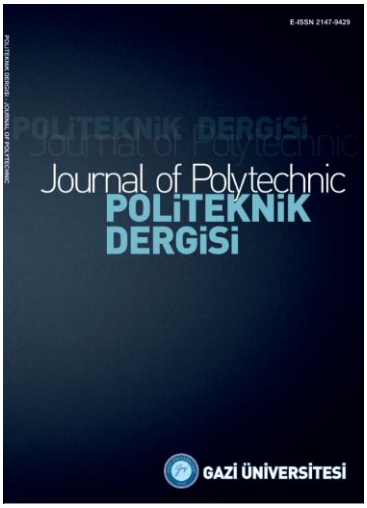

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Identification of Pure and Mixed Urine Stains Through Surface Enhanced Raman Spectroscopy Using Gold Nanorods & Silver Nanoparticles

Highlights

- *Fast and non-destructive analyses were performed with a small amount of sample.*
- *A simulated forensic crime scene was prepared and analyzed.*
- *SERS signal was enhanced with silver nanoparticles and gold nanorods and their effects were compared.*
- *The study is multidisciplinary and involves forensic sciences, chemistry, and bionanotechnology.*

Graphical Abstract

This study focuses on the detectability of urine by SERS in gold nanorod/silver nanoparticle doped mixtures containing urine and apple juice in different concentrations. Known for their strong plasmonic properties, silver nanoparticles provide more signal enhancement compared to gold nanorods.

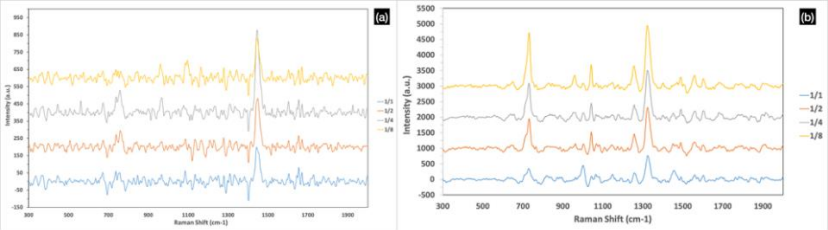

Figure. Comparison of SERS spectra of 1:1, 1:2, 1:4, and 1:8 concentrations of urine and apple juice mixture (a) with Gold nanorods and (b) Silver nanoparticles

Aim

This study aims to demonstrate the analyzability of urine with SERS using gold nanorod and silver nanoparticle in a complex forensic crime scene.

Design & Methodology

Raman analyses of the urine sample taken from a volunteer over 18 years old were first performed on a substrate and the urine was diluted at different concentrations until an acceptable peak was obtained. In the next step, Raman analyses were repeated by mixing apple juice, which has physical similarities with urine, with different concentrations of urine in equal proportions. Finally, the added mixtures were dried on a gold SERS substrate, and gold nanorods and silver nanoparticles were added separately and left to dry. Afterwards, Raman analyses were performed again.

Originality

This study aimed to detect urine from a complex crime scene with SERS. Besides that, the effect on the signal level of gold nanorods and silver nanoparticles used to amplify the SERS signal was compared. No such comparison was seen in similar studies.

Findings

It was observed that the noise ratio decreased with the dilution of the urine concentration. Strong peaks were seen at 942, 1040, 1465, and 1612 nm especially. It was determined that the intensity of some peaks decreased after mixing with apple juice. It was observed that the SERS signal level approximately 4 times with the addition of gold nanorods and approximately 20 times with the addition of silver nanoparticles increased.

Conclusion

The obtained data showed that urine can be detected both alone and in a mixed crime scene using SERS. It was also revealed that the gold nanorods and silver nanoparticles forming the SERS surface suppressed the fluorescence effect, and the peaks of the analyte were seen. As a result, Raman and SERS techniques seem to give a fast and precise result in the analysis of body fluid.

Declaration of Ethical Standards

At the meeting of the Non-Interventional Clinical Research Ethics Committee at Hacettepe University held on May 4, 2021, with the decision numbered 2021/10-01, it was approved to take biological samples from volunteers.

Identification of Pure and Mixed Urine Stains Through Surface Enhanced Raman Spectroscopy Using Gold Nanorods & Silver Nanoparticles

(This study was presented at ICMATSE 2022 conference.)

Research Article

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ABSTRACT

In forensic science laboratories and crime scene investigation applications, analyses of discovered stains are crucial. In order to carry out an effective investigation by determining the variables related to the crime and those involved in the event, it is necessary to analyze the samples quickly, in small quantities, and even in the form of mixtures. Therefore, it is of high importance to analyze evidential materials with non-destructive, fast, cost-effective techniques. Spectroscopic methods have advanced significantly in recent years with important developments in light detectors and take place among forensic procedures. This study focused on Raman and Surface Enhanced Raman Spectroscopy (SERS) for detecting pure urine mixed stains. In order to reduce the fluorescence effect caused by the matrix components of the urine, dilution was made and Raman analyses were performed for four different concentrations. It was observed that as the concentration decreased, the noise ratio decreased and the peaks became more pronounced. A simulated crime scene containing stains of pure urine, diluted urine (1:2, 1:4, and 1:8), apple juice, mixture (apple juice and different concentrations of urine), and gold nanorod/silver nanoparticle doped mixtures were created to check the detectability of urine from a complex crime scene. Despite the low peak intensity in the Raman spectrum, the presence of urine was still detectable. The SERS spectrums of the stains obtained from the simulated crime scene were examined first with the addition of gold nanorods and subsequently with the addition of silver nanoparticles. Silver nanoparticles, known for their strong plasmonic properties, were found to provide greater signal enhancement compared to gold nanorods.

Keywords: Nanoparticles, surface enhanced raman spectroscopy, forensic sciences, bionanotechnology, identification of biological fluids.

Altın Nano Çubuklar ve Gümüş Nano Parçacıklar Kullanılarak Yüzeyde Zenginleştirilmiş Raman Spektroskopisi Yoluyla Saf ve Karışık İdrar Lekelerinin Tanımlanması

ÖZ

Adli bilimlerde, laboratuvar ve olay yeri inceleme uygulamalarında, bulunan lekelerin analizleri büyük önem taşımaktadır. Suça ve olaya karışan değişkenlerin belirlenerek etkin bir soruşturma yürütülebilmesi için numunelerin hızlı, küçük miktarlarda ve hatta karışım halinde analiz edilmesi gerekmektedir. Bu nedenle delil niteliğindeki materyallerin tahribatsız, hızlı ve uygun maliyetli tekniklerle analiz edilmesi büyük önem taşımaktadır. Spektroskopik yöntemler, son yıllarda ışık dedektörlerindeki önemli gelişmelerle birlikte önemli ölçüde ilerlemiş ve adli tıp prosedürleri arasında yerini almıştır. Bu çalışma, saf idrar lekelerini karışım durumunda saptamak için Raman ve Yüzey İyileştirilmiş Raman Spektroskopisi'ne (SERS) odaklanmıştır. İdrarın matriks bileşenlerinin neden olduğu floresans etkisini azaltmak için dilüsyon yapılmış ve dört farklı konsantrasyon için Raman analizleri yapılmıştır. Konsantrasyon azaldıkça gürültü oranının azaldığı ve piklerin daha belirgin hale geldiği gözlenmiştir. Saf idrar, seyreltilmiş idrar (1:2, 1:4 ve 1:8), elma suyu, karışım (elma suyu ve farklı konsantrasyonlarda idrar) ve altın nanoçubuk/gümüş nanopartikül katkılı karışımları içeren simüle edilmiş bir olay yeri, karmaşık bir suç mahallinden idrarın saptanabilirliğini kontrol etmek için yaratılmıştır. Raman spektrumundaki düşük tepe yoğunluğuna rağmen, idrarın varlığı tespit edilebilmiştir. Simüle edilen olay mahallinden elde edilen lekelerin SERS spektrumları önce altın nanoçubuklar ardından gümüş nanoparçacıklar ilave edilerek incelenmiştir. Güçlü plazmonik özellikleriyle bilinen gümüş nanoparçacıkların, altın nanoçubuklara kıyasla daha fazla sinyal zenginleştirmesi sağladığı bulunmuştur.

Anahtar Kelimeler: Nanoparçacık, yüzeyde iyileştirilmiş raman spektroskopisi, adli bilimler, biyonanoteknoloji, biolojik sıvıların tanımlanması

1. INTRODUCTION

One of the most critical components of forensic science investigations is the detection, collection, and identification of body fluids discovered at a crime scene. Body fluids, including blood, sweat, urine, semen, and saliva, constitute the most crucial evidence for forensic research dedicated to solving crimes [1,2]. In certain cases, the detection and identification of bodily fluid-related stains found at the crime scene can be enough to determine the identity of a person involved in a crime.

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However, depending on the complexity of the crime scene and the time of occurrence, the fluids found may not be clearly distinguishable. Moreover, the amount of samples collected from the crime scene is often quite small. Even if it is apparent what the liquid collected from the crime scene is, a series of analyses must be conducted and officially confirmed before it can be considered as evidence. For instance, urine is a prevalent bodily fluid at crime scenes, and its pungent odor allows for its instant recognition. Nevertheless, a series of tests are required to detect the urine and the person it belongs to. There are specific tests designed to confirm the presence of urine; however, most of these tests are tailored only to detect urine, and after analysis, the sample becomes unusable for another test. Therefore, it is crucial for the analyst to know which test to perform for urine accurately. Otherwise, a test designed for a different bodily fluid may provide inaccurate results and degrade or destroy the urine sample. Additionally, longlisting laboratory procedures result in a delay of the administration of justice. Hence, there is a need for an analysis method that will not damage the collected urine stain/sample and will deliver fast and precise results.

In recent years, spectroscopic methods have significantly evolved thanks to significant innovations in laser technology [3]. With the development of new light detectors, they have become an essential tool in molecular structure characterization [4]. In this context, the application of Raman and Surface Enhanced Raman Spectroscopy (SERS) and other spectroscopic methods in the analysis of body fluids has increased in forensic sciences [5,6]. Originally used for clinical purposes, Raman and SERS have emerged as valuable methods for body fluid analysis and providing evidence in forensic sciences [7,8]. Moreover, SERS provides fast and cost-effective analysis with a small amount of samples [9,10]. There are several studies that utilize SERS-based techniques for the identification of urine [11-15].

Nanoparticles are commonly used to mitigate the fluorescence effect and suppress noise signals arising from the molecular structure of urine, thereby enhancing SERS signal. Their localized plasma surface properties enable them to increase the Raman scattering intensity and strengthen the SERS signal. Among various nanoparticles, gold nanorods and silver nanoparticles have been widely used in SERS analysis due to their easy synthesis and significant effect on SERS signal intensity [16-19].

The objective of this article is to demonstrate the applicability of SERS in the analysis of urine and at a complex crime scene. The study aims to quantitatively determine the presence of urine and compare the efficiency of gold nanorods and silver nanoparticle in enhancing the SERS signal. Based on the results, optimal conditions for the detection of urine using SERS, a selective and non-destructive method, will be established.

2. MATERYAL VE METOD (MATERIAL and METHOD)

In this study, Surface Enhanced Raman Spectroscopy (SERS) was utilized to identify and characterize urine commonly discovered at crime scenes. To improve SERS efficiency and overcome existing potential problems, nanotechnological signal enhancement was employed. Furthermore, we demonstrated that urine mixed with apple juice, which shares similar physical properties. To enhance Raman signals, gold nanorods, and silver nanoparticles were synthesized and their effectiveness was compared. Finally, a gold-plated ready-made substrate was used as the SERS substrate. Raman spectrophotometer (Delta Nu Reporter Portable Raman Spectroscopy 785 nm wavelength-WY, US) for SERS measurements, UV-Vis Spectrophotometer (Specord 50 Plus-Jena, Germany), centrifuge device (Eppendorf-Hamburg, Germany), deionized water device (Millipore Simplicity 185-Darmstadt, Germany), ultrasonic bath (Bandelin Sonorex-Berlin, Germany), precision balance (Shimadzu-Kyoto, Japan) and micropipette (1-10,10-100,100-1000 µl) were used in the experiments.

Sodium hydroxide (NaOH), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$), Cetyltrimethylammonium Bromide (CTAB), Chloroauric acid ($HAuCl_4$), and ascorbic acid (AA) were purchased from Sigma-Aldrich (Taufkirchen, Germany). Silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were procured from Merck (Darmstadt, Germany).

Preparation and Collection of Samples

The urine sample was collected from a healthy male volunteer over 18 years of age. To prepare the apple juice, the fruit was squeezed with a juicer, and the resulting juice was strained.

Nanoparticle Synthesis

The seed growth method is based on the synthesis of gold nanorods [20]. For the seed solution, 7.5 ml of 0.1 M CTAB and 250 µl of 0.01 M $HAuCl_4$ were mixed. 600 µl of 0.01 M $NaBH_4$ prepared in the ice bath was quickly poured into the previous solution. After 5 minutes, spherical nanoparticles to be used as seed solution was formed. CTAB, $HAuCl_4$, and $AgNO_3$ solutions were blended in another vial for the growth solution: 4.75 ml of 0.1 M, 1 ml of 0.01 M, and 60 l of $4x10^{-3}$ M, respectively. 250 µl of 0.1 M AA was added to the resulting orange mixture, mixed well and a colorless growth solution was obtained. 5 µl of the seed solution was added to the growth solution prepared in the vial at room temperature and left for 3 hours. Finally, purple-colored gold nanorod particles were formed. Absorption spectra of gold nanorods in the range of 400-900 nm were acquired using a spectrophotometer.

For silver nanoparticles, $3.3x10^{-3}$ M NaOH and $1.68x10^{-3}$ M hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) were mixed in 90 ml of distilled water with a magnetic stirrer. The 0.01 M $AgNO_3$ solution was quickly poured into 90 ml of solution used as the reducing agent. It was observed that the clear solution quickly turned into a metallic gray

color. Thus, the synthesis of colloidal silver nanoparticles was carried out. Silver nanoparticles were centrifuged before use. The absorption spectrum of silver nanoparticles in the range of 200-900 nm was obtained using a spectrophotometer.

As the SERS surface, a ready-made Si surface on which Au metal was deposited was wielded.

Procedure

In the first step, the Raman spectrum of the blank surface was gathered. Urine was then poured onto the surface. After waiting for about 2 hours to dry, Raman spectrum analyses were performed. Spectra were taken from 10 points of the 1 cm x 1 cm surface area of the stain. The Raman spectrum was measured in the range of 300-2000 nm. After the measurements, the noise effect in the data was reduced with the NuSpec program, and the baseline correction was applied. In order to show the reproducibility, the means and standard deviations of the urine spectra taken from 10 points were calculated and given in Table 1. Spectrum measurements were repeated in the same manner for the 1:1, 1:2, 1:4, and 1:8 diluted urine concentrations. The dilution of the concentration was carried out with deionized water. Pure apple juice was also analyzed after urine. No dilution was performed for apple juice.

In the second step, 100 µl of undiluted juice and 100 µl of urine were mixed at different concentrations in four centrifuge tubes. These four mixtures prepared were poured onto the surfaces, and SERS analyses were performed after two hours of drying time. In the third step, silver nanoparticles were added to the four mixtures prepared for signal enhancement. It was left to dry for 1 hour and SERS analyses were performed. In the last stage, all analyses performed in the third stage were made by adding gold nanorods to the samples. In the second, third, and last stages, SERS spectra were measured from 10 different points for each mixture. Spectra were gathered between 300-2000 cm^{-1} in 1 cm^{-1} steps. The noise effect was reduced and the baseline was corrected. Means and standard deviations were calculated for obtaining values between 300-2000 cm^{-1}. In addition, the change in signal intensity after dilution was also compared at all stages.

3. RESULTS

As a result of the analysis, the baseline of the spectra was corrected using the NuSpec program to reduce noise effects in the acquired data. Spectra were obtained using a 10x objective and 60-second integration time, with the best results obtained during trial measurements.

Initially, the spectrum of the gold-plated substrate used in the analysis was examined. The substrate exhibited six strong bands at 452, 532, 820, 1279, and 1517 cm^{-1} (Figure 1).

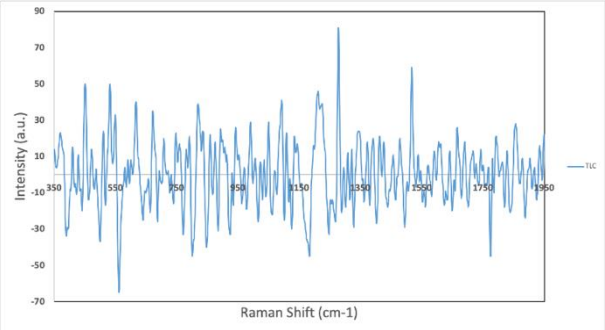

Figure 1. Raman Spectra of gold-plated TLC substrate

In the next step, analyses were performed on four different urine concentrations. Upon exclusion of peaks from the substrate, dominant signals were observed at 875, 942, 1040, 1120, 1224, 1330, 1465, and 1612 cm^{-1} (Figure 2a).

Afterward, the spectrum of undiluted apple juice was examined. As seen in Figure 2b, peaks were observed at 622, 662, 692, 735, and 1459 cm^{-1}.

Subsequently, four different urine concentrations and pure apple juice were mixed, and spectra were obtained for each of the four mixtures. Measurements were taken from ten different points for each mixture. In the resulting spectrum shown in Figure 2c, dominant peaks were observed at 484, 656, 735, 1130, 1224, 1460, 1583, and 1658 cm^{-1}.

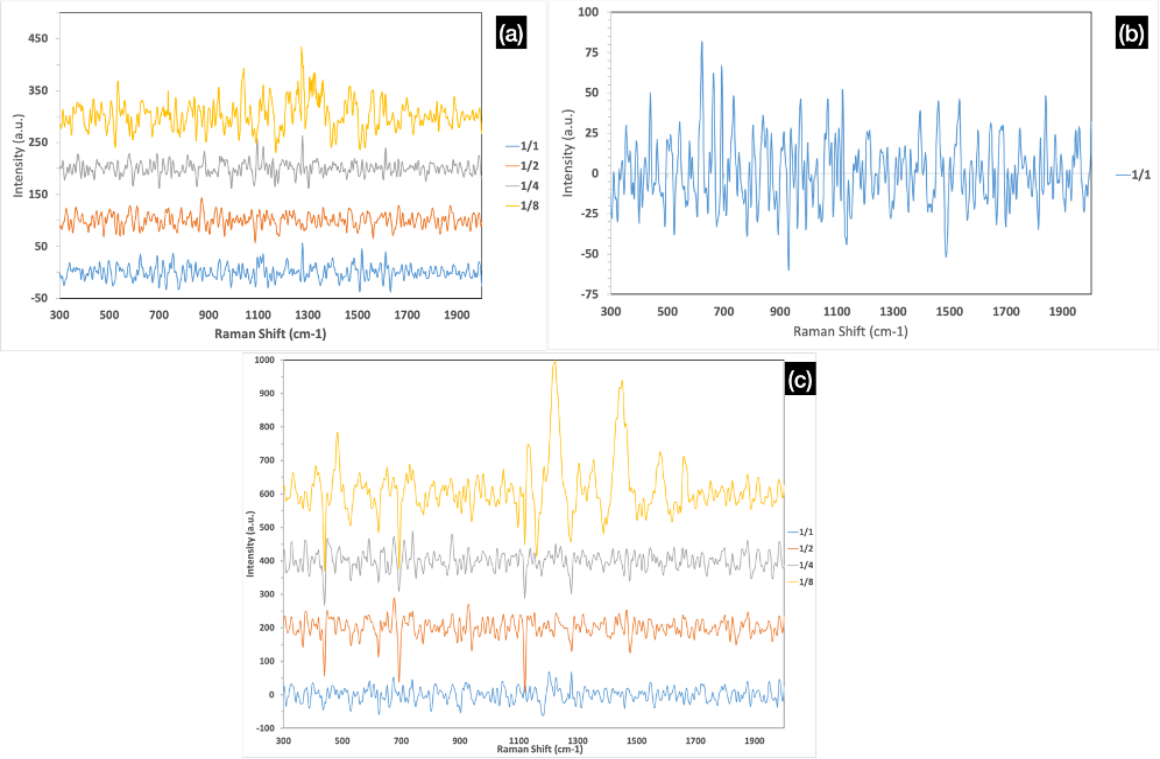

Figure 2. Raman spectra of (a) 1:1, 1:2, 1:4, and 1:8 concentrations of urine and (b) apple juice (c) mixed of urine and apple juice

Following, the absorption spectra were investigated for the characterization of the prepared gold nanorods and silver nanoparticles. The absorption spectra for the gold nanorods and the silver nanoparticles are given in Figure 3 and Figure 4, respectively. Upon examination of the spectrum of the gold nanorod, the peaks of the transverse and longitudinal modes are observed. These peaks align with the results reported by Li et al. As per the findings, the nanorods that had an average diameter of 13 nm and a length of 45 nm were obtained [21]. A study on the synthesis and UV-vis spectra of silver nanoparticles was conducted by Bhui et al. in 2009. In the study, the absorption spectra of silver nanoparticles synthesized from $AgNO_3$ at different time intervals were compared. The peak value at about 400nm seen in Figure 4 is in agreement with the work of Bhui et al. [22].

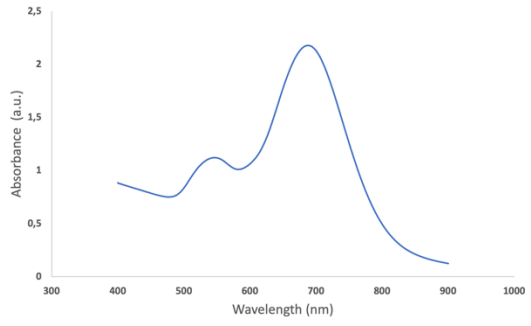

Figure 3. UV-Vis Spectrum of Gold nanorods

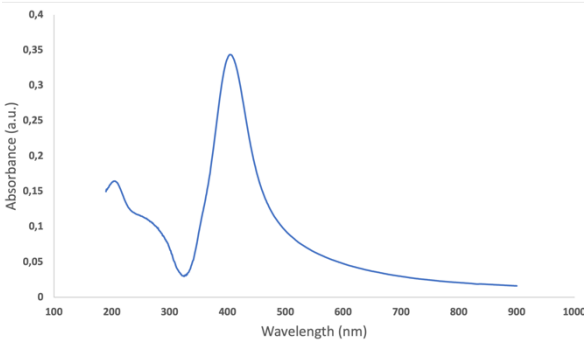

Figure 4. UV-Vis Spectrum of Silver nanoparticles

Gold nanorods were added to the mixture containing four different concentrations of urine prepared in the previous step, and measurements were taken from ten different points for all four samples after drying. Urine and apple juice SERS signals were seen at 667, 735, 775, 958, 1100, 1452, 1597, and 1654 cm^{-1} (Figure 5a).

Finally, silver nanoparticles were added to four mixtures containing different concentrations of urine. Again, in the SERS spectra taken from 10 different points, peaks were observed at 657, 735, 962, 1009, 1043, 1260, 1330, 1492, 1562, and 1607 cm^{-1} (Figure 5b). As seen in Figure 4, the spectra taken for different concentrations of urine appear to be reproducible.

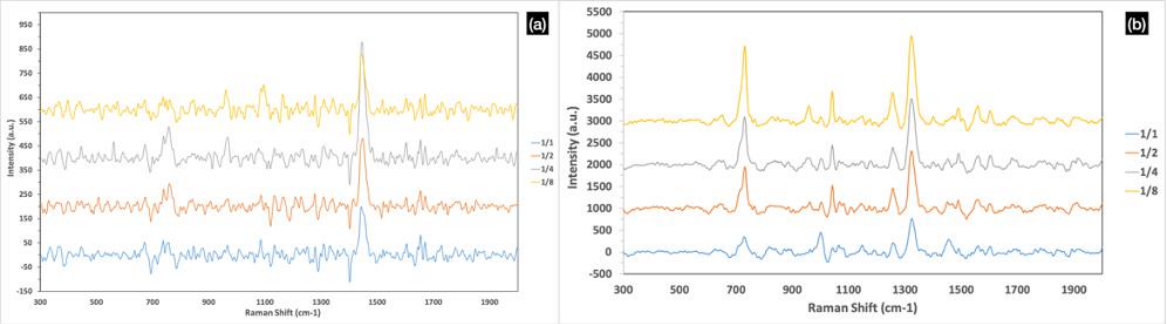

Figure 5. Comparison of SERS spectra of 1:1, 1:2, 1:4, and 1:8 concentrations of urine and apple juice mixture (a) with Gold nanorods and (b) Silver nanoparticles

Table 1. Percentage Standard Deviations of SERS spectra (a) Urine and apple juice mixture (b) With gold nanorods (c) With silver nanoparticles

(a)

		Raman Shift (cm^{-1})							
		484	**656**	**735**	**1130**	**1224**	**1460**	**1583**	**1658**
The concentration of urine in the mixture	**1:1**	24,9	27,0	23,3	23,2	17,6	27,2	19,0	20,4
	1:2	28,7	17,3	23,7	15,2	17,0	12,4	10,2	5,1
	1:4	22,6	8,8	12,9	14,7	12,6	19,9	14,6	14,6
	1:8	12,4	16,7	15,6	8,7	10,4	7,6	8,3	10,8

(b)

		Raman Shift (cm^{-1})							
		667	**735**	**775**	**958**	**1100**	**1452**	**1597**	**1654**
The concentration of urine in the mixture	**1:1**	19,7	11,9	5,4	14,2	11,5	13,0	17,5	14,8
	1:2	15,5	4,8	8,0	16,8	4,6	5,8	12,8	10,8
	1:4	8,0	6,3	6,4	11,4	5,9	5,3	4,9	3,9
	1:8	6,5	8,0	4,9	1,9	14,4	7,0	9,2	8,2

(c)

		Raman Shift (cm^{-1})									
		657	**735**	**962**	**1009**	**1043**	**1260**	**1330**	**1492**	**1562**	**1607**
The concentration of urine in the mixture	**1:1**	5,2	6,5	3,7	3,6	5,4	5,8	8,9	4,7	1,7	5,5
	1:2	16,6	14,9	11,3	6,5	2,0	11,0	4,9	6,8	8,2	7,4
	1:4	7,1	7,0	7,1	5,0	6,2	3,2	2,0	4,0	3,5	3,4
	1:8	6,4	5,5	5,6	4,2	5,9	3,5	10,3	5,5	7,7	7,3

To demonstrate the reproducibility of the spectra results obtained from ten different points in each of the prepared mixtures, standard deviation calculations were performed. Standard deviation calculations were performed for the Raman shifts of urine and apple juice seen in the above SERS spectra. The resulting standard deviation data for the urine-apple juice mixture, the mixture with added gold nanorods, and the mixture with added silver nanoparticles are given in Table 1. It is difficult to give a specific average standard deviation

value for SERS (Surface Enhanced Raman Scattering) spectra, as it can vary depending on various factors such as the quality of the substrate, the strength of the incident laser and the type of analyte being studied. However, in general, the standard deviation of SERS spectra is expected to be relatively low due to the enhancement of the Raman signal by the plasmonic properties of the substrate and nanoparticles. Standard deviation values in the range of 1-7% have been reported in some studies, but these studies often only considered the expected signal wavelengths and substrate, not the entire spectrum. The percentage of standard deviation reaches up to 30% at randomly selected wavelengths [23-25]. When Table 1 is examined, the standard deviation decreases to 2% for both the silver nanoparticle and the gold nanorod mixture. For the mixture containing only urine and apple juice, a standard deviation of about 15% is observed on average. It is observed that when the urine concentration is high the standard deviation tends to increase, cause was thought to be due to the fluorescence effect. The high standard deviation rate seen in some peaks is thought to be related to the mixture homogeneity and surface as well as the fluorescence effect.

3. DISCUSSION

Initially, the spectra of the substrate, urine, and apple juice used for analysis were examined separately. Although the noise coefficient was high, repeatable peaks were observed. Due to the fluorescence effect, which is typical of biological fluids, the urine spectra had relatively low signal-to-noise ratios. However, as urine concentration was diluted, stronger SERS signals were observed. These results obtained are consistent with previous studies [26-34]. In the case of apple juice, reproducible signals were obtained in the analysis; however, the signal intensities were relatively weak, likely due to fluorescence and noise effects. Few studies have investigated the SERS spectra of apple juice, and the results obtained in this study are in agreement with those reported in the literature [35-37]. The addition of gold nanorods and silver nanoparticles resulted in a substantial enhancement of SERS signal intensities in the urine-apple juice mixture. This can be attributed to the plasmonic properties of the nanoparticles, which increase the electromagnetic field near the surface and enhance the Raman scattering effect [38-40]. The absorption spectrum of the gold nanorods, as in Figure 3, revealed two peak values which come from the transverse and

Table 2. Peak positions and compound assignments of the Raman bands in human urine [11,15,45,46]

Ramanshift (this work) (cm^{-1})	Ramanshift (reference) (cm^{-1})	Molecular Vibrations	Compound assigned	Reference
775	772	-	Alanine	Chen et al. (2020)
875	846	-	Creatinine	Chen et al. (2020)
1009	1006	N-C-N stretching	Urea	Hoccart et al. (1993)
1040-1043	1050	-	Creatinine	Furukawa et al. (2006)
1100	1090	-	Phosphate, Histidine	Lin et al. (2020)
1120	1124	-	Uric acid	Cael et al. (1974)
1130	1130	C-O	Uric acid	Lin et al. (2020)
1260	1263	C-H bending	Amide	Cael et al. (1974)
1452	1456	-CH2	Hydroxybutyrate	Gelder et al. (2007)
1460-1465	1460	-CH2	Urea	Cael et al. (1974)
1492	1490	-	Creatinine	Premasiri et al. (2001)
1597	1596	C-O	Alanine, Serine	Lin et al. (2020)
1607-1612	1608	-	Urea	Frost et al. (2000)
1654-1658	1650	H-O-H bending mode	Water	Mossoba et al. (1999)

longitudinal plasma bands of the nanorods. These peak values can vary depending on the aspect ratio of gold nanorods, and the graph shows that good nanorods are obtained in consistent with similar studies [21,41,42]. Furthermore, the absorption spectrum of the silver nanoparticle, as in Figure 4, confirmed that particles with a size of ~30nm were successfully obtained, as previously revealed in reference studies [22,43,44].

The signal enhancement for SERS could theoretically reach the order of 10^{14}. However, this may vary depending on several factors such as the size and shape of the nanoparticle, the excitation wavelength, the distance between the nanoparticle and the analyte molecule, and the chemical properties of the molecule. Fluorescence and noise effects were suppressed in the mixture containing silver nanoparticles, leading to a signal increase of approximately 20 times. It was observed that the signals became more pronounced in the SERS spectrum with the addition of gold nanorods, however, the signal enhancement remained at approximately 4 times. Standard deviation analyses were performed to clarify the effect of gold and silver on the SERS signals. It was determined that the mean standard deviation decreased with the dilution of the urine concentration and very similar results was obtained in repeated measurements. As shown in Table 1, the lowest standard deviation was observed in the silver nanoparticle added mixture, where the strongest signals were obtained.

Considering all three cases in Table 1, it is seen that the deviation decreases as the signal strength increases. The results obtained are compatible with similar studies in the literature [23-25]. Upon examination of the results, it was found that the peaks acquired for gold nanorods, silver nanoparticles, and only urine-apple juice mixture are compatible with each other. The molecular interactions of the observed urine peaks were compared with data collected from the literature, as shown in Table 2.

Furthermore, it should be noted that urine samples collected from crime scenes may not always originate from healthy individuals. The sample owner may be a patient or a drug user, which may add another dimension to the investigation and direct it into other analytical procedures. Raman spectra of urine obtained from a healthy individual and a drug user can differ significantly due to the presence of different chemical components and certain biomolecules. Human urine from drug users may contain various drugs or their metabolites that can be detected by unique Raman spectra. In a study published by West et al., in 2010, it was shown that different signals occur in the Raman spectra of urine from individuals who have used ketamine, MDMA, cocaine, and amphetamine compared to a healthy person. It was observed that all of the spectrums were different from each other and from a healthy individual [47]. As evident, signal levels have changed in some molecular interactions, and peaks have appeared at different wavelengths. These situations should also be taken into account. Moreover, it is important to note that spectral differences between the urine of a healthy individual and a substance-addicted individual may vary depending on the specific drugs or substances involved, as well as the experimental conditions used for Raman spectroscopy. Several studies have compared the SERS of urine samples from healthy and drug-addicted individuals, revealing the differences in the spectra obtained [30,47–49].

4. CONCLUSION

Ensuring justice is a crucial issue in forensic medicine, and it is essential to analyze the evidence collected from the crime scene in a non-destructive manner as soon as possible. To this end, many studies have focused on Surface Enhanced Raman Spectroscopy (SERS), which is a promising method. The data obtained in this study demonstrated that urine can be detected both alone and in a mixed crime scene using SERS. Additionally, it was observed that the advanced plasmonic properties of gold nanorods and silver nanoparticles significantly increased SERS signal intensities. Therefore, SERS provided fast and accurate results in the non-destructive analysis of urine, even in a complicated and contaminated crime scene. It is suggested that the efficiency of SERS can be improved by optimizing the nanoparticles used for signal enhancement, and it can be used as a standard in the detection of urine in forensic crime scene analysis.

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DECLARATION OF ETHICAL STANDARDS

At the meeting of the Non-Interventional Clinical Research Ethics Committee at Hacettepe University, held on May 4, 2021, it was deemed appropriate to take biological samples from the volunteers with the decision numbered 2021/10-01.

AUTHORS' CONTRIBUTIONS

Uğur KÖROĞLU: Performed the experiments and analyse the results. Wrote the manuscript.

Necdet SAĞLAM: Determining the method used, setting up the model and interpreting the results.

Uğur TAMER: Performed the experiments and analyse the results. Wrote the manuscript.

Ramazan AKÇAN: Appropriateness of the methods and application process review, analyse the results and general review of the article.

CONFLICT OF INTEREST

There is no conflict of interest in this study.

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