STUDYING THE TRIVALENT ARSENIC ABSORPTION BY DE-PROTEINIZED HUMAN HAIR MATRIX

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CERTIFICATE

This is to certify that the thesis entitled, "STUDYING THE TRIVALENT ARSENIC ABSORPTION BY DE-PROTEINIZED HUMAN HAIR MATRIX" submitted by Ms. Sneha Kareer in partial fulfillment of the requirements for the award of the Bachelor of Technology in Biotechnology and Medical Engineering with specialization in "Biotechnology" at National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision and guidance.

To the best of my knowledge, the matter embodied in this thesis has not been submitted to any other University/ Institute for the award of any other Degree or Diploma.

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Declaration

I, **Sneha Kareer**, hereby declare that the thesis named "**Studying The Trivalent Arsenic Absorption By De-proteininzed Human Hair Matrix**" is my original research work. This work includes the valuable contribution of others and every effort is being highlighted with due reference of literature and acknowledgment of collaborative research and discussions.

This work was done under the guidance of **Dr. Sirsendu Sekhar Ray**, Dept. of Biotechnology and medical Engineering, NIT Rourkela.

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Abstract

Arsenic is a well known toxic substance in drinking water and its intake can cause severe pathological conditions. It is present in majorly in trivalent and pentavalent form, where the former is difficult to eliminate as it exists as neutral species in water. It is generally oxidised to pentavalent state before subjecting it to any other removal techniques. Here we use human hair matrix, after extracting proteins from it, to study absorption of trivalent arsenic. A solution of arsenic trioxide is used as test solution to ensure the presence of arsenic in trivalent form. The proteins from hair are extracted using Shindai method. The effect of pH of the solution used during the process is also studied within the range 7.5 to 9.5. Also heating using microwave is done instead of incubation and its result on absorption is recorded.

Introduction

1.1 Arsenic contamination

Arsenic is a well known toxic material and a common contaminant in ground water. The acceptable level as defined by WHO for maximum concentrations of arsenic in safe drinking water is 0.01 mg/L. Many countries like Bangladesh, united states and argentina are affected by arsenic contamination in ground water. Arsenic poisoning is a medical condition caused due to increased amount of arsenic in the body. The organs of the body that are usually affected by arsenic poisoning are the lungs, skin, kidneys, and liver. Symptoms of this include headache, confusion, convulsion, diarrhoea, vomiting, and in severe cases coma and death. Arsenic poisoning occurs due to prolonged consumption of arsenic affected water. Arsenic interferes with cellular longevity by allosteric inhibition of an essential metabolic enzyme pyruvate dehydrogenase (PDH) complex, which catalyzes the oxidation of pyruvate to acetyl-CoA byNAD^+ Due to this inhibition, the energy system of the cell is disrupted consequentially causing cellular apoptosis. Inorganic Arsenic trioxide in ground water particularly affects voltage-gated potassium channels, disrupting cellular electrolytic function resulting in neurological disturbances, cardiovascular episodes such as prolonged Q-T interval, neutropenia, high blood pressure, central nervous system dysfunction, anemia, leukemia, and death.

1.2 Treatment methods for arsenic containing water

Arsenic exists in water in arsenic(V) and arsenic (III) form. The former exists as monovalent and divalent cations ($H_2AsO_4^-$ and $HAsO_4^{2-}$) while latter occurs in predominantly neutral form (H_3AsO_3). This makes the removal of arsenic(V) much more effective. Most of the technologies used for arsenic removal are effective when the contaminant is present as

arsenic(V) ions. In case of arsenic (III) it can be converted to arsenic (V) and then can be removed. Some of the common technologies include coagulation and filtration, ion exchange, reverse osmosis, nanofiltration etc. The efficiency of coagulation depends on several factors like the nature and amount of coagulant used and initial concentration of arsenic. But it is not considered appropriate for small scale systems and also poses a problem of sludge disposal. Ion exchange is effective in arsenic removal but various other ions such as sufates, selenium, nitrate and fluoride can interfere with the process and increase the running length considerably. Also suspended solids can cause coagulation of the beds. Although reverse osmosis is an acceptable method for small scale water treatment but on very large scale it becomes expensive. And also reject water disposal is a problem. Initial tests using nanofiltration show positive results but it still needs to be optimised for practical usage as the recovery ranges from 15-20%. Some other methods are also being worked upon for purification of water. For example rice husk has been successfully used as water purifying material. If arsenic is specifically targeted, neutralised activated red mud, iron(II) ions or sand coated with iron oxide etc. are also being developed. Also, arsenic absorption in a column made up everyday waste material like coconut ash, newspaper brick ash etc. has been studied. Metal oxides also show tendency to adsorb arsenic from solution. Some metal ions like iron can assist in oxidation and adsorption simultaneously.

Further in this report basic laboratory tests are conducted to determine the absorption of arsenic from water using de-proteinized human hair extract. Chemical treatment of human hair was done to remove proteins and the remaining hair extract was utilized to study the arsenic absorption. A solution of arsenic was prepared using arsenic trioxide and was passed through the extract. The difference in the initial and final arsenic concentrations indicated successful absorption.

1.3 Structure of human hair

A hair can be defined as a slender, thread-like outgrowth from a follicle in the skin of mammals. Composed mainly of keratin, it has three morphological regions—the cuticle, medulla, and cortex.

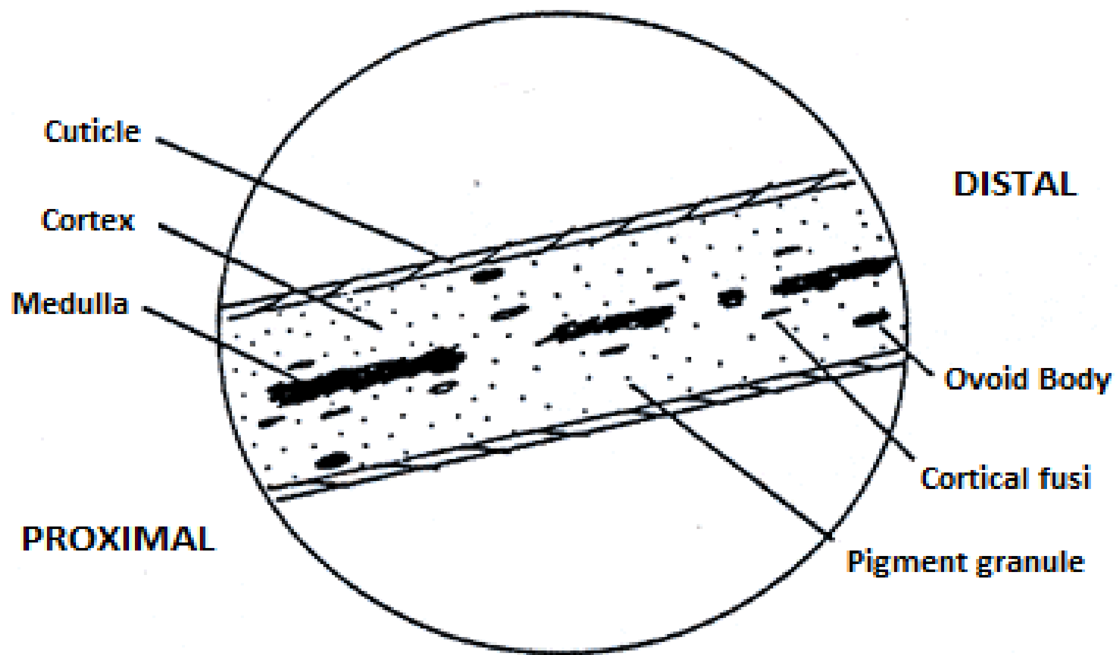

Figure 1: Basic hair structure

Cuticle

The cuticle is a translucent outer layer of the hair shaft consisting of scales that cover the shaft. Human hair consists of imbricate type of scale structure that is overlapping scales with narrow margins.

Medulla

The medulla is a central core of cells present in the hair. The medulla in human hair is generally amorphous in appearance. The structure of medulla in human hair can be described as—fragmentary or trace, discontinuous or broken, or continuous.

Cortex

The cortex is the main body of the hair composed of elongated and fusiform (spindle-shaped) cells. It may contain cortical fusi, pigment granules, and/or large oval-to-round-shaped structures called ovoid bodies. Pigment granules are small, dark, and solid structures that are granular in appearance and considerably smaller than cortical fusi. Pigment granules in human hair are commonly distributed toward the cuticle.

Hair follicles

Hairs start growing in a sac-like structure called the hair follicle that surrounds the hair root and is found below the skin. Sebaceous glands surrounding the hair follicle secrete sebum and oils into the hair follicle canal. At the base of a hair follicle is the hair root. During the growing phase, the follicle has a bulb-shaped bottom, in the centre of which is called the dermal papilla. The papilla contains small blood vessels, which bring it food and oxygen and takes away waste. Pigmented cells growing on top of the dermal papilla determine the color of hair. Hairs in humans are generally consistent in color and pigmentation throughout the length of the shaft. The pigmentation is evenly distributed, or slightly denser toward the cuticle.

Almost 80% of the hair structure is composed of proteins majorly keratin. Keratin is a hard fibrous structural protein whose monomers assemble into bundles to form intermediate filaments, which are tough and insoluble and form strong unmineralized tissues.

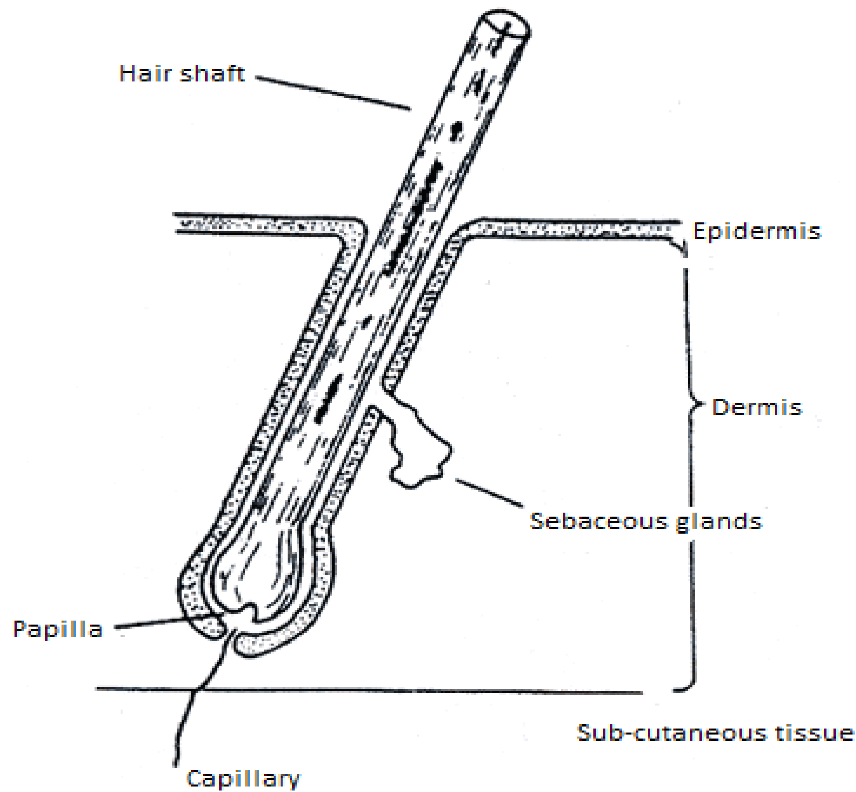

Figure 2: Hair root in skin

Fibrous molecules of keratin supercoil to form a very stable left-handed super-helical motif. These motifs multimerise to form filaments consisting of multiple copies of the keratinmonomer. In addition to intra- and intermolecular hydrogen bonds, keratins have large amounts of the sulfur-containing amino acid cysteine, required for the disulfide bridges that confer additional strength and rigidity by permanent, thermally-stable crosslinking. Human hair is approximately 14% cysteine. Hair consist of α-helically-coiled single protein strands (with regular intra-chain H-bonding), which are then further twisted into superhelical ropes that may be further coiled. In the organization of a single hair, three α-helices are twisted together to form a protofibril. This is the first fibril structure of the hair. Nine protofibrils are then bundled in a circle around two or more to form an eleven-stranded cable known as the

microfibril. These microfibrils are embedded in an amphorous unorganized protein matrix of high sulfur content, and hundreds of such microfibrils are cemented into an irregular fibrous bundle called a macrofibril. These macrofibrils are grouped together to form the cortex layers of the hair fibre. Packed dead cells surround these structures to form the cuticular layers. The medullary canal lies in the centre of these structures.

Protein extraction from hair was done by shindai method. Hair were delipidized and then chemically treated. Finally filtered such that the filtrate contained the extracted proteins and de-proteinized hair sample was obtained on the filter paper. Arsenic solution when passed from this layer of hair sample, showed diminished amount of arsenic. Modified Shindai method is also studied. The effect of pH on the process was studied and also the incubation replaced with microwave treatment was experimentally accomplished to study its effect.

Literature review

2.1 Treatment technologies for water contaminated with Arsenic

Arsenic treatment is a major step of water purification process in the areas where water is rich in arsenic ion concentration. Arsenic majorly exists as arsenic (III) and arsenic (V) ions in water. Arsenic (III) is both more toxic and more (Ferguson and Gravis, 1972) and more mobile than arsenic (V) (Amin et al. 2006). Most arsenic removal technologies are most effective at removing the pentavalent form of arsenic (arsenate), since the trivalent form (arsenite) is predominantly non-charged below pH 9.2. [1] Thus treatment systems include oxidation step which is coupled with removal processes such as coagulation, adsorption and ion exchange. Direct oxidation of arsenite is possible using a number of chemicals such as gaseous chlorine, hypochlorite, ozone, permanganate, hydrogen peroxide etc. Oxidation using direct UV radiation and ozone can also be done. After oxidation of arsenic (III) to arsenic (V), removal of ions is done. Coagulation and filtration are most common methods used for this purpose. Aluminium salts like alum and ferric salts like ferric chloride and ferrous sulphate can be used. During coagulation and filtration arsenic can be removed by three mechanisms (Edwards, 1994):

i. Precipitation: formation of insoluble compounds.
ii. Co-precipitation: incorporation of soluble arsenic species into a growing metal hydroxide phase.
iii. Adsorption: electrostatic binding of arsenic species to the external surface of insoluble metal hydroxides.

Use of various innovative adsorptive agents for arsenic is reported. A few such agents can be named as chelating resins in ferric iron form (Chanda et al. 1988a, 1988b) and Fe^{2+} treated activated carbon (Huang and Vane 1989). Metal oxides have strong affinities for arsenic and

can serve as effective sorbents. In some cases these oxides can also serve as oxidants. Any granular media such as quartz sand can be made highly sorptive by coating it with metal oxides. Sand fron ganges river which is rich in iron coating is effective in arsenic absorption (Vaishya). Iron oxide coated sand (IOCS) is also an excellent absorber both for arsenate and arsenite (Joshi and Chaudhary) [2]. A similar coated sand material is prepared using manganese dioxide. Since MnO_2 is a good oxidant, this material is also capable of removing arsenite as well as arsenate (Bajpai and Chaudhuri, 1999). Fuhrman et al. have used seawater activated- neutralised red mud (Bauxsol AB) as a novel adsorbent for removing inorganic arsenic from water. The study indicated that the bauxsol AB used in this study has excellent potential for use as an unconventional adsorbent that is comparable with commonly used pure adsorbents for As removal (e.g. Fe- and Al hydroxides) [3]. Another method used is ion exchange resins. These resins are based on a cross-linked polymer skeleton known as the matrix. Charged functional groups are covalently attached to this matrix and they form the exchange sites. Conventional synthetic ion exchange resins are the most commonly used media in ion exchange, though a variety of naturally occurring materials also have high ion exchange capacities, sometimes after pretreatment. The ion exchange capacity is actually a measure of number of exchange sites. Chitosan and chitin are natural polyaminosaccharides occurring in crustacean shells and have good ion exchange properties [1]. Elson and others investigated a mixture of chitosan and chitin, and found a relatively low arsenic removal capacity (Elson et al., 1980). Membrane filtration is another common method used for purification purpose. It is effective in removing bacteria, salts and heavy metals [1]. Two classes of membrane filtration techniques are identified, first low pressure filtration such as ultrafiltration and microfiltration and high-pressure filtration such as nanofiltration and reverse osmosis. The size of arsenic is in metal-ion size range thus, nanofiltration and reverse osmosis are suitable methods. The influent water for membrane filtration needs to be of high

quality because fouling of membranes is a common phenomenon. In water rich in arsenic is also rich in Fe or Mn ions, the conventional methods for Fe and Mn removal result in significant amount of arsenic removal as well. This occurs through co-precipitation or sorption into ferric or manganic hydroxides. Geroni et al. in their study highlight the potential of using iron (II) and citrate as a part of an industrial scale water treatment process [4]. A simple, low cost arsenic removal system was developed to treat arsenic contaminated ground Water by Samad, Rahman and Alam, 2010. The system decontaminates arsenic from water by sorption through fine particles of waste materials (Coconut husk.s ash, Refused brick dust, Stone dust and Waste newspaper) of multilayer. This column treatment system is appropriate and suitable homemade approach for arsenic removal in local areas, because of its simplicity and easy operation and handling [5].

2.2 Extraction of proteins from human hair

Proteins form almost 80% of hair structure. Two large groups of human hair proteins are known. One is hard a -keratins forming microfibrous intermediate filaments and the other is matrix proteins forming a nonfilamentous matrix as intermediate filaments-associated proteins. α-keratins are highly crosslinked structures with each other by disulfide bonds, enabling intermediate filaments to covalently crosslink with matrix proteins. Thus it is very difficult to obtain them in their native state. A convenient procedure for extraction of hair proteins in absence of detergent was developed in the Shinshu university. This is called Shindai's method [6]. Using this method it was also proved that phosphorylated species were also present in human hair [6]. Urea, thiourea and a reductant was used for the purpose of protein extraction. The maximum yield obtained was around 75%. The incubation at 50ºC was done for varying periods of time and the surface of hair was also visible after 48 hours

[6]. Shin S et al. modified the Shindai method by using microwave heating instead of normal incubation at 50ºC. During extraction, samples were exposed to microwave radiation (600 W) for a specified incubation period (5-120 min). The extraction efficiency of samples that had been incubated for 60 min was similar to that of samples that had been heated at 50°C for 24 hr using the conventional Shindai method [7].

Materials and Methods

3.1 MATERIALS

3.1.1 Chemicals Required

Ethanol	2-Mercaptoetanol
Methanol	Tris- base
Chloroform	HCl
Urea	Arsenic trioxide
Thiourea	Distilled water

3.1.2 Glasswares Required

Beakers	Filter papers
Flat bottom flasks	Pipettes and pipette tips
Funnels	Spatulas

3.1.3 Labwares Required

Weighing balance	Centrifuge machine
Incubator	Dessicator

3.1.4 Arsenic testing apparatus

Quantofix Arsen 10 testing kit was used to compare the concentration of arsenic in the solution. This is a colorimetric analysis kit wherein the sample is taken in testing bottle and reagents are mixed. The test strip provided has the test area which is exposed to vapours arising from the occurring reaction. If the sample contains arsenic the test area changes coloration and it can be compared to the reference color chart available with the kit. The range of arsenic concentration can easily be known and also the concentration of arsenic in two or more solutions can be compared using this.

3.2 METHODS

Human hair are de-proteinized by Shindai method of protein extraction. The pH of solution is modified to study the effect of pH on arsenic absoption. The extracted hair sample is used as an absorption material. The detailed process can be described as follows:

3.2.1 Hair protein extraction

Delipidizing the hair

1. Hair is cut into small pieces such that the length of each piece is not more than 2 mm.
2. Hair is treated with ethanol for 30 seconds in order to clean.
3. Hair is delipidized using 2:1v/v solution of chloroform and methanol. The time of treatment is 24 hours.
4. The solution is drained and hairs are dried.

Protein extraction

5. Shindai solution is prepared using following constituents:

Urea	5M
Thiourea	2.6M
Mercaptoethanol	5%
Tris-base	25mM

6. Three flat-bottom flasks are filled with 50 ml solution prepared above. Their pH is adjusted to 7.5, 8.5 and 9.5 respectively.

7. 0.2 gm of hair is added to each flask. These are then incubated at 50ºC for 3 days.

8. After three days, solution is filtered using a filter paper. The filtrate contains the extracted protein while the extracted hair sample is obtained in the filter paper.

9. The filtrate can be centrifuged at 15000*g at room temperature for 20 minutes so that proteins are obtained in the supernatant.

10. The hair sample is dried and used for water treatment.

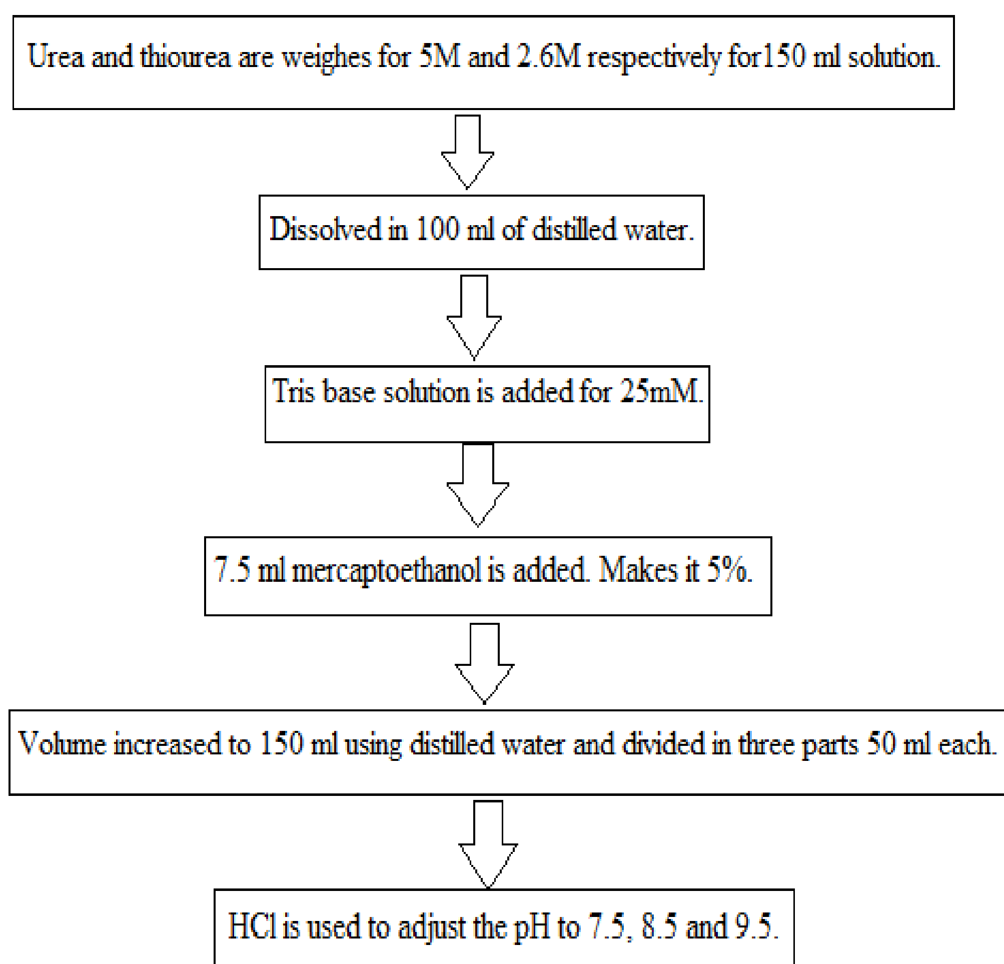

Figure 3: Preparation of Shindai solution

3.2.2 Modified Shindai Method

11. The hair immersed in Shindai solution was treated in a microwave oven at 600 W for 3 hours.

12. 1 hour treatment is equivalent to incubation at 50ºC for 24 hours.

13. This mixture was also processed and tested according to previous protocol.

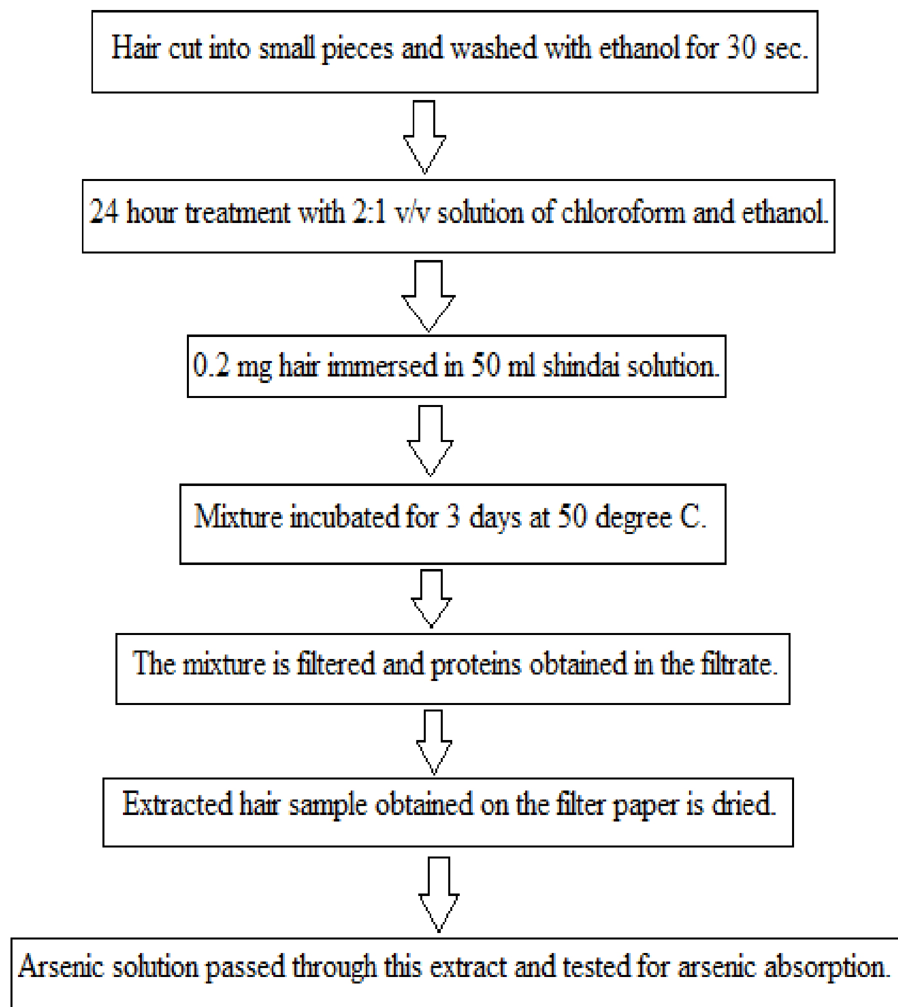

Figure 4: Shindai method of protein extraction and filtration of contaminated water

3.2.3 Preparation of arsenic solution

14. 0.5 mg of arsenic trioxide is weighed and dissolved in 1 litre of distilled water.

15. The solution is filtered to remove any suspended particles.

16. The strength of the solution is measured and recorded as initial solution concentration.

3.2.4 Testing the extracted sample for arsenic absorption

17. 25 ml of solution is passed through each extracted sample.

18. A control is set up using only the filter paper without extracted sample.

19. Filtered water from each flask and also from control set up is collected and tested for concentration of arsenic ions. The solutions were tested using *Quantofix Arsen 10* testing kit.

20. The results were recorded and compared.

Results

- Arsenic solution was prepared using arsenic trioxide. This was to ensure the presence of trivalent arsenic ions in the solution as this anionic form of arsenic is more difficult to remove.
- The initial concentration of solution was observed to be approximately 0.2mg/L.
- A control was setup using a filter paper without the hair extract. The concentration of this filtrate was fairly equal to that of the initial solution.
- It was then filtered through the obtained hair extract The results obtained were:

  For extract from pH=7.5 sample   0.05 – 0.1 mg/L

  For extract from pH=8.5 sample   0.05 – 0.1 mg/L

  For extract from pH=9.5 sample   0.05 – 0.1 mg/L

  Although the range of arsenic concentration in all the three cases was found to be the same but the coloration of the test strips indicate that within the pH range 7.5 to 9.5, increase in pH facilitates the arsenic absorption.

  Absorption with pH 9.5 > with pH 8.5 > with pH 7.5

- The solution was also filtered using extract from microwave assisted protein extraction and the arsenic concentration was found to be 0.05 mg/L

- The results can be tabulated as under

Solution source	Concentration in mg/L
Initial arsenic solution prepared using arsenic trioxide	0.2 mg/L
Control	0.2 mg/L
Solution passed through hair extract with pH=7.5	0.05 – 0.1 mg/L
Solution passed through hair extract with pH=8.5	0.05 – 0.1 mg/L
Solution passed through hair extract with pH=9.5	0.05 – 0.1 mg/L
Solution passed through hair extract from microwave heating	0.05 mg/L

Table1: Results for concentration of arsenic in tested solutions

Conclusion and Discussion

It is successfully demonstrated that matrix of human hair after extraction of proteins absorbs trivalent arsenic ions from the solution being passed through it. Since it is difficult to remove trivalent arsenic ion, this observation can be worked upon further so that it can be used as an effective part of water purification. The nature of interaction between arsenic ions and hair matrix is yet to be determined. This can indicate the efficiency of arsenic removal and also that of reuse of the matrix. Characterization of extracted proteins can also be done by doing SDS PAGE analysis in order to know the exact fraction of proteins that were extracted. During the process of extraction 2-mercaptoethanol as used as a reductant which is extremely carcinogenic. It should be replaced with another reductant such DTT (dithiothreitol) which is not as harmful as the previous one. Also the method of extraction aims at preventing the denaturation of proteins during the process which is no major concern of this research. Thus, the method can be modified to suit the requirements of this particular project creating a possibility of using inexpensive and less harmful chemicals. A study on the effect of other anionic species generally present in contaminated water on rate and degree of absorption is also required to determine the efficiency of system. Various column test can be done to study the effect of number of cycles of absorption, flow rate of water, retention time in the matrix packing, density and porosity of the packing initial concentration of arsenic ion etc. Hence, this preliminary experiment can be exploited further in various ways so that an efficient arsenic removal system can be developed.

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