A simple cost-effective microfluidic platform for rapid synthesis of diverse metal nanoparticles: A novel approach towards fighting SARS-CoV-2

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ABSTRACT

The latest addition to the family of Coronaviruses, SARS-CoV-2, unleashed its wrath across the globe. The outbreak has been so rapid and widespread that even the most developed countries are still struggling with ways to contain the spread of the virus. The virus began spreading from Wuhan in China in December 2019 and has currently affected more than200 countries worldwide. Nanotechnology has huge potential for killing viruses as severe as HIV, herpes, human papilloma virus, and viruses of the respiratory tract, both inside as well as outside the host. Metal-nanoparticles can be employed for biosensing methodology of viruses/bacteria, along with the development of novel drugs and vaccines for COVID-19 and future pandemics. It is thus required for the nanoparticles to be synthesized quickly along with precise control over their size distribution. In this study, we propose a simple microfluidic-reactor-platform for in-situ metal-nanoparticle synthesis to be used against the pandemic for the development of preventive, diagnostic, and antiviral drug therapies. The device has been fabricated using a customized standard photolithography process using a simple and cost-effective setup. The confirmation on standard silver and gold metal nanoparticle formation in the microfluidic reactor platform was analysed using optical fiber spectrophotometer. This novel microfluidic platform provides the advantage of in-situ synthesis, flow parameter control and reduced agglomeration of nanoparticles over the bulk synthesis due to segregation of nucleation and growth stages inside a microchannel. The results are highly reproducible and hence scaling up of the nanoparticle production is possible without involving complex instrumentation.

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1. Introduction

The world is still fighting the battle against COVID-19which began in late 2019. As per the latest data of WHO (Fig. 1), 1.7 million deaths have been reported worldwide [1]. Fig. 1 shows the number of deaths since September 2020. Researchers all over the globe are looking for novel ways to stop the contagion are on their way to provide help to the human community in terms of diagnostic kits, protective gear such as masks, sanitizers, detection kits, and vaccines to cure the infected people. Antiviral drugs available in the market usually target specific viral infections and focus on inhibiting their further development, instead of killing them.

Various nanomaterials have been used successfully as nanomedicine, namely, nanopolymers [2], dendrimer, oligomers, NPs, liposomes, and small molecules [3–7]. Except for nanoparticles, all other nanomaterials lose efficacy when the virus-compound complex disintegrates. NP based broad-spectrum drugs can be synthesized in such a way that the virion is irreversibly damaged. In cancer research, combined therapeutics and diagnostics have been extensively explored; however, considerable efforts have been made in the past few years to expand the scope of this approach to other areas which includes infectious diseases [8].

One important technology used in the preparation of antimicrobial drugs is nanotechnology. It utilizes the physical and chemical properties of the nanomaterials to control their shapes, sizes, and
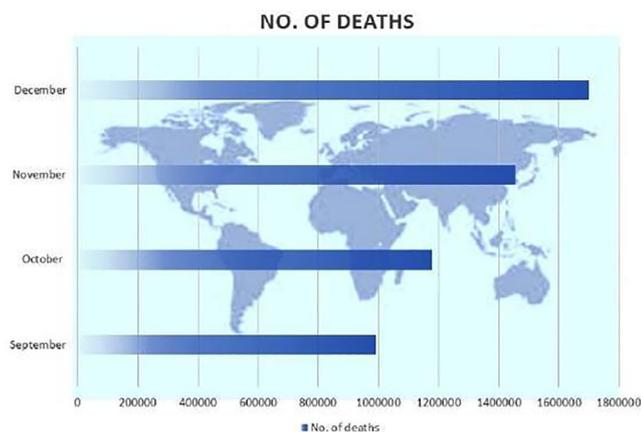
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Fig. 1. Chart showing number of deaths worldwide since September [1].

other surface parameters. Metal nanoparticles, to be specific, seem to be highly favourable about the subject under consideration. Various detection schemes and therapies are being conducted all over the world for the present pandemic. Silver and Gold nanoparticles are continuously being exploited for their antiviral properties. The size of the nanoparticles lies in the same range as the COVID-19 virus [8]. Silver nanoparticles are well known for their antibacterial properties, and their antiviral nature has also been proved to be effective against several viruses such as Hepatitis B, Human Immunodeficiency Virus (HIV), Respiratory Syncytial Virus, and Monkeypox Virus [2]. Disinfectants make use of nanomaterials for killing pathogens. Colorimetric nano-biosensors with built nanoparticles can identify explicit cell types for various illness determination [9]. Gold nanoparticles (AuNPs) are utilized generally in different organic applications because of their special optical properties. AuNPs are simple and affordable to work with, because of their moderately simple amalgamation, effortless surface science, excellent biocompatibility, remarkable properties, and distinctive surface plasmon resonance (SPR) phenomenon [10].The use of nanoparticles is not restricted to detection as they are already being used by researchers in developing antiviral drugs for the SARS-CoV-2 virus [11]. The nanotechnology domain can contribute essentially to the battle against COVID-19 by becoming a bridge between diagnosis and therapy. Nanomaterials have been utilized for the advancement of point-of-care diagnostics, transporters for therapeutics, and antibody improvement. The research, innovation and its deployment are the key weapons that can help us to fight with the current pandemic.

Nanodevices can be adjusted to recognize, treat, and prevent this illness from further spreading [12]. With sufficient emphasis towards the virus, it should not go unmentioned that nanoparticles are not only restricted to their usage infighting COVID-19 but play a variety of roles as biological tags in biosensors, as antibacterial coatings, in paints, optics, and many more. So, there is a need to synthesize these nanoparticles in a fast, controlled as well as in a closed and safe environment. In this paper, we propose a microfluidic based platform for in-situ nanoparticle synthesis towardsSARS-CoV-2 virus detection, its use in various preventive measures being taken as well as for therapy principles. The idea is to build a cost-efficient platform for closed, contamination-free synthesis of metal nanoparticles. The technique used to develop the platform is easy to replicate and capable to be extended on a large scale in parallel integration. The work presented here is unique in its design as reported to date with continuous circular serpentine to achieve high mixing efficacy; allows for a time separated nucleation and growth stages giving way for minimal agglomeration of nanoparticles and provides control of synthesis

parameters such as flow rate, flow velocity, the concentration of reagents, temperature, etc.

2. Theoretical principle

2.1. Nanoparticle synthesis in microfluidics

The synthesis protocol for inorganic nanoparticles comprises nucleation and growth stages. During batch fabrication, both these stages along with agglomeration of nanoparticles occur together due to lack of control over the process parameters, leading to high irregularities in nanoparticle size [13].

In contrast, the microfluidic synthesis of nanoparticles allows for spatial separation of the nucleation and growth stages of nanoparticles, better control of experimental parameters such as size distribution, enhances mixing, high reproducibility, high surface-to-volume ratio leading to improved sensitivity, and compatible integration with an online interface for optimization and feedback control which is otherwise not possible with the conventional batch fabrication process. Fig. 2 shows the different stages of nanoparticle synthesis inside the fabricated device [14].

2.2. Microfluidic mixing

The two kinds of mixing strategies adopted at microscale are Passive mixing and Active mixing. Since Reynold's number, defined as the ratio of inertial flow to viscous flow is very small at micro domain, i.e., $Re \ll 1$ since viscosity dominates inertia, the microfluidic devices essentially work in the laminar flow regime. Under those conditions, diffusion becomes the primary phenomenon for two or more fluids to mix. The time to mix, τmix, inside a continuous microfluidic channel is calculated using the following equation:
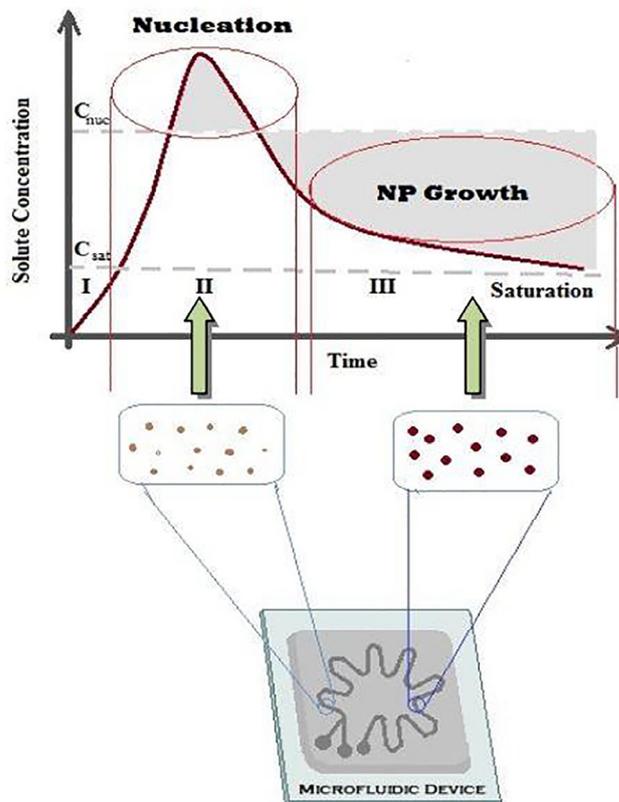

Fig. 2. The LaMer model of nucleation and growth in a microfluidic platform.

$$\tau_{mix} \sim \frac{w_f{}^2}{4D} \approx \frac{w^2}{9D(1 + 1/R^2)}$$

where w is the channel width, w_f is the width of the focused stream, D is the diffusivity of the solvent in the core stream and R is the ratio of the core stream rate to the total flow rate of surrounding streams [13].

3. Design and fabrication

The device is designed in a continuous circular serpentine in microscale, that ensures the reducing agent and metal salt mix together well, resulting into monodispersed metal nanoparticles. The design consists of two inlets for the two reactants and an outlet for the collection of synthesized nanoparticles with a channel width 300 μm, height 200 μm, and total length of 70 mm was designed to ensure proper mixing of the reagents inside the microchannel. Being minute in volume and scale, the microchannels provided a supportive site for the reaction to occur between the metal salt and the reducing agent leading to the formation of nanoparticles. The photomask was designed using Autodesk Auto-CAD2019 software (Fig. 3a) customized soft lithography technique was adopted for the fabrication of the device, as reported in our earlier studies [15]. The customized protocol for fabrication was adopted which involved the following steps: Firstly, the photoresist, SU-8 50, was spin-coated for 20 s at 1500 rpm to achieve the desired channel thickness. Then, the photoresist coated substrate was prebaked with a ramped temperature of 65 °C to 95 °C for 5 min and 20 min, respectively. Next, the spin-coated resist was exposed with UV-light (365 nm) after contact alignment of printed mask with photoresist. After the exposure, the Post-exposure bake was done with a ramped temperature of 65 °C to 95 °C for 1 min and 5 min, respectively. Finally, it was dipped in SU-8 developer to remove then on-cross linked part of the resist to obtain the mould. Further, Polydimethylsiloxane (PDMS) polymer was used for creating the channel replica via the soft-lithography process. A mixture was prepared by mixing elastomer and its curing agent (Dow Corning) in the 10:1 ratio. The bubbles generated while mixing, were removed by desiccating it and then it was poured over the mould. Further, the poured PDMS mould can be placed on the hot plate at 80 °C for 20 min or can be left overnight to harden the PDMS.

Then PDMS replica was removed from the substrate and inlet–outlet holes are punched followed by sealing it on a glass substrate by air plasma treatment to prepare the final device (Fig. 3b).

4. Experimental set-up

The device has been designed in a way that it provides a platform to properly follow the nanoparticle synthesis procedure as done on the macroscale with precise control over the synthesis parameters. Generally, two reagents, a metal salt, and a reducing agent are required to synthesize metal nanoparticles followed by stabilizing the synthesized nanoparticles by using a stabilizing agent, so, as to prevent agglomeration. Two Cole-Parmer syringe pumps were set up to control the flow rates of the two reactants to synthesize the nanoparticles (Fig. 3a).

The nanoparticles were then collected from the outlet of the device into the Eppendorf tube (Fig. 4a). The microfluidic device presented here takes the input of two solutions via inlets i.e., the metal salt solution and the reducing agent (Fig. 4b). These react inside the microchannel and result in the formation of metallic nanoparticles. The nanoparticles thus synthesized are collected and further analysed for confirmation using spectrometry. The synthesis parameters selected for gold and silver nanoparticles are taken from the previously reported work [16,17].

4.1. Silver nanoparticles

The silver nanoparticles were synthesized chemically using silver salt ($AgNO_3$), a reducing agent ($NaBH_4$), and NaOH was used to stabilize the synthesized silver nanoparticles simultaneously. Two solutions, (i) Solution A: 10 mL of 10 mM NaBH4 in 30 mM NaOH and (ii) Solution B: 2 mL of 1 mM $AgNO_3$ were freshly prepared in Milli-Q ultrapure water (Millipore) having a resistivity of 18.2 MΩ-cm. In a closed cabinet maintained at room temperature, Solution A and Solution B were fed to the inlet of the microchannel with a flow rate of 50 μl/min and 10 μl/min respectively (Fig. 4a). The synthesized product was collected at the outlet (Fig. 5a).

4.2. Gold nanoparticles

The gold nanoparticles were synthesized chemically using gold salt (HAuCl4), a reducing agent sodium citrate ($Na_3C_6H_5O_7.2H_2O$), and L-lysine was used for capping the synthesized gold nanoparti-
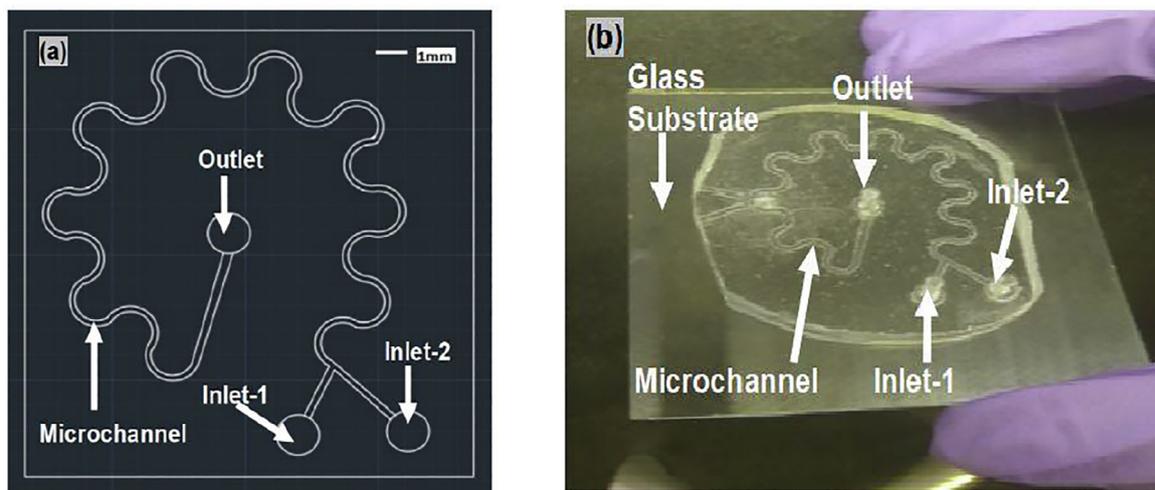

Fig. 3. (a) Representation of UV Photo-Mask design created via AutoCAD software. Width: 300 μm, total length: 70 mm and height: 200 μm. (b) Image of the actual fabricated device. The device is fabricated via soft-photolithography process.
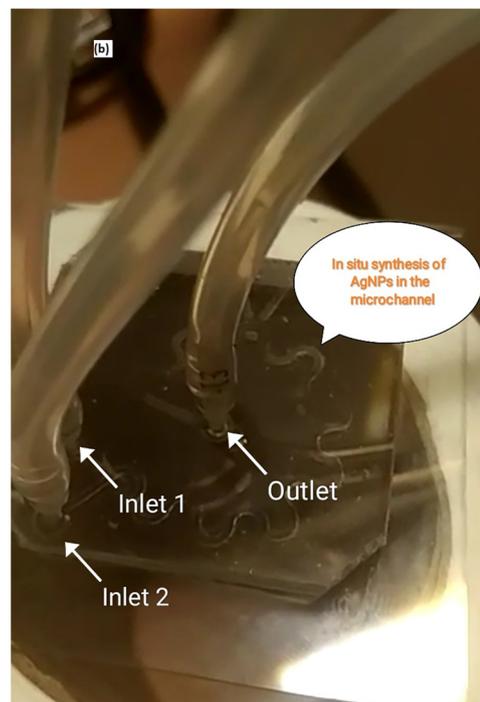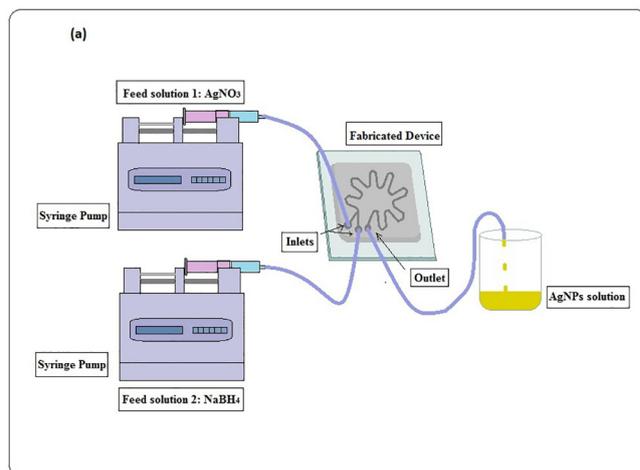
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Fig. 4. (a) Schematic diagram of the experimental setup. Two syringe pumps are pumping reagent solutions inside the microfluidic device through different inlets at precise flow rates. The desired nanoparticles are synthesized inside the channel and are obtained in an Eppendorf tube via outlet. (b) The final microdevice connected with the inlet and outlet tubings for the defined experimental set-up.
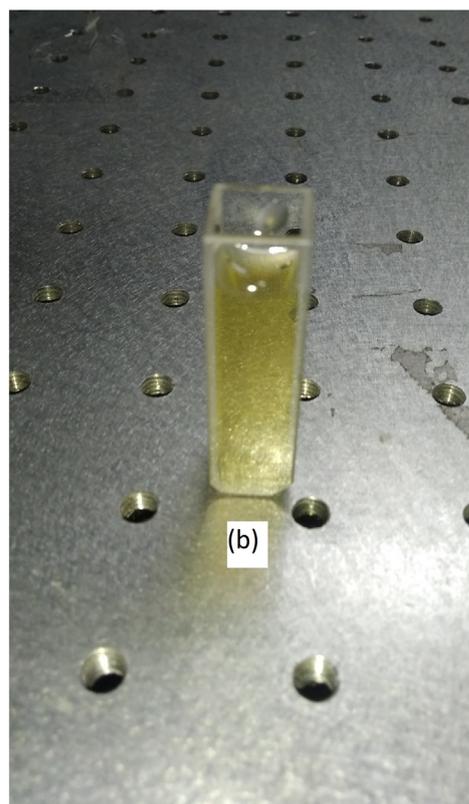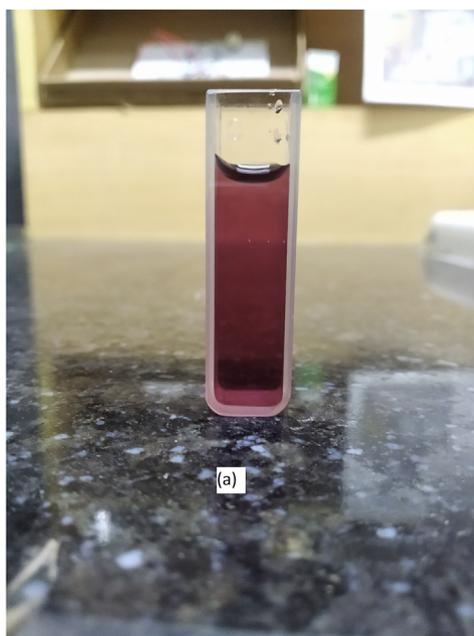

Fig. 5. a) Synthesized AuNPs (b) Synthesized AgNPs.

cles simultaneously. Two solutions, solution C: 10 mL of 22 mM $Na_3C_6H_5O_7.2H_2O$ with L-lysine and solution D: 2 mL of 2 mM

$HAuCl_4$ were freshly prepared in Milli-Q ultrapure water (Millipore) having a resistivity of 18.2 MΩ-cm. Solution C and D were
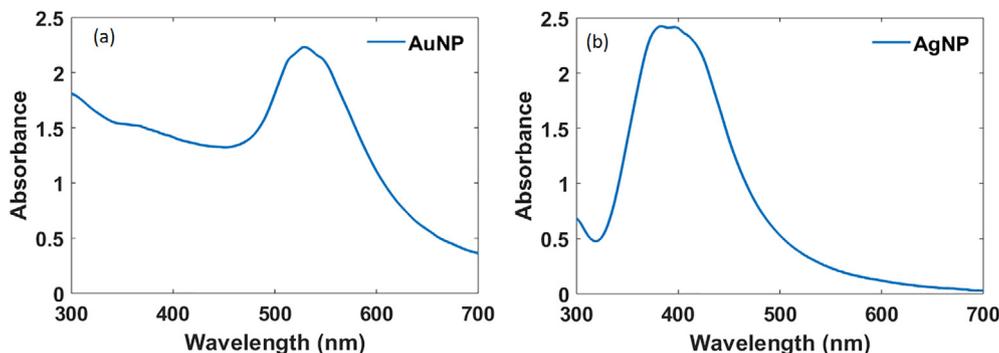
Fig. 6. (a) UV–Vis absorbance graph for synthesized AuNPs (b) UV–Vis absorbance graph for synthesized AgNPs. The spectrum was obtained using the Ocean-view Fiber optics spectrophotometer.
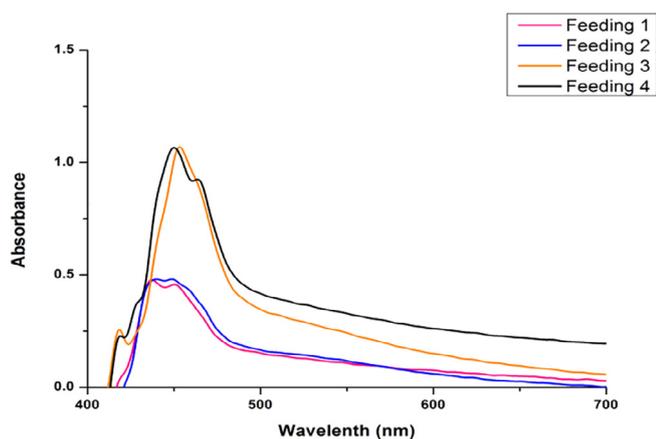

Fig. 7. UV–Vis absorbance graph for synthesized AuNPs at different flow rates [Table 1].

then fed to the inlet of the microchannel with a flow rate of 20 μl/min and 4 μl/min respectively (Fig. 4a). The synthesized product was then collected at the outlet (Fig. 5b).

4.3. Controlled synthesis of gold nanoparticles with varying flow rates

Two solutions, Solution 1: 5 mL of 0.5 mM HAuCl4mixed with 5 mL of 0.1 M CTAB solution and Solution 2: 1 mL 0.01 M NaBH4 were made freshly in DI water and feed with different flow rates as shown in Table 1.

5. Results and discussion

5.1. Experimental results

After the collection of the synthesized silver and gold nanoparticles, the UV–Vis spectroscopy was performed using the Ocean optics instrument. The absorbance peak for gold nanoparticles and silver nanoparticles was observed at 525nm (Fig. 6a) and 390 nm (Fig. 6b) respectively, which verifies with the literature

Table 1
Flow rates of reagents used for different feedings.

Feeding	Solution 1 [Flow rate] [μL/min]	Solution 2 [Flow rate] [μL/min]
1	10	1
2	20	2
3	60	6
4	80	8

[18,19]. Also, by varying the reaction times, temperatures, mixing efficiency, and reagent concentrations, the quality of the nanoparticles can be controlled [20]. The inlet reagents flow rates at different feedings are shown in Table 1; and their effects on the size of synthesized nanoparticles have been presented in Fig. 7, via the UV–Vis absorbance graph for synthesized AuNPs.

5.2. Applications

Noble metal nanoparticles find their use in a variety of industries because of the wide range of bactericidal and viricidal properties. The optical property exhibited by the silver and gold nanoparticles owing to the Surface Plasmon Resonance (SPR) effects has opened avenues for them in spectroscopy techniques. Another important application of metal nanoparticle is in qualitative and quantitative detection in biosensors.

The proposed in-situ synthesized nanoparticle can also be utilized as coatings in developing masks, sanitizers, inside detection kits, as well as in vaccines, keeping the current pandemic situation in mind. The whole process requires a basic fabrication laboratory setup and developing this solution will be a quick and effective procedure.

6. Conclusion

. In this paper, a novel microfluidic platform for metal nanoparticle synthesis has been investigated. Gold and Silver nanoparticles synthesized through the proposed microdevice were characterized via the UV-Visible spectroscopy process. Further, the procedure described in this paper can be easily adapted to synthesize other metal nanoparticles of different compositions, shapes and sizes, also, by controlling various experimental parameters such as flow rates and concentration of the diverse reactants. The physical, chemical and optical properties of nanoparticles change with their change in shapes and sizes and thus can be exploited in a variety of applications. In the proposed study, microfluidics provides an excellent platform for uniform and monodisperse nanoparticle synthesis. Additionally, the synthesised nanoparticles may be widely used in (bio)sensing, imaging, drug delivery, therapeutics, and diagnostics applications. Metal nanoparticles, especially noble metals, may have the potential in playing a crucial role in controlling the COVID 19 pandemic by aiding in its detection, diagnosis, drug development measures, as well as, by the photocatalytic inactivation of the SARS-CoV-2 virus. The proposed microdevice and in-situ method thus serve the purpose of faster nanoparticle synthesis which will, in turn, enhance the rate of research in this respect.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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