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DEVELOPMENT OF THE RECYCLING PROCEDURE FOR RAPID ANTIGEN TESTS

RAZVOJ POSTOPKA RECIKLIRANJA HITRIH ANTIGENSKIH TESTOV

Rebeka Rudolf[1,2*], Darja Feizpour[3], Žiga Jelen[1,4], Peter Majerič[1,2], Tilen Švarc[1], Matej Zadravec[1], Timi Gomboc[1], Aleksandra Kocijan[3]

[1]University of Maribor, Faculty of Mechanical Engineering, Maribor, Smetanova ul. 17, Slovenia
[2]Zlatarna Celje d.o.o., Kersnikova ul. 19, Celje, Slovenia
[3]Institute of Metals and Technology, Lepi pot 11, Ljubljana, Slovenia
[4]Znanstveno in inovacijsko središče Pomurje, Lendavska ulica 28, 9000 Murska Sobota, Slovenia

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The article presents the problem of rapid antigen tests when they become mass waste after use. Based on this, the hypothesis was made that rapid antigen tests can be recycled. Rapid antigen tests, which were used in the Covid-19 epidemic to quickly detect infections in the population or to confirm the presence of the Sars-Cov 2 virus in patients, were intended to limit the spread of the epidemic. To confirm the hypothesis of recycling for rapid antigen tests, the LFIA-REC ATP 150 project was prepared, which was selected for co-financing by the Norwegian Fund.

Rapid antigen tests consist of a sample and conjugate pad, detectable or nitrocellulose membranes and absorbent pads and a plastic case. The function of the sample pad is to evenly absorb the sample (mucus, blood) and lead it to the conjugate pad with a steady flow. Gold nanoparticles (labels) are deposited on the conjugate pad. The key is that the gold nanoparticles are conjugated with capture molecules capable of binding to potentially present antibodies or virus in the sample.

The scope of the research problem thus required the inclusion of various characterization techniques that must be applied to the individual material in the rapid antigen test to subsequently develop an efficient recycling process for the rapid antigen tests. The result of the research presented in this paper represents a newly developed algorithm of characterization techniques, which includes a recommended description of the preparation of samples of key materials from rapid antigen tests. This algorithm successfully achieved the characterization of gold nanoparticles from rapid antigen tests. Based on the developed algorithm, the final part of the project will validate the recycling process of rapid antigen tests, so that they can be recycled, i.e. gold nanoparticles or plastic used in new products. The paper presents the algorithm of characterization techniques with a description of the preparation of material samples from rapid antigen tests.

Keywords: rapid antigen tests, recycling, characterization, nanogold, plastic

V prispevku je predstavljena problematika hitrih antigenskih testov, ko le-ti postanejo po uporabi masovni odpadek. Na osnovi tega je bila postavljena hipoteza, da je hitre antigenske teste možno reciklirati. Hitri antigenski testi, ki so se uporabljali v epidemiji Covid 19 za hitro odkrivanje okužb v populaciji oziroma za potrditev prisotnosti virusa Sars-Cov 2 pri testirancih, so bili namenjeni za zamejitev širjenja epidemije. Za potrditev hipoteze recikliranja hitrih antigenskih testov je bil pripravljen projekt LFIA-REC ATP 150, ki je bil izbran za sofinanciranje s strani Norveškega fonda.

Hitri antigenski testi so sestavljeni iz vzorčne in konjugatne blazine, zaznavne oz. nitrocelulozne membrane in vpojne blazine ter iz plastičnega ohišja. Funkcija vzorčne blazine je, da enakomerno absorbira vzorec (sluz, kri) in ga z enakomernim tokom vodi do konjugatne blazine. Na konjugatni blazini so nanešeni zlati nanodelci (oznake). Ključno je, da so zlati nanodelci konjugirani z lovilnimi molekulami, ki so se sposobne vezati na potencialno prisotna protitelesa ali virus v vzorcu.

Področje raziskovalnega problema je tako zahtevalo vključitev različnih tehnik karakterizacije, ki jih je potrebno uporabiti za posamezen material v hitrem antigenskem testu, da bi lahko kasneje razvili učinkovit postopek recikliranja hitrih antigenskih testov. Rezultat raziskave, ki je predstavljen v tem prispevku, predstavlja na nov razvit algoritem tehnik karakterizacije, kjer je vključen priporočljiv opis priprave vzorcev ključnih materialov iz hitrih antigenskih testov. Ta algoritem je uspešno dosegel karakterizacijo nanodelcev zlata iz hitrih antigenskih testov. Na osnovi razvitega algoritma bo v sklepnem delu projekta validiran postopek recikliranja hitrih antigenskih testov, tako, da bodo lahko reciklati t.j. nanodelci zlata oziroma plastika uporabljeni v novih proizvodih.

Ključne besede: hitri antigenski testi, recikliranje, karakterizacija, nanozlato, plastika

1 INTRODUCTION

The Recycling of Rapid Antigen Lateral Flow Immunoassay (LFIA) Tests (COVID-19) (LFIA-REC) is aimed at mitigating climate change and adapting to it. The main focus of the project is not financial profitability but also aligning with leading guidelines for sustainable development.[1] The project contributes to the Development Strategy of Slovenia by 2030 by demonstrating pathways for the efficient recycling of rapid antigen LFIA tests, enabling the use of recyclables as secondary raw materials. The use of advanced technologies for the selective refinement of nanogold (AuNP) and plastic recycling is anticipated, supporting the central goal of the strategy, which is the quality of life for all through balanced development in economic, social, and environmental terms.[2]

*Corresponding author's e-mail:
rebeka.rudolf@um.si (Rebeka Rudolf)

The project is also in line with the 2030 Agenda for Sustainable Development, particularly Goal 12, which calls for sustainable production and consumption practices by reducing waste associated with rapid antigen LFIA tests.[3] Reuse of recyclables and Au nanoparticles for creating new products is also planned. Simultaneously, the project supports the European Green Deal[4] by advocating for a sustainable and green economy and promoting recycling and the circular economy in European efforts. Additionally, the project aligns with the goals of the Paris Agreements in Articles 4, 6, 7, 9, and 10.[5]

The End of the Federal COVID-19 public-health emergency in the USA was declared on May 11th 2023,[6] and represents the end of the COVID-19 pandemic in the western world, and with it all the precautions related to it slowed down or completely stopped. This resulted in large quantities of unused rapid antigen tests in storage facilities that are due to expire, which are worth millions and need to be recycled [7].

The recycling of LFIA tests for COVID 19 involves an innovative approach to extract valuable AuNPs from the conjugate pads, contributing to sustainability and resource optimization. In this process, the used LFIA test strips are collected, and the conjugate pads, which contain embedded gold nanoparticles, are carefully separated.

The LFIA test strip consists of a number of porous pads and membranes, such as the sample pad, conjugate pad, filter pads, nitrocellulose chromatographic membrane, and an absorbent pad.[8] These pads vary in composition, depending on the specifics of each LFIA test. However, the most common choices for them are cellulose fibres, polyester fibres, glass fibres and nitrocellulose. The basis of each LFIA test is a trapping molecule or bioreceptor and a nanomaterial label. Typically, nanomaterials such as AuNPs, magnetic, polymer, silver, carbon and fluorescent nanoparticles, as well as quantum dots are most often used for this purpose, with AuNPs being by far the most common.[9] The test strip is encased in a polymer or paper cassette, which both protects the test strip and ensures good contact between the various pads and membranes. These are typically composed of polystyrene (PS) or acrylonitrile butadiene styrene (ABS) plastic. Both the AuNPs contained in the test strip and the plastic of the cassette casing represent the highest recycling value in the whole LFIA test. However, to effectively recycle both the AuNPs and plastics it is necessary to separate them.

The separation device that is being developed will use mechanical grinding to break apart the membranes saturated with AuNPs and plastic casings. The resulting grindings will be directed to a vibrating table, which will sort the heavier fraction (plastic) and the lighter fraction (membranes). Both fractions will be prepared for further processing, such as recovering AuNPs from the membranes, which is an important aspect of the rapid test recycling. Several methods were developed to recover gold nanomaterials in a laboratory setting,[10,11] while in this research, the recycling of AuNPs was included in an established industrial refining process for gold.

This recycling initiative serves dual purposes: reducing environmental impact by diverting LFIA test components from traditional waste streams and repurposing AuNPs for potential reuse in various applications. The recovered AuNPs, once purified, can be utilized in new LFIA test production or redirected to other fields, such as catalysis, sensing technologies, or nanomaterial-based applications. By incorporating recycling strategies into LFIA test disposal practices, the scientific community contributes to both environmental conservation and the sustainable utilization of precious materials, aligning with global efforts to promote circular economies and responsible resource management.

To ensure efficient recycling, the key is the separation of the individual materials and the complex components. Currently, suitable devices for this purpose are not available, making close collaboration with industry and a swift response essential.

Nanotechnology has opened a realm of possibilities in various scientific domains, and the development and characterization of nanogold nanoparticles stand as a crucial area of research. These minute entities, ranging from 1 to 100 nanometers in size, exhibit unique optical, electronic, and catalytic properties, making them highly versatile in fields such as medicine, electronics, and materials science. Understanding the algorithm of characterizing nanogold nanoparticles is pivotal in harnessing their potential applications.

The characterization of nanogold nanoparticles involves a comprehensive algorithm combining various techniques to elucidate their physical, chemical, and structural properties. Understanding these properties is crucial to tailoring the nanoparticles for specific applications in fields like targeted drug delivery, imaging, catalysis, and sensor technology.[12–15] As research in nanotechnology advances, refining and expanding these characterization methods will continue to unveil the full potential of nanogold nanoparticles in various scientific and technological domains.

In this paper the protocol and algorithm are established, ensuring accurate transmission electron microscopy (TEM) and scanning TEM (STEM) characterization of recycled AuNP, while minimizing potential sample damage.

2 EXPERIMENTAL PART

The mechanical separation of LFIA tests involves the opening of the cassette housing using free spinning hammers in a modified SM-300 cutting mill (Retsch GmbH, Germany). This breaks open the whole test, while keeping the cassette halves and LFIA test strips mostly intact. This was followed by vibration sifting on a cus-

tom-made, linear-vibration sifter, which separates the cassette halves from the LFIA test strips.

The chemical refining of gold requires melting the gold scrap to be melted with silver, with the final metal containing about one fourth of gold. This metal is granulated and put in heated nitric acid (HNO_3) at 80 °C to dissolve the silver and other metals from the gold. The silver-containing solution is filtered form the remaining gold, which is then dissolved with aqua regia (nitric and hydrochloric (HCl) acids in a volume ratio of 1:3) at 70 °C. Sodium metabisulphite is then used to precipitate the gold from the acid solution, obtaining pure gold. In an industrial setting, the used acid solution is then neutralized, and a flocculant is added, producing an organic sludge which still contains a small amount of gold metal and other metals, dissolved by the acids during the refining process. This sludge is pumped inside a filter press, which removes the excess liquids and produces dry cakes of the sludge material, which can then be further refined to obtain the smaller amounts of gold within the cakes. A manual filter press model 500 × 500 with 30 inserted plates (EUROTecniche Srl, Mussolente, Italy) was acquired to facilitate this process in the project for the recovery of gold from the rapid antigen tests. For recovering AuNPs from the rapid-antigen-test membranes, a procedure was proposed for using aqua regia to dissolve the gold from the organic components of the membranes. Fume hoods are required to capture any fumes or gases generated during this procedure, two of which were acquired as a part of the project (EUROTecniche N. 2 Moplen Hood, EUROTecniche Srl, Mussolente, Italy). The gold-containing membranes, obtained from rapid antigen test separation, are kept in the fume hoods and soaked in aqua regia for 2 hours to ensure total dissolution of the gold in the acid. The produced acid is filtered from the rapid test membranes and can be used further with sodium metabisulphite to precipitate and obtain the gold metal content. The sodium metabisulphite reacts with the gold chloride in the acid solution, reducing the ionic gold to its metallic form.

In the framework of the project, the gold-containing acid can be used as a solution for Ultrasonic Spray Pyrolysis (USP), for the production of new AuNPs. This refining procedure can then be considered as reusing the initial AuNPs from the membranes for the production of new, recycled AuNPs, which can be further used in lateral flow tests or other products.

A schematic presentation of the recycling process for rapid antigen tests is shown in **Figure 1**.

The remaining membranes from the gold recovery can be thermally processed in a furnace to destroy the organic components and additionally retrieve any potentially remaining gold on the membranes. As part of the project, an ammonia dissociator (model P-ASP-0750, Millivolt GmbH, Donzdorf, Germany) was acquired for producing a mixture of hydrogen and nitrogen gases from ammonia, to facilitate the thermal processing in an industrial setting.

The chemical analyses of the samples obtained after the recycling procedure for the rapid antigen tests were performed using inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent 5800 VDV).

Electron microscopy, particularly transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM), offers high-resolution imaging of nanogold particles. These techniques help visualize the morphology, size, and dispersity of the particles. Additionally, selected-area electron diffraction (SAED) and fast Fourier transform (FFT) can provide insights into the crystalline nature of the nanoparticles. The electron-diffraction pattern provides information about the crystalline structure of the nanoparticles and enables identification of the crystal lattice, aiding in understanding the composition and atomic arrangement of the nanogold particles.

The sample preparation for TEM involved conventional TEM preparation and using the drop-casting method by which a portion of the sample was placed in a small centrifuge container of a few ml containing absolute ethanol or deionized water. Following this, a mixture of ethanol or deionized water and the sample was applied to the carbon formvar B film on the 200 mesh Cu TEM grid using a dropper. Subsequently, the sample dried overnight in a desiccator before the analysis. The examination and analysis were conducted using a JEM-2100HR and ARM 200 CF (JEOL, Tokyo, Japan) TEM with an attached energy-dispersive X-ray spectrometers (EDS) JED-2300T and solid-state detector (SSD) (JEOL, Tokyo, Japan), operating at 80 kV, 100 kV and 200 kV, under conditions that did not damage the samples. The analyses encompassed imaging, electron diffraction, and elemental composition assessment, with EDS conducted at standard acquisition settings: high resolution, live time mode, a 200-second acquisition time, and a probe size of either 10 nm or 25 nm at ×100,000 magnification.

3 RESULTS

The Au was successfully recovered from the samples before and after the recycling procedure of rapid antigen tests, which was confirmed using the ICP-OES technique. The measured concentrations of Au are stated in **Table 1**.

Table 1: Concentrations of Au after the recycling procedure of rapid antigen tests.

Sample	c_{Au} (μg/mL)
Grinded LFIA soaked in aqua regia	2.4
Au solution from the membrane pad soaking in aqua regia	39.6
Au solution after evaporation of excess aqua regia	247.7
Refined AuNP suspension after USP synthesis	17.0
AuNP suspension after rotary evaporation	197.5

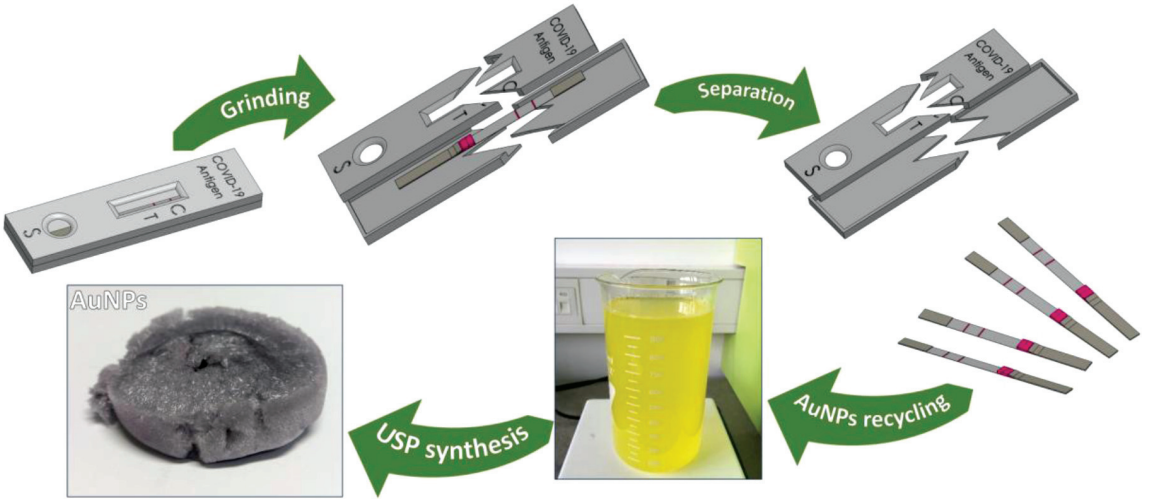

Figure 1: Schematic of the recycling process for rapid antigen tests

Further, after using ICP-OES to confirm the presence of Au in nanoparticles, the recycled AuNPs were characterized using TEM and STEM. During the TEM study of different samples for the recycling procedure of rapid antigen tests containing AuNPs a protocol and algorithm were established as follow:

Transmission Electron Microscopy (TEM) Protocol for Polymer Fiber Samples Containing Gold Nanoparticles

Sample Preparation:	
Polymer Dissolution:	Dissolve the polymer fibers containing gold nanoparticles in a suitable solvent, such as distilled or deionized water or ethanol. Ensure complete dissolution for effective nanoparticle extraction.
Centrifugation:	Transfer a representative portion of the polymer solution to a centrifuge tube and spin it at an appropriate speed and duration to separate the gold nanoparticles from the polymer matrix.
Resuspension:	Re-disperse the extracted gold nanoparticles in the chosen solvent to form a homogeneous solution.
TEM Grid Preparation:	Apply a droplet of the nanoparticle solution onto a TEM grid with a thin carbon formvar B film (200 mesh, Cu). Allow the sample to adsorb onto the grid surface.
Desiccation:	Place the TEM grid with the sample in a desiccator to air-dry overnight, ensuring the formation of a stable and representative sample.

TEM Analysis:	
Microscope Setup:	Set up the TEM microscope (e.g., JEM-2100HR) for analysis, ensuring proper alignment and calibration of the instrument.
Accelerating Voltage Selection:	Choose an appropriate accelerating voltage (kV) to minimize sample damage caused by the electron beam. Consider using lower kV settings for sensitive samples.
Imaging:	Acquire TEM images of the gold nanoparticles at various magnifications. Optimize imaging parameters for resolution and contrast.
Electron Diffraction (SAED):	Conduct Selected-Area Electron Diffraction (SAED) to determine the crystal structure of the gold nanoparticles. Select representative areas for diffraction analysis.
Chemical Composition Analysis (EDS):	Utilize the Energy-Dispersive X-ray Spectrometer (EDS) JED-2300T to perform elemental composition analysis. Ensure proper calibration of the EDS detector for accurate results.
Optimization for Sensitive Samples:	If the sample is sensitive to the electron beam, use a lower electron dose during imaging and analysis. Minimize exposure time and optimize imaging conditions accordingly.
Data Interpretation:	Analyse the TEM images, electron diffraction patterns, and EDS spectra to obtain information about the size, shape, crystal structure, and elemental composition of the gold nanoparticles.
Reporting and Documentation:	Document the experimental details, imaging parameters, and analysis results. Include representative images and diffraction patterns in the final report.

By following this protocol, polymer fiber samples containing AuNPs using TEM can be effectively prepared and analysed, ensuring accurate characterization while minimizing potential sample damage.

Transmission Electron Microscopy (TEM) and Scanning TEM (STEM) Protocol for Rapid Antigen Test Samples Containing Gold Nanoparticles

Sample Preparation:	
Particle Size Selection:	Choose the appropriate milled particle size (250 µm or 125 µm) of polymer and conjugate pads for analysis. This may depend on the desired resolution and distribution of gold nanoparticles.
Centrifugation (Optional):	If necessary, conduct a centrifugation step to isolate the gold nanoparticles from the milled particles. Use a suitable solvent for this step, and carefully separate the supernatant for further analysis.
Resuspension (Optional):	Re-suspend the isolated gold nanoparticles in a solvent of choice (e.g., distilled water or ethanol) for improved dispersion and uniformity on the TEM grid.

Deposition on TEM Grid:	Deposit the milled polymer and conjugate pad particles onto a TEM grid with a thin carbon formvar B film (200 mesh, Cu) using a dropper, utilizing distilled or deionized water or ethanol for easier deposition. Allow the sample to adsorb onto the grid surface.
Desiccation:	Place the TEM grid with the sample in a desiccator to air-dry overnight, ensuring the formation of a stable and representative sample.

TEM/STEM Analysis:	
Microscope Setup:	Prepare the TEM microscope (e.g., JEM-2100HR or ARM 200 CF) for analysis, ensuring proper alignment and calibration of the instrument.
Accelerating Voltage Selection:	Choose an appropriate accelerating voltage (kV) to minimize sample damage by the electron beam. Consider using lower kV (e.g., 80 kV or 100 kV) settings for sensitive samples.

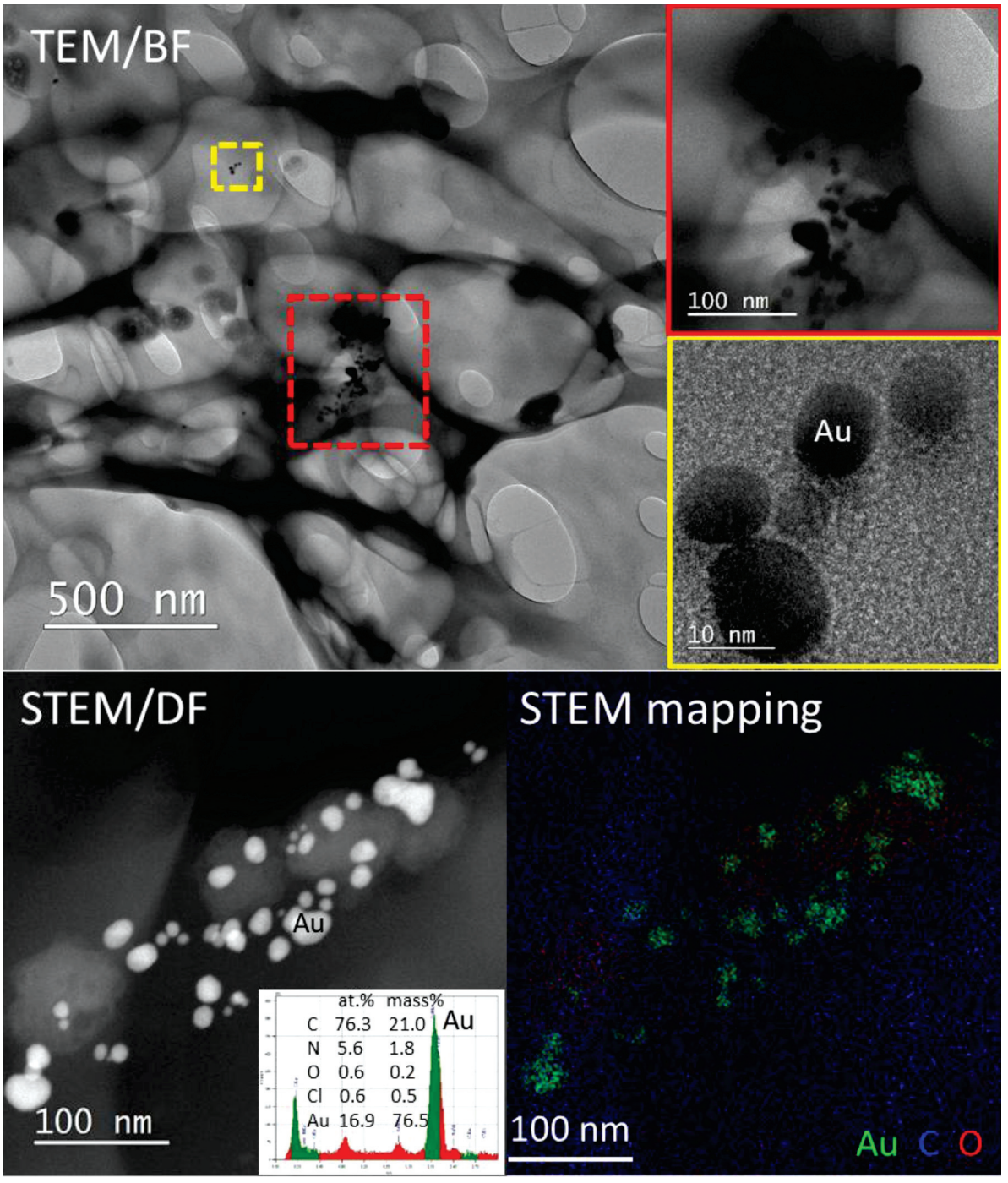

Figure 2: TEM bright-field (BF) image (top left at lower magnification and top right at higher magnifications of individual Au particles in the milled material of rapid antigen test sample) and STEM dark-field (DF) with EDS point analysis in atomic/weight % (bottom left) and with EDS mapping analysis (bottom right) in the milled material of rapid antigen test sample with milled particles' size 250 µm.

Imaging:	Acquire TEM/STEM images of the gold nanoparticles on the milled polymer and conjugate pad particles at various magnifications. Optimize imaging parameters for resolution and contrast.
Electron Diffraction (SAED):	Perform SAED to determine the crystal structure of the gold nanoparticles. Select representative areas for diffraction analysis.
Chemical Composition Analysis (EDS):	Employ the EDS JED-2300T or SDD to conduct elemental composition analysis. Calibrate the EDS detector for accurate results.
Optimization for Sensitive Samples:	If the sample is sensitive to the electron beam, use a lower electron dose during imaging and analysis. Minimize exposure time and optimize imaging conditions accordingly.
Data Interpretation:	Analyze the TEM images, electron-diffraction patterns, and EDS spectra to obtain information about the size, shape, crystal structure, and elemental composition of the gold nanoparticles.
Reporting and Documentation:	Document the experimental details, imaging parameters, and analysis results. Include representative images and diffraction patterns in the final report.

By following this protocol, effective preparation and analysis of rapid antigen test samples containing AuNPs using TEM can be achieved, ensuring accurate characterization, while minimizing potential sample damage.

The result of considering a protocol and algorithm written above produced TEM and STEM results presented in the **Figure 2**.

4 DISCUSSION

Using the gold-containing acid solution from the refining of rapid test membranes in USP has produced irregular and spherical AuNPs, as well as triangular, pentagonal and hexagonal particle shapes [16]. ICP-OES (Table 1) and TEM/STEM/EDS (**Figure 2**) confirmed the presence of Au in recycling nanoparticles from the rapid antigen tests. Further TEM and STEM analyses (**Figure 2**) revealed that the size of the AuNPs predominantly ranged below 100 nm, with some reaching dimensions in the few-hundred-nanometer range. The average size of particles falling below 100 nm ranged from approximately 10 nm to 50 nm. Among the AuNPs with a size below 100 nm, the majority exhibited a spherical morphology, while larger particles exceeding 100 nm displayed irregular shapes.

The feasibility of the proposed refining process and re-using the gold-containing acid solutions in USP for new AuNP production was confirmed. However, there are several areas where gold loss can occur in the complete process, from the test milling, membrane separation and gold recovery from the membranes. In the rapid test milling and membrane-separation process, the gold loss is not known due to the different types of rapid tests available, which have differing AuNP contents. As the AuNP markers adhere quite strongly on the test membranes, we expect the gold loss in this part of the overall rapid test recycling to be marginal. The larger portion of gold loss is expected in treating the membranes with acids. As the membranes are highly absorbent for liquids, they need to be drained off, for obtaining the maximum amount of gold. Even when drained off, some gold content may remain in the membranes. Additionally, a study for evaluating the AuNP recovery from test membranes has shown about 17 % gold loss during the acid heating [16], due to evaporation of the acids and the gold chloride inside the fume hood. A modification of the process is proposed, where less acid volume is used and the gold-containing membranes are filtered several times through the acid solution. In this way, the heating of the acids and gold loss are avoided, as there would be less acid and gold chloride evaporation from the obtained solution. A negative factor of such a process is gold adherence to the membranes and not being able to completely drain the membranes, resulting in less gold in the produced solution.

The proposed use of the aqua regia with dissolved gold from membranes in the USP production of new AuNPs is a new development in the conventional gold-refining process, resulting from the rapid antigen test recycling project. In using the prepared solution directly in the USP process, the gold precipitation with sodium metabisulphite used in the conventional refining is not necessary, resulting in less potential gold loss. However, some additional steps are required for preparing this gold solution for the USP process. It was shown that high acid volumes detrimentally affect the AuNP formation in the USP process, obtaining less-uniform AuNP sizes and shapes than with lower acid volumes.[16] The proposed modification of filtering the test membranes several times through the acid solution is also expected to reduce the acid volumes in the final solution to be used in USP, thus optimizing the AuNP formation to produce more uniform particle sizes and shapes.

5 CONCLUSIONS

The process of grinding and breaking apart rapid antigen LFIA tests makes it possible to separate with AuNP saturated membranes from the plastic housing and use them for USP precursor preparation. The refining process successfully utilizes USP to prepare recycled AuNPs from LFIAs.

The algorithm for characterizing the AuNPs integrates diverse techniques to reveal their properties, crucial for customizing their applications in medicine, imaging, and sensor technology as nanotechnology advances. The algorithm for characterization of AuNPs is presented and proposed, and with following this algorithm, characterization of recycled AuNPs from LFIAs was successfully achieved.

This study offers guidance for establishing recycling processes, applicable not only to AuNPs but also for other metal nanoparticle residues that have minimal or no acid content.

Acknowledgment

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Abbreviations

Acrylonitrile butadiene styrene (ABS), energy dispersive X-ray spectrometers (EDS), inductively coupled plasma optical emission spectroscopy (ICP-OES), lateral flow immunoassay (LFIA), nanogold (AuNPs), polystyrene (PS), scanning electron microscopy (SEM), Selected Area Electron Diffraction (SAED), solid-state detector (SSD), STEM dark-field (DF), TEM bright-field (BF), transmission electron microscopy (TEM), Ultrasonic Spray Pyrolysis (USP).

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