. Hence, the pre-concentration factor achieved was 125.

Effect of eluent type and flow rate

The elution studies were performed in order to understand the recovery of Au(III) from AOAS sorbent column. Series of experiments were conducted using different concentrations of HCl and thiourea. Quantitative recovery was accomplished with 1:1 mixture of (0.2 M HCl + 0.2 M thiourea). This mixture was selected as an eluent since preliminary studies indicated that higher concentration of HCl damages biomass of the column.

Au(III) recovery was studied using 5.0 – 15.0 mL of mixture. It was found that 5.0 mL of mixture resulted in 84.3 ± 0.4 % recovery whereas; 10.0 mL and 15.0 mL of mixture gave quantitative recovery. Hence the effect of flow rate was investigated from 0.1 – 1.0 mL min⁻¹ with 10.0 mL mixture. Using of 0.1 mL min⁻¹ and 0.2 mL min⁻¹ flow rates the recovery was 99.4 ± 0.1 % and 98.3 ± 0.1 % respectively. While, at 1.0 mL min⁻¹ flow rate, the recovery decreased to 84.7 ± 0.1 %. Therefore, 10.0 mL of mixture at a flow rate of 0.2 mL min⁻¹ was used as an eluent for all subsequent studies.

Adsorption equilibrium isotherm

Adsorption equilibrium isotherm was studied in terms of following models.

Langmuir Adsorption Isotherm

Langmuir model represents the equilibrium distribution of metal ions between the solid and liquid phases.³³ The Langmuir model is valid for monolayer adsorption on the surface of a finite number of sites. Based upon these assumptions, Langmuir equation³⁴ is given eqn. (1) and (2).

$$\frac{C_a}{q_e} = \frac{1}{q_0 b} + \frac{C_a}{q_0} \quad (1)$$

$$R_L = \frac{1}{1 + bC_0} \quad (2)$$

where

C_e =equilibrium concentration of adsorbate (mg L⁻¹)

q_e =amount of metal adsorbed per gram of the adsorbent at equilibrium (mg g⁻¹),

q_0 =maximum monolayer coverage capacity (mg g⁻¹),

b =Langmuir isotherm constant (L mg⁻¹) and

R_L =separation factor.

The magnitudes of Langmuir constants can be determined from the linear plot of C_e/q_e versus C_e delineated in Figure 3a. Langmuir constant is related to the affinity of binding sites (mL mg⁻¹). It is a measure of energy of adsorption

indicating the adsorption nature to be either unfavorable if $R_L > 1$, linear if $R_L = 1$, favorable if $0 < R_L < 1$ and irreversible if $R_L = 0$. The data obtained indicated that Langmuir isotherm was favorable as value of b was greater than 0 but less than 1. Maximum monolayer coverage capacity (mg g⁻¹) from the Langmuir Isotherm model was obtained to be 9.5328 mg g⁻¹, b was 0.315 L mg⁻¹, R_L (the separation factor), as mentioned in Table S1, was calculated from above equation 2 and the R_L value calculated was 0.936 which indicated that the sorption data fitted well with the Langmuir Isotherm model.

Freundlich Adsorption Isotherm

The Freundlich model assumes a multilayer of adsorption of Au(III) on *Aspergillus oryzae* cell surface. According to Freundlich, the ratio of the amount of solute adsorbed on the biomass to the concentration of solute in the solution is not constant at different concentrations.³⁵ According to this theory, the empirical Freundlich equation based on sorption on a heterogeneous surface area is as follows:

$$q_e = K_f (C_e)^{\frac{1}{n}} \quad (3)$$

where

q_e is the amount of Au(III) adsorbed at equilibrium (mg g⁻¹),

C_e is the equilibrium concentration (mg L⁻¹).

K_f and n are Freundlich constants related to sorption capacity and adsorption intensity respectively.

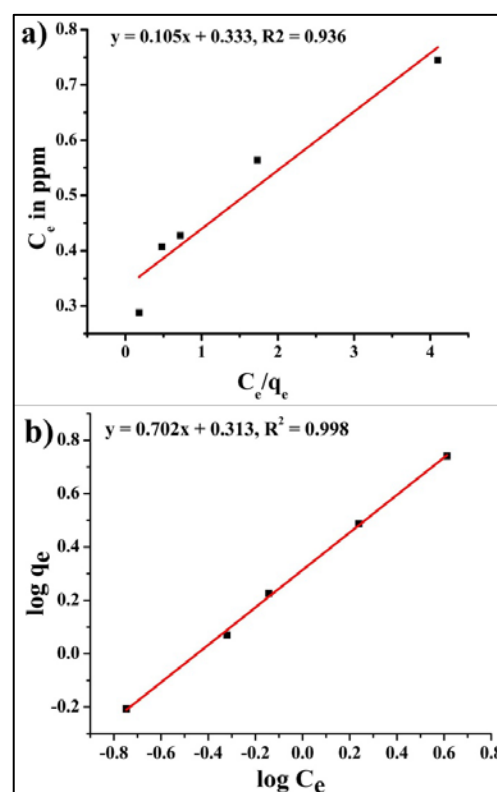


Figure 3. Adsorption isotherms for adsorption of Au (III) and AOAS (a) Langmuir (b) Freundlich.

Eqn. (3) can be linearized in logarithmic form as follows.

$$\log\left(\frac{x}{m}\right) = \log K_f + \left(\frac{1}{n}\right) \log C_e \quad (4)$$

where K_f and $1/n$ are capacity of the adsorbent for the adsorbate and adsorption intensity, respectively.

A linear regression plot of $\log q_e$ versus $\log C_e$ slope gives n value whereas intercept gives K_f value. The sorption capacity constant for Au(III) (K_f) and adsorption intensity of Au(III) (n) were found to be 0.5045 and 1.425 respectively. The value of n was observed to be between 1 to 10, indicating a favourable adsorption of Au(III) on AOAS. The correlation coefficient for the plot of $\log q_e$ vs. $\log C_e$ delineated in Figure 3b was found to be 0.998 indicating a very good fit of the experimental data.

Temkin adsorption isotherm

Temkin adsorption isotherm assumes that binding energy decreases linearly with increasing amount of metal bound to the surface of adsorbent.³⁶ Temkin isotherm has been applied in the form of Eqn. (5).

$$q_e = \frac{RT}{b} \ln A + \frac{RT}{b} \ln C_e \quad (5)$$

where

- b = Temkin constant related to heat of sorption (J mol^{-1}),
- A = the equilibrium binding constant (L g^{-1}),
- R = gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and
- T = absolute temperature (K).

As implied in the logarithmic form of the equation, uniform distribution of binding energies (up to some maximum binding energy) was carried out by plotting the quantity sorbed q_e against $\ln C_e$ delineated in Figure 4a and the constants were determined from the slope and intercept. The slope and intercept gives the value of Temkin constant related to heat of sorption and equilibrium binding constant respectively. In the present work, the values $A_T = 4.413 \text{ L g}^{-1}$, $RT/b = 3.574 \text{ J mol}^{-1}$ suggested a physical adsorption process whereas correlation coefficient (R^2) was found to be 0.911.

Dubinin–Radushkevich isotherm

Dubinin and Radushkevich (D–R) isotherm³⁷ commonly has been based on adsorption theory applied in the form of Eqn. (6) and its linear form can be shown in Eqn. (7).

$$q_e = q_{\max} \exp\left(-K_{DR} \varepsilon^2\right) \quad (6)$$

$$\log q_e = \log q_{\max} - K_{DR} \varepsilon^2 \quad (7)$$

where

K_{DR} ($\text{mol}^2 \text{ kJ}^{-2}$) is a constant related to the mean adsorption energy,

q_{\max} is the theoretical saturation capacity and ε is the Polanyi potential,

which can be calculated using the equation:

$$\varepsilon = RT \log\left(1 + \frac{1}{C_e}\right) \quad (8)$$

The intercept of the plot of $\log q_e$ versus ε^2 gives adsorption capacity, q_{\max} (mg g^{-1}) and the slope yields the K_{DR} ($\text{mol}^2 \text{ kJ}^{-2}$). R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$) and T is the absolute temperature in Kelvin.

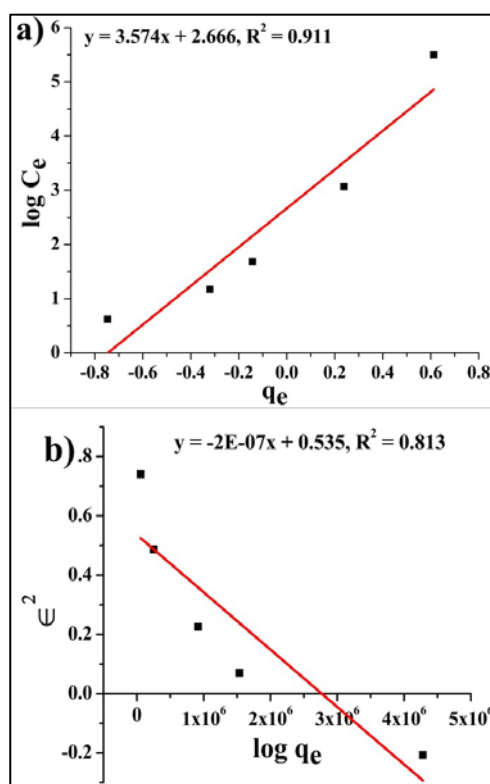


Figure 4. (a) Temkin (b) Dubinin and Radushkevich isotherms for adsorption of Au (III) and AOAS

The mean adsorption energy (E) can be calculated using the following relationship³⁷:

$$E = \frac{1}{\sqrt{-2K_{DR}}} \quad (9)$$

Some parameters were calculated theoretically from the D-R isotherm for Au(III) from Figure 4b. The theoretical saturation capacity (q_{\max}) was found to be 3.43 mg g^{-1}

Au(III) of AOAS, Constant K_{DR} obtained was $2 \times 10^{-7} \text{ mol}^2 \text{ kJ}^{-2}$, mean adsorption energy (E) calculated for Au(III) was 50 kJ mol^{-1} .

Harkin–Jura adsorption isotherm

The Harkin-Jura adsorption isotherm³⁸ can be expressed as eqn. (10).

$$\frac{1}{q_e^2} = \frac{B_2}{A} - \frac{1}{A} \log C_e \quad (10)$$

Harkin-Jura adsorption isotherm is applicable to multilayer adsorption which can be explained with the existence of a heterogeneous pore distribution, where B_2 is Harkin-Jura adsorption constant. Plot of $1/q_e^2$ vs. $\log C_e$ (Figure S2) gives slope and intercept which yields the value of A and B_2 respectively. For the present work the value obtained for $A = 2.3585$, $B_2 = 0.592$ and $R^2 = 0.740$. The lower value of correlation coefficient (0.740) indicated poor applicability of the H-J isotherm.

The adsorption isotherms such as Langmuir, Freundlich, Temkin, Dubinin and Radushkevich (D–R), Harkin-Jura were effectively followed (Table S2).

Mechanism for sorption and desorption of Au(III) on AOAS

During sorption mechanism, pH of the solution plays a major role for adsorption of Au(III) on sorbent. In first step of preparation of biomass activated on silica showed FTIR peak between $3600\text{--}3200 \text{ cm}^{-1}$ which get suppressed, confirming that -OH group is involved in bonding with fungal biomass. Whereas in the case of gold sorption on AOAS bonding was confirmed by SEM as well as disappearance of IR peak of 1644.63 cm^{-1} of amine group. During desorption, thiourea and HCl formed metal complex with gold where thiourea get linked to the central metal through the sulphur atom rather than nitrogen.³⁹ Hence gold shows +1 oxidation state rather than +3. The probable mechanism is shown in Figure 5.⁴⁰

Analytical performances of the method

The limit of detection (LOD) and limit of quantification (LOQ) of the proposed method for the determination of Au(III) was studied under the optimum experimental conditions. The limit of detection is defined as $CLOD = 3Sb/m$, where Sb is the standard deviation of replicate blank signals, and m is the slope of the calibration curve. The pre-concentration and limit of quantification based on ten times the standard deviations of the blank⁴¹ for Au(III) obtained were $0.88 \mu\text{g L}^{-1}$ and $2.93 \mu\text{g L}^{-1}$ respectively.

Real sample analysis

A tap water sample was analyzed for Au(III) using the standard addition method. The sample was spiked with different amount of Au(III) and passed through the AOAS sorbent packed column under the general procedure for

Au(III) determination. Good agreement was observed between the added and found amount of Au(III) (Table 1). The proposed method was also applied to determine Au(III) in a dummy sample (Table 1).

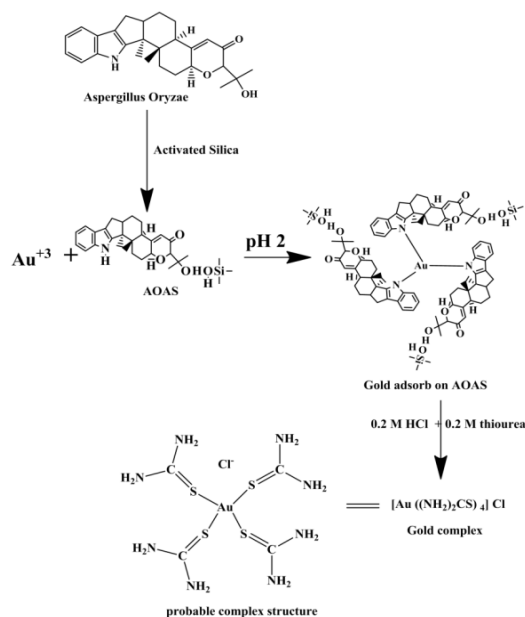


Figure 5. Probable mechanism for sorption and desorption of Au(III) on AOAS.

Table 1. Applications of AOAS method in the recovery of Au(III) in different samples.

Spiking of Au (III) in Tap Water sample			
Added amount	Found amount	Percent Recovery	
1.306± 0.12 mg	1.301± 0.18 mg	99.67± 0.07	
5.176± 0.15 mg	5.125± 0.22 mg	99.01± 0.32	
11.601± 0.11 mg	11.321± 0.30 mg	97.59± 0.40	
Spiking of Au (III) in Dummy sample			
Added volume	Added amount mg	Found amount mg	Percent Recovery
1 mL	0.424± 0.72	0.40± 0.62	94.42± 0.45
5 mL	2.087± 0.02	1.870± 0.85	89.79± 0.82
10 mL	4.027± 0.20	3.47± 0.61	86.25± 0.79
Determination of Au (III) in ayurvedic formulated product			
Item	ICP results mg	AOAS results mg	Percent Recovery
Baidyanath Swarnamalini Basat	84.34±0.025	83.73±0.039	98.09±0.05
Baidyanath Swog Chintumani	66.08±0.065	56.23± 0.311	85.09±0.35

During sorption, as the volume of dummy sample increases, matrix inserted in the sample was directly proportional to the volume of Au(III), which resulted in the decrease in recovery of Au(III). The data obtained with this method for the ayurvedic formulated product are presented in Table 1. The results of analysis of real samples indicated that the proposed method can be used for determination of Au(III) in different types of matrices.

Conclusions

An efficient method for determination of Au(III) adsorption on AOAS using SPE is reported. Preparation, characterization and adsorption properties have been studied. It is a simple, cost effective method which makes use of easily available and low cost biomass. The method is comparable with the procedure for limit of detection, pre-concentration factor and reusability. The reusability of the *Aspergillus oryzae* on activated silica for 29 cycles minimizes the waste generation. Application to the ayurvedic formulated product, dummy sample and tap water gave satisfactory results. The method can be applied for five different adsorption isotherms among that Langmuir and Freundlich were very well fitted.

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