Characterization to Estimate the Size of Gold Nanoparticles Deposited in Pregnancy Tests Using Diffuse Reflectance Spectroscopy

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ABSTRACT: When using a pregnancy test, a urine sample is deposited in one side of the nitrocellulose strip, which moves by capillary action to the other side of the test, where there are immobilized particles or biomarkers that indicate a positive or negative test result. Based on the manufacturer's data sheet, these are usually colloidal gold nanoparticles. This paper describes the results of a characterization study using diffuse reflectance spectroscopy on qualitative pregnancy tests. The importance of this study resides in the possibility of finding invalid tests or false negatives in the results, at the same time, the approximate size of the nanoparticles can be determined indirectly by analysing the spectra obtained experimentally.

KEYWORDS: Gold Nanoparticles, Pregnancy Tests, Spectroscopy.

INTRODUCTION

The development of the rapid test strip, also known as lateral flow immunoassay (LFIA), is the result of several threads that can be traced back to the 1950s. However, after numerous investigations, the first lateral flow products were introduced to the market in the 1980s [1]. Basically, a diagnostic cassette is a test for the qualitative detection of elevated levels of the hormone chorionic gonadotropin (hCG) in urine. The hCG hormone is a glycoprotein hormone synthesized by the placenta and detectable in urine early after implantation of a fertilized ovum in the chorionic tissue. It is the main signal and specific marker to indicate pregnancy [2]. When a test is performed, the urine sample is applied to one side and absorbed into the membrane. The sample then flows laterally across the nitrocellulose due to capillary action, and the hCG hormone in the urine sample combines with colloidal gold nanoparticles (AuNPs), moving chromatographically across the membrane [2, 3]. Metallic nanocomposites may act as chemical transducers in which the signal may be collected by distinct analytical methods. This class of nanomaterials has shown to be very useful as biosensing platforms, mostly due to the Localized Surface Plasmon Resonance effect (LSPR) [4]. The appearance of a purple line in the test area indicates a positive result, signaling the presence of hCG. The absence of this line indicates a negative result or the absence of hCG (figure 1.a). This is because nanoparticles larger than 70 nm absorb red light and reflect blue light, producing blue-purple solutions [5]. Figure 1.b shows an example of the absorption spectrum for metallic nanoparticles [6].
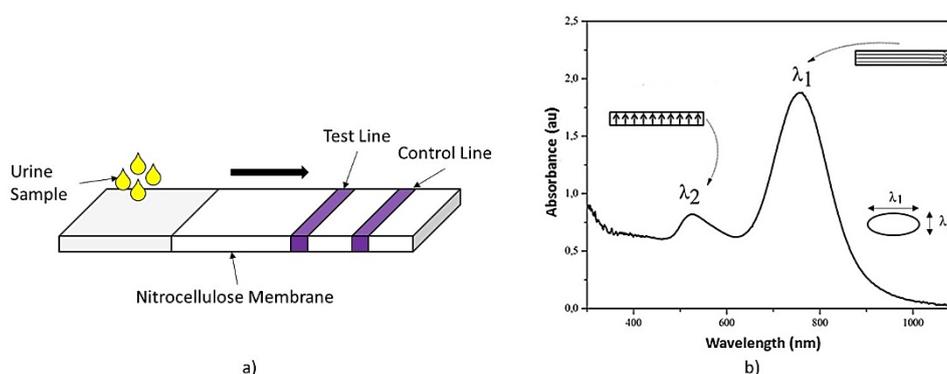

Figure 1. a) Configuration of a lateral flow test strip, b) Absorption spectrum for metallic nanorods, λ1 corresponds to the longitudinal oscillations and λ2 corresponds to the transversal oscillations of the surface plasmons.

EXPERIMENTAL SETUP

When an electromagnetic field interacts with the surface of metallic nanoparticles, a collective oscillation of the particle's surface electrons occurs [4, 6]. Light incident on a sample of matter, a certain amount of photons can be transmitted through the sample and the rest is reflected, or absorbed by some covalent links that act as longitudinal oscillating springs that couple with the exact frequency or wavelength of the incident light radiation [7]. Absorption is selective and depends on the molecular groups concerned. Thus, light absorption is estimated by the difference between incident light Li and reflected light Lr, or transmitted light Lt. In a spectrometer, when operating in reflection mode and using a sample that is sufficiently opaque for the transmission light Lt to be zero, the light absorption La can be calculated from the difference [7]

$$La = Li - Lr \tag{1}$$

The total energy reflected by a sample is the sum of specular (surface) reflection and diffuse reflection, which is temporarily absorbed and then re-emitted by the sample. Only the diffuse form of light provides useful information about the nature or composition of the sample. Diffusely reflected light already carries information about the sample [8]. This is very useful because it allows the diffuse reflectance spectrum to be obtained using a spectrometer. This study uses an Ocean Optic USB-4000 spectrometer (345 nm - 1100 nm) connected to a computer through a USB port and controlled by Spectrasuite software, which in turn is connected to an HL-2000 tungsten-halogen light source (360 nm - 2000 nm) using a bifurcated probe, as shown in Figure 2.a. The measuring probe that is directed towards the sample contains seven optical fibers, six of which emit light from the halogen source, the central optical fiber collects a fraction of the diffusely reflected light and sends it to the spectrometer's diffraction grating, where it is separated into wavelengths ranging from 100 nm to 1100 nm.
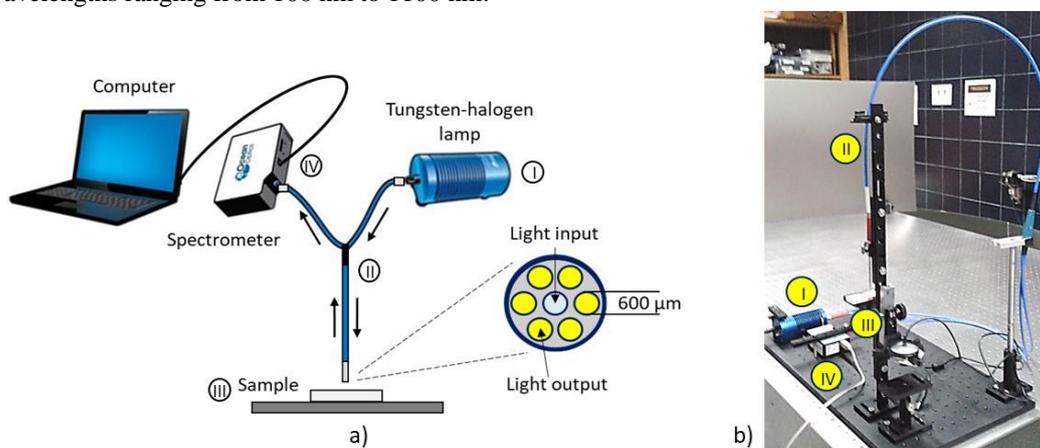
Figure 2. a) Connection of components for signal acquisition, b) Experimental setup: (I) Light Source, (II) Bifurcated Optical Fiber, (III) Pregnancy Test, (IV) Spectrometer.

A system of photodetectors that turn the optical signals into electrical signals, which are then digitized and sent to the computer for processing. The core of the fiber is pure silicon and the coating is doped fused silicon. The optimal operating bandwidth of the fibers goes from 400 nm to 2000 nm. The experimental setup is shown in Figure 2.b. The Spectrasuite software, in the diffuse reflectance option, operate within the range of the visible to near-infrared spectrum (350 nm to 1000 nm) and use the following equation to obtain the reflectance curves [8]:

$$R_{t\lambda} = \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \times 100\% \tag{2}$$

where S_λ is the intensity of the sample at a certain wavelength, D_λ is the intensity of the darkness spectrum, and R_λ is the intensity of the reference used.
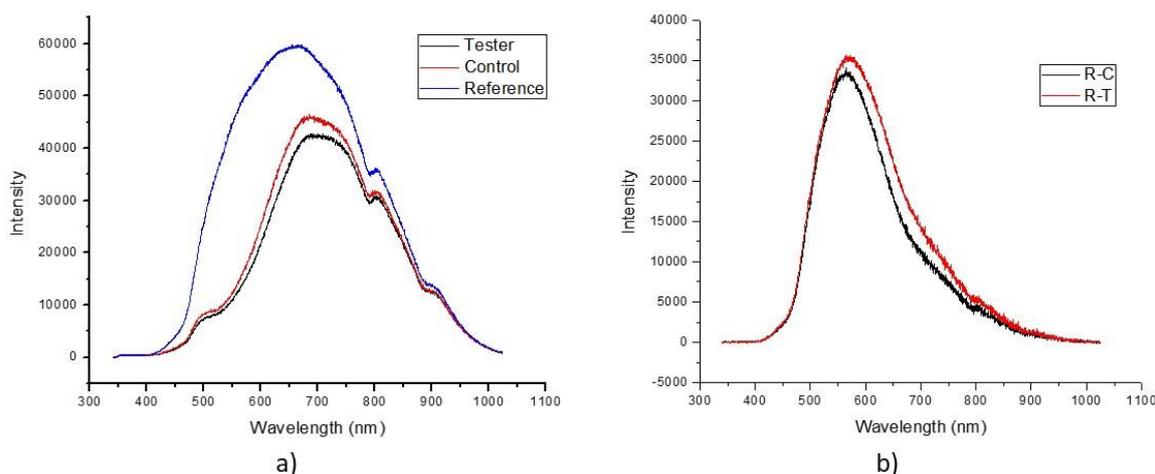
ACQUISITION OF REFLECTANCE SPECTRA

As indicated in the user manual, the spectrometer is calibrated by using its reference standard [8], selecting the reflectance option in the spectrometer software along with using equation 2, which ensures that the experimental setup works properly. Next step is to measure the selected areas of interest. We placed a test cassette for hCG detection on a flat surface (figure 3), a plastic pipette was used to apply some drops of urine (from a patient 27 weeks pregnant, verified by laboratory analysis) to the circular "S" hole on the test. The liquid is allowed to move until the control and test bands change color, with the latter indicating whether the urine sample is positive.
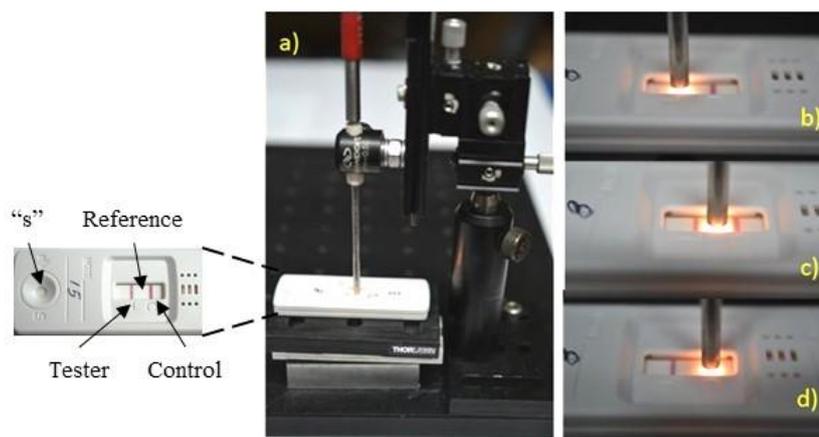
Figure 3. a) Place the cassette in the experimental setup and position the tip of the probe approximately 4 mm above the test strip to obtain a signal with the spectrometer, b) signals are acquired on the Tester line, c) after which the probe tip is placed on the white Reference area and finally, d) on the Control line.

The graph in figure 4.a shows that there is a difference between the two reflectance signals, indicating that part of the light is absorbed by the control and tester lines. Using the principle of equation 1, an operation (subtraction) is used to obtain the difference between the Reference–Control (R-C) and Reference–Tester (R-T) signals (figure 4.b), these signals are very similar but only the R-T graph will be used because the spectrum that provides information on the presence or absence of hCG is that of the Tester.

Figure 4. a) Diffuse Reflectance Spectral Response, b) result of the subtraction operation.

For this experiment, six kits with 25 pregnancy tests (cassettes) are used, for a total of 150 pregnancy tests to perform the measurements, but only some graphs from a single test kit will be shown, given the similarity in obtaining the graphs and because they are the most relevant to the study. The data obtained are plotted using Origin software.

RESULTS

Figure 5.a shows the graphs of the data obtained when performing the R-T operation. There is a signal with attenuation (pregnancy test cassette #22), which is inconsistent with the results of the other graphs. By visual inspection, the cassette does not show purple coloration on the tester line, which may indicate the presence of a false negative. The R-T spectra show different intensities and different central wavelengths, so they are normalized to compare them with the absorption spectrum of the nanoparticles. Comparing the graphs obtained (figure 5.b) with the information provided in [4, 6], it can observed that the results don't show effect LSPR, so this method may also be useful for determining (in the future) if colloidal gold nanoparticles are circular or nanorods. This can be useful when the manufacturer of pregnancy tests does not provide further information about the colloidal gold nanoparticles used, or when their form is unknown.
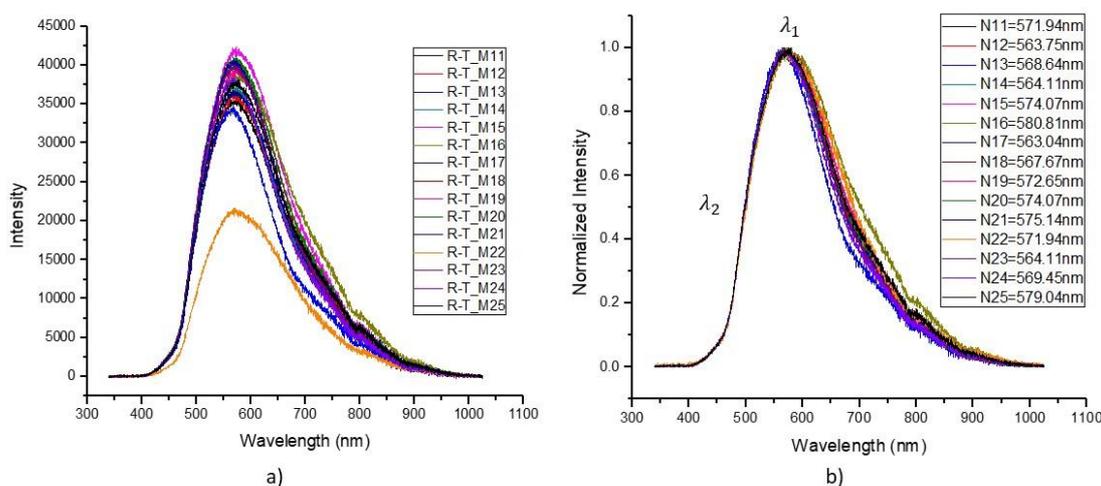

Figure 5. a) Graph with the R-T operation showing a possible false negative, b) data normalized to 1, the LSPR effect was not detected.

Finally, the information provided in [5] indicates that the size of nanoparticles corresponds to a specific wavelength, as shown in figure 6.a. The wavelength peaks obtained experimentally are between 563.04 nm and 580.81 nm (figure 6.b). Therefore, when comparing these values with the data shown in figure 6.a, it can be determined that the size of the nanoparticles is within a range of approximately 80 nm to 100 nm.
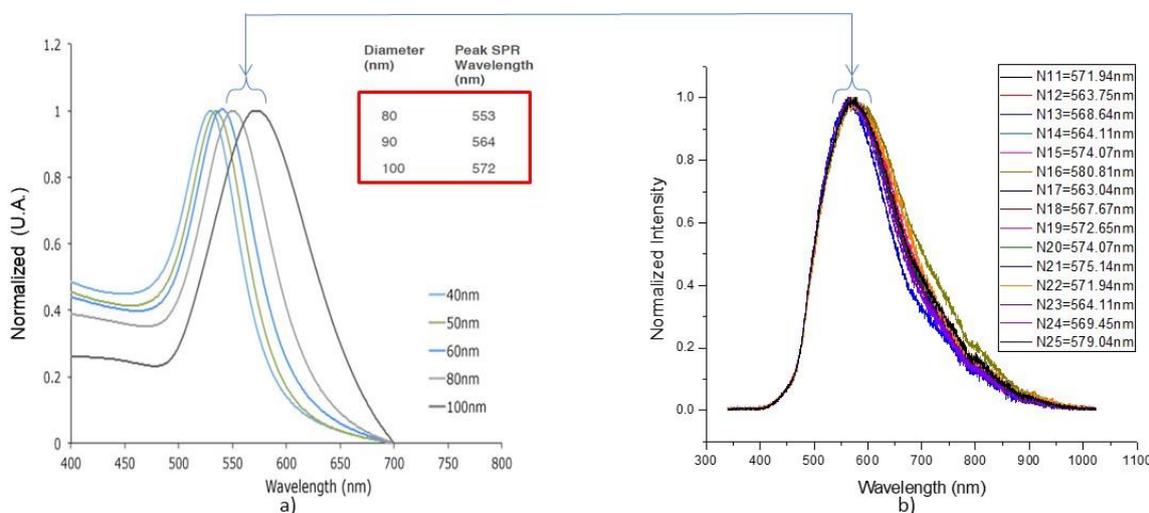

Figure 6.

CONCLUSION

Diffuse reflectance can be used to estimate the size of colloidal gold nanoparticles with hCG deposited in pregnancy tests. The absence of the LSPR effect in the graph data suggests that the nanoparticles are circular in shape. Within the 150 pregnancy tests, only one test was found with a significantly attenuated signal, which was not consistent with the manufacturer's instructions, in other words, a false negative was found.

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