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Multifunctional nano-magnetic particles assisted viral RNA-extraction protocol for potential detection of COVID-19

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ABSTRACT

The enduring outbreak of the corona virus disease (COVID-19) originated from China illustrates global concerns owing to their infective nature and time-consuming incubation. Even though diagnostic tools based on RT-PCR are being extensively employed at present, well-timed and precise diagnostics are still restricted because of the long-period and exhaustive man-power requirement. To tackle this problem, we herewith report the fabrication of the surface functionalised magnetic nanoparticles (MNP's) and viral RNA-extraction protocol for potential detection of COVID-19. The zinc ferrite nanoparticles were prepared by combustion synthesis and its surface was functionalised by silica and carboxyl-modified polyvinyl alcohol. The MNP's were characterized to confirm the nano-scale appearance and functionalization. The proposed model may provide the ability to extract the viral RNA from several specimens through automation process. In light of ease and proficiency, this protocol may significantly lessen the operation period and necessities for the present molecular-level diagnostic of COVID-19.

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1. Introduction

Recently, the epidemic infective virus disease named as 'Corona Virus Disease (COVID-19)' impacted the human life resulting in more than 309 k deaths over the globe [1]. The Corona virus is a novel virus associated with the equivalent group of viruses as 'Severe Acute Respiratory Syndrome (SARS)' and some kinds of regular cold [2]. Recent attempts have been exaggerated into the clinical diagnostic of COVID-19, together with critical patients and treatments of already infected individuals. Even though the genomic sequentially of COVID-19 has been entirely exposed and a variety of Reverse-transcription polymerase chain reaction (RT-PCR) assisted diagnostic kits have been investigated, clinical level diagnostic of COVID-19 is still a demanding task [3].

RT-PCR is recognised as a high quality and consistently employed tool to analyse and quantify the different RNA's in the labs and clinical diagnostics due to elevated sensitiveness in RNA amplification. Subsequent to the occurrence of COVID-19, numerous techniques assisted with RT-PCR for the detection of COVID-19 have been reported [4,5]. Even though, RT-PCR assisted techniques have been extensively applied in COVID-19 detection, their applicability in precise detection of virus and outbreak control is relentlessly hindered due to time taking processes and man-power requirement. The pure quantification of nucleic acid extracts from the complexive specimens are the main requirement for potential RT-PCR analysis [6]. The lower efficiency of the extraction may result in the unfortunate indications through the amplification process and consequently appears in the wrong diagnosis. Conventional techniques for nucleic acid binding consist many steps which are lengthy, time taking and susceptible to impurities [7]. In the supervision and controlling of impulsive epidemics like COVID-19, these conventional techniques may consume heavy man-power, however, lacking efficient detection with a higher risk of cross-infections. Therefore, rapid, expedient and automotive techniques for the extraction of nucleic acid are extremely necessary not only in COVID-19 detection but also for other diseases.

To tackle these drawbacks, magnetic nanoparticles (MNP's) assisted extraction techniques are convenient, simple and companionable with automotive processes [8,9]. The surface functionalised MNP's adsorbs the nucleic acid from the lysis solution and are quickly isolated from most of the contaminations with the help of external magnetic field. Following this quick process, nucleic acid can be further isolated from the functionalised surface of MNP's by desorption process in eluent. Even though this technique is much easier and quicker than conventional techniques, MNP's assisted extraction process still consists of several steps, which is insufficient for practical detection.

In light of this, a more simple and modernise MNP's assisted RNA-extraction protocol is proposed for potential extraction and RT-PCR-based diagnosis of COVID-19. The MNP's of zinc ferrite (ZNF) were fabricated by the cost-efficient sol-gel auto-combustion route and subsequently, its surface was functionalised with carboxyl containing polymers (CPoly). Among the magnetic materials, zinc ferrite was chosen due to its high chemical stability, soft magnetic behaviour, easy preparation and biocompatible nature [10,11]. Due to the powerful interface among nucleic acids and carboxyl groups, the surface functionalised MNP's facilitate speedy and potential adsorption of viral RNA's. Due to simple and cost-effective nature of this technique, it may provide a capable substitute for conventional techniques.

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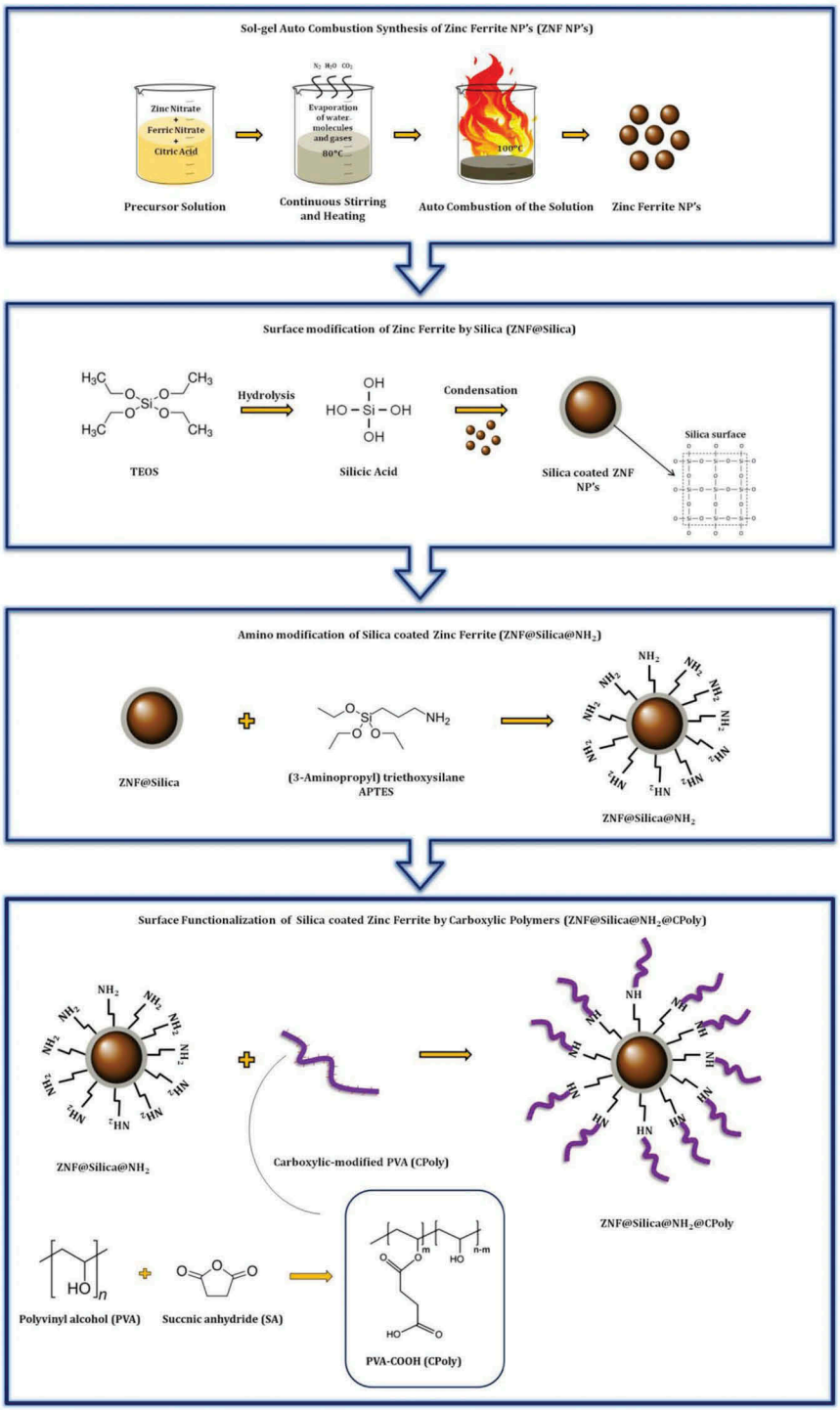

Figure 1. Schematic procedure for preparation of surface functionalised zinc ferrite NP's.

2. Experimental

The pristine zinc ferrite nanoparticles (ZNF NP's) were prepared by sol-gel auto-combustion technique [12,13]. In brief, nitrates of metal ions (Zn^{2+}, Fe^{3+}) along with citric acid ($C_6H_8O_7$) were dissolved in DD water and were continuously stirred for half an hour. The pH of the solution then adjusted to the 7 by the addition of liquid ammonia. Further, the pH adjusted solution was continuously stirred and heated at 80°C until it gets converted into gel. After the formation of gel, the heat was raised to 120°C which resulted in the auto combustion of the gel to form a fluffy loose powder. The powder was then grinded and sintered at 600°C for 4 h to acquire good crystallinity. The sintered ZNF NP's then used to prepare silica-coated ZNF NP's (ZNF@Si). For this, 0.5 g of ZNF NP's, 20 mL DD water and 15 mL NaOH were inserted into 200 mL of ethanol and ultrasonicated for 15 min at 27°C. Further, 1.5 mL of TEOS was then inserted drop-by-drop and continuously stirred for 3 h at 27°C to form as silica layer on the core of ZNF NP's. The silica modified ZNF NP's then accumulated using magnet and washed many times with DD water and ethanol to eliminate excessive TEOS. Afterwards, the APTES (0.25 mL) was added drop-by-drop in the solution, containing the mixture of 0.3 g of ZNF@Si and 100 mL isopropanol and ultrasonicated for 4 h to modify the surface of ZNF@Si with NH_2. The NH_2 modified ZNF@Si (ZNF@Si@NH_2) then accumulated using magnet and washed many times with DD water and ethanol to eliminate excessive APTES. Further, 0.1 g of

ZNF@Si@NH_2 was inserted into the DMSO solution and subsequently mixed with 3 g of carboxyl-modified PVA (CPoly) and then ultrasonicated for 15 min. Further, the NaOH solution was added into the mixture and exposed to the sonication of 30 min under continuous stirring. The CPoly functionalised ZNF@Si@NH_2 (ZNF@Si@NH_2 @CPoly) then accumulated using magnet and washed many times with DD water and DMSO to eliminate excessive CPoly. The schematic procedure for this whole process is given in Figure 1. The necessary information of the characterisation techniques used for the present investigations is provided in Table 1.

3. Results and discussion

Figure 2(a) displays the TEM image of the surface functionalised ZNF NP's, which shows the spherical shape morphology of the particles. Some kind of agglomeration was observed in the TEM image, which may be due to the magnetic interface between the particles. The silica layer on the core of ZNF NP's can be observed in the TEM image, which confirms the successful attachment of silica on ZNF NP's. Figure 2(b) displays the particle size distribution of the surface functionalised ZNF NP's. The average particle size was found to be 10.46 ± 1.5 nm. This shows the nanocrystalline nature of the prepared surface functionalised ZNF NP's.

The nanocrystalline appearance of the pristine ZNF NP's and surface functionalised ZNF NP's was determined via recording XRD patterns as shown in Figure 2(c). Both the XRD patterns show single phase formation of MNP's with formation of spinel cubic structure. No significant changes was observed in the XRD pattern of surface functionalised ZNF NP's. However, slight background noise was observed in second XRD pattern due to the amorphous nature of PVA and silica. The crystallite size values determined from 'Debye-Scherrer's formula' was found in the range is 12–14 nm showing the nanoscale formation of both the pristine ZNF NP's and surface functionalised ZNF NP's.

The TGA curves for both the pristine ZNF NP's and surface functionalised ZNF NP's are shown in Figure 2(d). The same kind of initial loss in weight below 250°C was observed for both the samples, which is appeared due to the removal of hydroxyl groups and water molecules remained during synthesis process. The total weight loss of 2.98% was observed for pristine ZNF NP's. No further weight loss was observed above 400°C for pristine ZNF NP's. The two-step loss in the weight up to 500°C was observed for surface functionalised ZNF NP's which credited to the decomposition and consequent elimination of coating agents. This weight loss of 16.24% mainly originated for the silica and polymer coating content present over the ZNF NP's. It assures the total % of coating layer presented on the core of ZNF NP's. Moreover, TGA analysis confirms that coating agent bonded chemically with the ZNF NP's core and not by means of adsorbing the surface of ZNF NP's [14,15].

The Zeta potentials of pristine ZNF NP's, ZNF@Si, ZNF@Si@NH_2 and ZNF@Si@NH_2@CPoly were measured to authenticate the surface functionalisation of ZNF NP's. Figure 2(e) displays Zeta potentials of pristine ZNF NP's, ZNF@Si, ZNF@Si@NH_2 and ZNF@Si@NH_2@CPoly. It is noted fromFigure 2(e) that average zeta potential of pristine ZNF NP's is −12.37 ± 0.92 mV which changes to 15 ± 0.85 mV for silica modified ZNF NP's. This change in zeta potential can be due to the change in nanoparticles surface from hydrophilic-to-hydrophobic by the silica surface. This zeta potential values further increases to 23.87 ± 0.79 mV for NH_2 modified MNP's. The NH_2 groups available on silica modified ZNF NP's makes it more hydrophobic to increase the zeta potential value. The significant decrease up to −41.67 ± 0.65 mV in zeta

Table 1. Characterisation techniques employed for current work.

Characterisation tool	Make and model	Specification
'Transmission Electron Microscopy (TEM)'	Make: PHILIPS; Model: CM-200	Energy: 20–200kV; Resolution: 2.4 Å
'X-ray diffraction (XRD)'	Make: Rigaku; Model: Ultima IV	Room Temperature; 2θ range: 20–70°; Cu-K_α, λ = 0.154 nm; step size: 0.040°/ 76.80 s
'Thermogravimetric analysis (TGA)'	Make: Shimadzu; Model: TGA-51	Room temperature to 800°C;
'Dynamic light scattering (DLS) and Zeta potential'	Make: Horiba; Model: SZ-100	Room temperature; Accuracy: ±0.30 mV and ±1.25 nm;

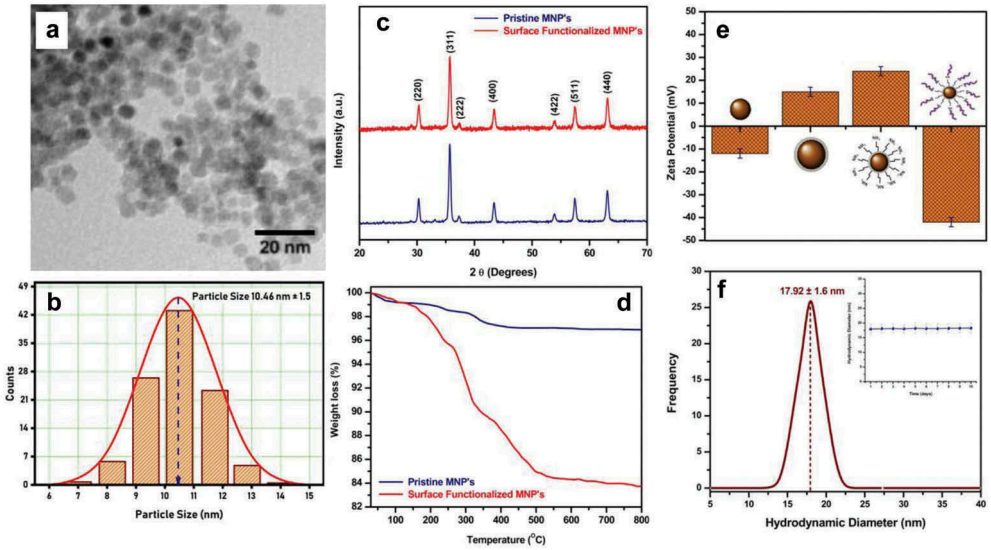

Figure 2. (a) TEM image (b) Particle size distribution (c) XRD patterns (d) TGA plots (e) Zeta potential and (f) hydrodynamic diameter of surface functionalised zinc ferrite NP's.

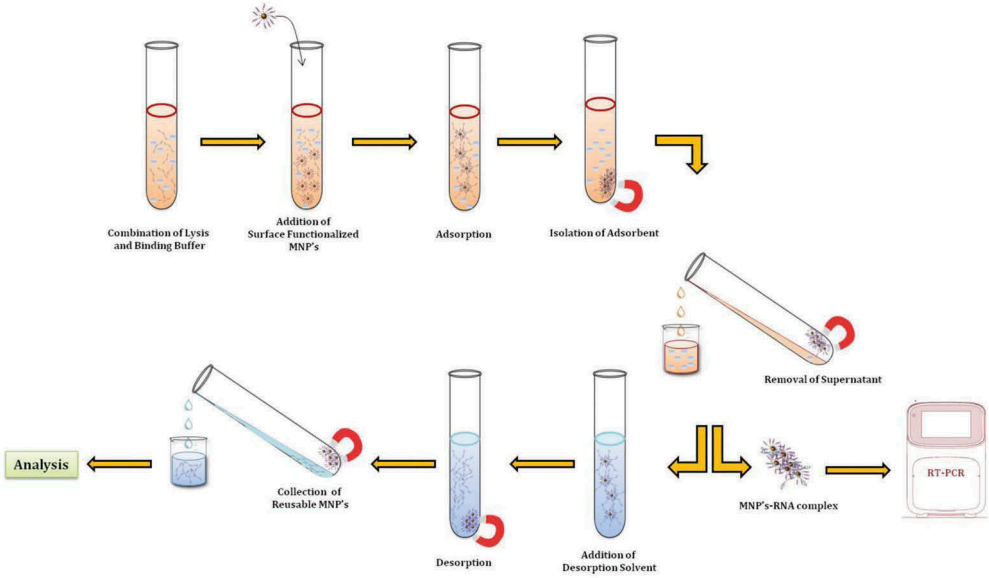

Figure 3. Schematic procedure for surface functionalised MNP's assisted RNA-extraction protocol.

potential value was noted for the surface functionalised ZNF NP's, which signifies the successful CPoly functionalisation of ZNF NP's. In addition to this, the noteworthy change in zeta potential values indicates the enhanced colloidal stability. Figure 2(f) displays the HDD size of the surface functionalised ZNF NP's calculated using the dynamic light scattering (DLS). The HDD size of the surface functionalised ZNF NP's was noted as 17.92 ± 1.6 nm with no noticeable aggregation. The insignificant changes in HDD size were observed even after 10 days (inset of Figure 2(f)), which displays the enhanced stability of the surface functionalised ZNF NP's.

Figure 3 gives the schematics of the surface functionalised ZNF NP's assisted RNA-extraction protocol for potential detection of viral RNA.

The protocol contains some steps such as the combination of lysis and binding buffer, the addition of the surface functionalised ZNF NP's, adsorption, removal of supernatants, desorption, the collection of RNA sample for RT-PCR and recovery of MNP's. The steps following the adsorption can also be omitted to lessen the time required for further processes. Thus, MNP's-RNA complexes can be directly used for the amplication in the RT-PCR process. As this process does not include any centrifugation process, the same RNA-extraction protocol can be automated as shown in Figure 4. This automation process tackles the disadvantageous centrifugation process of the conventional approach. Studies related to the real-time RNA-extraction process and RT-PCR amplification are in progress and will be presented in our upcoming reports. Figure 5 shows the

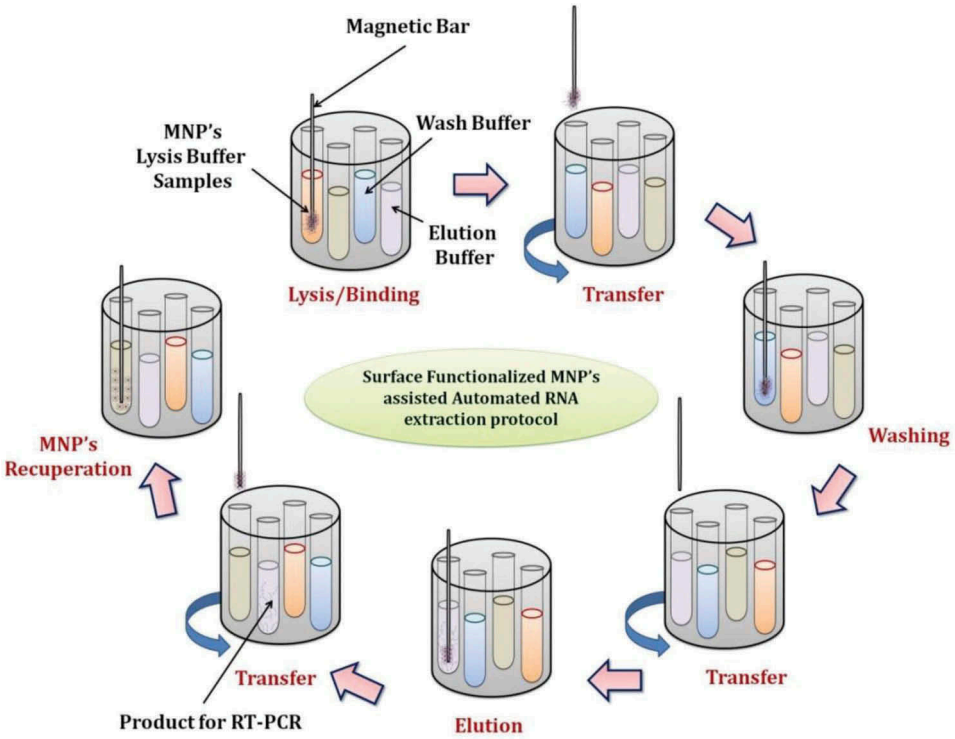

Figure 4. Surface functionalised MNP's assisted automated viral RNA-extraction protocol.

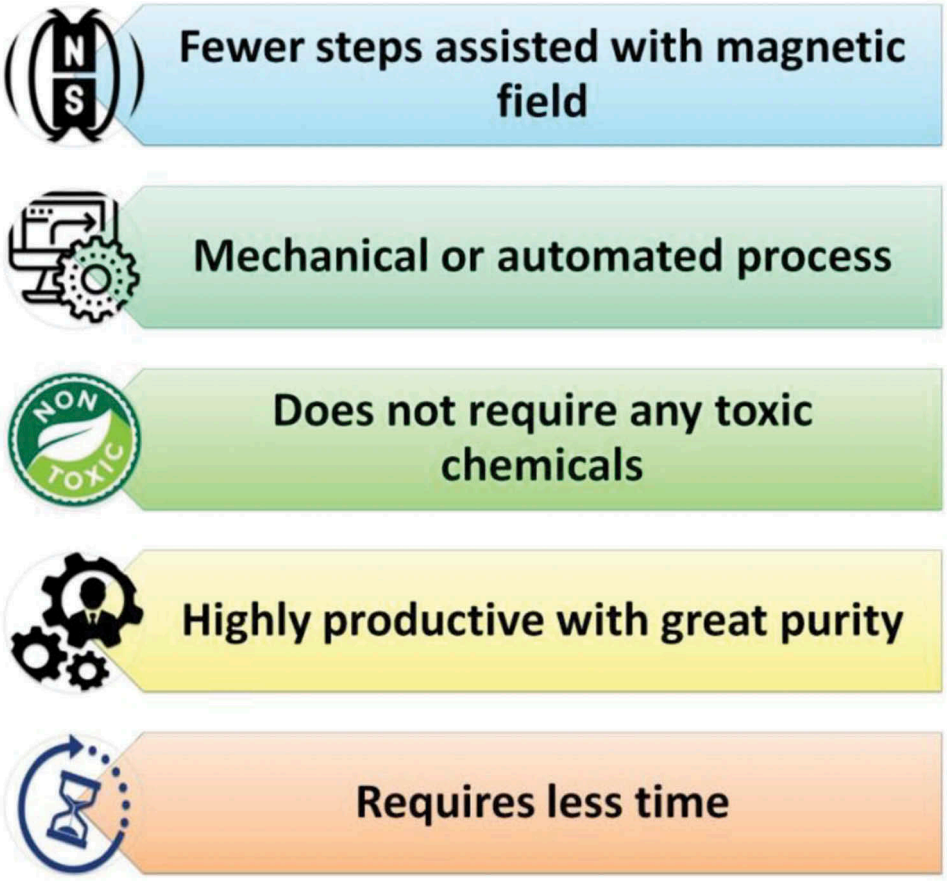

Figure 5. Merits of the current protocol proposed for viral RNA-extraction process.

merits of the current protocol proposed for viral RNA-extraction process.

4. Conclusions

The surface functionalised zinc ferrite NP's were fabricated successfully. The nanocrystalline nature and spherical shape morphology of the fabricated NP's were investigated through TEM and XRD. The successful functionalisation and stability of the fabricated NP's were verified through zeta potential and HDD size determination. The surface functionalised zinc ferrite NP's assisted uncomplicated but potential RNA-extraction protocol was proposed for active detection of COVID-19. The simple and cost effective nature of this protocol may offer capable substitute in near future for conventional techniques to tackle the tedious time taking procedures and man-power requirement, and therefore demonstrates applicability in active COVID-19 detection. More studies are required to employ this protocol in clinical diagnostics of COVID-19.

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Disclosure statement

There are no conflicts to declare.

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