Research Article

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Incorporation of silver stearate nanoparticles in methacrylate polymeric monoliths for hemeprotein isolation

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Abstract: A unique method was used to synthesize extremely stable silver stearate nanoparticles (AgStNPs) incorporated in an organic-based monolith. The facile strategy was then used to selectively isolate hemeproteins, myoglobin (Myo) and hemoglobin (Hb). Ethyl alcohol, silver nitrate, and stearic acid were, respectively, utilized as reducing agents, silver precursors, and capping agents. The color changed to cloudy from transparent, indicating that AgStNPs had been formed. AgStNP nanostructures were then distinctly integrated into the natural polymeric scaffold. To characterize the AgStNP–methacrylate polymeric monolith and the silver nanoparticles, energy-dispersive X-ray (EDX), scanning electron microscopy (SEM), and Fourier-transform infrared (FT-IR) spectroscopy were used. The results of the SEM analysis indicated that the AgStNP–methacrylate polymeric monolith’s texture was so rough in comparison with that of the methacrylate polymeric monolith, indicating that the extraction process of the monolith materials would be more efficient because of the extended surface area of the absorbent. The comparison between the FT-IR spectra of AgStNPs, the bare organic monolith, and AgStNP–methacrylate polymeric monolith confirms that the AgStNPs were immobilized on the surface of the organic monolith. The EDX profile of the built materials indicated an advanced peak of the Ag sequence which represented an Ag atom of 3.27%. The results therefore established that the AgStNPs had been successfully integrated into the monolithic materials. Extraction efficiencies of 92% and 97% were used to, respectively, recover preconcentrated Myo and Hb. An uncomplicated method is a unique approach of both fabrication and utilization of the nanosorbent to selectively isolate hemeproteins. The process can further be implemented by using other noble metals.

Keywords: silver stearate nanoparticles, integration, monolithic hybrid solid, hemeproteins, hydrophobicity

1 Introduction

The unique electrical, optical, mechanical, heat conduction, and catalytic features of metals have made them extremely noticeable in recent years [1–5]. The metals therefore have a number of uses in different fields, for instance antibacterial uses, improved version of Raman spectroscopy, catalysis [6,7], storage of data [8,9], lubrication [10], and electronics. The consistent distribution and fine particle sizes of the silver nanoparticles make them significantly important in microelectronics and electronics, especially because they have unique features like high opposition to oxidation and high thermal [11] and electrical conductivity [12], microwave irradiation [13], ultraviolet radiation [14], and electron irradiation [15], atom beam sputtering [16], alongside photochemical strategies [17], the sonochemical approach [18], and the chemical reduction [19,20] which have been used to develop the unique forms of nanomaterials. The fine particle sizes of the silver nanoparticles make them significantly important in microelectronics and electronics especially because it has unique features like high opposition towards oxidation and high thermal and electrical conductivity.

The simplicity of procedures, devices, and the chemical reduction strategies make them the most popular and suitable to be utilized. For most chemical reduction strategies, the development of silver nanoparticles regularly involves two steps, which include growth and nucleation. The van der Waals forces result in massive aggregates of the particles, which also aggregate irreversibly, and they use a high-energy surface throughout the growing stage [21]. A lower initial metal concentration of metal ions is applied because it results in smaller-sized fine particles of silver.

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The conduction are, however, unfavorable when producing silver nanoparticles that contain controlled properties because they should not be fabricated on a large scale. The oxidation of silver nanoparticles is also very easy at the formation stage, resulting in a limited number of applications in various industries. To find a solution, the primary step is to use suitable stabilizers. Different compounds and fatty acids have been utilized in forming metal nanoparticles through a unique medium [22]. The synthesis method that utilizes fatty acids is a significant area of interest because it is possible to integrate oleic acids, lauric acids, stearic acids, and palmitic acids, which are examples of fatty acids, into the metal nanoparticles [23,24].

The silver nanoparticles are secure under standard conditions such as dry powder, films, and solutions because they have long-chained molecules that are typically engrossed on the surface [25–27]. No study has however reported the utilization of stearic acid as a capping agent during the silver nanoparticle synthesis when the water-phase system is used. Some of the advantages of monolithic materials include high porosity [28–33], thermal stability, and mechanical strength. During the last two decades, the polymer monoliths have presented well-known features that have resulted in the development of synthetic approaches of the monolithic stationary stage where they have large pores and homogenous structures. Various natural solvents or toluene was utilized in the procedure, and they could cause biological hazards or environmental toxicities. The process also utilized various solvents such as toluene and butylamine which are organic and natural. The photoinitiated or thermal and radical polymerization of styrene-based and methacrylate monomers is one of the most used approaches that utilize the porogenic mixture [34,35].

The polymer monoliths present a significant advantage in that they can utilize various methodologies to attain the standard chemistry that is recommended for the polymer surface. Some of the approaches include the utilization of monomers that have specific functions [36] like working on chemical surfaces of the original monoliths [37], grafting polymerization [38–41], and the integration of the nanostructures of the polymeric scaffold [32,42,43]. The strategy in which the inclusion of nanostructures is performed increases the binding capacity and the adjustment of the surface chemistry as significant impacts of the huge surface-to-volume ratio that are features of the nanostructures. Two distinct methods can be utilized in the monolithic column functionalization of the nanoparticles. Nanoparticles that have a specific functional group can be flushed through columns that have complementary functionalities [44] with monolithic capillaries based on a coating method. The approach utilized polymer-based monoliths, which were functionalized using gold alongside negatively or positively charged nanoparticles. Alternatively, the nanostructures can be directly added to the polymerization mixture, which would result in an in situ polymerization reaction; then the nanoparticles would be implanted into the polymeric structure. The strategy was mainly used on the functionalization of monolithic columns that had amine-modified silica nanoparticles, hydroxyapatite, graphene oxide nanosheets, carbon nanotubes, and the natural metal framework [45–48]. An uninterrupted embedding of the nanostructures was applied for specific material types to solve the low binding capacity problem that is experienced with monolithic cryogels. For instance, the diatomite, magnetic, and sporopollenin particles have been seized inside the monolithic cryogels. Furthermore, the molecularly imprinted pieces have been condensed into both polyvinyl [49–51] and acrylamide-based alcohols.

The human organs, functions, cell regulation, structure, and tissues are significantly dependent on proteins. It is however extremely challenging to purify proteins from the biological matrices during the separation of biomolecules. The study emphasizes on the 64,500 Da, hemoglobin (Hb), and the brown color of Hb, a protein that is grouped into four hemes and is also excessively present in the red blood cells. Ferric cations are present in all heme groups, and oxygen transportation is dependent on them. Due to the oxygen transportation properties that are present in Hb, it is crucial in producing blood substitutes (instead of transfusion) in extreme situations for people who have unusual blood types. Hb must, therefore, be isolated in all prerequisite stages of similar investigations.

Apart from adding Hb, hemoproteins with various isoelectric points or pI values and molecular weights were utilized in the hemoprotein isolation process of the myoglobin (Myo, brown color and 16,951 Da), the polymer of silver stearate nanoparticle (AgStNP)–methacrylate. It was reported that Myo and Hb are both neutral.

The microporous monolith elements can identify large sizes of pores, and they are therefore administered on the base support to isolate various compounds [53] from mixed samples, perform cell separations [54], and finally the cell culture's three-dimensional polymeric scaffolds. There are deficient levels of efficiency which are obtained from such structures, therefore, making them disadvantageous. For this study, a unique
hydrophobic monolithic preconcentrator was developed alongside its use in the preconcentration of hemeproteins. The stage was the first trial in which the AgStNP–methacrylate polymeric monolith was synthesized in glass micropipettes. To assemble AgStNPs in water-phase structures, the study utilized a one-step response model where stearic acid was used as a capping agent. For reductant purposes in all the experiments, ethyl alcohol, which is less dangerous and also less costly in comparison with the NaBH₄, was used. To restrain the agglomeration and oxidation of the silver nanoparticles, stearic acid was utilized as a capping agent. The nanostructures were then directly embedded into the natural polymer monoliths. The scanning electron microscopy (SEM) analysis, energy-dispersive X-ray (EDX) analysis, and the Fourier-transform infrared (FT-IR) analysis are the main techniques that were used to characterize the fabricated materials. The preconcentration of hemeproteins such as Myo and Hb was conducted based on the micropipette format of the AgStNP–methacrylate polymeric monolith.

2 Experiment

2.1 Reagents and chemicals

Silver stearate synthesis was performed using ethyl alcohol, stearic acid (99%), and silver nitrate (99%), 3-(Trimethoxysilyl)propyl methacrylate, ammonium acetate buffer, acetonitrile, and trifluoro-acetic acid (TFA) were bought from Loughborough’s Fisher Scientific, UK. Ammonium acetate buffer, sodium hydroxide solution (NaOH), methanol (MeOH), aceto-nitrile (ACN), hydrochloric acid (HCL), and glacial acetic acid were bought from Nottingham’s Scientific Laboratory Supplies, UK. Butyl methacrylate (99%, BuMA), ethylene dimethacrylate (98%, EDMA), benzophenone (99%), Myo from the horse heart, and bovine Hb were bought from Poole’s Sigma-Aldrich, UK. Deionized water (18 MΩ cm⁻¹) was used to clean all polyethylene containers and glasses before the study.

2.2 Instruments

The study utilized a micropipette that was bought from London’s Lab Warehouse, and the electrical tape was acquired from the Onecall shop at Leeds. The study further utilized an S360 scanning electron microscope that was acquired from Cambridge’s Instrument Shop and 365 nm UV lamp from Leeds’s Spectronic Analytical Instruments. The hot-plate stirrer was purchased from VWR International LLC, Welwyn Garden City, UK. The FT-IR spectra used in the present study, were the diamond attenuated total reflection and diffuse reflectance infrared Fourier transform attachment, which were obtained from Perkin Elmer, Buckinghamshire, UK. The microtight adapters were obtained from the Kinesis shop at Cambridgeshire, UK. EDX spectrometer was bought from Tokyo in Japan. Some instruments were also purchased from USA, and they include the pH meter, which was obtained from Fisherman Hydrys 300 (Thermo Orion, Beverly, MA, USA). The syringe pump was also purchased from West Lafayette’s Bioanalytical Systems Inc. The UV-vis spectrophotometer (GENESYS 10S) was purchased from Thermo Scientific™ (Toronto, ON, USA).

2.3 Synthesis of AgStNPs

An aqueous AgNO₃ solution of 0.05 M was poured into a sterilized conical flask, and a magnetic stirrer was used in dissolving it into about 100 mL of ethyl alcohol. The process was used for the synthesis of the AgStNPs ([CH₃(CH₂)₉COOAg]). At 65°C all the reactants got heated, and a solution of equimolar stearic acid was dropped into the silver nitrate solution in a systematic manner. Each drop made the solution cloudy, and stirring the mixture for 40 min was the last step before the reaction was complete.

2.4 Synthesis of AgStNP–methacrylate polymeric monolith

To prepare the polymer-based monolith at room temperature and within the micropipette, radical polymerization, which is photoinitiated, was employed. An activation was initiated within the micropipette’s inner walls before the organic polymer-based monolith could be fabricated [52].

Before using deionized water to clean the micropipette, it was washed using acetone. A syringe pump was later used to wash it in a 0.1 M solution of NaOH at a 20 µL min⁻¹ flow rate for 60 min and then it was cleaned using deionized water. The micropipette was then rinsed
by pumping a 0.1 M solution of HCL at a 20 µL min$^{-1}$ flow rate through it for 60 min to extract the alkali metal ions and also neutralize them. Before the last rinse, the micropipette was washed with deionized water.

The PVA solution was used to silanize the micropipette’s inner surfaces. To achieve the required degree of silanization, the micropipette was cleaned using a solution that had 95% ethanol and PVA of 20% (v/v) at a pH level of 5 (the pH level was altered by a pH meter on glacial acetic acid). A syringe pump was then used to infuse the solution into the micropipette for 60 min at a 2 µL min$^{-1}$ flow rate. Before using nitrogen gas to dry the micropipette and leaving it overnight, its surface was vinylicized and then rinsed using acetone. After approximately 1 day, the micropipette was prepared for the polymerization reaction.

A binary mixture containing 2-propanol and methanol of a 50:50 ratio, BuMA, EDMA, and benzophenone made up the polymerization mixture. At room temperature, 0.02 g of AgStNPs was added to the mixture, and a stirrer was used to mix them for 15 min. A syringe pump was later used to infuse the blend into the micropipette. Both ends of the micropipette were covered using the blue tack then it was exposed to 365 nm of UV light at a distance of approximately 3 cm. The UV radiations reached only 2 cm of the micropipette because it was protected from forming a monolith by concealing it with an electrical tape, and the whole test was completed in 25 min. A MeOH syringe pump was flushed in the inner surface of the micropipette for 12 h in order to complete the polymerization reaction. It was aimed at freeing the AgStNP–methacrylate polymeric monolith from the porogenic solvents and any unreacted compounds.

2.5 Identification of the formed monoliths

The SEM analysis was used to characterize the dried monoliths by applying the scanning electron called the Cambridge S360, which was secured from Cambridge Instruments, UK. A 100 pA probe current and a 20 kV accelerating voltage were utilized when acquiring the images from an extremely high vacuum mode. A slim accelerating voltage were utilized when acquiring the images.

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2.6 Study of hemeprotein extraction

Hb and Myo were utilized for the extraction process, and both of them had a 75 mM concentration. The performance of the micropipette containing the AgStNP–methacrylate polymeric monolith was studied. Three hundred microliters of 10 mM ammonium acetate buffer solution with a pH of 7.3 were used for equilibration after activating the monolith elements at 300 µL ACN. Eight hundred microliters of the protein specimen were injected into the micropipette. Eight hundred microliters of ammonium acetate buffer solution with a pH of 7.3 were used in cleaning the micropipette following the completion of the extraction process. TFA (0.1%) and 500 µL of ACN (20%) were used to elute the proteins from the micropipette through injection. The eluent was accumulated and stored in the Eppendorf tube and then the spectrophotometer was used to analyze it.

The Myo wavelength of 409 nm and the Hb wavelength of 412 nm are the most significant features which were used to determine and compare the absorption measurements of the protein concentrations that were in the aqueous supernatant. The UV-vis which is a scanning spectrometer was utilized in identifying the standard calibration curves for all the proteins in this stage. The extraction recovery (ER) is calculated by the following equation:

\[
ER = \frac{C_{elu} \times V_{elu}}{C_{eq} \times V_{eq}} \times 100\%,
\]

where $C_{elu}$ and $C_{eq}$ are acquired from the peak area attained with the solid phase extraction and deprived of purification, $V_{eq}$ and $V_{elu}$ represent the volumes of sample solution and eluent, respectively.
3 Results and discussion

3.1 Fabrication of AgStNPs integrated organic monolith

The study aimed at fabricating integrated values of AgStNPs and the ones for methacrylate polymeric monolith within the micropipette mainly to enhance the significant features of the monolithic sorbent, so that it could be used for the extraction of the hemeprotein.

The process of preparing the poly(ethylene glycol) diacrylate cryogels, which are composite and in capillary form that could be applied in the disconnection of the acknowledged proteins that are of the hydrophobic interaction chromatography mode (Arrua et al.) [55], takes place. It was reported that insertion of the neutral nanoparticles into the polymeric scaffold resulted in an enhanced performance during the biomolecule separation. The neutral nanoparticles had non-polar features, which indicated that it was possible to integrate a substantial percentage of them into the polymeric design. It was, therefore, impossible for the analyte biomolecules and the deeply buried nanoparticles to interact at the mobile stage.

The level of stability of the silver nanoparticles is critical in determining some of their applications. For instance, if the procured silver nanoparticles precipitate or aggregate quickly, most of their users become limited. For this study, stearic acid was utilized in fabricating the AgStNPs because of its high level of surface inactivity. It is protected from being oxidized or aggregated because it belongs to an organic functional group that is not easily assimilated into the silver nanoparticles, and it can, therefore, be utilized as a surfactant. The narrow distribution, outstanding stability, and the tiny sizes of silver nanoparticles can be obtained when stearic acid is used. The alignment of the stearate’s silver ions occurs in shells while the localization of the silver atoms takes place in cores. It was suggested that the lattice structure was to be located at the nanoparticles’ center, which was a good suggestion for the silver crystal’s design [56].

According to Gorup et al. [57], the development of diamine silver complexes which are soluble is significant in the stabilization and also the synthesis of the small-sized and metallic silver nanoparticles. Zhang et al. [58] further utilized 1.0 g excess capacity of ammonia for the fabrication of silver nanoparticle’s stabilized stearic acid. Immediately after mixing ethyl alcohol and silver nitrate, the solution formed a diamine silver complex whose formation was significant in stabilizing the silver nanoparticles through lowering the growth stage’s reduction rate. To simultaneously develop considerable amount of nanoparticles, it was essential to increase the reaction temperature. For this study, stearic acid was poured into the heated suspension which was maintained at a temperature of 65°C. The silver nanoparticle’s slow growth process and swift nucleation were reported earlier than it is achieved at room temperature. The sum of available ions is decreased through the rapid process of nucleation, which fabricates consistent particles; therefore, the growth of the particles is slowed down. The growth rate of the particles decreased when the diamine silver complexes were formed and the tiny-sized silver nanoparticles also became narrowly distributed. After storing stearic acid or the stabilized nanoparticles of silver, sedimentation was not reported. The excellent stabilizing feature of stearic acid, therefore, ensures that the collected colloids of silver nanoparticles are incredibly stable.

The analysis of protein further barred the adsorption levels and resulted in the detection limit that demanded a low level of concentration. It was also more vital to restrain the adsorption of the proteins in the capillary walls or the micropipette; therefore, the surface had to be deactivated. γ-Methacryloxypropyl trimethoxysilane (γ-MPTS) was used to pretreat the surface of the micropipette, so that the monoliths could be prepared with the required standards and they could further be anchored through a process called silanization. The previous studies [59] also reported that the proteins become easily adsorbable when the surfaces are treated. Different strategies of modifying surface were therefore investigated to suppress any form of wall interaction or the production of unwanted analytes. The monolithic stability was attained by modifying the inside surface of the pipette, which also resulted in its compatibility with the proteins. γ-MPTS was used to form a permanent coating inside the micropipette, a procedure which had been used by Gilges et al. [60] A satisfactory surface for casting monoliths called BuMA-co-EDMA and also analyzing proteins was attained through the use of PVA. Even with the application of high voltage and pressure, the monolith was extremely stable therefore it failed to move. The PVA coating had thus formed a rough surface with extreme adhesion for the monolith. The results are, therefore, consistent with the study that had reported [61] the impacts of roughening glass surfaces through using boiled and deionized water as a secure attachment of the monolith.

Scheme 1 presents the steps of preparation of AgStNP–methacrylate polymeric monolith. A radical
polymerization that is photoinitiated freely was utilized in preparing the polymer-based monolith that was synthesized in the micropipette depending on the UV radiation and room temperature levels [62]. The previous experiment’s strategy was utilized in forming the polymer-based monoliths, except for a few changes [65]. A 50:50 mixture of 2-propanol and methanol, benzo-phenone, BuMA, and EDMA made up the polymerization mixture. A stirrer, 0.02 g of AgStNPs, 15 min at room temperature were all that were required for mixing the solution. Before the polymer formed or got precipitated, the polymerization solution was added into the micropipette under the lamp that produced UV lights. A syringe pump was used for extracting the monomeric materials that failed to react, the solvents that resulted in the formation of pyrogenic structures, and the soluble oligomers that were stuck in the pore after they got extracted from the micropipette. The process was called a hydrodynamic approach, which is based on a 2 µL flow rate for 80 min, and it was aimed at preventing the excessive polymerization reaction because of the high affinity of the crosslinks and the monomers, which forces them to extend the polymerization reaction. The nanostructures (AgStNPs) were then directly embedded into the natural polymer monoliths in order to form the AgStNP–methacrylate polymeric monolith. In Figure 1, the original appearance of the organic rod before the reaction is shown on the left, and the right side reports its state after the integration with the AgStNPs. Furthermore, the AgStNPs’ color changes to gray.

Figure 1: The appearance of the organic monolith before (left) and after (right) incorporation of AgStNPs.

3.2 Identification of materials

Figure 2 shows the SEM images of AgStNPs, methacrylate polymeric monolith, and AgStNPs integrated into the methacrylate polymeric monolith. Figure 2a displays AgStNPs, which are in the form of single and not aggregated particles. Figure 2b, on the other hand, presents the methacrylate polymeric monolith that is made up of a uniformly distributed network of pores. A homogenous figure was also reported for the monolith’s cross-section morphology. Figure 2c presents the structure of the AgStNP-organic monolith which is not similar to those of the methacrylate polymeric monolith and the AgStNPs. Furthermore, the standard structure of pores was detected, and it
presented a network that was interrelated and homogeneous based on the open pores. In comparison with the methacrylate polymeric monolith, the texture of the polymeric monolith of the AgStNP–methacrylate was rougher. It could, therefore, enhance the efficiency required when extracting the monolithic materials.

Figure 2: SEM micrographs of (a) AgStNPs, (b) bare organic monolith, and (c) AgStNP–methacrylate polymeric monolith.

FT-IR spectroscopy was utilized in analyzing the changes that had occurred in the structure of the chemical for both the converted and untransformed methacrylate polymeric monoliths. This technique was preferred because of its capability of following the variations that occur at the network and also the surfaces of the methacrylate polymeric monoliths. In Figure 3a, the features of the stearate's IR bands have been highlighted as (718, 1,337, 1,467, 1,508, and 1,517 cm\(^{-1}\)) for the lower frequency figures and (2,849, 2,916, and 2,957) for the high-frequency figures. For all the trans-extended chains, the asymmetric \((\nu_2(CH_3))\) mode and the symmetric one of \((\nu_2(CH_3))\) are located at the standard ranges of between 2,915–2,918 and 2,846–2,850 cm\(^{-1}\) [63]. For the disordered chains, [64] they are situated in figures ranging between 2,924–2,929 and 2,854–2,856 cm\(^{-1}\). The two significant peaks are therefore 2,916 and 2,849 cm\(^{-1}\) which are associated with the \((\nu_2(CH_3))\) asymmetric value and the \((\nu_2(CH_3))\) symmetric values, respectively. Additionally, the methylene group's rocking and scissoring modes were reported to be 718 and 1,467, respectively, which indicate that a triaxial system would make up AgStNPs on two chains for each unit cell [65]. The remaining bands were sequentially assigned to different Ag-stearate modes as follows: 1,508 cm\(^{-1}\) to \((\nu_4(COO^-))\), 1,517 cm\(^{-1}\) to \((\nu_4(COO^-))\), and 2,957 cm\(^{-1}\) to \((\nu_2(\text{CH}_3))\). The results of the FT-IR analysis are valid because they were proven when the AgStNPs displayed hydrophobic behavior because the topmost surface is made by the methyl group materials. Figure 3b further presents two bands at the FT-IR spectrum of methacrylate polymeric monolith. A broad band was observed at 3,345 cm\(^{-1}\) which was corresponding to the O–H stretching variation and the remaining band was reported at 1,726 cm\(^{-1}\), a figure that was from the carboxyl group, represented by the C=O stretching mode [66]. When a comparison is made between methacrylate and AgStNP–methacrylate, which are polymeric monoliths, and the AgStNPs’ FT-IR spectra, it was reported that the immobilization of the AgStNPs’ surface was reported as shown in Figure 3c.

To confirm the composition of produced materials, the EDX analysis was performed on the fabricated materials. The EDX spectra were taken for AgStNPs, and Figure 4 shows a 0.525 keV for oxygen, 2.983 keV for silver, and 0.227 value of carbon at the peak. The stearyl groups are reported at 1,726 cm\(^{-1}\), 1,337, 1,467, 1,508, and 1,517 cm\(^{-1}\) to \((\nu_4(COO^-))\) and their corresponding values of 1,726 cm\(^{-1}\), 1,337, 1,467, 1,508, and 1,517 cm\(^{-1}\) to \((\nu_4(COO^-))\), and 2,957 cm\(^{-1}\) to \((\nu_2(\text{CH}_3))\). The results of the FT-IR analysis are valid because they were proven when the AgStNPs displayed hydrophobic behavior because the topmost surface is made by the methyl group materials. Figure 3b further presents two bands at the FT-IR spectrum of methacrylate polymeric monolith. A broad band was observed at 3,345 cm\(^{-1}\) which was corresponding to the O–H stretching variation and the remaining band was reported at 1,726 cm\(^{-1}\), a figure that was from the carboxyl group, represented by the C=O stretching mode [66]. When a comparison is made between methacrylate and AgStNP–methacrylate, which are polymeric monoliths, and the AgStNPs’ FT-IR spectra, it was reported that the immobilization of the AgStNPs’ surface was reported as shown in Figure 3c.

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### 3.3 Extraction of hemeproteins

The primary aim was to utilize the monolith that was micropipette based to reduce the number of solvents and
protein samples, which would have otherwise been wasted. The micropipette additionally fastened the analysis process and the contents of the methacrylate polymeric monolith of the micropipette were used for extracting the hemeproteins. The micropipette’s monolith bed was well fabricated, after which its performance level was tested [67].

To preconcentrate the hemeprotein, 300 µL of ACN and 300 µL of 7.3 mM ammonium acetate buffer solution were equilibrated to activate the micropipette’s monolith bed. The standard amount was 800 µL of the hemeprotein sample that was applied to the micropipette’s inner surface. One hundred microliters of 10 mM ammonium acetate buffer solution with a 7.3 pH level were used to clean the micropipette after completing the extraction process. TFA (0.1%) and 500 µL of ACN (20%) were used to eluate the hemeproteins that were in the glass micropipettes. The hydrodynamic method, the ethylene tetrafluoroethylene (ETFE) tubing, and a syringe pump on the side of the micropipette were applied when injecting the solutions into the micropipette and 5 µL min⁻¹ was set as the required solution flow rate. In Figure 5, the images of cuvettes that have (A) standard and non-processed Hb, (B) a preconcentrated value of Hb using bare organic monoliths, and finally (C) preconcentrated Hb with the AgStNP–methacrylate polymeric monolith are presented. The Hb color was intensified after the preconcentration process by making use of methacrylate polymeric monolith containing AgStNPs confirming the preconcentration of the hemeprotein.

The left-hand side of Figure 6 presents an organic monolith, and the right-hand side shows the AgStNP–methacrylate polymeric monolith that were micropipette’s contents when preconcentrating the Myo. It, therefore, established that the brown color of Myo got attached to the monolith in a preconcentrated state that can be seen in the sorbent material’s color change. Additionally, the AgStNP–methacrylate polymeric monolith had a difference in the intensity of its color.

Figure 3: FT-IR spectra of (a) AgStNPs, (b) bare organic monolith, and (c) AgStNP–methacrylate polymeric monolith.
Furthermore, its performance was confirmed to be better when compared to that of organic monolith.

The standard and non-processed heme proteins (Myo and Hb) have their UV-vis spectra presented in Figure 7. The peaks of the standard and non-processed heme proteins were much lower than the standard heme proteins’ peak establishing that the monolith was modified using AgStNPs and preconcentration which increased the efficiency levels. An interaction was established between the AgStNPs present at the organic monolith’s surface and the heme group’s ferrous atoms. In Figure 8, the different (standard and non-processed) heme protein peaks that were attained after enrichment using both the AgStNP–methacrylate polymeric monolith and the organic monolith are shown. Improvement is noticeable in the peak levels of the heme protein heights that occurred after preconcentration. Additionally, for the heme proteins, the enhancement in the peak levels’ height is more, mainly when the AgStNP–methacrylate polymeric monolith is utilized.

Figure 4: EDX of (a) AgStNPs, (b) bare organic monolith, and (c) AgStNP–methacrylate polymeric monolith.
Table 2 shows that the heme proteins had a higher ER (92% of Myo and 97% of Hb) after the use of the AgStNP–methacrylate polymeric monolith. After analysis it is evident that the highest levels of extraction are attained when the values of the pI and those of the pH of all proteins are close. The likelihood of extraction using AgStNPs is, therefore, higher when the pH and the pI values are closer to each other. The proteins get positively or negatively charged when the pI value is below or above the pH value. A hydrophobic condition is attained when the pI and pH values are equal to the hydrophobic feature of proteins which eases their likelihood of being absorbed. The success of the approach is, therefore, highly independent on the hydrophobic interactions.

To assess the level at which the performance of the fabricated AgStNPs-organic monolith could be reproduced, the relative standard deviations (RSDs) at the highest level
of the peak of the preconcentrated hemeproteins were calculated. In Table 2, the comparison of extraction efficiency when a micropipette with four runs was used and when an $n = 3$ micropipette was used is presented. The results indicate that the hemeprotein preconcentration procedure could be reproduced because the RSD values (from 2.7% to 3.3%) were able to attain a suitable run-to-run reproducibility. The RSD ranged from 4.3% to 5.7% for the reproducibility of micropipette-to-micropipette to be accomplished. The reduction in experiment time and the reagent and sample volume was attained when the micropipette monolith was utilized, and it, therefore, ascertains that the avenues can be considered when developing a similar system and its applicability and the use of real samples and analysis can be confirmed in future studies.

### 4 Conclusion

The study’s main aim was to establish a micropipette for extracting hemeproteins by using organic monoliths that are modified by the hydrophobic nanostructures. The reduction process of chemicals was used to synthesize stable AgStNPs. The oxidation of silver nanoparticles and agglomeration were prevented by using stearic acid, which can also act as a capping agent to procure silver nanoparticles, which are in an aqueous medium. After storing the colloids of the silver nanoparticles for 60 days, they did not present any sedimentation signs. To attain a hemeprotein sorbent, the silver nanoparticles were utilized in dispersing highly soluble solutions then they were integrated into the natural monoliths that had been formed inside the micropipette. The preparation of the study sample could be viewed as a bottleneck in chemical analysis structures. The method can be used to experiment on noble metals such as Pd, Pt, and Au. The extraction of Myo and Hb was more selective when the AgStNP–methacrylate polymeric monolith was used, hence resulting in extraction efficiencies that could be reproduced.

**Ethical approval:** The conducted research is not related to either human or animal use.

**Conflict of interest:** Authors declare no conflict of interest.

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