

Graphene as a Surface Enhancement Raman Scattering (SERS) Substrate

Hleko Chauke^{1,2}, Sanele Nyembe¹, Nikiwe Mhlanga¹, Nosipho Moloto², Lucky Sikhwivhilu^{1*}

¹DSI/Mintek Nanotechnology Innovation Centre, Advanced Materials Division, Mintek, Randburg, 2125, South Africa

²Department of Chemistry, University of Witwatersrand, Braamfontein, Johannesburg, 2000, South Africa

*Corresponding author

Lucky Sikhwivhilu, DSI/Mintek Nanotechnology Innovation Centre, Advanced Materials Division, Mintek, Randburg, 2125, South Africa.

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Abstract

The discovery of surface-enhanced Raman spectroscopy (SERS) presents an opportunity for the development of ultrasensitive biosensors. SERS allows for fingerprint detection of biomolecules at low concentrations and can perform single-molecule detection. The most used SERS substrates are Plasmonic metal nanostructures such as Au and Ag mainly because of their superior electrical conductivity. The Plasmonic metal nanoparticles enhance the Raman signal of the target biomolecules through the electromagnetic field mechanism. Despite the successful use of metal nanostructures, alternative materials have been sought which can exhibit similar or better properties than their metal counterparts. Materials such as graphene which has excellent electrical properties and high surface area are good candidates for SERS applications. Graphene is a 2D nanomaterial consisting of sp² hybridized carbon atoms. The use of graphene in SERS is termed graphene-enhanced Raman spectroscopy (GERS) and is reported to use a charge transfer mechanism. Both graphene and metal nanostructures can be used together as SERS substrate and the synergistic effect of their mechanism can improve the enhancement factor and the sensitivity of the biosensor; however, the use of graphene as a SERS substrate is still at an early stage. GERS can be applied in disease diagnosis, detection of food additives, and biological species biosensors. The use of GERS in biosensors such as glucose biosensors presents an opportunity for ultrasensitive biosensors which can greatly improve the lives of individuals living with diabetes. In this review insights will be given on how to successfully circumvent the challenges associated with small Raman cross-section of glucose detection for use in medical application.

Introduction

The development of ultra-sensitive biosensors for medical diagnostics application is of significant importance not only for saving lives but also for the optimization of patient-treatment strategies and providing insights into the effectiveness of the treatment [1]. There is a growing interest in research towards the development of ultrasensitive sensing technologies such as electrochemical techniques, chromatography techniques, optical techniques, etc. [2]. Amongst the techniques, the optical techniques are the most promising techniques due to their intrinsic molecular specificity and multiplexing capabilities [3]. There are several types of optical techniques such as near-infrared (NIR), mid-infrared (MIR), Raman spectroscopy (RS), etc. [4]. The RS has drawn much attention as it can provide molecular fingerprint information. Furthermore, this technique can be used in non-invasive sensors. The RS has potential for use in ultrasensitive sensors because it can provide desirable characteristics such as rapid detection, label-free, simple sample pretreatment, and high stability during sensing [5]. The main drawback of RS is the low Raman scattering of biological molecules which limits its applicability in biosensors. However, several efforts have been made to improve the Raman scattering of biomolecules. These efforts include the use of different dimensions of nanomaterials such as zero-dimensional (0D), one dimensional (1D), two dimensional (2D), and three dimensional (3D) nanomaterials [6,7].

Notably, the most studied materials for improving Raman scattering are metallic nanostructures such as Au, Ag, Cu, etc. due to their high electrical conductivity and Plasmonic properties [8,9]. Studies have shown that the Raman activity of biological molecules can be significantly improved by the presence of metallic substrates [10-12]. This phenomenon was named surface-enhanced Raman scattering (SERS) and has been intensively researched due to its ability to detect low analyte concentrations [2]. The mechanism of enhancement using metallic nanoparticles is termed electromagnetic enhancement (EM) as it uses the surface Plasmon resonance to increase the local electromagnetic field around the biomolecule [13]. There have been attempts to improve the effectiveness of the nanoparticles by changing their morphology, texture, size, etc. which can improve the enhancement factor (EF) [14,15]. Despite their wide applicability in SERS, metallic nanoparticles have drawbacks such as high cost and low adsorption ability towards biomolecules [15]. The drawbacks limit the extent of the applicability of these materials and there is a need to explore other materials. Alternative nanomaterials are being studied and 2D materials have attracted much attention due to many unique properties with some properties similar to those of metallic nanoparticles.

In 2D nanomaterials, the electrons could be confined in a 2D structure which enables good transport of electrons without

interlayer interaction [16]. The ultra-thin size of each layer of 2D nanomaterials provides them with a relatively high specific surface area which enables adsorption of biomolecules [17]. The atomic thickness and strong chemical bonds give them high flexibility, strong mechanical strength, and good optical properties [17]. Furthermore, the large fraction of surface atoms allows for surface modification to enhance the intrinsic properties [13]. Due to these advantages, 2D materials have become the material of choice for most applications including SERS. One such 2D nanomaterial that sparked much interest is graphene due to its relatively high electrical conductivity and strength when compared to other 2D nanomaterials [5].

Graphene is a one-atom-thick two-dimensional (2D) carbon nanomaterial with a hexagonal packed sp² bonded carbon atom arranged in a honeycomb lattice [18]. Since its discovery in 2004, graphene has been at the forefront of 2D nanomaterial exploration for various applications such as energy storage, chemical and biological sensors, etc. [19]. Recently graphene has been applied as a SERS substrate which presented an opportunity for ultrasensitive SERS sensors. The mechanism of Raman scattering enhancement using graphene is attributed to the charge transfer between graphene and the analyte [13]. Factors that determine the Raman scattering enhancement include the graphene Fermi level, analyte molecular structure, and laser excitation wavelength [14].

The synthesis methods for graphene can be classified into two categories namely the top-down and bottom-up techniques. Top-down approaches involve the breaking down of bulk materials by overcoming the interlayer van der Waals forces into nano-sized particles or structures [20]. Top-down techniques include the mechanical force-assisted exfoliation method [21]. It is worth noting that the main drawback of top-down is the imperfection of the surface structure [22]. On the other hand, graphene can be synthesised using bottom-up techniques. Bottom-up approaches involve the building up of material from the bottom i.e. atom by atom, molecule by molecule or cluster by cluster [23]. The bottom-up techniques include the chemical route such as chemical vapour deposition (CVD), etc. [23,24]. Unlike top-down methods, bottom-up techniques are used in practical applications and offer a wide range of advantages as they are more economical, offer better control during synthesis, are easily scalable, and

are reproducible [25]. In subsequent sections, different SERS substrates will be discussed with emphasis on graphene, SERS mechanism and application in biosensing.

SERS Enhancement Mechanisms

The mechanism of sensing in SERS is still debatable, however, two mechanisms are well accepted throughout the science community and these include the electromagnetic (EM) and chemical enhancement (CE) as shown in Figure 1a [26,27]. The EM has been reported to induce Raman enhancement as a result of localised surface Plasmon resonance (LSPR) and is the main enhancement contributor for most metallic SERS measurements. The Raman scattering signal increases when a Raman active molecule is confined within a range of the electromagnetic field upon excitation of visible light [28]. The nanoparticles which are the SERS substrates are excited by the visible light [29]. The excitation causes electron oscillation inside the nanoparticles, which in return enhances the electromagnetic field as shown in Figure 1b. The EM mechanism can enhance the SERS signal to the magnitude of 10⁶-10⁷ times [14].

The 2nd proposed mechanism is the CE, which is mainly due to the charge transfer between the surfaces of the nanostructures such as graphene and the adsorbed molecule [30]. Figure 1c depicts the possible contributors to the CE mechanism. The first is the charge transfer which can occur in the direction from the metal to the molecule or from the molecule to the metal depending on the location of the Fermi level relative to the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The excitation light can excite the electrons from the HOMO to the LUMO indirectly through the metal [31]. The second one occurs when the excitation light is in resonance with the metal complex whereby the electrons can move from the HOMO directly into the unoccupied molecular orbital as shown in Figure 1c. [31-33]. The magnitude of enhancement of CE is 10-10², however, this number can be improved significantly by the choice of synthesis methods and functionalisation of the substrates [34,35].

To realise a complete mechanism and improved performance, the substrate should have vast SERS-active hotspots to ensure sensitivity, selectivity, uniform size, morphology, and stability [2].

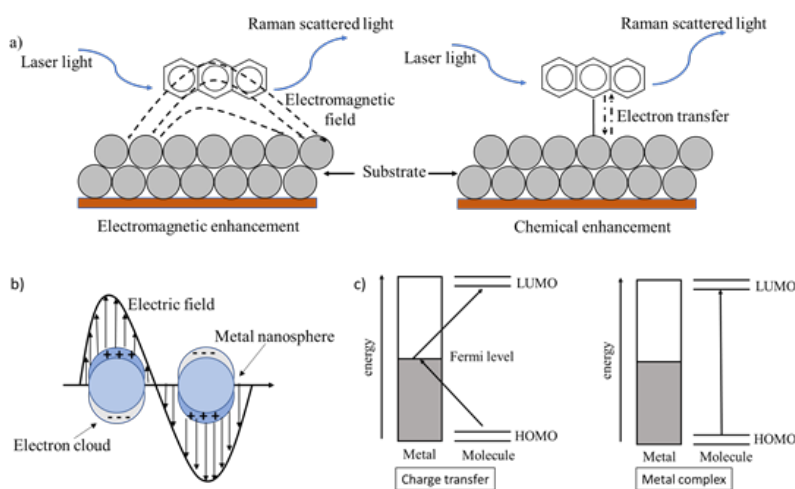


Figure 1: a) Schematic diagrams of electromagnetic and chemical enhancement. Adopted from [27].
 b) Schematic diagram showing excitation of LSPR. Adopted from [27,32].
 c) Chemical enhancement contributors. Adopted from [31].

Metallic nanoparticles and surface Plasmon resonance

The most used metallic nanoparticles in SERS are gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) because of high EF [34]. The TEM images of these nanoparticles are depicted in Figure 2 (a and c). The success of SERS is dominantly dependent on the interaction between the adsorbed molecules and the surface of the Plasmonic nanostructures [36]. Most Raman measurements occur around the visible and near-infrared wavelength range. Au, Ag and Cu nanostructures have LSPR that covers most of the aforementioned wavelength range as depicted in Figure 3, making these metals to be suitable SERS substrates [36]. Ag nanoparticles have been reported to have a high SERS EF when compared to other metallic nanoparticles such as Au and Cu, however, their reactivity prompts the use of stabilizing agents such as a polymer or graphene support [37]. Furthermore, redox reaction usually interferes during the SERS measurements when using Ag. It is worth mentioning that Ag nanoparticles show better EF compared to Au nanoparticles, however, different morphologies of Au nanostructures can produce better EF than Ag [8,38]. Au nanostructures have been proven to be the best candidate for application as SERS substrate because of their great stability, good biocompatibility, versatile morphologies (which affect the EF), etc. [5,9]. Au nanostructures also provide reproducible and reliable SERS measurements, allowing tests to be carried out in different conditions [39]. The reproducibility of SERS measurements is affected amongst other factors by the size distribution of the metallic nanostructures. A good size distribution leads to a better signal and reproducible measurements. The size distribution histograms of AuNPs and AgNPs are depicted in Figures 2 (b and d). The narrower size distribution indicates uniformity which reduces the susceptibility of a sensor to give false results. The average particle size of the depicted AuNPs and AgNPs are 14nm and 31 nm respectively. It is worth noting that the size of the nanoparticles must be smaller than the wavelength of the light to produce LSPR, however, if it is too small it results in the poor polarization of the nanoparticle and then poor plasmon resonance [11].

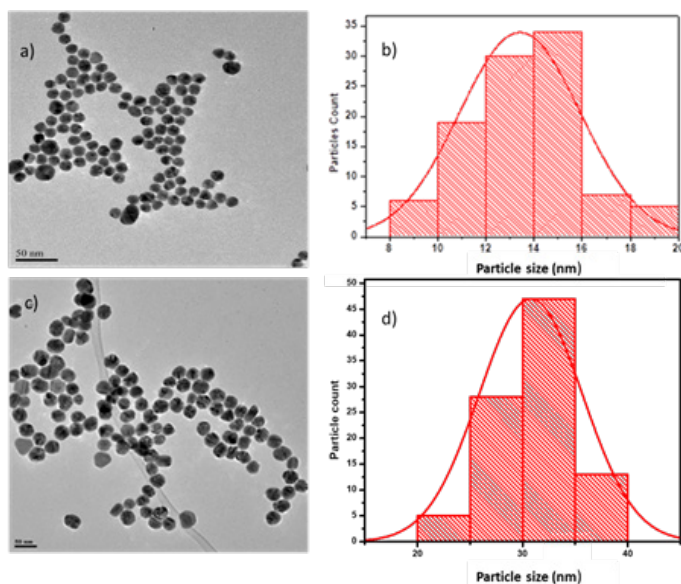


Figure 2: a) TEM image of AuNPs, b) Size distribution histogram of AuNPs, c) TEM image of AgNPs and d) Size distribution histogram of AgNPs

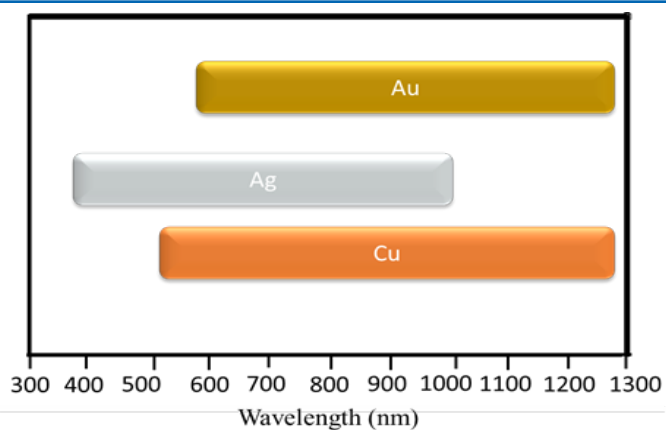


Figure 3: Wavelength range of Cu, Au, and Ag [36]

The chemistry of metallic nanostructures has been a subject of many research publications, for this reason, this section will focus on the plasmonic properties of metallic nanostructures as SERS substrate. The colours of some metallic nanostructures are due to their plasmon absorption band, which forms part of the basis for their application in SERS. The absorption band is formed when the light source is in resonance with the excitation of the conductive electrons of the particle. This effect is known as LSPR [40]. The LSPR of Au and Ag usually occurs at wavelengths between 520-800 nm and 420-780 nm respectively as depicted in Figure 3. The excitation of the LSPR leads to tuneable and enhanced electromagnetic fields, light absorption, and scattering based on the elemental parameters of the nanostructures [41]. AuNPs and AgNPs have relatively strong LSPR responses when compared to other metallic nanostructures, hence, they are the best candidates for SERS studies. The shape, morphology, size, etc. of the metallic nanostructures are some of the factors that affect the LSPR [14]. Strongly enhanced electromagnetic fields can be observed at the junctions, sharp edges, and narrow gaps of nanostructures [42]. Furthermore, the packing densities and inter particles gaps also affect the LSPR [43]. These localised electromagnetic fields are termed “hotspots” and to get a high enhancement factor, the occurrence of hotspots must be increased [8]. This leads to more efforts being made to fabricate Plasmonic nanoparticles with controllable sizes and morphologies to increase the hotspots and reproducibility of the signal [44]. Hence, various shapes can be synthesised such as nanoplates, nanocubes, nanochains, nanoflowers, nanostars, nanorods, nanocaps, nanoclusters, and hexagonally structured patterns [15,21,29,45,46]. These hotspots can be classified into three generations. The first generation of hotspots is generated from single nanostructures. When the nanostructures are dispersed in a homogeneous solution, a single nanostructure can produce hotspots. The first-generation hotspots exhibit moderate SERS activity [30]. The second-generation SERS hotspots are generated by nanostructures with controllable nanogaps. This generation of hotspots produced by nanogaps shows relatively good SERS activity with average orders of magnitude around 10^2 - 10^4 and can be used to detect single molecules [47]. The third-generation hotspot is generated by hybrid nanostructures. The nanostructures consist of plasma nanostructures and probe material. This generation of the hotspot is suitable for all surface analysis of materials when compared with the first two generations hotspot which are only suitable for some analysis [15].

Graphene

2D nanomaterials, especially graphene presents an opportunity for an alternative SERS substrate to the traditional metal substrates. Graphene is made of carbon atoms arranged in two intersecting triangular lattices which form a honeycomb pattern as shown in Figure 4a. The structure of graphene is unique when compared to its bulk counterpart, graphite [13]. Graphene exhibits Dirac cones where the conduction and valence band meet which are the six locations in the vertices of its hexagonal Brillouin zone [35]. The vertices are divided into two non-equivalent sets of three points. The points are labelled as K and K' as shown in Figure 4c. This causes low-energy electron excitations to behave as massless Dirac fermions, which exhibits the quantum Hall, Shubnikov-de Hass and Klein tunnelling effects that play a role in graphene applicability [33,48-50]. This can be explained using the linear Π band electronic dispersion for graphene given by

$$E = \hbar v_F K \dots \dots \dots 1$$

Where \hbar is Planck's constant and v_F are the electron Fermi velocity in graphene. This equation leads to the description of carriers in graphene as "massless Dirac Fermions" [50]. The electronic properties of graphene can be determined by the bonding of Π and antibonding of Π^* orbitals forming the electronic valence and conduction band of graphene as shown in Figure 4 (b and c). Graphene became the material of interest for use in SERS primarily because of its electrical conductivity [51]. Graphene is a gapless semiconductor carbon nanomaterial with the carrier systems changing at the Dirac point from electrons to holes and vice versa in a single structure [52]. This gapless or small bandgap nature of graphene makes it behave in a metallic nature with respect to electrical conductivity where the Fermi level is always in the conduction or valence band [33]. Graphene behaves the same as metallic nanostructures in terms of electrical conductivity, however, it has a better surface area (2600 m²/g) which makes it a competitive alternative nanomaterial for SERS application. Graphene also has a relatively high electrical current density when compared to some metallic nanoparticles [53]. Some metallic nanoparticles are known to have poor stability (which shortens the sensor lifespan), poor biomolecules absorption (which affects the sensitivity), weak biocompatibility which can lead to false signals, etc [33,54]. Graphene-based substrate overcomes these drawbacks and has a better affinity towards biomolecules due to the π - π stacking [6].

The structural defects of graphene have been reported to promote electron mobility rate by reducing the electron scattering during transport [50]. Graphene has low Johnson noise which is the noise generated by the thermal vibrations of the carriers inside the electric conductor [50]. Graphene possesses relatively low resistivity, approximately 10⁻⁶ $\Omega \cdot \text{cm}$ which is lower than that of Ag nanoparticles and Cu. [7,54,55]. The electrical conductivity of graphene can increase up to 10⁶ S/m. Graphene is by far the thinnest, lightest, strongest, best heat and electricity conducting material ever discovered. And if this is anything to go by, graphene is expected to revolutionise the use of ultrasensitive biosensors for disease diagnosis amongst other many applications.

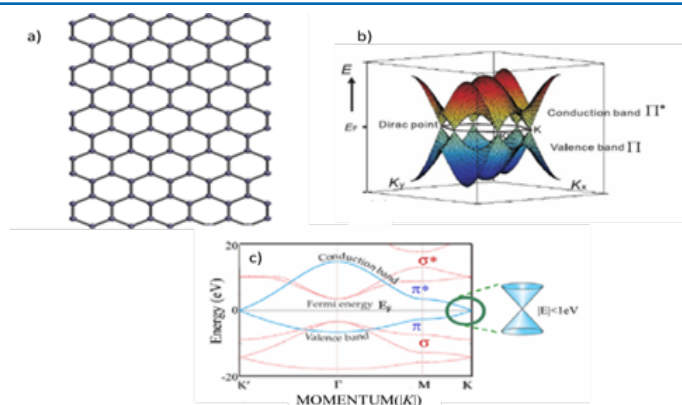


Figure 4: a) Flat sheet of monolayer graphene [33]. b) Stereoscopic graph representation of electronic band structure of graphene in the first Brillouin zone. Reproduced from [50]. © 2011Giannazzo F, Sonde S, Raineri V published under CC BY 3.0 license. Available from <http://doi.org/10.5772/15258>. c) Graphical representation of the electronic band structure of graphene in the first Brillouin zone. Reproduced from [50]. © 2011Giannazzo F, Sonde S, Raineri V published under CC BY 3.0 license. Available from <http://doi.org/10.5772/15258>.

Graphene Synthesis and Electronic Properties

The mobility of graphene has been reported in the literature to be in a wide range of approximately 10⁴ cm²v⁻¹s⁻¹ depending on the synthesis method and substrate choice [50]. This shows that the excellent electron mobility of graphene is dependent on the appropriate synthesis methods. Attempts are already being made to gain consistent control over the electronic properties. Notably, the control of density and structure through doping has been a promising way to control the electronic properties during synthesis. Different synthesis methods have been intensively studied over the past decade. Mechanical exfoliation is one of the earliest methods for the fabrication of graphene [56]. This method can successfully be used to fabricate pure graphene. However, it has several drawbacks such as long processing time, low reproducibility, low yield, limited application and requires trial and error approach [20]. Another method is the Epitaxial growth of graphene on silicon carbide (SiC) substrate. This method produces few-layer graphene, however, it is associated with several drawbacks such as relatively high synthesis temperature, high cost of SiC substrate and requires high vacuum [57,58]. The graphene produced using mechanical exfoliation shows poor SERS measurements because of the relatively poor quality of graphene while the graphene produced by the epitaxial method shows better SERS measurements. However, the cost of synthesis is high which limits the applicability [59].

Chemical methods which include chemical exfoliation and chemical vapour deposition (CVD) have also been studied. The chemical exfoliation of graphene using modified Hummer's methods is one of the widely studied chemical methods [60]. This method produces graphene with a controllable number of layers [61]. However, the graphene produced possesses significant structural defects which are induced by the reduction of graphene oxide [20]. Furthermore, the graphene produced has relatively poorer electrical conductivity and mechanical properties due to defects

which make it less applicable in some applications such as SERS biosensors [33]. The CVD technique has been reported to produce high-quality graphene with a controllable number of layers (mono and multilayer graphene) [62]. Moreover, this technique allows doping and making desired graphene structures with consistent electronic properties [23,63]. Substitutional doping using CVD can be achieved through the introduction of heteroatoms within the graphene lattice which tune the Physico-chemical properties of graphene [64]. The introduction of nitrogen in graphene is known to change the electronic properties by shifting the Fermi level and tune the bandgap [65,66]. The doped nitrogen also enhances the sensing properties by changing the charge distribution around the dopants [67]. As much as functionalization during CVD process can improve certain properties, several factors need to be controlled during the CVD process to achieve the desired graphene and these include catalytic substrates (typically Cu and Ni), reaction temperature, nature of precursor, base pressure, and gas composition [13]. The main drawback of this technique is transferring the graphene from the substrate into the insulating substrate for analysis. However, this is already being solved by using substrates that have weak interaction with graphene such as Cu foil substrates with weak van der Waals forces, making it easier to transfer to a non-conducting substrate [22,23].

Graphene-Based SERS (GERS)

The first use of graphene as a SERS substrate was reported in 2010 by Ling and co-workers [68]. In their study, they deposited common Raman molecules such as phthalocyanine (Pc) and rhodamine 6G on graphene and SiO₂/Si substrates using vacuum evaporation method. The Raman signal intensities of monolayer graphene were reported to be stronger than the SiO₂/Si substrate. Furthermore, graphene detected even low concentrations of the molecules (~10⁻⁸ mol/L). The reported results showed an enhancement factor of 10² which was slightly lower than that of metals which is 10⁶-10⁷. Ling and co-workers demonstrated that graphene can enhance the Raman signals of the absorbed molecules and that it is a promising candidate for future use in SERS to detect trace species [68]. The most likely mechanism that takes place during GERS is the CE mechanism, this is because the EM mechanism is ruled out as a possible contributor to GERS because the plasmon resonance of graphene lies in the terahertz (THz) region, while the excitation wavelengths for the Raman measurements using metallic nanostructures are in the visible [30,33,50]. Graphene provides an ideal flat surface and chemical interaction with many biological molecules [43]. Graphene also makes it possible to study the CE mechanism independently in a SERS platform [11].

The CE SERS mechanism is characterized by 2 CE methods, namely; the excited and ground state CE. Ling and coworkers investigated GERS CE using Raman excited profiles of phthalocyanide (CuPc) molecules [69]. Non-GERS and GERS samples Raman intensities were obtained whereby the non-GERS was the Raman excitation profile of CuPc and the CuPc deposited on a graphene surface was the GERS sample. The obtained Raman intensities were fitted into a Lorentzian function which revealed a ground-state charge transfer. Zheng and coworkers also

studied the GERS mechanism using CuPc as a probe molecule and electrochemically deposited graphene on TiO₂ as a GERS sample [70]. The graphene-TiO₂ nanocomposites GERS mechanism was studied experimentally and theoretically and a charge transfer between graphene-TiO₂ and CuPc driven by the TiO₂-induced fermi level shift of graphene was observed.

Graphene as a special kind of SERS substrate offers several advantages when compared with traditional SERS substrates and these advantages include uniformity, reproducibility, high sensitivity, and low detection limit leading to a controllable and quantitative GERS [71]. Furthermore, graphene possesses fluorescence quenching effects [72]. GERS overcomes some drawbacks of conventional SERS substrates and broadens the applications of SERS in many fields. The metallic nanoparticles show good optical properties whereas graphene exhibits relatively lower optical properties with 2.3% light absorption from visible light to terahertz [54]. However, graphene has the advantage of having a broad range of light absorption when compared to metallic nanoparticles. The percentage light absorption can be adjusted by changing the Fermi position. Graphene possesses ideal optical properties such as tunable absorption and polarization dependant effects. A GERS-based sensor has several advantages such as high spatial resolution, accurate detection, fast detection, and unlabelled samples detection [54]. These advantages show the competitive nature of graphene over metallic nanoparticles in SERS applications. The factors that form part of GERS can be divided into three categories: molecule, graphene, and excitation laser energy [18]. The categories should work together to give optimum sensing measurements. Factors that influence GERS system include graphene number of layers, graphene layer dispersions, molecular selectivity, and graphene fermi levels [52]. The molecular selectivity in GERS is one of the important properties and is depicted in Figure 5. The GERS system can be selective based on two main molecule characteristics, namely, molecular energy level and molecular structure [71].

The EF involving the molecular energy level requires the energies of the lowest unoccupied orbital and the highest occupied orbital to be in an allowable range relative to the graphene Fermi levels [30,37]. The molecular structure EF effect involves the molecular symmetry and the substituents which are similar to the graphene structure [33]. The validity of the EF through the molecular structure can be explained by the charge transfer interaction between biomolecules and graphene, this also gives insights into CE mechanism of Raman enhancement [73]. The controlled introduction of defects in the graphene, which changes the band alignment between biomolecule and graphene, consequently, leads to a change in electronic properties which affect the EF [50]. Huh and co-workers first demonstrated that changes in defects can affect the EF. They exposed graphene to UV treatment and showed that the intensity of the absorbed rhodamine B was greater than in pristine graphene. They reported an EF of 10⁴ on UV-treated graphene which was two magnitudes greater than that of pristine graphene [74]. This increase was attributed to the increased polarizability of the surface due to the oxygen molecules induced by the UV treatment. This enhanced the interaction between the molecule and the surface of graphene [33].

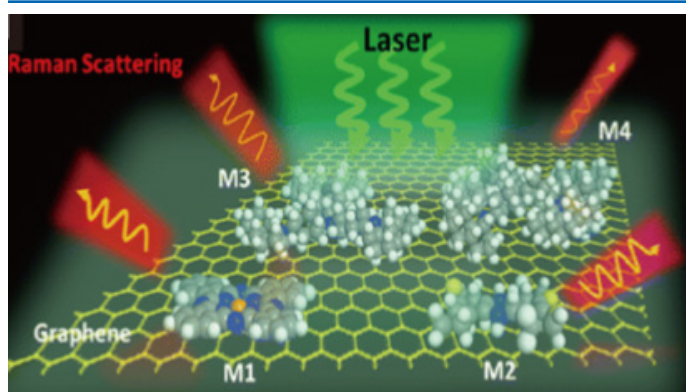


Figure 5: Schematic diagram of molecular selectivity in GERS. Reproduced from [71].

More studies have focused on the development of 2D nanomaterials and the immobilization of metallic nanoparticles on the surface to exploit the Physico-chemical properties [75]. The CE mechanism of graphene has had limited enhancement capabilities to date, with the EF relatively lower than metallic nanostructures, therefore, having a substrate with both mechanisms is desirable. In 2012, Xu and co-workers decorated a few graphene layers with SERS active metal nanoparticles [43]. They reported a significant improvement in Raman enhancement with reproducible signals which is attributed to the Plasmonic metal Nano island arrays arranged in a flat graphene creating an atomically smooth surface with electromagnetic hotspots. Metallic nanoparticles like AuNP are known to be Plasmonic and have become an ideal component in the detection of glucose, enzymes, microorganisms and cancer biomarkers [9]. The cooperative effect of graphene and metallic nanostructures can greatly improve sensitivity and selectivity. Furthermore, the flat sheet of graphene increases the stability of the plasmonic nanostructures such as Ag [37,43]. Graphene with the necessary modification

can show improved biocompatibility and reduced cytotoxicity in biosensing applications. Graphene and gold nanoparticles composites can be used to fabricate a highly stable sensor. Moreover, the composite exhibits excellent catalytic activity and improved biocompatibility [76].

It is worth noting that 2D nanomaterials can load more biomolecules due to relatively high specific surface area. Moreover, SERS active metal nanoparticles can easily be functionalised into the surface of 2D nanomaterials to make a composite. This composite has been reported to show a relatively high EF as compared to individual components, furthermore, the use of Plasmonic nanomaterials such as gold nanostructures with graphene has demonstrated superior properties such as improved chemical stability, biocompatibility, excellent catalytic activity, and remarkable surface chemical properties [37,46,71,77]. The 2D nanomaterial adsorbs more molecules on the surface and the arranged metallic nanoparticles offer more “hotspots”. Hence the system of graphene and Plasmonic metal nanoparticles can capture more molecules and adequately enhance the SERS signals [37]. Therefore, the enhancement effect of the GERS/metal nanoparticle comes from the sum of EM-based on the electromagnetic field from the plasma nanoparticles and the CE through the charge transfer and chemical bonding of graphene with the biomolecules [78]. The synergistic effect of these two mechanisms makes graphene/metal nanostructures an ideal substrate for SERS measurements. Other graphene derivatives such as reduced graphene oxide (rGO) have been used in a composite with Plasmonic nanomaterials [79]. Graphene/Plasmonic nanomaterials systems have been developed and applied in different diseases as shown in Table 1. Different morphologies of metal nanostructures have been mobilized on graphene surfaces for applications in the detection of various analytes. This shows that graphene with Plasmonic metal nanoparticles as SERS substrates can detect variety of analytes [80].

Table 1: Summarizes some of the graphene/Plasmonic nanostructures studies.

SERS substrate	Analytes	EF	Detection limit	REF
G/Au	Adenine	1,2 X10 ⁷	10 ⁻⁷ M	[11]
G/Au nanohexagons	cancer stem cells	—	10μg/L	[46]
CVD-G film/AuNPs	multiplex DNA	—	10pM	[35]
G/Fe ₃ O ₄ NPs	Glucose	-	0,8μM	[76]
Ag-Cu ₂ O/rGO	Glucose	—	10 ⁻⁸ M	[77]
rGO/AgNPs	S. aureus/E.coli	-	10 ⁵ CFU/cm ³	[80]

SERS Application in Glucose

SERS can detect chemical and biological species in different fields such as biochemistry, biosensing, electrochemistry, catalysis, etc. SERS biosensors have been used for the detection of various diseases such as Alzheimer, diabetes, cancer, and Parkinson’s diseases [7]. This section highlights the application of SERS in biosensors, specifically focusing on biosensors for the detection and quantification of glucose levels. The world health organization (WHO) reported that the number of people living with diabetes increased from 108 million in 1980 to 422 million in 2014 globally among adults over 18 years [81]. In South Africa (SA) diabetes mellitus accounts for 4.8% of the total deaths in the country, only second to tuberculosis according to WHO report [82]. These alarming statistics channeled more research towards finding faster, reliable, and accurate ways to diagnose diabetes at early stages such as the use of ultra-sensitive SERS biosensors [4].

Diabetes mellitus is a chronic disorder that requires the regulation of glucose levels in the human body [83]. A cure or a preventative treatment for diabetes is yet to be developed, making glucose monitoring one of the most effective ways of managing diabetes [84]. Glucose monitoring, regular exercise, and diet have been proven to prolong the life expectancy of the affected individuals [83]. Glucose monitoring can optimize patient-treatment strategies and provide insights into the effectiveness of the medication, exercise, and diet [85]. Failure to maintain the required glucose levels in the body results in secondary health complications such as kidney failures, nerves, and circulatory systems malfunction [86]. There are two major types of diabetes, namely, Type 1 and Type 2. Type 1 is a chronic condition whereby the pancreas produces little or no insulin and Type 2 is a chronic condition that affects the way the body processes blood glucose [85,87]. Type 1 accounts for 5-10% while Type 2 accounts for 90-95% of people living with diabetes [84]. The most

possible cause for Type 1 is both genetic and environmental factors while Type 2 is lifestyle dependent (i.e. lack of exercise and being overweight) [81]. The development of an ultrasensitive glucose sensor (sensitive enough to detect glucose from other bodily fluids such as saliva) that allows for real-time monitoring of glucose levels would greatly improve the lives of diabetic patients.

One of the first reported glucose SERS measurements was conducted using silver film on nanospheres (AgFON) as a substrate. Glucose is known to have a poor affinity for silver, hence, the results of SERS measurements on bare AgFON are relatively poor. The introduction of a layer such as the self-assembled monolayer (SAM) to bring the glucose closer to the electromagnetic enhancement of AgFON improved the glucose signal [88]. Introducing a SAM or a biological linker protects the substrate from oxidation. The SAM is stable, and the surface structure of the SAM can be fabricated for a specific application. However, the use of SAM or biological linkers results in complex systems. Direct sensing is desirable (i.e. using substrates that show a good affinity for glucose). Gold films over nanostructures (AuFON) also present an opportunity to improve the SERS measurements. The use of AuFON unlike AgFON allows for the direct sensing of glucose because Au has a better affinity for glucose. Studies have shown an improved EF of 10^7 [89]. It is desirable to have a substrate that will have a detection which is in line with the WHO standard (11.1 mmol/L) for glucose sensors [89,90].

Bimetallic systems can also be used as SERS substrates and can improve the EF factor. Bimetallic systems such as Au@Ag as SERS substrates give more electromagnetic field hotspots which result in improved detection limit, EF factor, and sensitivity [91]. Graphene as an alternative SERS substrate for the detection of glucose is a relatively new concept and is still being explored. One of the earliest applications of graphene as SERS substrate was conducted by Huang and co-workers who synthesized monolayer graphene using mechanical exfoliation in a silicon wafer substrate [29]. They used graphene as the SERS substrate for the detection of hemoglobin in the blood. The hemoglobin on the silicon wafer showed no Raman activity. After using a graphene-based substrate, it enabled facile observation of the hemoglobin fingerprints. Moreover, this study showed that the Raman signals of biomolecules can be enhanced when measured in direct contact with graphene without a linker or Plasmonic nanoparticles. Similar applications have been conducted using graphene as SERS substrates, which was also the basis of using graphene in glucose detection and quantification [92]. The graphene produced by the CVD method has high electrical conductivity and improved quality. Hence, this graphene can show impressive SERS performance towards glucose [73].

Graphene has also been used as a support to Plasmonic nanostructures in the measurements of glucose. Gupta and co-workers fabricated Ag@AuNP/GO functionalized with mercaptophenyl boronic acid as a SERS substrate [93]. The developed biosensor exhibited a glucose detection linear range of 2-6 mM and a detection limit of 0.33 mM. Zhu and co-workers synthesized silver-coated gold nanorods (AuNRs@Ag) in graphene oxide (GO) as a SERS substrate for the detection of glucose in urine [94]. The study showed successful detection of glucose in synthetic urine. The detection of glucose in the urine shows that SERS can be used to detect glucose with other bodily fluids and not just blood.

Current developed GERS sensor substrates include the use of GO/Au nanorods as SERS substrate and measures glucose concentration through the change in Raman activity of ascorbic acid [95]. GERS offers a possibility of ultrasensitive biosensors for glucose levels monitoring, which can be done in a non-invasive manner. There is still room for improvements regarding the EF and detection limit so that the GERS glucose sensor can be competitive when compared to other glucose sensors

Challenges and Perspective

The use of GERS in biosensors has shown excellent performance, however, there is still much to be done in the fabrication of the substrate and better understanding of sensing mechanisms. The GERS substrate encounters some challenges such as lack of proper graphene modification with desirable properties for SERS measurements, little understanding of the mechanisms during the SERS measurement, lack of understanding of the synergistic effect of the EM and CM mechanisms, reliable and efficient large-scale preparations of graphene and its composite, effective designing of stable substrates with multifunctional properties and precise control of hotspot to maintain the sensitivity and EF [97]. Apart from challenges associated with substrates, some challenges associated with the bio-applications of GERS still need to be addressed [51]. Graphene stability in complex real sample analytes is still a challenge, the large surface area of graphene could cause nonspecific adsorption which can lead to disruptions in measurements. The cytotoxicity of graphene has been a debate in the past decade, therefore, the toxicity of graphene-based substrates for SERS still needs to be carried out on graphene and its hybrids [98]. The balance between reproducibility, sensitivity, and selectivity remains a significant part of the performance of SERS.

Conclusion

The use of 2D graphene sheets as SERS substrates opens new possibilities in biosensing. SERS is an ultrasensitive technique that allows for the fingerprint detection of biomolecules at low concentrations. SERS provides significant structural information of bio-samples such as blood. Graphene presents an excellent substrate for SERS because its surface area allows for strong adsorption of target species. The synergistic effect of graphene and Plasmonic nanoparticles can improve EF and sensitivity. Finally, the use of graphene in SERS overcomes the limitations associated with the conventional SERS substrates and with proper modification, GERS can lead to practical applications of SERS. The use of GERS can lead to a portable and non-invasive sensor. For the GERS to strive it needs to compete with some of the commercial glucose sensors such as the commercial glucose meter with a detection limit of 0.6 mM. Other sensors include the electrochemical sensor with a detection limit of 0.035 and the plasmonic metal nanoparticles SERS.

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