Fabrication of Monolithic Silica Microchip for Efficient DNA Purification

Eman Alzahrani

Abstract - The demand for high purity deoxyribonucleic acid (DNA) is still increasing. The aim of this work is to fabricate a microchip that has the ability to preconcentrate DNA from biological samples with a high extraction efficiency compared to commercial DNA extraction kits. This was achieved by fabrication of monolithic materials, followed by placing the monolithic silica disk inside the extraction chamber of the polycarbonate microchip. The formation of the mesopores on the silica skeleton was achieved by treating the monolithic silica rod, using different concentrations of aqueous ammonia solution, mainly 0M, 1M, 5M, and 7M, while all other parameters involved in the fabrication of the monolithic silica rods were kept identical. The fabricated materials were studied using EDAX analysis, TEM analysis, and the SEM analysis. Based on the results, 5 M ammonia solution was chosen for optimisation of fabrication of silica-based monolith. Moreover, the benefit of integrating solid-phase nucleic acid extraction techniques into a microfluidic system was to get highly efficient isolation of target analytes due to beneficial surface area characteristics. In this study, isolation of nucleic acids from mouse liver was achieved using a silica-based monolith, onto which nucleic acids were adsorbed onto a solid support; the residual biological matrix and any exogenous contaminants were then removed by washing the monolithic materials with 80% ethanol, and finally the purified DNA was eluted from the microchip using 200 µL of 10 mM tris-EDTA buffer solution (pH 8.5). The data showed that the UV absorption ratio of A_{260}/A_{230} was 1.75±0.05 and the absorbance ratio of A_{260}/A_{280} was 1.70±0.04 for the fabricated microchip, showing a good degree of purity. It would be interesting to investigate the use of the fabricated microchip for purification of DNA from forensic samples.

Index Terms—deoxyribonucleic acid (DNA); extraction method; monolithic materials; polycarbonate microchip; sol-gel method.

I. INTRODUCTION

DNA analysis of biological samples has found widespread application in the field of forensic science and clinical diagnostic applications [1]. The isolation of DNA from biological samples constitutes the first step in a variety of bioanalytical techniques; for example, the polymerase chain reaction (PCR), which performs optimally when using purified DNA free from potential inhibitors of the amplification reaction [2-4]. In many instances, such as forensic investigations, biological samples are limited both in terms of quantity and quality; therefore, retrieving the maximum amount of DNA possible from the original sample is crucial [5-7]. DNA extraction can be performed by using commercially available kits such as the QIAamp DNA Micro kit and Analytik Jena DNA kit.

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They are widely used for DNA extraction and provide the gold standard. However, conventional DNA extraction kits are quite expensive [8]. In addition, they rely on bulky equipment for centrifugation DNA analysis of biological samples. Commonly, recovered DNA samples are limited in terms of the amount of the biological sample. As a result, methodologies that enable concentration of DNA samples as well as isolation from the complex biological matrix offer distinct advantages and increase the likelihood of achieving positive results during downstream analyses [9]. Solid phase extraction (SPE) is an extraction method that is based on using a stationary phase and a liquid phase to isolate interesting analytes from interfering materials [10].The selection of sorbent is the key factor in SPE, which depends on the properties of the target analytes, the kind of sample matrix, and the materials that need to be removed [9-12]. The ideal sorbent material to be used for SPE should not be degraded by micro-organisms when using a biological sample and it should not be affected by high temperature or storage. The SPE sorbent should be chemically stable and not react with the cleaning, conditioning, washing, or eluting solvents. In addition, one of the most important properties is a high surface area that is proportional to the capacity of the sorbent, in order to increase its loadability [13-15]. Another important property is the permeability of the SPE sorbent, in order to decrease the processing time by increasing the flow rate and decreasing the backpressure. In addition, a suitable sorbent for SPE should be able to separate desired from undesired components, and give batch-to-batch reproducibility [16, 17]. Recent materials that have been used as SPE sorbents are monolithic, which means a continuous bed consisting of a single piece of solid porous material [18, 19]. The inorganic monolithic materials have many advantages, such as mechanical strength, relatively high thermal stability, high porosity, and immunity to organic solvents. Moreover, the silica-based monoliths contain a distribution of both macropores that can increase the liquid flow through the monolith without increasing the backpressure, and mesopores that can increase the surface area, resulting in good interaction with analytes and maximization of the loadability of the sorbent [20-24]. Synthetic silica-based monolithic materials have been introduced as porous monolithic separation media in high-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrochromatography (CEC). In addition, they have been used as immobilised enzymatic reactors and as sorbents in SPE [25, 26]. Although silica-based monoliths are becoming increasingly popular as sorbents, there are few papers describing their use as materials for extraction.

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The trend in clinical analysis toward microfluidic devices is due to the decrease in sample volume, reduction in analysis time, decreased reagent consumption, portability of the microfluidic device, and the ability to integrate multiple analytical processes onto a single microfluidic device [27-31]. The aim of this study is to fabricate a microchip containing a monolithic silica disk for DNA isolation. The silica-based monolith was fabricated using the sol-gel method, and the porosity of the fabricated materials was optimised using different concentrations of ammonia solutions. After fabrication of the monolithic material, it was placed in the extraction chamber of the polycarbonate microchip for use in DNA extraction. The performance of the fabricated microchip containing the monolithic disk was compared with the commercial DNA extraction kits in terms of ability to preconcentrate DNA from mouse liver tissue.

II. Experimental

A. Chemicals and materials

Polyethylene oxide (PEO), with an average relative molecular mass MW = 10000 Da, tetraethylorthosilicate 99% (TEOS), proteinase K, tris-EDTA buffer solution (TE buffer), and guanidine hydrochloride (GuHCl) were purchased from Sigma-Aldrich (Poole, UK) and used as received without any further purification. Nitric acid, ethanol, and ammonia were obtained from Fisher Scientific (Loughborough, UK). Distilled water was employed in the preparation of all solutions and reagents. Polytetrafluoroethylene (PTFE) thread seal tape was purchased from ARCO Ltd. (Hull, UK). Disposable plastic syringes (2 mL) were purchased from Scientific Laboratory Supplies (Nottingham, UK). The ethylene tetrafluoroethylene (ETFE) tubing (1/16" x 0.17 mm i.d.) was purchased from Thames Restek Ltd. (Saunderton, UK). Double Bubble Mix and Fix epoxy resin was purchased from Bondmaster limited (London, UK), and the DNA extraction kit from Analytik Jena Ltd. (Jena, Germany).

B. Instrumentation

Scanning electron microscope (SEM) Cambridge S360 from Cambridge Instruments (Cambridge, UK), transmission electron microscopy (TEM) from JEOL Ltd. (Welwyn Garden City, UK), TEM images were acquired with a Gatan Ultrascan 4000 digital camera attached to a JEOL 2010 transmission electron microscope, energy dispersive X-ray (EDAX) analysis was performed using an INCA 350 EDAX system (Oxford Instruments, Abingdon, UK), a hot plate-stirrer from VWR International LLC (West Chester, PA, USA), UV-Vis spectrophotometer from Thermo Scientific™ GENESYS 10S (Toronto, Canada), a syringe pump from Bioanalytical System Inc. (West Lafayette, USA), a furnace from Wisd Laboratory Instrument (Wertheim, Germany), and a Remi Centrifuge from IndiaMRT (Uttar Pradesh, India) were used in the study.

C. Preparation of monolithic materials

The silica-based monoliths were fabricated by adding 0.564 g PEO to 5.074 mL of 1 M nitric acid, 0.582 mL of distilled water, and 4.512 mL of TEOS. The mixture was stirred using a stirrer for 50 min while immersed in an ice bath to promote a hydrolytic reaction. Then, the mixture was sonicated to remove any bubbles that may have formed during mixing. The resulting homogeneous mixture was poured slowly into 2 mL disposable plastic syringes. The thin end of the syringes was sealed using PTFE thread seal tape. The plastic syringes were placed in an oven and gelation occurred within 2 hours. The gelled sample was aged for 24 hours at 40 °C to give white solid rods. The monolithic silica rods were slowly tapped out of the plastic tubes because they were quite brittle at this step. The monolithic silica rods were then soaked in a water bath for 2 hours. The effect of ammonia concentration on the formation of the monolithic silica rods was investigated. Different concentrations of ammonia solution (0, 1, 5, and 7 M) were utilised for fabricated silica monolith at an elevated temperature of 85 °C for 24 hours. The monolithic rods were then washed with deionised water until a neutral pH was obtained. The monolithic silica rods were placed in the oven for 24 hours at 40 °C, followed by another 24 hours at 100 °C.

D. Fabrication of the polycarbonate microchip

The design of the polycarbonate microchip consisted of two plates, as can be seen in Figure 1.

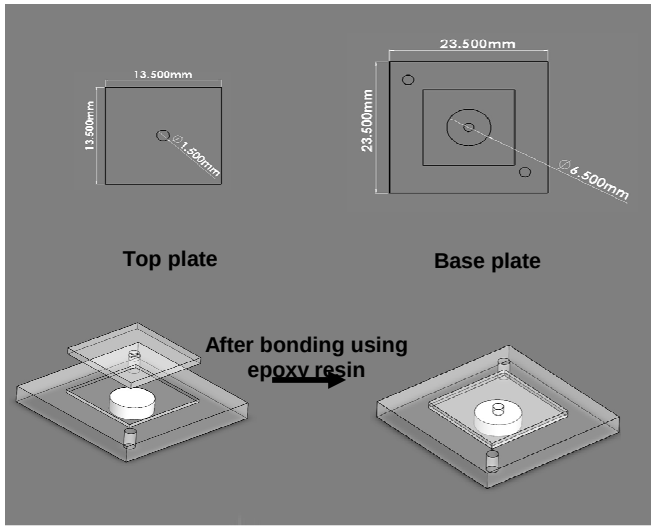

Fig. 1 Schematic diagram of the polycarbonate microchip

The dimensions of the top plate were 13.5 mm length and width, and a thickness of 1 mm with an access hole (1.5 mm). The base plate had a length and width of 23.5 mm with a thickness of 2.8 mm, and contained the extraction chamber (6.5 mm width and 2 mm depth) that was milled using a CNC (computer numerical control) milling machine, and a 1.5 mm access hole in the centre of the chamber. The monolithic silica disk was cut and placed on the extraction chamber. The two plates were fixed using epoxy resin and the ETFE tubing was fixed into the access holes drilled into the microfluidic device using epoxy resin. After bonding the two plates, the microchip was used for DNA extraction.

E. Monolithic material characterisation

The morphology of the dried monolith was characterised by scanning electron microscopy (SEM) using a Cambridge S360 scanning electron microscope, and images were obtained using an accelerating voltage of 20 kV and a probe current of 100 pA in high vacuum mode. The samples were coated with a thin layer of gold-platinum. EDAX analysis was used to find the chemical composition of monolithic materials. The formation of mesopores in the silica-based monolith was confirmed by using TEM. The samples were crushed, mixed with 1 mL acetone and sonicated for 4 min. 5 µL aliquot was put onto lacy carbon-coated 3 mm diameter copper grids. TEM images were acquired with a Gatan

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Ultrascan 4000 digital camera attached to a JEOL 2010 transmission electron microscope running at 20 kV.

F. Preconcentration of DNA

DNA extractions were carried out on mouse liver tissue. 50 mg of sample tissue was transferred to a pestle, 400 µL of lysis buffer solution (10% SDS, 0.1 mM NaCl, 0.5 mM Tris-HCl, pH=8) was added and the sample was ground up. Then, 25 µL of proteinase K was added. The sample solution was vortexed for 1min and the solution was incubated for 1 h at 50 ºC. The sample was centrifuged and the supernatant was transferred to a 1.5 mL Eppendorf tube. 500 µL of binding buffer, which was 5 M GuHCl in 10 mM TE buffer solution (pH 6.7), was added to the sample solution. The monolithic material inside the microchip was washed with 10 mM TE buffer solution (pH 6.7) for 10 min using a syringe pump at a flow rate of 5 µL min^{-1}. Then, the sample solution was flowed through the monolith at a flow rate of 2 µL min^{-1}. The monolith was washed with 80% ethanol at a flow rate 3 µL min^{-1}. Finally, the preconcentrated DNA was eluted from the microchip using 200 µL of 10 mM TE buffer solution (pH 8.5) at a flow rate of 1 µL min^{-1} and the solution was collected inside a 1.5 mL Eppendorf tube. DNA isolation from mouse liver tissue was also carried out using a DNA extraction kit according to the protocol described by manufacturers. The performance of the fabricated microchip in isolating DNA was compared to the performance of the DNA extraction kit. The purity of the isolated DNA was determined based on the UV absorption, and the absorbance of the samples before and after purification were determined at three wavelengths (230, 260, and 280 nm).

III. RESULTS AND DISCUSSION

A. Preparation of monolithic material

In this study, the silica-based monolith was fabricated using the sol-gel method, which involves three steps: hydrolysis, condensation, and polycondensation. Firstly, an alkoxy silicon derivative that was TEOS is hydrolysed to form reactive silanol groups (Si-OH), followed by condensation in order to form polycondensed species containing siloxane linkages (-Si-O-Si-) between two silane molecules [32]. The hydrolytic reaction occurred in the presence of PEO, utilised to promote the phase separation between silica and water as well as the formation of macropores and micropores in the materials. Nitric acid was utilised to start the hydrolysis and condensation reactions while distilled water was utilised as a solvent, since it is a good for reactants as well as being non-toxic and affordable [33]. In the condensation step, alcohol and water were removed in order to form a dimer, and finally the gel was formed after polycondensation reaction. Figure 2 shows a photograph of the prepared monolithic silica, showing that the monolithic silica rod was successfully fabricated through the casting method. In addition, it can be seen that the formed monolithic rod was crack-free.

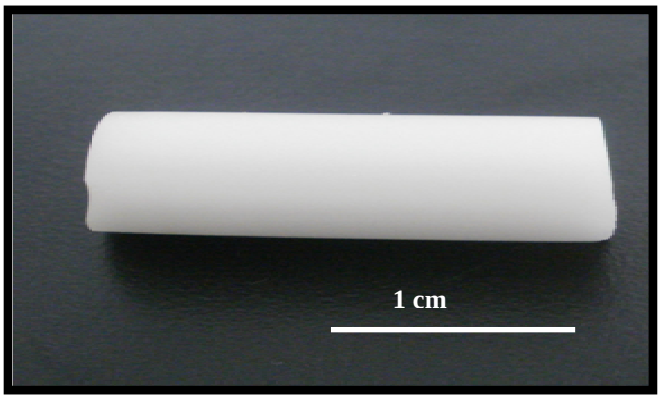

Fig. 2 shows the appearance of the fabricated monolithic silica rod

The main purpose of using a pore-tailoring medium (ammonia solution) is to increase the surface area of the fabricated monolith by converting the micropores that have a low surface area to mesopores that have a high surface area without affecting the macropores. In this work, the effect of the concentration of ammonia solution on the porosity and morphology of the fabricated materials was investigated. This was performed by using different concentrations of ammonia solution (0, 1, 5, and 7 M) while keeping the other parameters constant in order to obtain satisfactory monolithic materials. The reaction of the thermal decomposition was 24 h only, since a long period of treatment can cause a decrease in the surface area by increasing the pore size.

B. Characterisation of the prepared silica monolith

In this work, different characterisation methods were used for studying the prepared monolithic materials: EDAX analysis, TEM analysis, and SEM analysis.

EDAX analysis

The fabricated monolithic silica rods prepared using different concentrations of ammonia solution were characterised using EDAX analysis. Figure 3 shows the EDAX spectrum of the fabricated silica-based monolith. The results show that silicon peak (Si) at 1.8 keV, and oxygen (O) at 0.5 keV only without showing any peaks, which confirms that the sample did not contain any contamination. Besides, from EDAX spectra of all prepared monoliths in this study, it was found that the concentrations of ammonia solution did not affect the compositions of the prepared materials as expected.

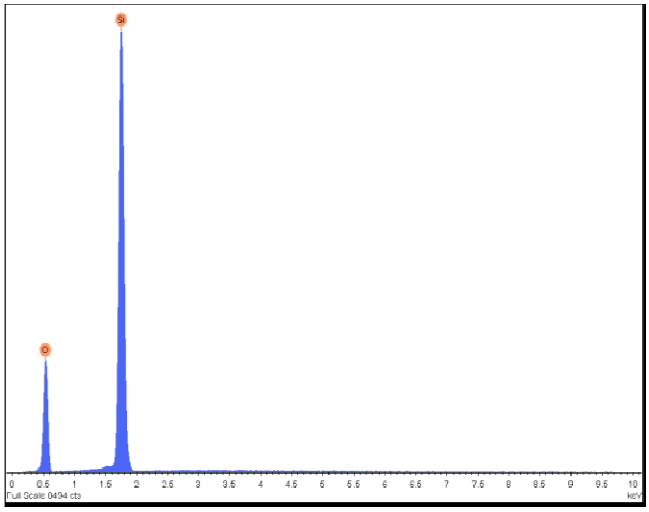

Fig. 3 EDAX image of silica-based monolith

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TEM analysis

The fabricated materials were also characterised using TEM analysis, since it can provide images of very small structures. TEM images of the prepared silica monoliths using different concentrations of ammonia solution show the high homogeneity of the all fabricated silica-based monoliths (Figure 4). In addition, TEM micrographs showed that the formation of the mesostructured silica monoliths, except the monolithic material that was fabricated without using the pore-tailoring medium, which showed the absence of the mesopores in the monolithic structure, Figure 4 (A).

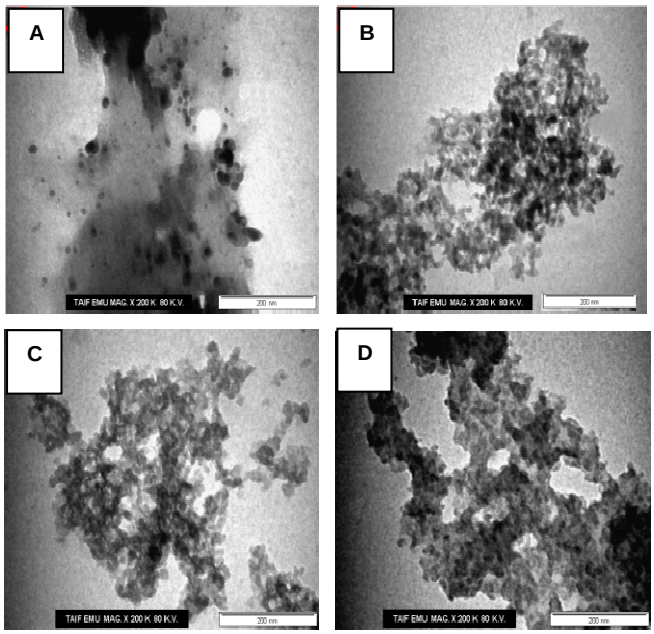

Fig. 4 TEM images of the fabricated silica monoliths using different concentrations of ammonia solution: (A) 0 M, (B) 1 M, (C) 5 M, and (D) 7 M

SEM analysis

The fabricated materials were further studied by SEM analysis at different magnifications (200X and 500X) at 15kV, as can be seen in Figure 5. It is obvious that all the fabricated materials have high homogeneity. The SEM image of the silica-based monolith prepared without using the ammonia solution illustrated that it did not contain enough macropores compared with monolithic materials prepared using ammonia solution. In addition, it was found that the size of pores depends on the concentration of ammonia solution, and the macropores of monolithic materials increased by an increase the concentration of ammonia solution from 1M to 5M; however, the morphology of the silica-based monolith was changed when the concentration of ammonia solution was increased to 7M.

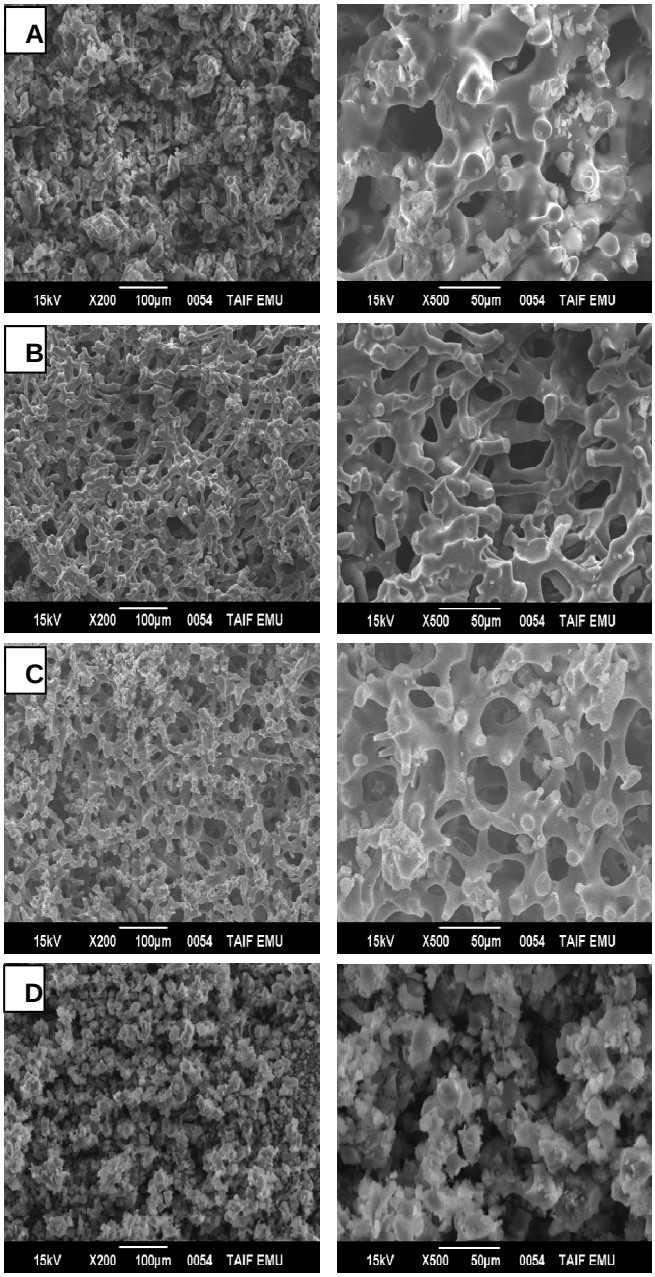

Fig. 5 SEM micrographs of the fabricated silica-based monoliths using different concentrations of ammonia solution:(A) 0 M, (B) 1 M, (C) 5 M, and (D) 7 M

Based on previous results, it was found that it is very important to use ammonia solution as the pore-tailoring medium to create the mesostructured silica monolith. It was found that the suitable concentration of ammonia solution to prepared silica-based monolith in this study was 5M since it can form a monolithic material with high macropores, allowing for high flow rates.

C. Extraction of DNA

The aim of the DNA purification is to remove interfering materials and preconcentrate DNA. As a result, the signal intensities of DNA will be improved, and the quantities of DNA can be easily measured. In this work, the prepared monolithic disk was cut and placed on the extraction chamber on the base plate of the polycarbonate microchip and then the top and base plates were fixed together using epoxy resin. Microchip are used to reduce the volume of reagents and the sample. Additionally, the reaction time can be decreased and reactions can be carried out more safely since the size of the

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reactors are small. Moreover, the ratio between the inner surface area of the chamber to the volume of solution is higher than that of conventional systems. This can increase the interaction between the fabricated materials used and the analyte [34]. After preparation of the microchip, it was ready for use in DNA extraction. In the present investigation, DNA was isolated from mouse liver (50 mg). Before injection of the sample, the monolithic material inside the polycarbonate microchip was pretreated with 10 mM TE buffer solution (pH 6.7) for 10 min using a syringe pump at flow rate of 5 µL min^{-1}. This step is very important at this stage in order to activate the surface of silica-based monolith inside the microchip and produce optimal DNA isolation. After this pretreatment step, 500 µL of the sample was injected inside the microchip using a syringe pump at a flow rate of 2 µL min^{-1}. The monolith was washed with 80% ethanol at a flow rate of 3 µL min^{-1} to remove any remaining proteins and cellular contaminants. The preconcentrated DNA was eluted from the microchip using 200 µL of 10 mM TE buffer solution (pH 8.5) at a flow rate of 1 µL min^{-1} and collected in 1.5 mL Eppendorf tube. During the experiment, it was found that the monolithic material inside the extraction chamber of the microchip showed high permeability and high flow rate could be used without leakage. The purity of the isolated DNA from the commercial kit and the fabricated microchip was determined based on the UV absorption, and the measurements were repeated three times. The purity was assessed at the 260/230 nm and the 260/280 nm absorbance ratios and the averages of the measurements were taken to measure the efficiency and presence of proteins and RNA contamination; the results are presented in Table 1.

Table 1: Purity ratios of extracted DNA from mouse liver by the commercial kit and the fabricated microchip. The values are averages ± standard errors based on three replications

Method	Crude DNA A_{260}/A_{230}	Crude DNA A_{260}/A_{280}	purified DNA A_{260}/A_{230}	purified DNA A_{260}/A_{280}
Commercial kit	0.89±0.09	0.68±0.06	1.26±0.04	1.13±0.02
Fabricated microchip	0.97±0.07	0.74±0.08	1.75±0.05	1.70±0.04

The UV absorption ratio of A_{260}/A_{230} was 1.26±0.04 for commercial kit and 1.75±0.05 for fabricated microchip, while the UV absorption ratio of A_{260}/A_{280} was 1.13±0.02 for commercial kit and 1.70±0.04 for fabricated microchip. These results indicate that DNA extracted via the fabricated microchip containing the monolithic material yielded DNA that was relatively free from protein contamination compared to the commercial kit.

IV. Conclusion

In this study, fabrication of the inorganic silica-based monolith using a sol-gel method was investigated. The fabricated silica-based monolith placed in a polycarbonate microchip for DNA extraction was performed. It was concluded that using a pore-tailoring medium, which was aqueous ammonia solution (5M), was suitable to fabricate the monolithic materials in this study. The results of this study indicate that the silica-based monolith had high porosity, offered high surface area, and had lower backpressure. In addition, the results showed that the silica-based monolith was permeable, has the ability to remove impurities, and the data showed a good degree of purity when using the microchip.

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