Surface-Enhanced Infrared Attenuated Total Reflection Spectroscopy based on Carbon Nanomaterials

DISSERTATION
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Abstract

This cumulative dissertation focuses on studying the potential of carbon nanomaterials in surface-enhanced infrared absorption (SEIRA) spectroscopy, which is based on four peer-reviewed journal articles. The introduction of this dissertation outlines the motivation and theoretical background. Namely, fundamentals and challenges of SEIRA as well as conventional Fourier transform infrared (FTIR) spectroscopy are introduced. As promising enhancing materials serving as SEIRA substrates, carbon nanomaterials, and particularly graphene, graphene oxide, and carbon-based nanodots are highlighted in respect of their extraordinary properties and advantages vs. commonly used noble metals. Given the advantage of analyzing aqueous samples with enhanced sensitivity, infrared attenuated total reflection (ATR) configurations are beneficial to SEIRA applications with the basic principles and merits likewise introduced. Latest progress, applications, and challenges in SEIRA spectroscopy utilizing graphene-based nanomaterials as the signal-enhancing substrate are reviewed in an associated journal article.

The research presented in this dissertation provides innovative platforms for SEIRA sensing, in particular in-situ and real-time SEIRA monitoring of molecular species in aqueous environments.

Firstly, graphene-decorated ATR prisms serving as a novel and efficient platform for simultaneously enhancing multiple characteristic IR bands of molecules in aqueous phase
was successfully developed for the first time, and also enabled recording the temporal evolution of the entire molecular vibrational fingerprints. Generic applicability of the proposed platform was verified via various molecules with aromatic moieties.

Secondly, readily obtained water-dispersible carbon nanodots (CNDs) that were drop-casted onto the ATR waveguide surface have demonstrated the pronounced ability to notably enhance the IR signals of a variety of analytes in aqueous solutions. The potential of this CNDs-based SEIRA was further confirmed by quantitatively assaying adenine solutions at low concentration levels in a label-free fashion.

Thirdly, graphene oxide (GO) with distinct advantages vs. hydrophobic graphene including excellent water dispersibility, ease of preparation, and capability to interact with a wider range of molecules based on non-covalent interactions has been proved an even more universally applicable material inducing selective chemical enhancement in SEIRA scenarios. In addition, GO eliminated potential interferences from dispersing agents or processing residues common to procedures involving graphene.

Consequently, carbon nanomaterials-based SEIRA methods established in this dissertation have demonstrated (i) pronounced IR signal enhancement effects, (ii) simple experimental procedures, and (iii) convenient operation free from toxic agents, harsh experimental conditions, and sophisticated fabrication/sampling routines. Last but not least, these studies are of particular importance for facilitating fundamental understanding on the chemical enhancement mechanisms in SEIRA spectroscopy.
# Table of Contents

Peer-reviewed Publications ........................................................................................................2
List of Abbreviations ..................................................................................................................4
Aim and Overview of the Dissertation .....................................................................................6
Introduction ...............................................................................................................................8
  Motivation and Theoretical Background .............................................................................8
  1.1 Surface Enhanced Infrared Absorption (SEIRA) .........................................................10
  1.2 Carbon Nanomaterials .................................................................................................16
     1.2.1 Graphene .............................................................................................................17
     1.2.2 Graphene Oxide ..................................................................................................21
     1.2.3 Carbon-based Dots .............................................................................................24
  1.3 Infrared Attenuated Total Reflection Spectroscopy ....................................................26
Results and discussion ............................................................................................................30
References ................................................................................................................................33
List of Figures ..........................................................................................................................42
Journal Articles .......................................................................................................................43
  Paper I. Graphene-based Surface Enhanced Vibrational Spectroscopy: Recent
          Developments, Challenges, and Applications ..............................................................43
  Paper II. Versatile Analytical Platform Based on Graphene-Enhanced Infrared
          Attenuated Total Reflection Spectroscopy ....................................................................78
  Paper III. Surface-Enhanced Infrared Attenuated Total Reflection Spectroscopy via
          Carbon Nanodots for Small Molecules in Aqueous Solution .......................................92
  Paper IV. Selective Chemical Enhancement via Graphene Oxide in Infrared
          Attenuated Total Reflection Spectroscopy ..................................................................108
Acknowledgments ...................................................................................................................133
Curriculum Vitae .......................................................................................................................134
Declaration of Authorship ........................................................................................................137
Eidesstattliche Erklärung ..........................................................................................................137
Peer-reviewed Publications

The present dissertation is based on the following papers, which have been published in (or submitted to) international peer-reviewed journals:

I. Graphene-based surface enhanced vibrational spectroscopy: recent developments, challenges, and applications

Yuan Hu, Ángela I. López-Lorente, and Boris Mizaikoff.

ACS Photonics, 2019, in review.

II. Versatile analytical platform based on graphene-enhanced infrared attenuated total reflection spectroscopy

Yuan Hu, Ángela I. López-Lorente, and Boris Mizaikoff.

ACS Photonics, 2018, 5 (6), 2160–2167.

DOI: 10.1021/acsphotonics.8b00028.

III. Surface-enhanced infrared attenuated total reflection spectroscopy via carbon nanodots for small molecules in aqueous solution

Yuan Hu, Qiong Chen, Lijie Ci, Kecheng Cao, and Boris Mizaikoff.

Analytical and Bioanalytical Chemistry, 2019, 411 (9), 1863–1871.


Featured as: “Paper in Forefront” and “Journal Cover”
IV. Selective chemical enhancement via graphene oxide in infrared attenuated total reflection spectroscopy

Yuan Hu, Kecheng Cao, Lijie Ci, and Boris Mizaikoff.


Furthermore, the PhD research documented herein has contributed to the following studies:

V. Emerging biosensor platforms for the assessment of water-borne pathogens

Nishant Kumar,† Yuan Hu,† Suman Singh, and Boris Mizaikoff.

(†Equal contribution for first authorship)

*Analyst, 2018*, 143 (2), 359–373.

DOI: 10.1039/C7AN00983F.

*Featured as:* “The top 10 most downloaded articles published in Analyst in 2018”
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Explanation</th>
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<tbody>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>MIR</td>
<td>Mid-Infrared</td>
</tr>
<tr>
<td>ATR</td>
<td>Attenuated Total Reflection</td>
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<tr>
<td>SEIRA</td>
<td>Surface-Enhanced Infrared Absorption</td>
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<tr>
<td>SERS</td>
<td>Surface-Enhanced Raman Scattering</td>
</tr>
<tr>
<td>GEIRA</td>
<td>Graphene-Enhanced Infrared Absorption</td>
</tr>
<tr>
<td>IR-ATR</td>
<td>Infrared Attenuated Total Reflection Spectroscopy</td>
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<tr>
<td>R6G</td>
<td>Rhodamine 6G</td>
</tr>
<tr>
<td>CNDs</td>
<td>Carbon Nanodots</td>
</tr>
<tr>
<td>GO</td>
<td>Graphene Oxide</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal-to-Noise Ratio</td>
</tr>
<tr>
<td>QCL</td>
<td>Quantum Cascade Lasers</td>
</tr>
<tr>
<td>EF</td>
<td>Enhancement Factor</td>
</tr>
<tr>
<td>IRRAS</td>
<td>Infrared Reflection-Absorption Spectroscopy</td>
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<tr>
<td>EM</td>
<td>Electromagnetic Mechanism</td>
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<tr>
<td>CM</td>
<td>Chemical Mechanism</td>
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<tr>
<td>CNT</td>
<td>Carbon Nanotube</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>2D</td>
<td>Two-Dimensional</td>
</tr>
<tr>
<td>HOPG</td>
<td>Highly Oriented Pyrolytic Graphite</td>
</tr>
<tr>
<td>SiC</td>
<td>Silicon Carbide</td>
</tr>
<tr>
<td>CVD</td>
<td>Chemical Vapor Deposition</td>
</tr>
<tr>
<td>PAHs</td>
<td>Polycyclic Aromatic Hydrocarbons</td>
</tr>
<tr>
<td>FET</td>
<td>Field Effect Transistors</td>
</tr>
<tr>
<td>0D</td>
<td>Zero-Dimensional</td>
</tr>
<tr>
<td>GQDs</td>
<td>Graphene Quantum Dots</td>
</tr>
<tr>
<td>CQDs</td>
<td>Carbon Quantum Dots</td>
</tr>
<tr>
<td>TIR</td>
<td>Total Internal Reflection</td>
</tr>
<tr>
<td>IRE</td>
<td>Internal Reflection Element</td>
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</table>
Aim and Overview of the Dissertation

The present dissertation aims at establishing efficient analytical strategies for enhancing infrared (IR) absorption features of molecules using carbon nanomaterials. As conventional IR absorption techniques usually suffer from low sensitivities, their practical applications are restricted, especially when sensing minute amounts of analytes. Surface-enhanced infrared absorption (SEIRA) spectroscopy enables enhancing molecular IR signals rendering even low concentrated analytes IR-spectroscopically visible. To date, most reported SEIRA methods are based on noble metal nanostructures for analyzing solid samples or solids residues from initially liquid samples. Obtaining these nanostructures frequently involves sophisticated fabrication schemes, and limits to a relatively narrow spectral window for analyte detection. Therefore, it is desired to exploit novel broadband SEIRA sensing approaches for analyzing aqueous samples using more accessible, cost-efficient, and environmentally friendly non-metallic materials, i.e. herein, carbon nanomaterials. Consequently, the research in this cumulative dissertation is focused on three main topics:

I. A graphene-enhanced infrared absorption (GEIRA) platform was developed and optimized based on infrared attenuated total reflection spectroscopy (IR-ATR), which significantly enhanced the characteristic IR bands of several exemplary aromatic molecules in aqueous solution. Using rhodamine 6G (R6G) as a model analyte, the potential enhancement mechanisms were revealed and discussed. The detailed results
of this study are summarized in the published journal article “Versatile Analytical Platform Based on Graphene-Enhanced Infrared Attenuated Total Reflection Spectroscopy” (Paper II).

II. Highly water-dispersible carbon nanodots (CNDs) were prepared via a ‘green’ electrochemical synthesis method for decorating an ATR waveguide surface, and exhibited remarkable SEIRA effects for a variety of analytes in aqueous phase. Moreover, the developed SEIRA strategy enabled the quantitative analysis of adenine in solution serving as an exemplary biomolecular analyte. The details of this study are presented in the published journal article “Surface-enhanced infrared attenuated total reflection spectroscopy via carbon nanodots for small molecules in aqueous solution” (Paper III).

III. Graphene oxide (GO) with its excellent water dispersibility and abundant oxygen-containing functional groups was deposited at an ATR prism surface via a simple drop-casting method, which enabled real-time and in-situ monitoring of IR signatures of various molecules in aqueous environment. Distinct chemical signal enhancement was observed across the entire spectral region of interest. This study is described in detail in the manuscript “Selective chemical enhancement via graphene oxide in infrared attenuated total reflection spectroscopy” (Paper IV), which is currently in review in an international peer-reviewed journal.
Introduction

Motivation and Theoretical Background

Fourier transform infrared (FTIR) spectroscopy serves a label-free, molecularly selective, and non-destructive analytical platform in this thesis for identifying molecular species and chemical compounds via directly probing their vibrational characteristics (Figure 1). The absorption bands in the mid-infrared (MIR) spectral regime ranging from approximately 4000 to 400 cm\(^{-1}\) (i.e. 2.5–25 μm) are directly derived from the chemical bonds associated with vibrational modes involving dipole moment changes. Thus, a ‘vibrational fingerprint’ of the functional groups and molecular bonds comprised within a molecule is generated within this spectral region. From a chemical perspective, the MIR spectrum can be segmented into four general regions: the X-H stretching region (e.g. O–H, C–H and N–H stretching; 4000–2500 cm\(^{-1}\)), the triple bond region (e.g. C=C and C≡N stretching; 2500–2000 cm\(^{-1}\)), the double bond region (e.g. C=C and C=O stretching; 2000–1500 cm\(^{-1}\)), and the fingerprint region (mostly bending and skeletal vibrations, 1500–400 cm\(^{-1}\)), in which the most pronounced vibrational features are located. Therefore, IR measurements provide highly discriminatory and quantitative chemical, structural, and compositional information on constituents in samples of any aggregation state. Despite their advantages and potential, the widespread application of conventional IR techniques in real-world trace analysis (e.g. ultrathin films, low-volume samples, ultra-trace concentration levels, etc.) is limited due to the inherently low sensitivity associated with the
modest molecular absorption cross-sections.\textsuperscript{1, 12} Given the significant mismatch between the incident MIR wavelengths (i.e. micrometers) and the (sub-)nanoscale dimensions of most probed molecules of interest (i.e. typically <10 nm), the photon–molecule interaction cross-section is extremely small.\textsuperscript{13} Besides, IR absorption is governed by the Beer–Lambert law, which requires either an extended absorption path or a high density/concentration of the target analyte within that absorption path length to achieve notable IR signals for a specific molecule.\textsuperscript{14} Resulting, detection of vibrational molecular fingerprints using FTIR spectroscopy imposes a rather high threshold on the minimum quantity of the investigated species.\textsuperscript{5} Hence, FTIR studies on minute amounts of analytes remain challenging given their prohibitively weak signals.\textsuperscript{15} To improve the signal-to-noise ratio (S/N), advanced highly brilliant light sources may be employed instead of the commonly used thermal light source such as quantum cascade lasers (QCL) or IR synchrotron radiation. However, synchrotrons are not readily accessible, and most IR lasers are expensive and subject to limited bandwidths thus facilitating target analyte detection rather than broadband applications.\textsuperscript{16-17} To overcome these limitations one may choose to apply signal-enhancing strategies with the utilization of surface enhanced infrared absorption (SEIRA) as the most commonly applied solution.\textsuperscript{18}
Figure 1. Characteristic infrared vibrations of exemplary molecular species. The hatched part indicates the fingerprint region containing skeletal vibrations. Figure reproduced with permission from ref 3. Copyright 2017 American Chemical Society.

1.1 Surface Enhanced Infrared Absorption (SEIRA)

Since Hartstein et al.\textsuperscript{19} first observed noticeably increased IR absorption features of molecular monolayers adsorbed at thin noble metal (Au or Ag) films in 1980, and Hatta et al.\textsuperscript{20} further confirmed this phenomenon, SEIRA spectroscopy has been an emerging field and extensively studied in analogy to surface-enhanced Raman scattering (SERS) spectroscopy. With different excitation mechanisms and selection rules,\textsuperscript{21} SEIRA and SERS are in fact spectroscopic techniques offering complementary vibrational information, hence, both are essential and indispensable in molecular analysis.\textsuperscript{22} More
specifically, IR absorption spectroscopy relies on transitions between molecular vibrational energy levels arising from the absorption of incident MIR radiation, i.e. the direct excitation of vibrational modes. In contrast, Raman spectroscopy is based on an inelastic scattering process, whereby the incident photons with higher energy transfer part of their energy to molecular vibrations and the remaining energy randomly scatters as photons with reduced frequencies. Generally, SEIRA is most pronounced for asymmetric vibrations of polar groups, whereas SERS favors symmetric vibrations of nonpolar groups. Despite nearly four decades of history, the overall development of SEIRA has received considerably less attention than SERS, which may in part be ascribed to the fact that SEIRA usually implies significantly smaller enhancement factors (EF; ranging up to \(10^3\), yet, mostly around 10–100) vs. SERS (with EFs up to \(10^{12}\)). Nevertheless, SEIRA provides some noticeable merits vs. SERS: (i) albeit being small, the IR absorption cross-section is several orders of magnitude higher than that of the Raman scattering cross-section (IR \(\sigma_{\text{abs}} \approx 10^{-20}\) cm\(^2\) per molecule; Raman \(\sigma_{\text{sca}} \approx 10^{-30}\) cm\(^2\) per molecule), and (ii) a potentially fluorescence background frequently accompanying SERS is not interfering with SEIRA signal. Hence, at ideal conditions the sensitivity/signal enhancement factor of SEIRA may in fact reach the levels of some SERS applications. Furthermore, SERS cannot provide the entire molecular chemical fingerprint, as it probes the change of polarizability during vibrations of molecules primarily at visible frequencies. Another advantage of SEIRA may be the reduced measurement times when compared to SERS, and even to conventional infrared
reflection-absorption spectroscopies (e.g. IRRAS). Therefore, the potential of SEIRA is undisputed, while not fully exploited to date.

Although not conclusively clarified to date, it is widely accepted that at least two different mechanisms contribute to the overall SEIRA enhancement: (i) chemical mechanisms (CM), and (ii) electromagnetic mechanisms (EM), i.e. similar to SERS. An IR absorption ($A_{IR}$) signal may be expressed as follows:

$$A_{IR} \propto |\partial \mu / \partial Q \cdot E|^2 = |\partial \mu / \partial Q|^2 |E|^2 \cos^2 \theta$$

(1)

where $\partial \mu / \partial Q$ represents the dipole moment derivative with respect to the normal coordinate ($Q$), i.e. derivative of the dipole moment $\mu$ along the normal direction; $E$ stands for the local electric field that excites the analytes, of which the intensity ($|E|^2$) at the substrate surface is different from that of the incident photon field; and $\theta$ denotes the angle between $\partial \mu / \partial Q$ and $E$. The CM enhancement is related to an increase of $|\partial \mu / \partial Q|^2$ (i.e. the absorption coefficient), which typically indicates that the IR signal is enhanced as a result of specific chemical interactions between analyte molecules and the signal-enhancing substrate or substrate surface; however, the fundamentals of CM remain poorly understood. CM therefore depends on the type of interaction between chemisorbed molecules and the substrate surface, and is generally short-ranged, since the probed species should reside within close distance from the substrate surface enabling chemical interactions. In contrast, EM enhancement originates from amplified local electric fields at the substrate surface, which is proportional to the square of the local
electromagnetic field in SEIRA (EF\(\propto|E|^2\)). In contrast, the overall EM enhancement factor of SERS displays a fourth-power dependency.\(^{24, 37}\) EM enhancement primarily results from the interaction of incident light with the free conduction electrons of the enhancing substrate material leading to the excitation of surface plasmon resonances (i.e. collective oscillations of free conduction electrons/charge carriers in conducting materials in resonance with an incident electromagnetic field). In the case of the IR spectral range, they may arise at the surface of plasmonic nanostructures,\(^{28}\) which generates a local electromagnetic field stronger than the incident photon field experienced by molecules adjacent to the surface.\(^{26, 36}\) In other words, the strongly enhanced electromagnetic field in the plasmonic substrate is localized (a.k.a. a ‘hotspot’) and results from the coupling of the incident photon field with the substrate surface. Thus, vibrational dipoles of molecules located inside these hotspots are subject to enhanced IR absorption signals.\(^{1, 38}\) Additionally, the enhanced field decays sharply with distance from the substrate surface, i.e. EM enhancement is restricted to the immediate vicinity of the surface.\(^{18, 26, 33}\) In general, the electromagnetic enhancement effect in SEIRA decreases as the detuning between the plasmon frequency and the vibrational frequency increases.\(^{13, 39}\) Accordingly, to achieve strong EM enhancement the excited surface plasmon should be resonant with the frequency of the target molecular vibrational mode.\(^5\) EM coexists with CM in many cases, however it is difficult to clearly separate their individual contributions in a single experiment, as EM is usually anticipated to contribute much more to the overall SEIRA enhancement, thereby obscuring the CM contribution. It should be noted that molecular
vibrational modes that have associated dipole moment derivative components (i.e. dipole changes) perpendicular to the substrate surface are preferentially amplified, a.k.a. the ‘surface selection rule’ of SEIRA.\textsuperscript{27, 40} Hence, straightforward information on the molecular orientation of the adsorbed species may be obtained by making full use of this surface selection rule.\textsuperscript{41} In fact, both physisorbed and chemisorbed molecules exhibit SEIRA enhancement, however, the latter generally imply more pronounced enhancement contributions attributed to CMs.\textsuperscript{6, 41-42}

To date, the majority of SEIRA studies are based on metallic signal-enhancing materials and structures, especially noble metals such as gold or silver.\textsuperscript{36} However, metal plasmonics suffer from high losses, especially in IR spectral regime. Hence, to realize strong plasmonic enhancement in the MIR, well-defined metallic nanostructures are engineered such as metamaterials with nanoscale dimensions, nanoparticles, nanoislands, nanoantennas, nanorods, nanoshells, etc.\textsuperscript{3-4, 28, 43} Ideally, specific plasmonic nanostructures are expected to present well-defined resonances offering strong nearfield enhancements and light confinement in a sub-wavelength volume with minimal energy loss in a more reproducible fashion.\textsuperscript{44} The intensity of substrate-based plasmon resonances that match the target vibrational frequencies to enhance light-matter interactions, i.e. the electromagnetic enhancement effects are strongly dependent on the properties of the plasmonic material in terms of surface morphology and composition, and of the dielectric properties of the surrounding environment.\textsuperscript{45} Usually, molecules reveal multiple vibrational bands in the MIR fingerprint region. However, plasmonic resonance supported
by a given metallic nanostructure only provides a limited bandwidth usually covering only one or few absorption lines/bands.\textsuperscript{46} As a consequence, usually only a few absorption features, i.e. those mostly located at the ‘red’ (i.e. long wavelength) side of the plasmonic resonance can be observed.\textsuperscript{47} Although the plasmonic resonance can be tuned to approach a selected vibrational frequency by optimized design of tailored substrate structures in advance, this may be insufficient and inconvenient for unambiguously identifying and characterizing various molecular species on a broadband scale. Moreover, plasmon frequencies of the commonly used metallic substrate are fixed after fabrication, i.e. lacks in external tunability. Thereby, the SEIRA-based detection of multiple vibrational modes in a broad spectral window using a single device is impossible. While post-fabrication tuning of plasmonic resonances may be possible by modifying the geometry/charge carrier density of the plasmonic metallic material, and/or changing the refractive index of the surrounding environment,\textsuperscript{3} this is difficult to control and requires sophisticated manipulations. Furthermore, the fabrication processes of specific noble metal nanostructures are frequently complex, expensive, and poor in reproducibility, thereby limiting the application of metal-based SEIRA sensing in practical scenarios. It is thus desirable developing adequate alternatives ideally based on non-metallic materials that are more accessible, reproducible, and cost-efficient, and facilitate large-scale and environmentally friendly synthesis for broadband enhancement of molecular IR fingerprints. Recent reports have demonstrated that carbon nanomaterials such as graphene could be favorable candidates for SEIRA sensing in this respect.\textsuperscript{1,48}
1.2 Carbon Nanomaterials

Carbon is among the most abundant natural elements on earth. Among the carbon-based materials, carbon nanomaterials including fullerenes, carbon nanotubes (CNT), graphene, carbon-based dots or pure carbon dots, nanodiamonds, etc. have attracted extensive attention owing to their extraordinary mechanical, electric, thermal, optical, chemical and biological properties (Figure 2). Most carbon nanomaterials with sizes typically ranging from 1 nm to 1 μm in one or more dimensions have been widely applied in areas including but not limited to energy conversion and storage, treatment of environmental pollutants, (photo)catalysis, clinical/biomedical applications, analytical applications, (electrochemical/photoluminescent) biosensors, bioimaging, etc. Clearly, these materials have their own limitations. For example, the preparation and separation of nanodiamonds is usually expensive and time-consuming, while fullerene and carbon nanotubes (CNTs) are insoluble in water, which again may limit their applicability. Graphene-based materials and carbon-based dots with potentially high surface areas, robustness, chemical inertness, and biocompatibility are therefore of substantial interest for a variety of analytical scenarios. More importantly, they have recently emerged as feasible substrates in surface-enhanced spectroscopies including SERS and SEIRA.
**INTRODUCTION**

Figure 2. Schematics of the structures of typical carbon nanomaterials including fullerenes, carbon nanotubes, graphene, carbon dots, and nanodiamonds. Figure adapted with permission from ref 52. Copyright 2015 American Chemical Society.

### 1.2.1 Graphene

Graphene (2010 Nobel Prize for Physics) comprises two-dimensional (2D) planar sheets of sp²-hybridized carbon atoms packed in a honeycomb crystal lattice with a single-layer or few-layer thickness (no more than ten layers). Graphene was for the first time successfully prepared by a simple micromechanical cleavage method in 2004 by Novoselov et al, in which a commercially available highly oriented pyrolytic graphite (HOPG) was peeled using scotch-tape and then deposited on to a silicon substrate. This approach allowed easy production of high quality single- and double-layered graphene flakes, but with irregular shapes. Conversely, graphite comprises stacked layers of...
adjacent graphene sheets, which are connected via weak van der Waals forces. Pristine graphene can thus be obtained from high purity bulk graphite by exfoliation and cleavage using mechanical or chemical energy to break the rather weak bonds and then extract individual graphene sheets, which implies a repeated peeling process. However, since the above-mentioned top-down micromechanical cleavage process is difficult to control and the throughput/yield is extremely low, this procedure is less suitable for mass production of graphene fulfilling the requirements of real-world devices. Alternative strategies for the efficient synthesis of graphene can be primarily categorized as (i) exfoliation of bulk graphite or its derivatives (e.g. chemically derived graphene from graphite oxide) in liquid phase, (ii) chemical vapor deposition (CVD) from C-containing gases on catalytic metal surfaces such as Ni and Cu, (iii) epitaxial growth at electrically insulating surfaces such as silicon carbide (SiC), (iv) total organic synthesis based on polycyclic aromatic hydrocarbons (PAHs), and (v) other processing routes (e.g. unzipping of CNTs) not further detailed herein. Each of these approaches has its advantages along with some drawbacks. For example, liquid-phase exfoliation, epitaxial growth, and CVD all enable large-scale production, however, the exfoliated products in solvents have extensive structural defects generated from the oxidation and reduction processes, leading to very poor electrical properties of the resultant graphene. By contrast, graphene sheets synthesized via the bottom-up epitaxial and CVD techniques exhibit uniformity, large area, and nearly no defects, but challenges remain in fine controlling the graphene film thickness (i.e. the number of layers).
Since the discovery of graphene, this material has extensively been studied as a ‘hot topic’ in multiple fields, and has been intensively investigated for taking advantage of its unique characteristics such as high aspect ratio (i.e. the ratio of lateral size to thickness), atomic uniformity, flexibility, special electron and photon structures, ultrahigh carrier mobility, etc. These merits result from the distinctive structure of graphene, as shown in Figure 3. A hexagonal unit cell of graphene consists of two equivalent triangular sub-lattices of carbon atoms joined together by strong in-plane $\sigma$ bonds with a carbon-carbon bond length of $\sim0.142$ nm, which constitutes the rigid backbone of the honeycomb net. All carbon atoms in the lattice have $\pi$ orbitals perpendicular to the plane, which substantiates a large delocalized network of $\pi$ electrons controlling interactions between graphene layers and other materials/molecules. Consequently, graphene is exceptionally stable compared to other nanosystems.

Figure 3. Schematic of the chemical structure of graphene.
Unlike metals with an abundance of free charges, graphene is a semimetal with a small density of states, and therefore has unique plasmonic properties.\textsuperscript{71} It features plasmons at MIR and THz frequencies that originate from the collective motion of massless Dirac fermions (i.e. charge carriers of zero effective mass).\textsuperscript{72-73} The two-dimensional and semimetal nature of graphene allows readily injecting free carriers, and more importantly, outstanding post-fabrication wide range dynamic tunability of graphene plasmonic resonances by modulating its carrier density (i.e. Fermi level) via either chemical doping or electrostatic gating. This is impossible using conventional noble metals.\textsuperscript{71} In addition, MIR graphene plasmons have long lifetimes, and give rise to high spatial field confinement with low damping/losses (i.e. confine the light in a subwavelength volume with minimal energy loss) in a broadband spectral window. Hence, plasmon wavelengths two orders of magnitude smaller than the free space light wavelength are obtainable via adequate doping.\textsuperscript{1, 74-75} These advantages, together with the previously mentioned intrinsic superior chemical and physical properties render graphene a promising signal-enhancing material in infrared sensing applications.\textsuperscript{12, 39, 76-77} Despite the distinct merits, graphene plasmonics in SEIRA applications also come with their own limitations.

Firstly, to tune graphene plasmon resonances to the MIR region and ensure overlap with the vibrational modes of interest, sophisticated external voltage loading for electrostatic gating and/or rigid, low-throughput and costly fabrication techniques of patterned periodic graphene nanostructures (or hybridized graphene with other materials) are usually required.\textsuperscript{48, 78-80} Secondly, the inherent MIR plasmonic resonances of monolayer graphene
are weak, which may be ascribed to the low oscillator strengths caused by the large momentum mismatch between external IR radiation and graphene plasmons, as well as the diminished carrier mobility arising from the deteriorated quality (i.e. defects, impurities, grain boundaries, etc.) of graphene nanostructures during the fabrication, patterning, and/or transfer process.\textsuperscript{44, 81-82} Thirdly, stable and controllable chemical doping may increase the carrier density, yet remains challenging.\textsuperscript{72} Consequently, utilizing graphene plasmonics for SEIRA enhancement appears far from practice, and has therefore been a major subject of the present thesis, as it has been shown that the SEIRA potential of graphene and its derivatives may be exploited taking particular advantage of chemical enhancement mechanisms resulting from its extraordinary chemical/physical properties, which has long been ignored in SEIRA, yet recently confirmed.\textsuperscript{59, 83}

1.2.2 Graphene Oxide

Graphene oxide (GO) is an important graphene derivative and provides a similar atomically thin structural lattice while bearing oxygen-containing functional groups.\textsuperscript{84} The lateral size of GO may range from tens of nanometers to several hundreds of micrometers, which renders it exceptionally suitable for practical use as a substrate material. Large quantities of GO can be readily produced by chemical oxidation of bulk graphite and subsequent exfoliation.\textsuperscript{68, 85} Such invasive chemical treatment inevitably generates amorphous structural defects in GO, which disrupt its electronic structure and change it into an insulating and disordered material, which is conceptually different from
highly conducting crystalline graphene. Graphene is endowed with an intrinsically hydrophobic nature showing a strong tendency to agglomerate in solvents, which severely restrict its applications in some areas. Preventing aggregation of graphene flakes in solution is thus essential to retain the unique properties of individual graphene sheets, for example by using dispersing agents. However, the presence of dispersing agents may also lead to a reduced performance in some applications. GO contains graphitic sp$^2$ carbon domains along with randomly distributed sp$^3$ hybridized carbons, due to the non-uniform coverage of its basal plane by oxygen functionalities, i.e. hydroxyl (−OH), and epoxy (−O) groups on either side of the basal plane of the carbon skeleton, while carbonyl (C=O) and carboxyl (−COOH) groups are mainly present at the edge sites, as shown in Figure 4. These abundant active oxygen sites are strongly hydrophilic, thus rendering GO well dispersed and stable in aqueous environments, which is in sharp contrast to graphene. Moreover, the oxygenated functional groups may act as reactive/anchoring sites for ionic and non-ionic interactions binding organic, polymeric, and biological molecules via non-covalent forces such as electrostatic forces, π−π stacking, hydrogen bonding, van der Waals forces, and hydrophobic interactions. Controlling the oxidation (i.e. the specific oxygen groups and their density) of GO provides a way to tailor its chemical, electronic, and/or optical properties, etc. Hence, GO displays advantageous characteristics to be used in a wide range of applications such as biosensing, bioengineering, drug delivery, imaging of cells, environmental pollutant scavengers, field effect transistors (FET), electrochemical sensors and energy storage.
Consequently, GO has been demonstrated as a feasible material in SERS benefitting chemical enhancement mechanism. However, in SEIRA only few reports discuss the utility of GO up to now. A detailed review on the governing principles, comparisons, developments, and applications of SERS/SEIRA based on graphene and graphene derivatives including GO via EM and CM enhancement has been published in Paper I, a review article entitled ‘Graphene-based surface enhanced vibrational spectroscopy: recent developments, challenges, and applications’.

Figure 4. Schematic of a representative structure of graphene oxide (GO).
1.2.3 Carbon-based Dots

Relevant carbon allotropes with graphitic structures apart from graphene and GO are zero-dimensional (0D) carbon-based dots, a.k.a. nanometer-sized particles such as graphene quantum dots (GQDs), carbon quantum dots (CQDs), and carbon nanodots (CNDs), which have been intensively investigated in the recent decade and widely employed, e.g. in biosensors. These carbon-based dots are extremely small in size (typical diameter of CNDs/CQDs < 10 nm; lateral dimension of GQDs < 100 nm), and mainly composed of sp²/sp³ carbon. They have abundant hydrophilic oxygen-containing groups at the surface, and/or other doped heteroatoms, thus intrinsically providing excellent water dispersibility, low toxicity, biocompatibility, ease of modification, and superior chemical stability. Here, CNDs are generally amorphous to nanocrystalline discrete quasi-spherical nanodots, which lack quantum confinement (Figure 5a; also named carbon dots or C-dots in literature), whereas spherical CQDs present quantum confinement and an evident crystal lattice (Figure 5b). GQDs basically refer to nanosheets of π-conjugated sp² carbons with lateral dimensions larger than the height (i.e. small fragments of one or a few layers of graphene with functional groups on the edges; Figure 5c), which show well-defined graphene lattice structures and are prepared from precursors consisting of graphene-based materials (e.g. GO), whereas CQDs can be synthesized from other carbon nanomaterials with crystalline structure (e.g. CNTs), or by high temperature pyrolysis of some organic molecules. Generally, CNDs are most likely comprised of a graphitic sp² carbon core and the outer part featuring sp³ hybridized
carbon atoms.\textsuperscript{96, 98} Their synthesis can be classified into two main routes: (i) top-down nanocutting methods entailing cleavage of bulk carbonaceous starting materials such as GO, CNTs, carbon fiber, carbon black, and graphite electrodes through arc-discharge, laser ablation, and electrochemical treatment, and (ii) bottom-up organic approaches based on the carbonization of precursors utilizing small molecules treated by external energy such as ultrasonication, microwave pyrolysis, hydrothermal, and heating.\textsuperscript{54, 99} Accordingly, the associated low cost, abundant sources, high-yield fabrication, and density of accessible binding sites further promotes the application of carbon-based dots, which are particularly adaptable in chem/bio sensing scenarios.\textsuperscript{95} Yet, studies on carbon-based dots for SERS/SEIRA sensing are still at the prototype stage, and predominantly focus on their utility in SERS and CM enhancement taking advantage of their adsorption/enriching capability for analytes at the surface.\textsuperscript{60, 100-102} However, SEIRA applications have been scarcely reported, and are therefore another focus of the present thesis.
Figure 5. Schematic of representative chemical structures of (a) carbon nanodots (CNDs), (b) carbon quantum dots (CQDs), and (c) graphene quantum dots (GQDs).

1.3 Infrared Attenuated Total Reflection Spectroscopy

To date, the majority of the developed SEIRA strategies based on graphene and graphene analogues are limited to the analysis of solid and occasionally gaseous samples, since they were mostly conducted in IR transmission mode. Since the IR absorption spectrum of liquid water is pronounced, it may obscure absorption features of the target analyte in aqueous environments.\textsuperscript{103} In principle, combining SEIRA with the Kretschmann attenuated total reflection (ATR) configuration could be an efficient way to solve this problem, which may further improve the sensitivity/enhancement effect via multiple reflections in the ATR prism (a.k.a. internal reflection elements, IREs) while suppressing background absorption signals from the aqueous matrix.\textsuperscript{1, 104}
IR-ATR spectroscopy (a.k.a. evanescent field absorption spectroscopy) in the MIR regime is a useful tool for analyzing thin films, solids, and liquids.\textsuperscript{105} Figure 6 depicts the fundamental principle of a representative ATR configuration, which is based on the generic concept of total internal reflection (TIR). Specifically, IR light is totally reflected at the interface between the ATR waveguide (i.e. optically denser medium, with refractive index \(n_1\)) and the adjacent medium/sample (i.e. optically rarer medium, with refractive index \(n_2\)) if the incident angle (\(\theta\)) exceeds the critical angle (\(\theta_c\)), which can be calculated as:\textsuperscript{106}

\[
\theta_c = \sin^{-1}\frac{n_2}{n_1}
\]  

(2)

where \(n_1 > n_2\). In TIR, part of the IR radiation propagating inside the MIR-transparent ATR waveguide leaks into the adjacent sample medium generating an evanescent field with the field intensity decaying exponentially away from the waveguide–sample interface. The penetration depth \((d_p)\) of the evanescent field given a certain incident light wavelength \(\lambda\) can be approximated as:

\[
d_p = \frac{\lambda}{2\pi \sqrt{n_1^2 \sin^2 \theta - n_2^2}}
\]  

(3)

Only IR active molecules located within the penetration depth (typically up to several micrometers) may absorb energy from the evanescent field, thereby attenuating the field intensity at the frequencies in resonance with the corresponding molecular vibrations, and producing an IR-ATR absorption spectrum (a.k.a. evanescent field absorption spectrum).
The absorbance of analyte generally increases with more internal reflections resulting in enhanced absorbance signals due to the extended nominal absorption path length. There is a wide variety of commercially available ATR crystals that support or single- or multi-reflections, which are made from zinc selenide (ZnSe), zinc sulfide (ZnS), germanium (Ge), thallium bromo-iodide (KRS-5), diamond, silicon (Si), etc. As a consequence, employing ATR elements in SEIRA applications appears advantageous, as the ATR prism surface may be coated with the signal-enhancing nanomaterials and/or other chemical modifications for enhanced extraction, enrichment, and recognition of molecular constituents. Hence, experimental manipulation is simplified while a higher surface sensitivity is maintained with virtually unrestricted mass transport.
Figure 6. Schematic representation of attenuated total reflection (ATR) comprising a waveguide ($n_1$), an adjacent sample medium ($n_2$, e.g. liquid or solid sample containing analytes), and a bottom/substrate medium ($n_3$). The refractive index: $n_1 > n_2, n_3$. 

INTRODUCTION
Results and discussion

The potential of carbon nanomaterials for enhancing infrared absorption signals of molecules in aqueous phase has been exploited in the present dissertation by combination of these materials with infrared attenuated total reflection (IR-ATR) spectroscopy. The obtained results have confirmed that carbon nanomaterials – and in particular graphene, graphene oxide (GO), and carbon nanodots (CNDs) – provide excellent chemical/physical characteristics for noncovalently binding a variety of chemically and biologically relevant molecules. The achieved signal enhancements furthermore confirm the utility of these materials as promising alternatives to conventional metallic substrates in SEIRA sensing. In addition to their general performance, these materials provide eco-friendly synthesis, low cost, abundant sources, simple manipulation, broadband enhancement, and suitability for a wide range of analytes with superior reproducibility. The most relevant results accomplished within this dissertation have been published in corresponding peer-reviewed journal articles, and are briefly summarized in the following paragraphs.

The first manuscript ‘Graphene-based Surface Enhanced Vibrational Spectroscopy: Recent Developments, Challenges, and Applications’ is a review article, presents the fundamentals of SEIRA and SERS, and highlights the state-of-the-art in graphene-enhanced vibrational sensing based on carbon materials. Recent advances in SEIRA and SERS utilizing graphene-based nanomaterials are summarized along with the
RESULTS AND DISCUSSION

different roles, challenges, and possible enhancement mechanisms of graphene-based nanostructures in enhancing molecular vibrations.

The article ‘Versatile Analytical Platform Based on Graphene-Enhanced Infrared Attenuated Total Reflection Spectroscopy’ for the first time established a simple and efficient method based on graphene-enhanced infrared absorption (GEIRA) for directly analyzing a variety of aromatic molecules in aqueous phase over a broad MIR spectral range by taking advantages of IR-ATR spectroscopy. After optimization of experimental conditions, remarkable enhancement effect of multiple IR bands with excellent reproducibility was achieved utilizing commercially available aqueous graphene dispersion. The GEIRA effect demonstrated dependence on the sample solution pH and concentration, and enabled investigation of the time evolution of GEIRA signals. The applicability of the method for quantitative analysis was evaluated with rhodamine 6G as model analyte. Chemical enhancement mechanisms (CM) predominantly based on the enrichment effect of graphene were confirmed as the dominating contribution to the observed GEIRA effect.

The article ‘Surface-enhanced infrared attenuated total reflection spectroscopy via carbon nanodots for small molecules in aqueous solution’ for the first time demonstrated that carbon nanodots (CNDs) could be promising candidates for signal-enhancing SEIRA substrates. Water-dispersible CNDs with uniform size were prepared via a green synthesis route, i.e. by direct electrolysis of graphite in deionized
RESULTS AND DISCUSSION

water. As-prepared CNDs dramatically enhanced IR signal of various aromatic dyes and purines in aqueous solution. The established CNDs-based SEIRA strategy was further confirmed by the successful quantitative determination of adenine solution, with the limit of detection (LOD) of 0.003 mg/mL. The influences of the amount and aggregation of CNDs as well as solution pH on the SEIRA responses of adenine were investigated. The observed CND-SEIRA enhancement can be attributed to the enrichment of molecules at the CND surface via noncovalent interactions giving rise to pronounced chemical enhancement effects.

The manuscript ‘Selective chemical enhancement via graphene oxide in infrared attenuated total reflection spectroscopy’ for the first time confirmed the effectiveness of graphene oxide (GO) as a universal enhancing material in ATR-SEIRA spectroscopy. GO could selectivity adsorb various tested molecules via electrostatic interaction and/or π–π stacking owing to its rich negatively charged sites and sp² domains enabling pronounced chemical enhancement of multiple molecular IR signals across the entire spectral region, as well as real-time and in-situ monitoring of evolving IR signatures in aqueous solutions. Compared to hydrophobic graphene, GO is endowed with excellent water dispersibility and more chemical moieties to interact with a wider range of molecules. In conclusion, GO may serve as an even more efficient substrate vs. graphene for inducing chemical enhancement in SEIRA sensing applications.
References


REFERENCES


REFERENCES


REFERENCES

3906-24.


81. Guo, Q.; Li, C.; Deng, B.; Yuan, S.; Guinea, F.; Xia, F., Infrared Nanophotonics Based


REFERENCES


List of Figures

**Figure 1.** Characteristic infrared vibrations of exemplary molecular species. The hatched part indicates the fingerprint region containing skeletal vibrations. Figure reproduced with permission from ref 3. Copyright 2017 American Chemical Society.

**Figure 2.** Schematics of the structures of typical carbon nanomaterials including fullerenes, carbon nanotubes, graphene, carbon dots, and nanodiamonds. Figure adapted with permission from ref 52. Copyright 2015 American Chemical Society.

**Figure 3.** Schematic of the chemical structure of graphene.

**Figure 4.** Schematic of a representative structure of graphene oxide (GO).

**Figure 5.** Schematic of representative chemical structures of (a) carbon nanodots (CNDs), (b) carbon quantum dots (CQDs), and (c) graphene quantum dots (GQDs).

**Figure 6.** Schematic representation of attenuated total reflection (ATR) comprising a waveguide ($n_1$), an adjacent sample medium ($n_2$, e.g. liquid or solid sample containing analytes), and a bottom/substrate medium ($n_3$). The refractive index: $n_1 > n_2, n_3$. 
Journal Articles

Paper I. Graphene-based Surface Enhanced Vibrational Spectroscopy: Recent Developments, Challenges, and Applications

The scope of the paper was determined in close communication with B. Mizaikoff and Á.I. López-Lorente. Y. Hu wrote and organized the manuscript. Á.I. López-Lorente contributed to the SERS part of the contents. B. Mizaikoff edited the manuscript for the final version.

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Graphene-based Surface Enhanced Vibrational Spectroscopy:
Recent Developments, Challenges, and Applications

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Abstract:
Surface enhanced vibrational spectroscopies including surface enhanced infrared absorption (SEIRA) and surface enhanced Raman scattering (SERS) selectively probe molecular vibrations with pronounced signal enhancements yielding improved sensitivity. In contrast to conventional noble metal enhancement, this review highlights latest progress and applications utilizing graphene as the signal-enhancing substrate, and summarizes recent advances in graphene-based SEIRA and SERS. Next to an introduction to the fundamentals of SEIRA and SERS, the different roles of graphene for infrared vs. Raman spectroscopy along with challenges in enhancing molecular vibrations are discussed. Considerations on distinct graphene structures and associated enhancement mechanisms along with selected application examples aim at inspiring the design of more effective SEIRA/SERS configurations utilizing graphene and its derivatives as key materials.

Keywords: graphene, surface enhanced infrared, SEIRA, surface enhanced Raman, SERS, graphene enhanced infrared, GEIRA, vibrational spectroscopy
1. Introduction

Raman and infrared (IR) spectroscopy are powerful analytical tools that nondestructively probe molecular vibrations providing spectroscopic fingerprint spectra of molecular species in solid, liquid, and gaseous samples for chemical identification/characterization, exploration of reaction mechanisms, monitoring of kinetic processes, and molecular quantification. Raman spectroscopy relies on the inelastic scattering of incident radiation yielding a response for molecules whose polarizability changes during the vibration, while signals in IR spectroscopy are based on direct and selective absorption of IR radiation by the molecule; if a net change in dipole moment is caused during vibrational, ro-vibrational or rotational transitions within the molecule. However, conventional Raman and IR techniques suffer from an intrinsically low sensitivity explained by their normalized relative molecular absorption/scattering cross-sections vs. other photon-molecule interactions, thus limiting their application in real-world analytical scenarios.\textsuperscript{1,2} Consequently, surface enhanced Raman scattering (SERS) and surface enhanced infrared absorption (SEIRA) spectroscopies have gained increasing attention since their discoveries in the 1970s\textsuperscript{3,4} and 1980s\textsuperscript{5}, respectively, whereby Raman and IR signals of molecules adsorbed or close to (i.e., at molecular diameter distances) specific substrate surfaces are substantially amplified.

It is widely accepted that two distinct mechanisms give rise to these vibrational signal enhancements, namely: so-called electromagnetic and chemical mechanisms. The electromagnetic mechanism (EM) is widely considered the dominant parameter in SERS originating from appropriate nanostructured substrates giving rise to an increased electromagnetic near field at the surface resulting from surface plasmons excited \textit{via} the incident light.\textsuperscript{6} In contrast to SERS, the surface plasmon should ideally be resonant with the frequency of the molecular vibrational mode in SEIRA,\textsuperscript{7} as the enhancement effect decreases with increasing detuning between the plasmon frequency and the vibrational frequency.\textsuperscript{6,7} Electromagnetic signal enhancement is restricted to the immediate vicinity of the substrate surface, as the electromagnetic field is highly localized and decays exponentially away from the surface, thus rendering SERS and SEIRA surface-bound methods.\textsuperscript{8}

The chemical mechanism (CM) is also short-ranged, as the target molecules need to be in close range of the substrate surface for chemical interactions to be effective.\textsuperscript{11-13} In most cases, EM is anticipated to contribute much more to the overall SERS/SEIRA signal enhancement vs. CM, although it is almost impossible to clearly separate their individual contributions in a single experiment; while EM enhancement is well described, the basis of CM remains poorly understood.\textsuperscript{14} In general, SERS usually entails overall enhancement factors that are orders of magnitude higher than observed in SEIRA, since SERS depends biquadratically on the local electromagnetic field, rather than quadratically in case of SEIRA.\textsuperscript{13,15,16} However and again on a normalized
scale, the molecular cross-section for IR absorption is higher by several orders of magnitude vs. the molecular cross-section for Raman scattering, although both of them are considered low when compared, e.g., to electronic transitions. In contrast to SEIRA, SERS may be interfered via an intense fluorescence background, which is considered a competing effect. From an analytical perspective, given the difference in excitation mechanisms, selection rules, and molecular absorption/scattering cross-sections, SERS and SEIRA should be considered complementary rather than competitive in term of vibrational information derived from the probed molecules.

To date, the vast majority of signal-enhancing materials for SERS/SEIRA are derived from metallic nanostructures, especially noble metals shaped as nanoparticles, nanorods, nanoshells, nanoantennas, etc. The obtainable enhancement effect greatly depends on the properties of the applied material, as generating and sustaining plasmons is essentially determined by the geometry and composition of the material, as well as its surrounding environment. However, plasmon frequencies of metals are essentially 'locked' after fabrication of the metallic substrate and obtaining a certain geometry, i.e., lacks in external tunability. This particularly limits their utility in SEIRA when aiming at utilizing multiple vibrational modes of a molecule (e.g., for identification) across a broad spectral range. In SERS, using metals as signal-enhancing substrate may give rise to unwanted reactions of the target molecule (i.e., either with the surface or via catalytic effects) at nanoscale structures, in addition to a potential fluorescence background, which may itself be enhanced (a.k.a., metal enhanced fluorescence; MEF). Last but not least, reproducible and low-cost fabrication of metallic nanostructures remains challenging. Hence, alternative non-metallic materials useful in both SEIRA and SERS applications are in demand.

Graphene is an atomically thin planar sheet comprising sp²-hybridized carbon atoms arranged in a honeycomb lattice, which is nowadays economically viable and conveniently obtained via various well-established synthesis routes including mechanical or liquid-phase exfoliation of graphite, chemical vapor deposition (CVD) from C-containing gases, reduction of graphite oxide, molecular beam epitaxy, etc. Based on its unique structure, graphene has unique electronic and optical properties, excellent mechanical strength, and a high surface area, while being considered biologically largely 'compatible', chemical inert, flexible, and atomically uniform. Given these characteristics, graphene-based nanostructures have recently emerged as promising signal-enhancing materials in surface enhanced spectroscopies exhibiting prominent advantages vs. conventional metallic substrates. For example, graphene shows superior adsorption affinities toward aromatic molecules and moieties attributed to its high surface-to-volume ratio and delocalized π bonds, thereby suggesting significant adsorption/enrichment capacities for appropriate analytes. Moreover, graphene may effectively quench the photoluminescence of fluorescent molecules via resonance energy transfer processes, thus significantly reducing or even eliminating the fluorescence background in SERS. Graphene is
also supporting highly confined surface plasmon waves at mid-infrared frequencies with low attenuation loss, thus resulting in stronger light-matter interactions in SEIRA. Lastly but not least important especially for SEIRA applications, graphene plasmonics may be dynamically tuned via electrostatic gating or chemical doping, thereby enabling post-fabrication tuning to the required wavelength(s) for achieving plasmonic enhancement across an entire frequency band.

This review summarizes recent progress and selected highlight applications of both SEIRA and SERS based on graphene and graphene-related materials along with potential challenges and future perspectives.

2. Graphene-based enhancement of infrared absorptions (GEIRA)

As previously introduced, for taking advantage of electromagnetic enhancement in SEIRA, the surface plasmon should be within the IR region such that vibrational dipoles of molecules located within the plasmonic hotspots (i.e., within the enhanced electromagnetic near-field) reveal enhanced optical absorption signals. Such near-field enhancement increases with increasing proximity of the vibrational frequency to the plasmon frequency. Graphene plasmon resonances may be tuned to the mid-infrared (3-20 μm; MIR) spectral range for precisely overlapping with desired vibrational modes by modulating the carrier density (i.e., the Fermi level) of graphene by electrostatic gating, chemical doping or similar handles. Alternatively or in addition, patterning of periodic structures of graphene (i.e., modifying shape, size and number stacking layers) is a viable strategy for maximizing the enhancement effect. Furthermore, integrating graphene with sophisticated photonic structures is another strategy for enabling strong graphene-light interactions. Apart from EM, potential CM for enhancing infrared signatures induced by graphene should not be neglected. In the following, typical examples of graphene-based SEIRA (a.k.a., graphene-enhanced infrared absorption; GEIRA) are highlighted.

2.1. Enhancement of infrared absorptions based on graphene structures

Graphene surface plasmons have been proven theoretically to induce strong near-field enhancement of light-matter interactions, i.e., surface plasmons sustained by graphene may significantly amplify the absorption of infrared vibrational modes of molecules. Hence, graphene nanostructures were predicted as potentially highly suitable SEIRA substrates. Li et al., were the first to experimentally realize graphene plasmon enhanced vibrational sensing of thin polymer films using infrared transmission spectroscopy. Graphene nanoribbon (GNR) arrays on SiO₂-covered Si substrate were prepared by electron beam lithography (EBL), and thin layers of poly(methyl methacrylate) (PMMA) and poly(vinylpyrrolidone) (PVP) were then analyzed. They found that the IR absorption of the polymer was significantly
enhanced in the presence of the GNR array, which was attributed to an enhanced near-field confined to the vicinity of the GNR surface. Moreover, the enhancement was inversely proportional to the detuning between the graphene plasmon and the selected vibrational frequencies, namely, the enhancement effect depended on the graphene ribbon width. To simultaneously enhance the strength of light–graphene interaction in GNRs, and to broaden its plasmonic spectral bandwidth, Deng et al. suggested a hybrid two-layer GNR array consisting of nanoribbons with two different widths exhibiting much stronger light absorption within the relevant MIR spectral range from 8 and 14 μm, which may be applicable to SEIRA sensing.

Likewise based on a graphene nanoribbon substrate, Rodrigo et al. reported a SEIRA biosensor for the label-free detection of proteins. An electrostatic field was applied via a varied bias voltage to dynamically control the Fermi level of the GNRs, thus, the plasmonic resonances were electrically tuned to selectively overlap with different vibrational bands of the protein absorbed at the GNR array (Figure 1a). As a result, SEIRA sensing across a broad spectral range with a single device was achieved. Additionally, they demonstrated that the SEIRA signals of the proposed graphene biosensor was higher than that obtained using metallic localized surface plasmon resonance (LSPR) sensors based on gold nanoantenna arrays. This was explained by the higher spatial confinement of graphene plasmons in the MIR regime.

The strong hybridization of graphene plasmons and surface phonons of conventional substrates such SiO₂ in the MIR may diminish the spectral tunability, as well as the near-field enhancement of graphene plasmons. This restrains the application of plasmonic graphene for SEIRA applications. Utilizing electron beam lithography and electron beam evaporation techniques, Hu et al. prepared a 300-nm CaF₂ thin-film-covered silicon (CaF₂/Si) substrate to support GNR arrays that were electrically connected via Au electrodes (Figure 1b). Thereby, substrate phonon effects were eliminated by virtue of the broadband transparency of CaF₂ in the MIR range. The 8-nm-thick polyethylene oxide (PEO) polymer film and residual discontinuous PMMA sub-monolayer film were analyzed using this device, and both revealed pronounced signal enhancement. The plasmon resonance frequency of graphene could be tuned in the range 900-1400 cm⁻¹ to selectively enhance target molecular vibrational modes by adjusting the graphene Fermi level via the gate voltage.

Integrating GNRs on a transparent mica substrate, and using an ion–gel applied to the top gate as an analyte Hu et al. fabricated a flexible and electrically tunable plasmonic device useful as a potential SEIRA substrate (Figure 1c). The authors showed that the plasmonic performance of the proposed device, i.e., the resonance frequency, intensity, and electrical tunability of the graphene plasmon remained almost unaltered after different bending radius (from 3.5 to 1 mm) and bending cycles (tested up to 1000 cycles) were applied. The infrared vibrational modes of the ion–gel film serving as exemplary analyte were investigated. The observed SEIRA
enhancement of the vibrational signals exhibited bending-independent features, and
was greatly increased once the plasmon resonance approached the vibrational modes,
which was again controlled via the Fermi level of the GRNs tuned using the applied
top-gate voltage.

Figure 1. Electrically tunable plasmonic SEIRA devices based on graphene
nanoribbons. (a) Conceptual view of the proposed plasmonic graphene biosensor with
electrical tunability, and the scanning electron microscope image of the fabricated
graphene nanoribbon array (W = 30 nm, P = 80 nm). (b) Schematic illumination of
the graphene plasmon enhanced molecular fingerprint device that can be tuned by
electrostatic doping via the gate voltage (V_g). (c) Schematic (top) and photograph
(bottom) of the proposed flexible and electrically tunable graphene–microporous
plasmonic device. Adapted with permission from ref63, ref67, and ref68. Copyright 2015 American
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Wiley-VCH.

Apart from quasi-solid-phase samples (i.e., polymer/protein layers) as mentioned
above, Farnier et al. detected vibrational modes in small quantities of gas-phase
molecules (e.g., C–O and C–C vibrations in acetone and hexane vapor) by taking
advantages of plasmon-induced transparency (PIT) enhancement features based on
plasmonic GNR arrays.69 This approach showed molecular specificity, since different
molecules could be differentiated from the band frequencies of PIT associated with
the vibrational modes. Here, the plasmonic excitation could be turned ‘on’ and ‘off’
by controlling the incident radiation polarization, as plasmons in GNRs were excited
only when light polarized perpendicular (but not parallel) to the ribbon length was
used. Thus, contributions from plasmonic excitation could be discriminated from
other possible radiation absorption effects. Besides, the authors tuned the plasmon
resonance of GNRs by the adsorption and desorption of gaseous nitrogen dioxide
(NO2), and determined that the PIT enhancement increased with decreasing energy
difference between the graphene plasmon resonance and the vibrational modes.
In addition to nanoribbon arrays, graphene nanohole arrays may also be suitable as plasmonic SEIRA substrates. Gopalan et al. have recently prepared a periodic nanohole array arranged in a square lattice on monolayer graphene, which was itself deposited onto a Si substrate with a thermally oxidized SiO$_2$ surface layer. Thereby, geometry-dependent multiband plasmonic resonances in the MIR region were obtained offering electrically connected surfaces for electrostatically tuning the plasmonic response (Figure 2a). In contrast to nanoribbons, the polarization of light would not affect the coupling with surface plasmons using graphene nanohole arrays, and thus, an unpolarized infrared source was sufficient. Specifically, this type of nanopatterning could be implemented via both electron beam lithography (EBL) and nanoimprint lithography (NIL) combined with laser ablation techniques, whereby the latter allowed for cost-effective and large-scale fabrication strategies.

Nanodots or nanodisks are alternative graphene nanostructures for plasmonic SEIRA substrates. Based on numerical simulations, Marini et al. studied the potentially achievable plasmonic SEIRA enhancement of pyridine serving as a generic molecule placed near a free-standing electrically doped graphene nanodisk (i.e., 300 nm in diameter). The obtained results suggested the utility of a spectrometer-free sensing approach to discriminate molecular resonances by examining graphene Fermi levels. Zhang et al. synthesized a graphene nanodot array (GNDA) at the surface of a silicon substrate via a block copolymer self-assembly strategy (Figure 2b). In contrast to predominantly used EBL, high-throughput fabrication at relatively low cost, and facile large-scale patterning are the main advantages. Compared to bare or continuous graphene-covered silicon substrates, vibrational signals of investigated exemplary analytes such as self-assembled monolayers (i.e., SAMs using a derivative of perchlorotriphenylmethyl radicals), and single-stranded DNA (ssDNA) oligomers adsorbed onto GNDA were enhanced by more than a factor of 10. The proposed substrate was also applied to detect and discriminate different metal ions by recording changes in SEIRA peak intensity and frequency shifts of the water-soluble molecular cages that could bind those ions. In particular, the authors observed SEIRA enhancement of vibrational signals at spectral regions far distant from the plasmonic resonance, which were ascribed to electric field enhancement of higher order harmonic resonances after finite element time-domain (FDTD) simulations.

Surface plasmons at doped graphene nanostructures provide extreme light confinement, and their frequencies are naturally located in the MIR range at appropriate doping levels. Considering the electrical tunability of graphene plasmons, Zundel et al. theoretically proposed finite arrays of graphene nanodisks divided into identical subarrays (i.e., termed 'pixels') with individual doping levels and independently controlled via electrostatic gating. By individually tuning the doping level of each 'pixel' within the array, the plasmon resonance could be sequentially and selectively activated to overlap with the vibrational modes of...
analytes. Therefore, by recording changes in the absorption spectrum analytes
deposited across the array could be identified, and their spatial locations could be
simultaneously detected with subwavelength spatial resolution determined by the
pixel size. The authors further proposed that using two graphene ribbons oriented
perpendicular to each other may be a viable alternative to graphene nanodisk arrays
for spatially resolved SEIRA sensing.

A more complex plasmonic Fano graphene metamaterial was designed by Guo et
al., which comprised a hybrid structure of a graphene disk inserted into a graphene
split-ring resonator (SRR) in unit cells arranged in a square lattice at a 300 nm CaF₂
film serving as a substrate (Figure 2c).53 By numerical calculation, the generated
higher-order Fano resonances created multiple resonant absorption peaks within a
rather wide spectral window, which was beneficial for enhancing multiple vibration
frequencies of analytes at any given point of time. An 8 mm-thick tert-butylamine
layer was tested as exemplary analyte with the IR signals revealing significant
amplification (up to 425-fold) once the Fano resonances were adjusted to overlap with
the vibrational modes of the analyte by tuning the Fermi level of graphene.

![Figure 2. Potential plasmonic SEIRA substrates based on graphene
nanoholes/nanodots arrays. (a) Schematic of the gate-tunable plasmonic graphene
nanodisk arrays. (b) Schematic fabrication processes of the graphene nanodot array
(GNDA) via the block copolymer self-assembly strategy. (c) Schematic of the
designed plasmonic Fano metamaterial consisting of a graphene disk and a graphene
split-ring resonator in a unit cell. Reproduced with permission from ref53, ref54,
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Society of Chemistry.]

So far, plasmonic SEIRA measurements were discussed mainly analyzing solid and
gaseous samples, since the experiments were mostly performed in transmission mode.

The pronounced IR absorption of liquid water operating in aqueous solution may
obscure absorption features of the target analyte. Hence, integrating an attenuated
total reflection (ATR) configuration into SEIRA sensing platforms minimizes water
interferences with the evanescent field establishing a quasi-thin-film cell, while further
improving the surface sensitivity. Hence, *in situ* analysis of aqueous solution is
enabled, and the IR-signatures of species present in vicinity of ATR waveguide
surface decorated with appropriate signal-enhancing structures can be probed.\(^{41, 64}\)

Zheng et al. proposed an IR biosensor for aqueous-phase molecules based on
chemically doped graphene, and an ATR element (Figure 3a).\(^{65}\) A boron-doped
graphene (BG) nanodisk array deposited at the surface of a ZnSe ATR prism was
fabricated by nanosphere lithography (NSL). The boron doping level and size of the
graphene nanodisks was adequately adjusted. Compared to unstructured BG films, the
observed absorbance of the band at 1260 cm\(^{-1}\) (i.e., P=O vibration) of the investigated
DNA solution was larger at the BG nanodisk array, thus suggesting a plasmonic
SEIRA enhancement induced by nanostructured BG. Taking advantage of the strong
affinity between an aptamer and a protein, this biosensor was applied to also monitor
specific protein recognition processes. The authors first immobilized an L-selectin
aptamer at the BG nanodisk surface via \(\pi-\pi\) interaction and van der Waals forces
between BG and aptamer. Subsequently, the target (i.e., L-selectin protein) was added
and gradually captured by the aptamer-modified ATR. The corresponding changes of
the IR-spectra were readily observed. However, in the case of detecting L-selectin it
should be noted that there was no evidence whether or not the IR signal of L-selectin
was enhanced.

Despite the beneficial features of graphene serving as a plasmonic substrate for
SEIRA, a critical drawback restricts its practical applications, i.e., that the plasmonic
response of monolayer graphene, which depends on the carrier density is relatively
weak.\(^{46}\) Chemical doping may be a convenient and feasible strategy to increase the
carrier density, however, stable and controllable chemical doping remains
challenging.\(^{46}\) High-quality graphene material provides less plasmon damping, and
definitely improves the achievable SEIRA effect. Last but not least, patterning
graphene into specific periodic nanostructures (i.e., nanoribbons, nanodisks, nanodots,
etc.) aims at compensating the large momentum mismatch between plasmonic waves
and light in free space such that graphene plasmons may be excited in the MIR.\(^{33, 60}\)

However, the fabrication processes involved in establishing patterned graphene layers
are usually sophisticated, low-throughput, costly, and may destroy the integrity of the
graphene structure.\(^{57}\) The existence of defects, impurities, and/or grain boundaries in
graphene will give rise to lower carrier mobility, which in turn diminishes the lifetime
and intensity of graphene plasmons.\(^{46-49}\) Conventional PMMA-assisted graphene
transfer procedures usually lead to PMMA residues that may further deteriorate the
SEIRA quality of graphene. Hence, exploring simple, yet, efficient graphene SEIRA
substrates is still of substantial interest.
The role of graphene as a molecular enrichment interface – in combination with its signal-enhancing properties – has been long neglected in SEIRA studies mainly focused on exploiting the plasmonic enhancement effects. Hu et al. have established a versatile and practical graphene-enhanced infrared absorption (a.k.a., GEIRA) platform for analyzing aqueous solutions by taking advantage of an ATR sensing configuration and the unique properties of graphene. Graphene dispersions were first drop-casted and dried at the surface of a diamond ATR prism prior to adding the sample solution (Figure 3b), which required only a few microliters of both graphene dispersion and sample solution. While this procedure may readily be automated using an appropriate microfluidic system, the simplicity and convenience of the manipulation vs. conventional graphene-transfer-involving procedures is immediately evident. Characteristic IR bands of several aromatic molecules (i.e., rhodamine 6G, rhodamine B, auramine, crystal violet, fuchsine, methylene blue, neutral red, and 4-hydroxybenzoic acid, etc.) in aqueous solution were significantly enhanced across the entire MIR spectral range using a graphene-deposited ATR prism. The proposed platform also enabled recording the temporal evolution of the GEIRA spectra in real time. The authors suggested a chemical enhancement mechanism, i.e., by π–π stacking interactions between aromatic moieties of molecules and delocalized π bonds of graphene, the investigated analytes were adsorbed onto graphene at the ATR waveguide surface, and thereby enriched within the penetration depth of the exponentially decaying evanescent field.

Similarly, graphene oxide (GO) was chemically reduced on the surface of a single reflection silicon (Si) triangular prism by Cao et al. resulting in stacked film structures with random size/morphology at nanoscale intervals, which was termed a “reduced graphene oxide (rGO) island film”. Methylene blue (MB) that was dried at thus prepared rGO island films deposited onto a Si prism, and showed enhanced IR absorptions (~8.9 times), which the authors mainly ascribed to graphene plasmons with multiple resonances as a result of the random morphology. Analyzing the time-resolved IR signals of MB aqueous solution measured with the rGO, they suggested that the recorded IR signals indeed derived from the adsorbed MB on rGO at the Si surface due to chemical adsorption corresponding to the pronounced π–π conjugation. However, it was not demonstrated whether the enhancement of MB in aqueous solution was predominantly derived from the enrichment (i.e., adsorption) effect or the plasmonic properties of rGO.
2.2. Graphene hybrids as SEIRA substrates

To further enhance the light–matter interaction for improved plasmonic SEIRA effects, graphene was hybridized with other materials. Chen et al. theoretically designed a hybrid SEIRA structure composed of graphene sandwiched between a crescent resonator (CR) with gradual change of gold ligaments and the dielectric substrate with a metal reflecton layer underneath. The proposed CR was similar to conventional metallic split-ring resonators (SRR), however, provided better field enhancement. The introduction of graphene further improved the field enhancement across a larger area, and endowed the hybrid structure with dynamic plasmon tunability. Using numerical simulations, Chen et al. also presented a nanoresonator established via a plasmonic graphene disk that was separated from a metallic substrate by a dielectric spacer with tunable nanoscale thickness supporting acoustic graphene plasmons (AGPs) originating from the hybridization of graphene plasmons and their corresponding mirror image. The electromagnetic field of AGPs was vertically highly confined inside the dielectric spacer, thus providing several advantages over...
conventional graphene plasmons including high sensitivity to the dielectric
permittivity inside the nanoresonator, and additional tunability of the resonance
frequencies via the spacer thickness apart from the graphene disk size. Combined with
system coupling a Fourier spectrometer and a scattering-type scanning near-field
optical microscope (s-SNOM)\textsuperscript{38, 73}, this AGP resonator could perform as a potential
SEIRA platform for sensing ultra-small quantities of analytes (e.g., a protein
monolayer) located within the resonator gap revealing enhanced vibrational
fingerprints (Figure 4a).

Guo et al. simulated a hybrid metal-graphene metamaterial integrating the gold
disk reflector and gold grating with a periodic array of graphene nanoribbons on a
CaF\textsubscript{2} substrate, which focused the MIR radiation at the graphene. Thereby, greatly
enhanced graphene plasmonic absorptions were derived owing to the interference
effect occurring within the reflector and the grating-induced lightning-rod effect.\textsuperscript{80}
The calculated electric field intensity was enhanced by 4 orders of magnitude, thus
should enable sensitive SEIRA detection of trace SO\textsubscript{2} gas with remarkable signal
elevation originating from molecules located within hotspots (i.e., at the graphene
ribbon edges). Moreover, Wu et al. theoretically investigated a plasmonic biosensor
consisting of a continuous graphene layer integrated on top of a patterned periodic
gold grating at a quartz substrate, whereby the plasmonic resonance frequencies could
be tuned by adjusting the graphene Fermi levels to couple with the molecular
vibrational modes.\textsuperscript{74} A continuous graphene layer offers competitive advantages over
patterned graphene nanostructures, such as suppressed edge scattering and improved
mobility, while the wave vector mismatch between free space radiation and graphene
plasmons could be compensated \textit{via} the Au grating. Experimentally, an 8 nm-thick
protein bilayer (i.e., recombinant protein A/G along with goat antioinmouse
immunoglobulin G) was placed above the graphene film and analyzed, whereby two
reflection peaks/transmission dips in the reflection/transmission spectra were
observed at suitable Fermi levels of graphene. This indicated evidence of enhanced
resonant absorptions of two main vibrational modes in the tested protein layer.
Similarly, Wei et al. designed a hybrid grating structure \textit{via} numeric simulation based
on a continuous doped monolayer graphene sheet transferred onto the patterned
periodic array of sub-wavelength Si gratings with an insulator buffer layer (e.g.
poly-hydroxyethylene, NFC) at the upper surface and an Au reflective layer underneath.
The low-permittivity buffer layer was introduced below graphene, thereby avoiding
the potentially increased interface scattering induced by the rough grating structures,
which may deteriorate the graphene mobility and subsequently degrade the plasmonic
performance. Simulations showed that an asymmetric Fabry–Pérot (FP) cavity was
formed between graphene and Au reflective layer enhancing the light-graphene
interaction. After depositing Au electrodes on the graphene surface, the proposed
cavity-enhanced plasmonic infrared sensor could operate within a broad IR spectral
window (i.e., 11.16–7.32 \textmu m) \textit{via} variation of the external gate voltage. A similar yet
more complex configuration serving as a potential SEIRA platform utilizing a
nanograting and a FP cavity was recently simulated by Tang et al.\textsuperscript{89} In this
configuration, the first graphene layer at a ZnSe nanograting and the insulating layer (KBr) with the second graphene layer underneath formed a capacitor structure to electrically tune the Fermi energy of the uppermost graphene interface. The spacer layer (e.g., KBr, ZnSe, or Ge) was positioned underneath the second graphene layer to control the distance between graphene and the bottom metal mirror (Au) that reflected the incident light back. Both acted as a FP cavity that could be optimized to strengthen the light-graphene plasmon interaction. In addition, Zhu et al. experimentally established a hybrid metasurface device comprised of a monolayer of graphene-coated Au nanorod antennas, which were placed onto a SiO₂ spacer-layer-covered Pt mirror forming an optical cavity on the Si substrate (Figure 4b), which improved the quality factor of the plasmonic resonance.²³ Owing to its flexibility, graphene could be suspended at the nanogaps (30 nm) established by the antennas across a large area, where the strongly confined and enhanced electric field interacted more effectively with the graphene-adsorbed molecules. This device has been used as a SEIRA substrate for the quantitative detection of drop-coated glucose revealing enhanced vibrational fingerprints at 1 nmol and 100 pmol levels. However, the likewise enhanced band of PMMA residues resulting from the graphene fabrication process were convoluting the SEIRA spectra, which may be problematic when probing vibrational modes of alternative analytes at adjacent frequencies.

Luxmoore et al. fabricated a complementary split-ring resonator (CSRR) that was split along its horizontal symmetry axis, and made from Cr/Au (5/80 nm) and Al (90 nm) for the lower and upper section directly contacting a graphene ribbon embedded within the capacitor gap (Figure 4e).²⁶ The proposed hybrid metamaterial acted analogous to a cavity enhancing the interaction of sample molecules with IR radiation. This assembly facilitated surface-enhanced sensing of a deposited PMMA layer (~20 nm) via photothermal electric detection based on CSRR-enhanced absorption within the graphene ribbon. The PMMA vibrational modes coupled to the CSRR resonant modes resulted in plasmon-induced transparency features that could be read out from the corresponding dips of the obtained photovoltage spectra. This hybrid device with strong enhancement of the electric field and tunability may be also compatible with SEIRA applications.

Although dynamic tuning of graphene plasmons by applying an external voltage is beneficial in many analytical scenarios, the sophisticated fabrication procedure restrains its practical application. Alternatively, graphene plasmons may be tuned by modifying the dielectric constant of the surrounding media, namely, altering the electrostatic environment. For example, Peng et al. deposited gold nanoparticles (AuNPs) on a graphene nanodot array (GNA) at a Si substrate to form a hybrid structure via a block copolymer self-assembly approach. Thermal annealing of the gold film concluded a cost-effective procedure maintaining high-throughput potential compared to conventional EBL techniques.²⁷ The introduced AuNPs enhanced the absorption of GNA plasmons, and contributed an additional plasmon resonance band
in a broader MIR window. Sub-monolayer polyethylene oxide (PEO) polymer was analyzed with this hybrid SEIRA substrate demonstrating a broadband signal enhancement. However, because of the random size distribution of AuNPs formed via the thermal annealing process, the spectral bandwidth may not be ensured, and the unfavorably low field confinement of metallic materials in the MIR regime may still exist. Accordingly, polystyrene (PS) particles in lieu of metal nanoparticles were introduced to the surface of the graphene layer at a ZnSe substrate by Liu et al., which was implemented by transfer from a water-decane interface or by drop-coating. The deposited PS beads with an average diameter of 1 µm could diffract the IR light, which facilitated matching of the momentum and frequency of the incident photons with the surface plasmon polaritons (SPPs) waves in graphene. Subsequently, the excitation of MIR plasmons of the unpatterned graphene was mediated. Besides, the excited surface plasmons could propagate a few micrometers along the graphene surface. Consequently, enhancement of the interaction between target molecules and IR radiation could be achieved across a relatively large area. Para-aminobenzoic acid (PABA), residual PMMA, and organics from the transfer process of graphene were probed with a low density of PS particles, thereby reducing the IR background resulting from PS absorptions while maintaining satisfying signal enhancement (Figure 4d).

Figure 4. Graphene-involved hybrids for SEIRA. (a) Schematic of the designed graphene plasmonic nanoresonator separated from a metallic film by a nanometric dielectric spacer. (b) Schematic of the hybrid metasurface consisting of monolayer graphene and Au nanoantennas with low-molecular-weight analytes adsorbed on the
suspended graphene. (c) Schematic of the hybrid metamaterial comprised of a graphene ribbon embedded within a Cr/Au split ring resonator. (d) Schematic of graphene substrate with a low deposition density of polystyrene (PS) particles for SEIRA sensing para-aminobenzoic acid (PABA). Reproduced with permission from ref\textsuperscript{22}, ref\textsuperscript{23}, ref\textsuperscript{26}, and ref\textsuperscript{34}. Copyright 2017 American Chemical Society, 2018 Springer Nature, 2016 American Chemical Society, and 2017 Elsevier.

3. Graphene-based enhancement of Raman scattering (GERS)

Conventional SERS phenomena based on plasmonic metallic substrates are associated with two enhancement mechanisms, as previously discussed: electromagnetic (EM) and chemical (CM) mechanisms, with the first contributing the majority of the enhanced signals. In contrast, graphene-enhanced Raman scattering (GERS) mainly relies on CM as a consequence of molecule–substrate interactions (e.g., π-π stacking between aromatic compounds and graphene substrate, etc.), since the optical absorption of graphene is rather low (~2.3%), and the intrinsic graphene plasmon is in the terahertz (THz) spectral regime rather than at visible frequencies.\textsuperscript{28,40} In turn, this implies higher molecular selectivity in GERS as compared to conventional SERS.\textsuperscript{79} The molecular selectivity arises from the different strength of analyte-graphene interactions, and from effective charge transfer between them.\textsuperscript{80} Thus, different molecules will show different GERS enhancement factors (EFs). Molecular selectivity of GERS has been investigated using a variety of molecules with different molecular properties observing that molecular symmetry and substituents similar to the graphene structure lead to higher EFs.\textsuperscript{79} Generally, EF contributed by CM is relatively low. However, due to fact that molecular enrichment of analytes via π-π interactions may occur resulting in detection limits of GERS comparable to those achieved with noble metal structures in conventional SERS (i.e., as low as $8 \times 10^{-10}$ M for the example of R6G).\textsuperscript{81}

Graphene along with other 2D materials has been extensively studied as substrates for SERS applications.\textsuperscript{11,29,82} Owing to its excellent Raman scattering properties related to its electronic and photonic structure, and control on the number of layers with atomic precision,\textsuperscript{83} graphene can be considered as a Raman probe for both to study SERS effects and SERS-related issues and for the investigation of the SERS features of graphene itself. For example, the deposition of AuNP islands changes the surface of graphene resulting in the occurrence of new bands in the SERS spectrum along with an increase in the Raman signal of graphene.\textsuperscript{84} In addition, graphene sheets have also been combined with other nanomaterials such that a synergistic effect was observed between the fluorescence reduction and increased adsorption capabilities of graphene. In addition, this provides an increase in electromagnetic field around metallic NPs leading to additional EM effects in GERS. In the following sections, selected relevant studies on GERS are discussed especially focusing on the latest developments, as - in contrast to GEIRA - reviews on graphene and graphene-based
hybrid films have already been published.\textsuperscript{28, 33, 34, 40}

3.1. Enhancement of Raman scattering based on graphene structures

GERS comprises the use of graphene as enhancing substrate for Raman scattering signal. As previously mentioned, fluorescence is a competitive excitation process frequently obscuring the Raman signal of analytes, as the normalized molecular cross-section of fluorescence is significantly larger than that of Raman scattering.\textsuperscript{85} First observations on the effect of graphene on the spectral features of molecules adsorbed at its surface were related to quenching effects on the fluorescence of dyes such as R6G or protoporphyrin.\textsuperscript{86} Fluorescent dyes are able to interact with the sp\textsuperscript{2} carbon sheets of graphene via π-π interactions, thus resulting in a decrease in the fluorescence by up to three orders of magnitude. Hence, graphene provides a truly interesting substrate platform for the analysis of Raman signals of fluorescent dyes without the interference of a fluorescent background of the analyte.

Advantageously, the π-π interactions between analytes and the 2D honeycomb structure of graphene not only leads to a reduction in fluorescence background, but also to an enhancement of the Raman signal, a.k.a., GERS effect. In 2010, Ling et al.\textsuperscript{81} discussed for the first time the use of graphene as substrate with Raman signal-enhancing properties, and have investigated the effect on the Raman features of common SERS probes such as phthalocyanine (Pc), R6G, protoporphyrin IX (PPP), and crystal violet (CV) as compared to bare SiO\textsubscript{2}/Si substrates. The observed enhancement was mainly ascribed to a charge-transfer-mechanism between graphene and analyte molecules attributed as a chemical enhancement mechanism. CM is usually described as a so-called ‘first-layer-effects’, i.e., the first layer of adsorbed molecules exhibiting a significantly larger SERS cross-section.\textsuperscript{87} In fact, they found that the EFs were different depending on the number of layers of graphene (i.e., monolayer vs. few layers vs. multilayer graphene sheets), and that the Raman intensity decreased as the number of layers increased.\textsuperscript{83} In addition, it has been demonstrated that the Raman scattering intensity of a molecule in a GERS and non-GERS system on a SiO\textsubscript{2}/Si substrate with the same SiO\textsubscript{2} thickness are comparable, since there is no interference observed.\textsuperscript{88}

As previously mentioned, in GERS it is considered that the main contribution to the observed enhancement arises from CM, while EM can be considered negligible. In contrast, in common SERS systems both EM and CM coexist with the latter being difficult to separate from the dominant EM. Thus, graphene is an ideal platform for investigating CMs of molecules adsorbed onto graphene and mapping out the effect of the chemical structure of the analyte species. Several fundamental studies have been carried out based on GERS to elucidate the detailed mechanisms specifically focusing on ‘first-layer-effects’ in graphene-based substrates.\textsuperscript{89} and on charge-transfer effects.\textsuperscript{90} The GERS intensity depends on several factors such as molecule concentration and
orientation,91 molecule-graphene distance, graphene thickness,92 number of graphene layers, Fermi level,93 nature of graphene derivatives, and excitation laser energy.94 A detailed description of these phenomena is beyond the scope of this contribution, and can be found elsewhere.28,37

Chemical enhancement mechanisms are based on the adsorption and interaction of molecules at the surface of graphene, thus, the chemical structure and surface properties of graphene-based materials will significantly influence the enhancement. Apart from graphene, alternative graphene-derived structures have shown GERS effects including doped graphene, graphene oxide (GO),94,95 graphene quantum dots (GQDs),94, and graphene meshes.97 Most of these structures indeed revealed larger EFs compared to exfoliated graphene. Generally, these materials contain defects in the graphene structure along with functional groups such as hydroxyl, carboxyl, and epoxy moieties, which will influence the CM enhancement of the Raman signal.

Doping of graphene has been evaluated as a strategy to improve the sensitivity in GERS measurements. The electronic structure of graphene (i.e., the band gap) can be modified by addition of extrinsic atomic species absorbed or intercalated within the graphene lattice. For example, nitrogen-doped graphene films prepared by ultraviolet pulsed laser deposition (PLD) in the presence of nitrogen have shown improved chemical enhancement for Raman spectra of adsorbed R6G, as compared with pristine graphene.98 The enhancement depends on the nitrogen content, since N-doping leads to an increased number of charge carriers.

Graphene oxide (GO) has a 2D structure composed of sp2 carbon domains surrounded by sp3 carbon structures and oxygen-containing functional groups including carboxyl, hydroxyl, and epoxy moieties. It has been shown that reduced GO (rGO) may significantly increase the CM enhancement by up to one order of magnitude for RbB, as compared to pristine graphene.95 The enhancement was reported to depend on the reduction time of GO. Highly electronegative oxygen species in GO are described to contribute to the enhancement of the signal, since they may impose a strong local electric field onto adsorbed molecules. However, local defects showed no positive correlation, pH-dependent enhancement effects of aromatic molecules on GO have also been reported related to the adsorption of positively charged molecules at the negatively charged oxygen groups in GO via electrostatic interaction.99 In addition, Huh et al.100 demonstrated that the oxidation of graphene via UV/ozone treatment introduced oxygen-containing groups in graphene, and induced structural disorder and defects at its surface, which resulted in an increase in EF observed for graphene from ~103 prior to doping to ~104 after the treatment (Figure 5).
Figure 5. (a) Scheme of the GERS substrate prepared via chemical vapor deposition (CVD) of large-scale graphene. Graphene is later oxidized and p-doped by the UV-induced ozone molecules (the inset depicts the down-shifting of the Fermi energy of graphene after UV/ozone treatment). Rhodamine B (Rhb) molecules are deposited on graphene surface after immersion of the substrate for 30 min in a 10⁻⁵ M dye solution. (b) GERS spectra of RhB before (0 min) and after ozone treatment (5 min). (c) GERS enhancement factor for RhB before and after 5 min ozone treatment. Figures adapted with permission from ref. Copyright 2011 American Chemical Society.

A monolayer of chemically reduced GO nanosheets bonded onto Si substrates have shown improved substrate quenching of fluorescence and improved enhancement of R6G, as compared to mechanically exfoliated graphene. Annealed paper-like films of rGO have also been described as flexible SERS substrates, which proved significant enhancement for R6G molecules. In another study, the GERS intensity was found to depend on the thermal reduction temperature. Both fluorescence quenching and π-π interactions with R6G molecules, which causes charge transfer, are responsible for the GERS signal. Flexible rGO film can be used for the detection of residual R6G molecules, e.g., at the surface of fruits. In addition, fluorination of rGO has revealed a strategy to improve the enhancement of rGO. By manipulating the F-content on rGO, the EF may be tuned based on surface-localized dipoles of F-containing groups hypothesized to induce local electric field, which are responsible for additional enhancement effects.

Graphene quantum dots are nanometer-sized fragments of graphene providing larger specific surface areas vs. conventional graphene materials, and more accessible
edges, which in turn enables the adsorption of more analyte molecules. While GQDs have been investigated as suitable GERS substrates, impurities occurring during their synthesis should be controlled such that no reduction of the charge-transfer properties are evident. Assembly of GQDs into nanotube arrays\textsuperscript{34} have shown their potential as GERS substrates ensuring efficient charge transfer between target analytes and GQDs. The organization of GQDs into nanotube arrays provides a nanoporous structure, which enables large surface areas for adsorption, as well as fine rough surfaces favoring signal enhancement process. These effects were observed to be significantly increased vs. drop-casted GQD films. For example, Liu et al.\textsuperscript{109} prepared substrates by depositing ~2 nm sized ultra-clean GQDs onto SiO$_2$/Si. The obtained EFs, which showed a dependence on GQD size were higher as compared to graphene sheets, which is ascribed to the large number of edges and the enhanced charge transfer between analytes and GQDs.

Finally, graphene nanomeshes have also been evaluated as alternative for improved chemical enhancement of the Raman signal. The amount of edges and their types evidently determine the electronic structure of the nanomesh. In the following, a p-type doped graphene nanomesh SERS substrate using Cu nanoparticles has been reported, which significantly improved the Raman signal of RhB molecules as compared to pristine graphene.\textsuperscript{97}

### 3.2. Graphene hybrids as SERS substrates

As previously discussed, CM is the dominating mechanism contributing to the signal enhancement observed in GERS. Thus, graphene does not immediately provide ideal characteristics of a SERS substrate owing to its low Raman scattering cross-section,\textsuperscript{40} and the lack of enhanced electromagnetic fields due to THz surface plasmons.\textsuperscript{49} The quest for further enhancement of the Raman signal has led to combining the attractive characteristics of graphene-based nanostructures including pristine graphene, GO, and GQDs with other nanostructures into a variety of useful hybrid SERS/GERS substrates in recent years.

Graphene is an ideal 2D platform for the functionalization and binding of nanoparticles, thus providing a platform that may take advantage of synergistic effects of both graphene nanomaterials and metal NPs. The introduction of graphene within such hybrid substrates has several advantages related to the different roles that graphene may play. Graphene can be used as matrix support of metal NPs, since it is a highly uniform and flat material suitable for the controlled deposition of the NPs facilitating high reproducibility and stability of the SERS signal, and avoiding NP aggregation. At the same time, suppression of the fluorescence background and a large surface area for molecular enrichment are beneficial.\textsuperscript{104} Analytes that adsorb weakly on conventional metallic SERS substrates may enrich well at graphene – an thereby close to the immobilized metallic NPs – due to the delocalized $\pi$-bond of graphene, which favours the adsorption of, e.g., aromatic molecules via $\pi$-$\pi$
interactions.\textsuperscript{40, 105} In such hybrid substrates, the hot spots created by metal NPs are able to pass through the graphene layer, thereby resulting exemplarily in atomically flat surfaces with enhanced electric fields supporting SERS effects.\textsuperscript{106} Graphene substrates decorated with metal nanostructures reveal both EM and CM; the synergistic effects of each nanomaterial may therefore lead to improved sensitivity, selectivity, and reproducibility in GERS. In addition to graphene, GO may be used taking advantage of the negatively charged oxygen moieties that may serve as anchors of positively charged metal NPs.

Graphene may also act as a cover layer for metal NPs, i.e., a shielding interface in order to protect nanoparticles from oxidation and corrosion, thus extending the lifetime and improving the stability of SERS substrates. At the same time, this may prevent undesired catalytic reactions of molecules at metallic NPs and may assist in avoiding photo-induced damages. These approaches are also summarized under the term ‘shell-isolated SERS’,\textsuperscript{107} which allows for passivated substrates with a minimum loss of electromagnetic enhancement.

Finally, graphene may act as sub-nanospacer during the preparation of separated metal NP substrates. Sandwich substrates of metal NPs included within a top and a bottom layer of graphene have also been described, whereby metal nanostructures are protected from oxidation in both directions.

A detailed description of the different approaches developed in this field is beyond the scope of this review, thus, a selected number of hybrid substrates combining graphene-based materials and a variety of additional nanostructures that have more recently been developed will be exemplarily highlighted.

As previously discussed, a drawback of SERS methods in general is the lack of or the poor reproducibility. Thus, analytical approaches overcoming this bottleneck are needed when applying SERS/GERS in quantitative measurement scenarios. In order to obtain SERS substrates that are uniform and stable across sizable sensing areas, graphene has been combined with metallic nanoparticles, and predominantly gold or silver\textsuperscript{108-109}, as well as alloy NPs comprising both metals.\textsuperscript{110} For example, a graphene-shielded periodic metallic structure comprising AgNPs has been described (Figure 6a) preserving the SERS activity of the substrate along with providing new features.\textsuperscript{108} In addition, Sutrović et al.\textsuperscript{111} have studied the mechanisms of combined SERS and GERS in a hybrid system established by AgNPs on a glass substrate overcoated with a single layer of graphene (SLG) for the target analyte (phthalocyanine, H$_2$Pc) (Figure 6b). Moreover, SERS substrates based on graphene-encapsulated AuNPs have been reported,\textsuperscript{112} which have been also combined with single-walled carbon nanotubes that effectively increased the number of surface functional sites.\textsuperscript{113}
Graphene oxide has been combined in different formats with metallic NPs. A variety of strategies have been described for the preparation of such hybrid substrates, e.g., using micro/nanostructured lotus-leaf-patterns as a template for the preparation of GO deposited at Ag micro-islands\textsuperscript{12} (Figure 6c). Furthermore, graphene quantum dot hybrid substrates have been reported, e.g., composed of AgNPs protected by nitrogen-doped GQDs (AgNP@N-GQD), which proved a significant improvement of the Raman signal enhancement of glucose vs. using only AgNPs. In addition, the SERS performance was preserved over a period of 20 days stored at wet and dry conditions.\textsuperscript{116}

In addition to spherical Au/Ag nanostructures, plasmonic nanostructures such as nanostars, nanocubes, nanowires,\textsuperscript{117} and nanoflowers have been combined with graphene materials. For example, a graphene/Ag-nanoflowers/PMMA\textsuperscript{115} substrate (Figure 6d) has been described comprising the presence of metallic NPs and polymers.
in a sandwich-type structure. Branched NPs such as nanostars are from a geometric perspective ideally suited as amplifiers of Raman signals because of the large number of hot spots distributed at their surface, which results in sizeable antenna effects. A hybrid SERS material prepared by seed-mediated synthesis of AuNSs supported on rGO (Figure 7) has been applied for the determination of doxorubicin (DOX). The localized surface plasmon resonances of the rGO-AuNSs hybrid may be tuned by modifying growth reaction parameters, which increases the versatility of the substrate. Superior SERS behavior was demonstrated when compared to bare AuNSs, as rGO prevented AuNSs from aggregation, and at the same time increased the number of interaction sites for aromatic DOX molecules.

![Figure 7](image_url)  
*Figure 7. Scheme of the preparation of a reduced graphene oxide-gold nanostars (rGO-AuNSs) SERS hybrid substrate for the SERS detection of doxorubicin. Reproduced with permission from ref19. Copyright 2014 American Chemical Society.*

As previously mentioned, graphene may play a variety of roles in hybrid substrates. Guo et al.19 developed a flexible SERS sensor comprising an AuNSs layer between two sheets of graphene. While the inner AuNSs offers a high number of hot spots with pronounced electromagnetic field enhancement, the presence of graphene improves the stability and reproducibility of the SERS/GERS signals. Another role is...
acting as a spacer of NPs. In this regard, GO has been coupled to anisotropic AuNSs, whereby GO acted as a nanospacer being sandwiched between two layers of closely packed AuNSs.\textsuperscript{121} This multidimensional plasmonic coupling (i.e., both horizontal and vertical) lead to an increased sensitivity, which was combined with additional CM and molecule adsorption capabilities at the GO interlayer.

Highlighting few examples of alternative metals, copper has suitable properties for the development of SERS substrates (e.g., core-shell graphene@CuNP\textsuperscript{s}) at significantly lower cost as compared to Au and Ag. However, these structures suffer from aggregation and oxidation of the Cu surface. Hence, graphene may play an important role in stabilizing materials such as CuNPs. In addition to copper, other metal oxide NPs have been coupled to graphene for establishing hybrid SERS substrates. The inclusion of TiO\textsubscript{2} or ZnO has demonstrated new functionalities such as catalytic degradation.\textsuperscript{123-124} For example, TiO\textsubscript{2}/GO nanocomposites with photocatalytically grown Ag nanocrystals have been reported.\textsuperscript{124} In such materials, graphene may assist charge separation, and may act as an electron carrier in the composite material modifying the photocatalytic performance of the composite. Likewise, the morphology of the NPs is affected, which directly correlates to the Raman signal enhancement. Last but not least, the addition of magnetic nanoparticles may assist in recovery of the substrate via magnetic forces during their analytic application.\textsuperscript{125-126}

4. Conclusions and outlook

This review briefly introduces the fundamentals of surface enhanced vibrational spectroscopies, namely surface enhanced infrared absorption (SEIRA) and surface-enhanced Raman scattering (SERS) with a specific focus on latest developments utilizing graphene and graphene-derived materials, and graphene hybrids as signal-enhancing substrates.

Despite significant progress in recent years, both SEIRA and SERS are still of limited use in practical applications owing to rigorous demands by quantitative optical/spectroscopic experiments and routines, as well as restrictions to selected molecules. SEIRA has clearly received less attention than SERS, which may in part be attributed to the relatively lower achievable enhancement factors and limited choice of signal-enhancing materials. This may change in future due to the unique chemical properties of graphene – and other 2D materials – along with pronounced plasmonic features in the mid-infrared regime rendering them promising alternatives as SEIRA substrates for directly enhancing molecular vibrational signatures. Despite numerous studies on graphene plasmonics, graphene-induced SEIRA – GEIRA – sensing remains rather exotic with nearly half of the published papers concerning theoretical simulations. A sure fact is that the intrinsic graphene plasmonic absorption/response is relatively weak in the MIR range, which arises predominantly from the required quality of the material, and the momentum mismatch between the external light and the plasmonic waves. In turn, graphene with reduced
defects/impurities providing strong absorbance, high carrier mobility, and extended
electron relaxation times is highly beneficial for GEIRA scenarios. In pursuit of an
improved performance of plasmonic GEIRA, patterned graphene nanostructures and
the integration into hybrid assemblies are feasible approaches, albeit at the expense of
sophisticated - and potentially expensive - fabrication routines, reduction of electron
mobility and quality factor, degradation of accessible wavelength window, and/or
field confinement. Besides, increasing doping levels and utilizing multilayer graphene
has revealed increased plasmonic SEIRA effects. Furthermore, the adsorption and
enrichment of molecules along with tailoring their chemical interactions with
graphene and its derivatives via π–π stacking and other driving forces should be
exploited more deeply in future, thereby also facilitating a more profound
understanding of the chemical enhancement mechanism in SEIRA. Most currently
applied GEIRA sensing platforms are based on transmission-absorption
configurations analyzing solid or gaseous samples. It is strongly anticipated the
emergence of more experimental studies using internal reflection techniques (e.g.,
ATR) in combination with GEIRA enabling advanced in situ real-time monitoring of
analytes and reactions in aqueous media will add to the more ubiquitous application
of graphene-enhanced IR spectroscopy in the near future, e.g., in biomaterial
scenarios.

As for graphene-based SERS – GERS - abundant studies have been reported, even
though its potential has not been fully unlocked yet. Graphene itself does not provide
ideal properties as signal-enhancing material in SERS applications, as it does not
support electromagnetic enhancement mechanisms in the visible regime. Nevertheless,
graphene supports Raman signal enhancement via chemical mechanisms resulting
from π–π interactions with appropriate analytes and functional moieties. Not only
graphene, but a variety of graphene derivatives have been explored promoting
enhanced chemical interactions due to structural or chemical defects. It is expected
that in the near future advancements of Raman excitation light sources from the deep
UV to the THz will enable further exploitation of the SERS properties of graphene
and related materials by combining both enhancement mechanisms. Notwithstanding,
the development of hybrid SERS substrates comprising graphene along with metal or
semiconductor nanoparticles has evolved remarkably, whereby graphene assist not
only in signal enhancement, but also in decreasing the fluorescence background,
acting as a corrosion barrier, and reducing photo-induced damage.

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References


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(27) Huang, Y. F.; Zhu, H. P.; Liu, G. K.; Wu, D. Y.; Ren, B.; Tinn, Z. Q., When the signal is not from the original molecule to be detected: chemical transformation of para-aminothiophenol on Ag during the SERS measurement. *J. Am. Chem. Soc.* **2010**, *132*, 9244-9246.

1757


1758


1759


1760


(43) García de Abajo, F. J., Graphene Plasmonics: Challenges and Opportunities. *ACS Photonics* 2014, *1*, 135-152.


2013, 13, 2541-2547.
(64) Hu, Y.; Chen, Q.; Ci, L.; Cao, K.; Mizakoff, B., Surface-enhanced infrared attenuated total reflection spectroscopy via carbon nanodots for small molecules in


(79) Huang, S.; Ling, X.; Liang, L.; Song, Y.; Fang, W.; Zhang, J.; Kong, J.; Meunier, V.; Dresselhaus, M. S., Molecular selectivity of graphene-enhanced Raman scattering.
Nano Lett. 2015, 15, 2892-2901.


(91) Ling, X.; Wu, J.; Xu, W.; Zhang, J., Probing the effect of molecular orientation on the intensity of chemical enhancement using graphene-enhanced Raman spectroscopy. Small 2012, 8, 1365-1372.


(110) Yu, J.; Ma, Y.; Yang, C.; Zhang, H.; Liu, L.; Su, J.; Gao, Y., SERS-active
composite based on rGO and Au/Ag core-shell nanorods for analytical applications. 


(112) Li, Y.; Burnham, K.; Dykes, J.; Chopra, N., Self-patterned of graphene-encapsulated gold nanoparticles for surface-enhanced Raman spectroscopy. 


(122) Yang, C.; Zhang, C.; Huo, Y.; Jiang, S.; Qiu, H.; Xu, Y.; Li, X.; Man, B., Shell-isolated graphene@ Cu nanoparticles on graphene@ Cu substrates for the application in SERS. Carbon 2016, 98, 526-533.


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Paper II. Versatile Analytical Platform Based on Graphene-Enhanced Infrared Attenuated Total Reflection Spectroscopy

Y. Hu performed all measurements, analyzed the data, and wrote the manuscript. The proposed method was designed in close collaboration with Á.I. López-Lorente. Á.I. López-Lorente and B. Mizaikoff contributed with revisions to the final version.

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Versatile Analytical Platform Based on Graphene-Enhanced Infrared Attenuated Total Reflection Spectroscopy

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Supporting Information

ABSTRACT: Graphene, with its unique properties including atomic thickness, atomic uniformity, and delocalized π bonds, has been reported as a promising alternative material versus noble metals for surface-enhanced spectroscopies. Here, a simple and effective graphene-enhanced infrared absorption (GEIRA) strategy was developed based on infrared attenuated total reflection spectroscopy (IR-ATR). The IR signals of a broad range of molecules were significantly enhanced using graphene-decorated diamond ATR crystal surfaces versus conventional ATR waveguides. Utilizing rhodamine 6G (R6G) as the main model molecule, the experimental conditions were optimized, and potential enhancement mechanisms are discussed. Aqueous sample solutions were directly analyzed utilizing graphene dispersions, which eliminates harsh experimental conditions, tedious sample pretreatment, and sophisticated fabrication/patterning routines at the ATR waveguide surface. The GEIRA approach presented here provides simple experimental procedures, convenient operation, and excellent reproducibility, promoting a more widespread usage of graphene in surface-enhanced infrared absorption spectroscopy.

KEYWORDS: graphene, surface-enhanced infrared absorption spectroscopy, attenuated total reflection, rhodamine 6G

Infrared (IR) absorption spectroscopy acquires molecular vibrational and rotational fingerprints associated with specific chemical, structural, and compositional information on molecular constituents by determining the absorption in the mid-infrared spectral regime (2.5–20 μm).1-11 Hence, rapid, non-destructive, highly discriminatory, and label-free detection of analytes is enabled.1-11 Nevertheless, conventional IR techniques suffer from comparatively low sensitivities, which restrict practical applications in trace and ultratrace analysis.1-11 Strategies for enhancing IR signals have received increasing attention since Hartstein et al. for the first time reported increased IR absorption features occurring at noble metal layers.1-11 To date, such enhancement effects have been observed on various nanostructured substrates,1-11 including metallic nanoslabs,1-11 nanoshells,1-11 nanowires,1-11 nanotubes,1-11 and so on, which are commonly known as surface-enhanced infrared absorption (SEIRA) effect in analogy to surface-enhanced Raman scattering (SERS) and surface-enhanced fluorescence (SEF).1-11 It is generally accepted that the enhancement of infrared absorption features at certain substrates may be attributed to at least two distinct mechanisms, that is, electromagnetic (EM) enhancement, an increase in the local electric field at the substrate surface, and chemical enhancement (CE), which is derived from chemical interactions between the target molecule and the substrate surface.1-11 The majority of reported SEIRA substrates are based on noble metals (e.g., Au, Ag),1-11 as their plasmonic resonances can be extended to the infrared region via rational design of specific nanostructures.1-11 However, the plasmon frequencies of metals are fixed after structuring, thereby limiting SEIRA detection of multiple IR bands across a broad spectral range.1-11 Additionally, the SEIRA effect is restricted to the immediate vicinity of the substrate surface1-11 and greatly depends on the surface morphology.1-11 Hence, to provide sensitive, reproducible, and reliable SEIRA signals, the reproducibility of nanostructures is essential, yet remains challenging.1-11 Consequently, more accessible, cost-efficient, environmentally friendly, and reproducible nonmetallic materials useful for broadband IR absorption enhancement is in demand.

Graphene is a two-dimensional (2D) semimetallic material made of sp²-bonded carbon atoms arranged in a honeycomb pattern.1-11 Recently, it has been proposed as a promising alternative material for enhancing infrared absorptions,1-11 as well as Raman scattering,1-11 owing to its remarkable advantages, such as the unique atomic thickness/uniformity, flexibility, chemical inertness, tight spacial plasmon confinement, extended plasmon propagation distances, exceptional electrical/optical tuning properties, and excellent ability to noncovalently bind to a wide variety of chemically and biologically relevant molecules. This clearly advantageously discriminates graphene from conventional metallic materials.1-11 Reports on graphene-enhanced IR absorption (GEIRA) remain rare to date and were
JOURNAL ARTICLES

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Achieved mainly via graphene modified by chemical doping, electrostatic gating, patterning into nanofibers, nanoflakes, and combining with noble metals. However, these methods usually require extended pretreatment procedures, sophisticated fabrication schemes, and rigorous control of the experimental conditions. Hence, infrared absorption techniques remain of limited utility if sophisticated surface nanostructures are required.

Even though SEIRA can be implemented in transmission IR spectroscopy, using an attenuated total reflection (ATR) configuration appears advantageous, as the shallow penetration depth of IR light into the sample/media (e.g., few micrometers) allows the analysis of targets dissolved even in strongly IR absorbing media, such as water, and ensures exquisite surface sensitivity. In addition, the surface of an ATR waveguide or crystal readily serves as supporting substrate for depositing signal-enhancing nanomaterials, which simplifies the experimental manipulation. Herein, we report a simple and efficient graphene-enhanced infrared absorption (GEIRA) approach, taking advantage of graphene and IR-ATR spectroscopy and avoiding harsh preparation conditions, toxic agents, and complex sampling operations. The experimental conditions were optimized using aqueous solutions of rhodamine 6G (R6G) as a model analyte. The generic applicability of the proposed GEIRA platform was verified via several additional analytes, illustrating its potential and versatility. Besides, mechanistic studies were performed by investigating the evolutions of GEIRA spectra with time and at a variety of analyte concentrations.

RESULTS AND DISCUSSION

A scheme of the developed GEIRA platform is shown in Figure 1. Rhodamine 6G (R6G), used as a model analyte herein, is in fact a water contaminant and toxic to human beings and animals. Figure 2A depicts the IR spectra of R6G solutions at different concentrations, levels recorded at the pristine diamond ATR waveguide. The IR absorbance of R6G remained quite weak, even at concentrations as high as 2.5 mg/mL, thereby indicating the rather low sensitivity of conventional IR-ATR measurements toward R6G. By contrast, in the presence of graphene the IR absorbances of R6G are significantly enhanced with excellent repeatability (error bars in Figure 2B) tests, as shown in Figure 2B. After optimizing the amount of deposited graphene (Figure 2C), the enhancement effect was increasing with increasing concentration of graphene (from 0.01 to 0.05 mg/mL) at the same initial volume of 2 μL. Herein, 2 μL was determined as the minimum volume of graphene dispersion required to entirely coat the diamond surface. Moreover, the enhancement of the IR absorptions associated with R6G was not further enhanced when increasing the graphene volume from 2 to 4 μL (Figure S1). Accordingly, 2 μL of 0.05 mg/mL graphene was selected for the subsequent experiments to ensure a maximized GEIRA effect.

The effectiveness of the developed graphene-coating method resulting in dried graphene flakes at the diamond ATR surface was further confirmed by comparing the spectral response of R6G with alternative procedures for depositing graphene: (i) the same amount of graphene dispersion was directly deposited at an identical diamond waveguide surface, yet without drying, and (ii) graphene grown via chemical vapor deposition on copper foil (i.e., CVD-graphene) was transferred onto a pristine diamond waveguide [for details see Supporting Information]. The IR spectrum of 0.5 mg/mL R6G solution revealed a stable GEIRA effect when graphene was applied via the three different procedures, among which dried-graphene decorated diamond prepared by the proposed drop-coat method revealed maximum signal enhancement (Figure 2D).

To meet the need of various measurement scenarios, the generic applicability of an analytical method is vital. Having this in mind, the developed GEIRA platform was tested for alternative molecules in aquatic solution at the same experimental condition. The concentration of the analyte solutions was fixed at 0.5 mg/mL (except 0.2 mg/mL for auramine due to its low solubility in water), enabling comparative studies. As shown in Figure 3, without the assistance of graphene, all the tested molecules provide only weak IR signatures. In contrast, the IR spectra of all tested analytes were prominently enhanced using the dried-graphene decorated diamond ATR waveguide confirming the generic performance of the GEIRA platform. The IR fingerprint patterns in Figure 3 were further normalized such that the characteristics of the spectra are comparable at the same scale as depicted in Figure S2. From the normalized spectra of a given analyte recorded with and without graphene, it is evident that the presence of graphene does not induce noticeable changes of the characteristic peaks (i.e., band position or shape), yet proportionally magnifies the absorption bands. The reliability of the proposed GEIRA platform is thereby verified. For a more straightforward comparison of the variation of peak intensity, an experimental enhancement factor (eEF) was calculated:

\[ eEF = \frac{I_{GEIRA}}{I_0} \]

whereby \( I_{GEIRA} \) and \( I_0 \) are the enhanced and non-enhanced IR signal intensities (i.e., peak height) of the considered band of the analyte analyzed via the GEIRA platform and the pristine diamond ATR crystal, respectively. In other words, the eEF reports a multiplication (i.e., enhancement) factor of the pristine absorption signal\(^{19,20}\). In this study, only bands that were also evident in the pristine IR spectra (i.e., recorded in absence of graphene) were considered. The calculated eEFs of thus considered bands and the corresponding assignments are listed in Table 1. Evidently, the eEF values of different peaks of one specific molecule are of the same order of magnitude. Taking auramine as an example, its characteristic bands around 1,907 and 1,378 cm\(^{-1}\) are attributed to the stretching vibration of the p-dimethylaminobenzene aromatic ring, and C–N in (C)=N–N, of which the eEF is 16.3 and 21.5, respectively. The molecular structures of crystal violet (CV) and fuchsin are similar, with very close eEF values at each peak position. Although methylene blue (MB) and neutral red (NR) have a lot in common, their spectra appear very different, and neither are their eEF values alike. Rhodamine B

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ACS Photonics 2018, 5, 2160–2167
(A) IR spectral signatures of R6G solution at different concentrations recorded via a prismatic diamond ATR waveguide. (B) Unprocessed IR spectra of 2.5 mg/mL R6G solution collected at the diamond waveguide with and without graphene coating (0.05 mg/mL graphene was first deposited and dried at the diamond surface). (C) Effect of graphene concentration on the IR absorption of 2.5 mg/mL R6G. (D) Influence of different graphene addition methods on the GIERA effect (R6G: 0.5 mg/mL).

Figure 3. Comparison of IR fingerprint patterns of (A) auramine, (B) crystal violet, (C) fuchsin, (D) methylene blue, (E) neutral red, and (F) thiazolene II recorded on dried graphene-decorated and bare diamond waveguide surfaces. The insets reveal the chemical structure of the molecules.

(RB) and R6G share the same core structure; however, they may be discriminated by their characteristic IR fingerprints. R6G provides higher eEFs, which is attributed to the nonoptimized measurement conditions of RB, as detailed below.

It should be noted that the experimental conditions were not particularly optimized for either specific molecule. Therefore, the calculated eEF may be further increased by appropriate optimization strategies. As an exemplary parameter, the pH value of the sample solution was investigated in more detail, as it potentially affects the interaction between graphene and the analyte. For instance, 4-hydroxybenzoic acid (4-HBA, 0.5 mg/mL pH 3.4) displayed a moderate enhancement effect on the dried graphene-modified ATR waveguide (Figure 4A). If the pH value of the sample solution was appropriately adjusted using...
Table 1. Assignment and eEF of Characteristic Peaks of Molecules Analyzed via the Developed GEIRA Platform*  

<table>
<thead>
<tr>
<th>molecule</th>
<th>wavenumber (cm⁻¹)</th>
<th>assignment⁵</th>
<th>eEF⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>anhydride</td>
<td>1597</td>
<td>a(νmax), p- substituted aromatic ring</td>
<td>16.5</td>
</tr>
<tr>
<td>crystal violet</td>
<td>1589</td>
<td>a(νmax), p- substituted aromatic ring</td>
<td>12.74</td>
</tr>
<tr>
<td></td>
<td>1366</td>
<td>a(υ(C=N)), (C=O)=N</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>1176</td>
<td>a(υ(C–N)), skeletal vibration</td>
<td>7.9</td>
</tr>
<tr>
<td>succinate</td>
<td>1358</td>
<td>a(υ(CO2)), p- substituted aromatic ring</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>1369</td>
<td>a(υ(C-O)), (C=O)=N</td>
<td>15.9</td>
</tr>
<tr>
<td>methylene blue</td>
<td>1601</td>
<td>a(υ(C=O)), 1,4-trisubstituted ring</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td>1357</td>
<td>a(υ(C=C)), exit = CH</td>
<td>15.1</td>
</tr>
<tr>
<td>neutral red</td>
<td>1506</td>
<td>a(υ(C=O)), 1,4-trisubstituted ring</td>
<td>48.2</td>
</tr>
<tr>
<td></td>
<td>1310</td>
<td>a(υ(C=O)), (C=O)=N</td>
<td>146.3</td>
</tr>
<tr>
<td>rhodamine B</td>
<td>1597</td>
<td>a(υ(C=O)), 1,4-trisubstituted ring</td>
<td>18.3</td>
</tr>
<tr>
<td></td>
<td>1318</td>
<td>a(υ(C=O)), Ph–N–(R)</td>
<td>28.0</td>
</tr>
<tr>
<td>rhodamine 8G</td>
<td>1602</td>
<td>a(υ(C=O)), C–N=N=C</td>
<td>44.9</td>
</tr>
<tr>
<td></td>
<td>1331</td>
<td>a(υ(C=O)), 1,4-trisubstituted ring</td>
<td>42.4</td>
</tr>
<tr>
<td></td>
<td>1503</td>
<td>a(υ(C=O)), 1,4-trisubstituted ring</td>
<td>47.6</td>
</tr>
<tr>
<td></td>
<td>1307</td>
<td>a(υ(C=O)), Ph–N–H–R</td>
<td>37.7</td>
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<tr>
<td></td>
<td>1706</td>
<td>a(υ(C=O)), Ph–COOH</td>
<td>42.0</td>
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<tr>
<td></td>
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<td>a(υ(C=O)), Ph–OH</td>
<td>44.9</td>
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<td></td>
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<td>a(υ(C=O)), Ph–COOH</td>
<td>26.4</td>
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<tr>
<td></td>
<td>1076</td>
<td>a(υ(C=O))</td>
<td>155.3</td>
</tr>
</tbody>
</table>

*Azurin: 0.2 mg/mL. Others: 0.5 mg/mL. ⁵υ = stretching, δ = bending. *Experimental enhancement factor (eEF).  


diluted HCl or NaOH solution, the highest band intensities were in fact achieved at pH = 1.9. In that case, the cycle of 2.5 mg/mL R6G solution, a noticeable GEIRA effect was only evident at the initially selected pH = 1.5 without improved enhancement after pH adjustment (Figure 4B). Figure 4CD exhibits the gain in eEFs of these exemplary molecules at optimized pH values. The possible reason for the variation of enhancement effect caused by sample solution pH value will be discussed later.  

In addition, it was observed that the peak intensity of the GEIRA spectrum was not constant with time. Figure 5A–E show how the GEIRA spectra for a selection of molecules evolved with increasing time after adding the sample solution onto the dried-graphene-decorated ATR crystal, and the corresponding intensities of their selected peaks as a function of time were plotted in Figure 5F. For molecules at low concentration, for example, R6G at 0.05 mg/mL (Figure 5A) and anhydride at 0.2 mg/mL (Figure 5D), the IR absorbance increased only slowly with time and almost reached an equilibrium value after approximately 4 min. If the sample concentration was increased to 0.5 mg/mL (i.e., R6G and neutral red, Figure 5B and E, respectively), the IR peak intensity kept increasing for the entire duration of the measurement (i.e., 10 min). As for R6G at a high concentration of 2.5 mg/mL (Figure 5C), the IR signal increased sharply until approximately 4 min, and then appeared to have reached saturation. For practical reasons, samples without and with graphene were both investigated for periods of 10 min, thereby ensuring a suitable compromise between maximizing the signal enhancement and maintaining a reasonable analysis time.  

As previously mentioned, electromagnetic enhancement and chemical enhancement are two mechanisms that may contribute to the total enhancement of the IR signal. The electromagnetic fields of graphene IR plasmons exhibit substantial subdiffractional confinement of IR excitation, that is, reportedly up to 2 orders of magnitude higher compared to metals.⁶⁶ Nevertheless, by virtue of the relatively high momentum mismatch between the surface plasmons and the IR photons,⁶⁶ excitation of surface plasmons in graphene flakes remains challenging. Hence, adequate modification of graphene is obligatory in order to overcome such mismatch.⁶⁶ However, apart from the electromagnetic contribution, graphene could also act as a molecular enrichment matrix, as reported in graphene-based SERIS system contributing to the chemical enhancement via π–π interactions and so on.⁶⁶,⁷⁰,⁷¹ Note that the graphene used in this study has not been specifically structured in size or doped, and the GEIRA effect has been observed within a broad range of IR frequencies. Taking into consideration the factors and the obtained experimental results, it is thus hypothesized that the observed GEIRA effect predominantly results from a chemical mechanism (CM), which in turn relates to adsorption processes playing a dominating role. More specifically, the tested molecules all comprise aromatic moieties, which benefit adsorption onto the graphene matrix via π–π stacking interactions.⁶⁶,⁷¹ Consequently, sample molecules are enriched at the graphene surface.⁷¹  

In general, the penetration depth of the evanescent field in this wavelength regime is typically on the order of magnitude of less than micrometers. The diamond ATR crystal covered by dried graphene via the drop-coat method revealed much more pronounced GEIRA effects versus CVD-graphene transferred.
Figure 5. Evolution of the GIERA signals from (A) 0.05 mg/mL R6G, (B) 0.5 mg/mL R6G, (C) 2.5 mg/mL R6G, (D) 0.03 mg/mL uranium, and (E) 0.5 mg/mL neutral red with increasing time after adding the sample solution onto the dried-graphene-decorated diamond ATR crystal. (F) GIERA signal intensities of selected peaks of the corresponding analytes vs time after the sample addition.

Figure 6. (A) Concentration-dependence of GIERA spectra of R6G obtained by measuring R6G solution with the developed GIERA platform. (B) The corresponding peak intensities at 1609, 1531, 1503, and 1307 cm⁻¹ as a function of the R6G concentration, the inset is the best-fit Langmuir–Freundlich isotherms of data at 1607 cm⁻¹. The error bars indicate the standard deviation in GIERA intensity for three independent experiments.

to the surface, and graphene dispersion mixtures (Figure 2D), respectively, as apparently more graphene flakes remain at or close to the ATR waveguide surface. Hence, more analyte molecules are concentrated via chemical interaction with graphene within the penetration depth of the evanescent field. Furthermore, the increase of the GIERA signals with time after addition of the sample solution (Figure 5) may indeed result from the adsorption process to reach equilibrium. A shift in solution pH apparently does not influence the characteristics of the IR finger pattern, as the normalized spectra of 4-HBA and R6G collected at different pH values demonstrate in Figure S3. Nonetheless, pH changes may still affect molecules such that the adsorption performance of graphene is altered, thereby inducing changes in the GIERA effect (Figure 4). To be specific, 4-HBA as a weak acid with a pKₐ value of 4.54 would partially dissociate in water. At a solution pH of 1.9 (Figure 4A), H⁺ release from 4-HBA was greatly suppressed. Conversely, dissociated 4-HBA (i.e., as negatively charged HBA) dominated as the pH increased to 6.6 or 8.4. The accumulation of dissociated 4-HBA at the graphene surface was thereby restrained via electrostatic repulsion between the negatively charged groups. The case of R6G appears more complex. As a cationic dye, R6G may dissociate in the aqueous solution with pKₐ of 18.72 (i.e., strongest acidic) and 2.34 (i.e., strongest base). If the solution pH was set at 1.7 (Figure 4B), R6G appeared mostly protonated (i.e., positively charged), while it appears deprotonated (i.e., negatively charged) beyond a pH of 5.2, thus, giving rise to strong electrostatic repulsion between charged R6G species adverse to their adsorption onto graphene. As a result, the most prominent SEIRA effect was apparent, if the majority of analytes were present as electronneutral molecules at the appropriate individual pH conditions (i.e., pH = 3.6 in this case). Additionally, owing to the π-π interactions between graphene and the aromatic moieties the distance between graphene and the molecules is small, and charge transfer may be facilitated. This likely contributes to the chemical enhancement. As evident in Figure 2A, the band intensity (I), here, of R6G, analyzed using conventional ATR varies with the sample concentration (C). Figure 6A displays the concentration-dependent GIERA spectra of R6G. The peak intensities of four
characteristic bands at 1608, 1531, 1503, and 1307 cm\(^{-1}\) obtained from the GEIRA spectra, as well as the IR-ATR spectra recorded without graphene are plotted against the R6G concentration in Figure 6B and S4, respectively. At each concentration, the peak intensities of the four non-enhanced bands were comparable, and increased with increasing concentration. Consequently, linear calibration functions were fitted to the experimental data (Figure S4), indicating that the signal intensity of the nonenhanced IR spectra was proportional to the concentration of R6G.

Evaluating the GEIRA spectra, the peak intensity was not linearly dependent on the R6G concentration (Figure 6B); instead, the experimental data were fitted using a quasi-Langmuir–Freundlich equation, which is generally applied for modeling multilayer adsorption processes:

\[
I = \frac{K \times C}{1 + n \times C}
\]

where \(I\) is the IR peak intensity, \(C\) refers to the ratio of IR peak intensity versus adsorption amount, \(K\) is the equilibrium concentration of adsorbate in solution, and \(n\) is a constant for a given adsorbate and adsorbent at constant temperature. Exemplarily, the values of the band at 1608 cm\(^{-1}\) along with the fitted curve are shown in the inset of Figure 6B, with \(K\) and \(n\) at 1,661 and 3.03, respectively, and a correlation coefficient \(R^2\) of 0.988. Here the value \((1/C)\) is used to replace the amount of adsorbate in the general Langmuir–Freundlich isotherm equation. \(Q\) of the fitting isotherm is 0.969, which indicates that the per unit amount of R6G contributes approximately 0.969 (a.m.) to the peak intensity in the GEIRA spectra. The remarkably good fit provided by the quasi-Langmuir–Freundlich model implies that R6G molecules are indeed adsorbed to graphene at or adjacent to the ATR surface via a multilayer adsorption process that, in turn, confirms that the adsorption of analyte at graphene is the main contribution to the observed GEIRA effect.

In conclusion, this study presents a simple yet effective method based on graphene-enhanced infrared absorption (GEIRA) spectroscopy for analyzing a variety of molecules containing aromatic moieties based on attenuated total reflection. Pronounced IR signal enhancement effects were observed for several characteristic bands across the entire IR-ATR spectrum with excellent reproducibility utilizing aqueous graphene dispersions. Given that chemical enhancement is the dominating contribution to the observed signal increase, the dependence of the GEIRA effect on the pH value of the sample solution was investigated and confirmed. Furthermore, using ATR spectroscopy facilitated the direct analysis of aqueous sample solutions providing additional insight on the temporal evolution of the GEIRA signal. Last but not least, these studies enabled a fundamental understanding on the chemical enhancement mechanism in GEIRA spectroscopy.

**METHODS**

Reagents and Materials. All chemicals were at least of analytical grade and applied without further purification. Ultrapure water was obtained from a water purification system (conductivity 18.0 MΩ cm\(^{-1}\), Igla Labwater, VWS Deutschland GmbH, Celle, Germany) and employed throughout this work. The sample solutions were prepared directly by dissolving the specific chemical in ultrapure water. The commercially available graphene dispersion (0.05 mg/mL, with 2% w/v of sodium chloride as surfactant) was purchased from NanoIntegris Inc. (Sloke, U.S.A., product name: PureSheets MONO). The graphene solution contains 27% single layer graphene, 48% double layer graphene, and 25% triple layer graphene with ~10000 nm\(^2\) average flake area.

Procedure. Infrared spectroscopic studies were carried out using a Bruker Vertex 70 FT-IR spectrometer (Bruker Optik, Ettlingen, Germany), equipped with a liquid nitrogen cooled mercury–cadmium–telluride (MCT) detector (Bruker Optik, Ettlingen, Germany) and an attenuated total reflection configuration (ConcentraIR2; Harrick Scientific Products, New York, U.S.A.) consisting of a diamond ATR (10 reflections) waveguide attached to a ZnSe optical coupling element. To investigate the GEIRA effect, the diamond crystal was decorated using drop-coating: 3 µl of graphene dispersion (0.05 mg/mL) was slowly dropped and distributed evenly across the surface of the diamond waveguide mounted in the ATR assembly and completely dried on site in open air under ambient conditions. Then, 20 µl of sample solution was added on top of the graphene-coated diamond ATR, spectra were acquired by averaging 300 sample scans within the spectral range of 4000–600 cm\(^{-1}\) at a spectral resolution of 2 cm\(^{-1}\), and repeated at least three times. Prior to sample measurements, background spectra of water (Blank) were recorded and subsequently subtracted from the sample spectra. Between each measurement, the ATR cell was thoroughly cleaned using cotton swabs along with ethanol and water. For comparison, samples were also measured at the same conditions at the identical diamond crystal, however, without graphene decoration. Data was collected and processed using the OPUS 6.5 software package (Bruker Optik, Ettlingen, Germany).

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsphotonics.8b00028.

Figure S1, effect of graphene dispersion volume on the IR absorption of R6G. Detailed descriptions of transfer of CVD graphene on diamond ATR. Figures S2 and S3, normalized spectra of different analytes measured on bare and dried-graphene-decorated diamond waveguide (at different pH values). Figure S4, IR peak intensity dependence on the concentration of R6G solution (PDF).

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The authors declare no competing financial interest.

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**REFERENCES**

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86
Supporting Information

A Versatile Analytical Platform Based on Graphene-Enhanced Infrared Attenuated Total Reflection Spectroscopy

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<table>
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<th>Yuan Hu, Ángela I. López-Lorente, Boris Mizukoff</th>
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<td>A Versatile Analytical Platform Based on Graphene-Enhanced Infrared Attenuated Total Reflection Spectroscopy</td>
</tr>
<tr>
<td>Number of pages</td>
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1. Effect of graphene dispersion volume on the IR absorption of R6G

![Graph showing IR absorbance vs. wavenumber for different volumes of graphene dispersion.](image)

Figure S1. Effect of graphene dispersion (0.05 mg/mL) volume on the IR absorption of 2.5 mg/mL R6G solution (graphene was first dropped onto the diamond ATR surface and dried on site at ambient conditions).

2. Transfer of CVD-graphene onto diamond ATR

Commercially available graphene (80% monolayer with flake size around 20 μm) grown on Cu foil by chemical vapor deposition (CVD-graphene, purchased from Graphenea S.A.) was transferred onto diamond ATR waveguide with a modified transfer method adapted from Gerardo Algara-Siller, *et al.* Specifically, CVD-graphene on the copper foil was dimensionally tailored to fit the dimensions of the diamond ATR surface, and then contacted with the diamond waveguide surface using isopropanol alcohol. Afterwards, the copper foil was etched away using ammonium persulfate (10%). The diamond was gently rinsed with water prior to analytical measurements.


3. Normalized spectra of different analytes measured on

Page S2
dried-graphene-decorated and bare diamond waveguide.

Figure S2. Comparison of normalized IR spectra of (A) auramine, (B) crystal violet, (C) fuchsine, (D) methylene blue, (E) neutral red, and (F) rhodamine B collected on dried-graphene-decorated and bare diamond waveguide.
4. Normalized spectra of analytes measured on bare diamond waveguide and dried-graphene-decorated at different solution pH values.

Figure S3. Comparison of normalized IR fingerprint patterns of (A) 0.5 mg/mL 4-HBA, and (B) 2.5 mg/mL R6G solution analyzed on bare diamond ATR and the dried-graphene-coated diamond ATR at different pH values.

5. Dependence of IR peak intensity on the concentration of R6G

Figure S4. Non-enhanced peak intensities of the characteristic bands at 1608 cm\(^{-1}\), 1531 cm\(^{-1}\), 1503 cm\(^{-1}\), and 1307 cm\(^{-1}\) vs. the concentration of R6G in solution
obtained at the pristine diamond ATR waveguide. The corresponding linear regression equations are listed below:

<table>
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<th>Wavenumber (cm(^{-1}))</th>
<th>Equation</th>
<th>(R^2)</th>
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<td>1608</td>
<td>(I = 0.0066C_{[\text{RGO}]} + 0.0029)</td>
<td>0.9865</td>
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Paper III. Surface-Enhanced Infrared Attenuated Total Reflection Spectroscopy via Carbon Nanodots for Small Molecules in Aqueous Solution

Y. Hu independently designed and performed all the experiments, conducted the data evaluation, and wrote the manuscript. Q. Chen and L. Ci contributed the carbon nanodots used in this work. K. Cao performed the high resolution transmission electron microscopy (HRTEM) test of the applied materials. B. Mizaikoff contributed with revisions to the final manuscript.

Trends and Critical Reviews
Nanoparticles for Bioanalysis
Guest Editors  Maria Carmen Blanco-López  Montserrat Rivas
Surface-enhanced infrared attenuated total reflection spectroscopy via carbon nanodots for small molecules in aqueous solution

Yuan Hu1 · Qiong Chen2 · Lijie Ci2 · Kecheng Cao3 · Boris Mizailoff1

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Abstract
In this study, carbon nanodots (CNDs) with excellent aqueous dispersibility, narrow size distribution, and oxygen-rich functional groups have been prepared via a green electrochemical method. Graphite electrodes were directly electrolyzed at ambient temperatures to form uniform CNDs in deionized water, which is free from additional oxidant/reductant. As-synthesized CNDs have been applied to coat an attenuated total reflection (ATR) waveguide enabling surface-enhanced infrared absorption (SEIRA) spectroscopic studies for detecting a variety of analytes in aqueous phase with remarkably enhanced IR band intensities. Finally, the proposed ATR-SEIRA strategy enabled quantitatively analyzing adenosine in aqueous solution after optimizing the amount of CNDs, the solution pH, and potential CND aggregation.

Keywords Carbon nanodots · Surface-enhanced infrared absorption spectroscopy · SEIRA · Attenuated total reflection · ATR · Adenosine

Introduction
Infrared (IR) absorption spectroscopy is a powerful analytical tool for substance identification via directly probing vibrational signatures of a wide range of dipole-active molecular species and compounds, thereby enabling non-invasive and label-free optical sensing [1]. Nevertheless, due to occasionally small IR absorption cross-sections, conventional IR measurements frequently require substantial amounts/concentrations of analytes. Additionally, weak signals for some peaks of interest (e.g., amide vibrations) may be obscured by rather pronounced IR absorptions of liquid water in the 3600–3000 and 1700–1550 cm⁻¹ spectral range [2, 3], which restricts conventional IR absorption techniques in aqueous environments [4]. To overcome these issues, surface-enhanced infrared absorption (SEIRA) strategies combined with attenuated total reflection (ATR) techniques have emerged as a viable concept [5, 6]. ATR-SEIRA promises an enhanced surface sensitivity, while interferences from bulk solution are reduced given the exponentially decaying strength of the evanescent field [7]. This approach has been successfully applied to in situ qualitative and quantitative analysis of various chemical species, and time-resolved monitoring of chemical reaction processes, as well as molecular interactions at the interfaces [8, 9]. Numerous SEIRA substrates with nanoscale morphologies have been designed and experimentally demonstrated to efficiently enhance IR signals with the vast majority relying on noble metal structures that usually require sophisticated fabrication schemes [2, 3]. In order to further expand the utility of SEIRA, more sustainable, cost-effective, and readily accessible materials providing the required signal enhancements are of interest.

The recent emergence of carbon nanomaterials, especially graphene, carbon nanotubes (CNTs), and carbon...
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nanodots (CNDs), has opened new avenues for surface-enhanced spectroscopies offering advantages vs. metallic nanostructures including low cost, low toxicity, high synthetic and functional flexibility, biocompatibility, and chemical stability [10–16]. Compared to rather hydrophobic graphene sheets and CNTs, CNDs are characterized by superior aqueous dispersibility, smaller dimensions, and more accessible binding sites [17]. Thus, more effective adsorption of target molecules—an essential prerequisite for taking advantage of their signal-enhancing properties—was beneficial [18]. Typical CNDs comprise discrete, quasi-spherical nanoparticles with diameters < 10 nm, have apparent p6 character, and provide abundant hydrophilic oxygen-containing functional groups at the surface [17, 19]. In addition, CNDs are readily obtained via a variety of straightforward, economically viable, and scalable synthesis methods [20, 21]. Among these strategies, electrochemical synthesis using universal bulk carbon sources such as graphite are most common given the fact that it is convenient, eco-friendly, and avoids hazardous chemicals as well as harsh experimental conditions. Generally, CNDs synthesized via an electrochemical routine may be used directly without any further modification [16]. Despite these advantages, studies on CNDs as signal-enhancing materials in SEIRA applications are still scarce, and predominantly focus on their utility in surface-enhanced Raman scattering (SERS) [22–24].

Adenine is a purine nucleobase found in both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecular structure, which plays a crucial role in a wide variety of biological processes. Abnormal changes of adenine levels could reflect deficiencies or mutations in the immune system or may be linked to metabolic disorders and diseases [25, 26]. A variety of methods for the detection and quantification of adenine have been developed, including chromatography, capillary electrophoresis, electrochemistry, mass spectrometry, fluorescence spectroscopy/sensing, and SERS [27–31]. In turn, only few reports discuss SEIRA-based analysis of adenine [32–35].

In the present study, highly water-dispersible CNDs were synthesized via one-step electrolysis utilizing two graphite electrodes in water. As-prepared CNDs were then deposited at the surface of an ATR waveguide, which exhibited a significant SEIRA effect during studies of several exemplary dye molecules. More importantly, the infrared absorption of adenine in aqueous solution appeared remarkably enhanced by the presence of CNDs, which is essential for investigating the potential of ATR techniques in assaying adenine in a label-free manner. After optimizing the experimental conditions, possible mechanisms of the observed SEIRA enhancement via CNDs were studied.

Experimental

Reagents and materials

All reagents were of analytical grade and used as-received without further purification. Deionized water was employed for all experiments. The sample solutions were prepared directly by dissolving the specific chemical in water. Diluted HCl and NaOH solutions were used to adjust pH values of certain sample solutions.

Instrumentation

High resolution transmission electron microscopy (HRTEM) imaging was performed using an image side Cs-corrected Thermo Fisher Titan 80–300 TEM (USA) operated at 80 kV. The TEM samples were prepared by drop-casting aqueous CND dispersions onto lacey carbon TEM grids. Infrared measurements were carried out on a Bruker Vertex 70 FT-IR spectrometer equipped with a liquid nitrogen-cooled mercury-cadmium-telluride (MCT) detector (Bruker Optics, Ettlingen, Germany). An attenuated total reflection unit (ConcentrIRM2; Harrick Scientific Products, New York, USA) comprising a diamond ATR waveguide (10 internal reflections) was utilized. IR spectra were collected at a spectral resolution of 2 cm⁻¹ averaging 100 scans within the spectral window of 4000–600 cm⁻¹. Data acquisition and processing was executed using the OPUS 6.5 software package (Bruker Optics, Ettlingen, Germany).

Preparation of CNDs

CNDs were synthesized in an electrolysis cell comprising two graphite plates serving as both the working and the counter electrode immersed initially in deionized water. The electrolysis process was initiated by applying a constant current of 0.42 A. During the electrolysis, the ion conductivity of the electrolyte solution continuously increased with reaction time, while the solution color gradually changed from colorless to brown, and eventually became black, thereby indicating the formation and accumulation of CNDs within the solution. The resultant homogeneously black solution was centrifuged at 8000 rpm for 10 min to remove large or agglomerated particles. Solid CND powders were then collected by freeze-drying the supernatant. The obtained CNDs were re-dispersed in distilled water at a concentration of 1.0 mg/mL with ultrasonic assistance for further characterization and usage.

SEIRA studies

For SEIRA studies, 4 μL of CND dispersion were drop-cast and distributed evenly across the diamond waveguide surface, and left to dry at ambient conditions. Subsequently, 20 μL of
sample solution were added on top of the CND-decorated ATR waveguide, and IR measurements were immediately executed. Prior to sample measurements, a background spectrum of 20 μL of water at the identical CND-decorated waveguide was recorded, and ratioed against each aqueous sample spectrum. Between each measurement, the sample cell was thoroughly washed with ethanol and water. For comparison, pure analyte samples of the same volume were analyzed at the identical diamond ATR waveguide in absence of CNDs.

Results and discussion

Morphological studies of the synthesized CNDs were carried out with HRTEM. Representative images of finely dispersed CNDs are shown in Fig. 1a, in which dots with obviously higher contrast are CND particles (example CNDs are indicated by red arrows). The size distribution of CNDs in the HRTEM image was calculated and plotted in the bottom right inset of Fig. 1a. It is evident that the CNDs are well-dispersed and quite uniform in size, with diameters mainly in the narrow range of 2–6 nm and only infrequent (<2%) agglomerates of relatively larger size (11–12 nm). To characterize the functional groups at the CND surface, the CND dispersion was dried at the surface of the ATR waveguide, and the IR spectrum was acquired, as shown in Fig. 1b. The broad infrared absorption band in the spectral region 3200 and 3053 cm⁻¹ can be attributed to the O-H stretching vibration, which may originate from surface hydroxyl groups and carboxylic acid moieties, as well as adsorbed water. The bands around 1693 cm⁻¹ and 1253 cm⁻¹ are assigned to the stretching vibrations of C=O and C-O, respectively, which are associated with ester and carboxylic acid groups conjugated to the aromatic carbon substrate. The peaks at 1595, 870, and 740 cm⁻¹ correspond to the stretching modes of aromatic benzene rings, and to the deformation of aromatic C-H. The deformation vibration of the O-H is observed at 1423 cm⁻¹. The IR absorption arising from C–O–C of esters and ethers appears at 1147 cm⁻¹. These characteristics confirm that graphite has been successfully electrochemically oxidized, broken up, and exfoliated into nanoscale CNDs. O and H are introduced into the graphitic network forming hydroxyl, ether, ester, carboxylic, and carboxylic acid groups decorating the CND surface, which contribute to the excellent water-dispersibility of thus obtained CNDs.

To investigate the SEIRA effect of CNDs, first several exemplary dye molecules were studied in aqueous solution at a concentration of 0.5 mg/mL (i.e., except 0.2 mg/mL for auramine due to its low aqueous solubility) at the same experimental conditions as later adenine; the results are shown in Fig. 2. The signals of dye solutions analyzed directly at the unmodified ATR waveguide surface, the IR signatures of auramine, fluorescein, methyl violet 2B, neutral red, rhodamine B, and rhodamine 6G were significantly amplified, if 4 μL of CND dispersion was dried at ATR waveguide surface beforehand. It was found that the SEIRA spectra and absorbance of all tested analytes remained almost unchanged already a minute after the sample solution was added onto the CND-decorated ATR prism, thus confirming rapid interaction of CNDs with analyte molecules. Electronic Supplementary Material (ESM) Fig. S1 illustrates the time evolution of SEIRA spectra for auramine, fluorescein, and rhodamine B. For ensuring a fair comparison of different analytes, the SEIRA spectra presented in Fig. 2 and the following figures were all collected after signal equilibrium has been reached (i.e., generally within 5 min). To evaluate the SEIRA effect, the experimental enhancement factor (eEF) was calculated, i.e., the ratio of the peak height of characteristic SEIRA bands and the corresponding band intensity recorded in absence of CNDs [11]. The calculated eEFs of the considered absorption bands along with the corresponding
assignments are listed in ESM Table S1. The eEs of the analyzed dyes were in the range of 5–20, thus demonstrating the effectiveness of using as-synthesized CNDs in ATR-SEIRA.

In addition to dye molecules, adenine was investigated as a biologically relevant analyte. The IR absorption of adenine in aqueous solution recorded at the bare ATR surface is so weak that the characteristic IR bands are barely discernible. In contrast, the signals appear significantly enhanced, if the same aqueous adenine solution is analyzed at a CND-decorated ATR diamond waveguide surface (Fig. 3). The normalized IR signatures of adenine in ESM Fig. S2 demonstrates that all characteristic bands in the CND-based SEIRA spectrum of adenine could also be found in the IR spectra of pure adenine solution analyzed in absence of CNDs, thus indicating the validity of the adenine SEIRA patterns. The SEIRA bands are readily identified despite some overlaps in the range of 1400 to 1200 cm⁻¹ that are predominantly related to skeletal ring stretching modes and some contributions of the C–H bending modes [15]. The band at 1698 cm⁻¹ and 1418 cm⁻¹ can be assigned to the stretching vibrations of C=O in the nitrogen heteroaromatic ring, and the band at 1581 cm⁻¹ is assigned to the aromatic pyrimidine ring stretching mode.

Further experiments were executed for manifesting the effect of the amount of CNDs on the SEIRA performance. Figure 3a depicts the IR signatures of 0.2 mg/mL adenine
solution collected after different volumes of CND dispersion were deposited at the waveguide surface, and the corresponding SEIRA spectra plotted against time are given in ESM Fig. S3. It was shown that the spectra of adenine remained nearly constant with increasing time after adding the sample solution. Moreover, increasing the volume of deposited CND dispersion beyond 6 μL seemed unfavorable to further improve the SEIRA effect, which may be attributed to the fact that the highest intensity of the evanescent field—the majority of the IR signal—was generated in the immediate vicinity of the waveguide surface [36]. Thus, an excess of CNDs may in fact adversely affect the interaction of the evanescent field with the analyte molecules. Hence, 4 μL of CND dispersion was used throughout all further studies.

The effect of the solution pH on CND-SEIRA was carefully examined by comparing the SEIRA responses of adenine solutions at different pH values. It is worth noting that adjusting the pH of the pure adenine solution (0.2 mg/mL) did not induce any notable IR signal change when analyzed at the pristine ATR waveguide, i.e., in absence of CND (see ESM Fig. S4). As shown in Fig. 3b—though at varying degrees—CNDs evidently enhance the IR absorption of adenine at all investigated pH values, i.e., in a range of 2.0–12.0. In turn, this indicates that CNDs have significant potential as infrared-enhancing nanostructures throughout a wide range of aqueous sensing scenarios.

More specifically, the SEIRA spectra at pH 2.0 appear slightly different with the maximum absorption band occurring at around 1256 cm⁻¹, which is attributed to the N-H stretching mode, while the most intense bands at all other pH values are located at approx. 1418 cm⁻¹. This may be explained as a consequence of protonation of adenine (pKₐ = 4.15, pKₐ = 9.8) at pH 2.0 [37]. The overall absorbance of adenine is most pronounced in the pH range of 4.1–8.5 where adenine exists in molecular form. The maximum enhancement of the adenine IR signature is observed at pH 4.1 with a calculated eEF of 50.0, 36.4, and 38.9 corresponding to the bands at 1698, 1581, and 1418 cm⁻¹, respectively. A sharp decline of the SEIRA signal intensity appears at pH 10.1 and higher, i.e., where deprotonated adenine is the dominating species. These observations indicate that the interaction between CNDs and adenine is not simply via electrostatic attraction alone. Considering practical application scenarios and the observed SEIRA performance, the solution pH of adenine was set at 6.6 for all subsequent experiments.

The CNDs prepared herein are nanosized and well-dispersed in water without assistance of surfactant or stabilizer, thus avoiding possible spectral interferences from additional reagents. In addition, they offer a large accessible surface area for interaction with analytes. Even after 2 months of storage, the CND suspension still appears homogenous without any evident precipitate. However, partial aggregation has likely occurred after storage for 1 month at room temperature, which was evident via HRTEM studies (Fig. 4a). After 2 months, the particles appear to form even larger agglomerates (Fig. 4b). Compared to freshly dispersed CNDs (Fig. 4a), the aggregation of CNDs apparently reduces the SEIRA efficiency, as shown for adenine (Fig. 4c), potentially owing to the reduction of available surface area. Consequently, to ensure maximum SEIRA effectiveness, freshly prepared CND dispersions are recommended.

To address the potential of CND-based ATR-SEIRA in quantitative analysis, the evolution of SEIRA spectra at varying concentrations of adenine at pH 6.6 was studied (Fig. 5a). The corresponding SEIRA peak intensities at 1418, 1581, and 1698 cm⁻¹ (absorbance, I₁₄₁₈, I₁₅₈₁, I₁₆₉₈) were plotted as a function of adenine concentration (C), as illustrated in Fig. 5b. The intensities of the three peaks all increase linearly within the investigated concentration range of 0.01–0.3 mg/mL. The corresponding linear regression equations are I₁₄₁₈ = 0.146C + 3 × 10⁻⁴ (R² = 0.9922), I₁₅₈₁ = 0.153C − 8.4 × 10⁻⁵ (R² = 0.9947), and I₁₆₉₈ = 0.0807C + 7.6 × 10⁻⁴ (R² = 0.9936). Furthermore, the limit of detection (LOD), which is calculated as three times the standard deviation of the blank.
Fig. 5  a Concentration-dependent SEIRA spectra of adenine in aqueous solution recorded at CND-modified ATR diamond waveguides. b Calibration functions plotting the intensity of the SEIRA bands at 1698, 1581, and 1418 cm⁻¹ vs. the adenine concentration. The error bars indicate the standard deviation in SEIRA intensity for the average of three individual measurements.

signal (n = 11) divided by the slope of the calibration curve was estimated at 0.003 mg/mL, i.e., average of the data obtained at the three bands evaluated above. These results corroborate the utility of CND-based ATR-SEIRA for quantifying adenine in aqueous solution.

Apart from adenine, purine and three purine derivatives (i.e., caffeine, guanine, and uric acid) were finally investigated at a concentration of 0.2 mg/mL. The purine and caffeine solutions were set at neutral pH, while the pH values of the uric acid and guanine solutions were forced to pH 12.5 and pH 13.3 by adding diluted NaOH due to their low solubility in water at neutral pH [38]. As evident in Fig. 6, regarding caffeine and uric acid there is no prominent change of signal intensity in the presence of CNDs. In contrast, SEIRA enhancement is evident in the case of purine and guanine with calculated eEFs of 4.2 and 9.2 for purine at 1414 cm⁻¹ and guanine at 1428 cm⁻¹, respectively. At similar conditions, adenine (pH 6.6, Fig. 3b) has an eEF of up to 34.5 evaluating the corresponding analyte.

Fig. 6  Comparison of the IR spectra of a uric acid, b caffeine, c guanine, and d uric acid recorded at CND-decorated vs. pristine ATR diamond waveguide surfaces. The insets show the chemical structure of the corresponding analyte.
the same peak (1418 cm\(^{-1}\)). It should be noted that the considered bands all arise from the C-N stretching mode in the nitrogen heterocyclic ring. The difference in CND-based SEIRA enhancement for different purines is most probably resulting from their structural differences. While there is no extended conjugated system in the molecular structures of caffeine and uric acid, purine, adenine, and guanine allow for substantially more pronounced delocalization of π-electrons, which is favorable for the affinity with the CND surface via π-π interaction. Besides, the NH\(_2\) groups in adenine and guanine may further strengthen the interaction affinity via the oxygen-containing moieties at the CNDs such as the carbonylic function via electrostatic attraction and hydrogen bonding [39]. Such interactions are limited in the guanine structure, which therefore explains the lower eE% vs. adenine. Based on the experimental findings herein, it is hypothesized that the observed CND-induced SEIRA effect may derive from the—more or less pronounced—intimate interaction of analytes with the CND surface, therefore suggesting a predominantly chemical enhancement mechanism (CM) [11], as CNDs may adsorb molecules via π-π stacking, electrostatic attraction, hydrogen bonding, etc.

Conclusions

A green synthesis route of water-dispersible CNDs with uniform nanoscale dimensions was accomplished via direct electrolysis of graphite in deionized water, as-prepared CNDs have shown pronounced IR signal enhancement effects for various aromatic dyes, and for purines and purine derivatives in aqueous solution. It has been demonstrated that such SEIRA effects may indeed be utilized for the label-free quantification determination of adenine in water at low concentrations (0.003 mg/mL). Confounding factors such as the amount and aggregate state of CNDs, as well as the solution pH were investigated vs. the obtained SEIRA response of adenine. After investigating various purine derivatives, the observed CND-based SEIRA enhancement was predominantly attributed to affinity of the investigated molecules to the CND surface via π-π interactions, electrostatic attraction, and hydrogen bonding, i.e., chemical enhancement effects. In summary, this study confirms that carbon nanodots are promising candidates serving as efficient SEIRA nanostructures, which may be of particular relevance for the development of label-free IR-based biosensors.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no competing interests.

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References


JOURNAL ARTICLES


Ke Cheng Cao is a PhD candidate supervised by Prof. U. Kaiser at Ulm University, Germany. He focuses on atomic-scale chemical and physical processes investigated by simultaneously applying an electron beam as stimulus and probe in aberration-corrected high-resolution transmission electron microscopy.

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Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Surface-enhanced infrared attenuated total reflection spectroscopy via carbon nanodots for small molecules in aqueous solution

Yuan Hu, Qiong Chen, Lijie Ci, Kecheng Cao, Boris Mizaikoff
**Fig. S1** Evolution of the SEIRA signals from (a) 0.2 mg/mL auramine, (b) 0.5 mg/mL fuchsin, and (c) 0.5 mg/mL rhodamine B with increasing time after adding the sample solution onto the CND-deposited ATR prism.
Table S1 Assignment and experimental enhancement factor (eEF) of characteristic peaks of analyte molecules investigated via the developed CND-based IR-ATR-SEIRA strategy

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<tr>
<th>Molecule</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Assignment</th>
<th>eEF</th>
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<td>Auramine</td>
<td>1595</td>
<td>(\nu) (ring), p-disubstituted aromatic ring</td>
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<td>1381</td>
<td>(\nu) (C–N), (C)(\equiv)N</td>
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<td>Fuchsine</td>
<td>1589</td>
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<td></td>
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<td>1182</td>
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\(^a\) Auramine: 0.2 mg/mL; Others: 0.5 mg/mL.

\(^b\) \(\nu\) = stretching, \(\delta\) = bending / deformation.
**Fig. S2** Normalized IR spectra of adenine solution (0.2 mg/mL) collected on dried-CND-deposited (red) and bare (green) diamond waveguide.

**Fig. S3** Time evolution of SEIRA spectra from 0.2 mg/mL adenine solution measured after the sample solution was added onto dried-CND-decorated ATR waveguide. The initial applied volume of CND dispersion is: (a) 2 μL, (b) 4 μL and (c) 6 μL.
**Fig. S4** IR signatures of pure adenine solutions at different pH values analyzed at pristine ATR diamond waveguide surfaces
Paper IV. Selective Chemical Enhancement via Graphene Oxide in Infrared Attenuated Total Reflection Spectroscopy

Y. Hu independently designed and performed all the experiments, analyzed the data, and wrote the manuscript. K. Cao conducted the high resolution transmission electron microscopy (HRTEM) and X-ray photoelectron spectroscopy (XPS) characterization of the applied material. L. Ci contributed the graphene oxide material used in this work. B. Mizaikoff contributed with revisions to the final manuscript.

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Selective chemical enhancement via graphene oxide in infrared attenuated total reflection spectroscopy

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Abstract: Graphene oxide (GO) as a readily prepared graphene derivative comprises negatively charged oxygenated functional groups, and a mixture of sp² and sp³ carbon domains. In comparison with hydrophobic graphene, GO is characterized by superior water dispersibility, and may non-covalently interact with a significantly wider range of molecules. Taking advantage of GO and infrared attenuated total reflection spectroscopy (IR-ATR), a universal and efficient strategy enabling unprecedented absorption signal amplification via chemical enhancement of various molecular species in aqueous environment was demonstrated in this study. The evolution of selectively enhanced molecular fingerprints across the entire mid-infrared spectral range was in situ monitored in real time, while retaining the characteristic molecular IR absorptions, and confirms the utility of GO as a suitable and versatile material in surface enhanced infrared absorption spectroscopy (SEIRA) scenarios.

Keywords: graphene oxide, surface enhanced infrared absorption spectroscopy, SEIRA, graphene enhanced infrared absorption spectroscopy, GEIRA, graphene oxide enhanced infrared absorption spectroscopy, GOEIRA attenuated total reflection, ATR, chemical enhancement
Introduction

Surface-enhanced infrared absorption (SEIRA) spectroscopy yields vibrational fingerprints of molecular species in the mid-infrared spectral region (2.5 - 20 μm; MIR), while significantly improving the intrinsically modest detection sensitivity of conventional infrared (IR) techniques.\(^1\) While the fundamental underlying mechanisms giving rise to SEIRA effects are still under debate, a combination of electromagnetic mechanisms (EM) and the chemical mechanisms (CM) is the widely accepted hypothesis.\(^3\) EM implies an enhanced electromagnetic near-field resulting from plasmonic excitations that is restricted to the immediate vicinity of the substrate surface.\(^4\) Complementarily, CM enhances IR signatures by chemical interactions between the analyte molecules and the substrate surface.\(^5\) To date, numerous papers have been published taking advantage of SEIRA effects via the excitation of MIR surface plasmons, i.e., the collective oscillations of conduction electrons in a variety of conductive nanomaterials.\(^6\) In general, plasmonic SEIRA enhancement increases with decreasing detuning between the plasmon resonance and the vibrational frequency.\(^7\) The plasmon frequencies are determined by the composition, surface morphology, and immediate environment of the applied plasmonic materials.\(^8\) Therefore, simultaneously enhancing multiple vibrational frequencies across a broad spectral range with a single plasmonic device still remains challenging.

Graphene comprised of sp²-bonded carbon atoms arranged in a 2D hexagonal lattice, has emerged as a promising material in plasmonic-based SEIRA owing to its unique structure and semi-metallic nature. Distinctive advantages vs. commonly used metal substrates include strong field confinement, and dynamic spectral tunability via electrostatic gating/chemical doping.\(^9\)\(^-\)\(^12\) However, to achieve appreciable SEIRA effects via graphene plasmons, sophisticated external voltage loading and/or rigorous nanopatterning techniques are usually involved.\(^13\)\(^-\)\(^15\) Moreover, the inherent IR plasmonic response of monolayer graphene is relatively weak, which may be ascribed to the sizable momentum mismatch between external IR radiation and graphene plasmons along with a diminished carrier mobility caused by the deteriorated quality (i.e., defects, impurities, etc.) of graphene nanostructures during the fabrication, patterning, and transfer process.\(^16\)\(^-\)\(^17\) Consequently, utilizing graphene plasmonics for SEIRA is certainly useful, yet limited in practical applicability. Conversely, the extraordinary chemical/physical properties of graphene and graphene derivatives may advantageously be exploited in surface enhanced vibrational spectroscopies capitalizing on chemical enhancement mechanisms, which has been confirmed during several seminal studies based on graphene and its derivatives.\(^18\)\(^-\)\(^19\)

Current implementations of graphene-based SEIRA - a.k.a., GEIRA - predominantly use conventional IR transmission spectroscopy modalities analyzing thin solid films or gaseous samples.\(^5\)\(^\text{a}\)\(^-\)\(^5\)\(^\text{b}\) This is mainly attributed to potential pronounced background absorptions of the matrix in liquid samples (e.g., when using water as solvent), which may obscure absorption features of the target species.\(^20\) In contrast, surface sensitive attenuated total reflection (ATR) configurations facilitate liquid phase IR spectroscopy.
especially in aqueous environments, as the rather shallow penetration depth of the exponentially decaying evanescent (i.e., few micrometers) constitutes a quasi-thin-film-cell.\textsuperscript{22-23} Previous work by our research team has demonstrated that graphene-decorated multi-reflection ATR waveguides may indeed serve as a versatile platform for the instantaneuous analysis of aromatic molecules in aqueous solution providing remarkably enhanced IR fingerprints across the entire MIR wavelength regime.\textsuperscript{24} The enhancement could be predominantly attributed to the enrichment of analytes at the ATR waveguide surface via \( \pi-\pi \) interactions occurring between graphene, and aromatic moieties of the analyte molecules, i.e., chemical enhancement has been the major contribution to the observed signal amplification. Similarly, ATR-integrated graphene-SEIRA strategies have been proposed using boron-doped graphene nanodisk arrays,\textsuperscript{25} and reduced graphene oxide.\textsuperscript{26}

Graphene oxide (GO) is an important graphene derivative that is readily prepared in bulk quantities via exfoliation of graphite oxide, which retains the graphene basal plane while containing abundant active oxygen functional groups (i.e., epoxide, hydroxyl, carbonyl, and carboxyl groups).\textsuperscript{27} The oxygenated lattice composed of both \( \text{sp}^2 \) and \( \text{sp}^3 \) hybridized domains favors GO vs. graphene by its excellent water dispersibility, and capability to interact non-covalently with a wide range of molecules, i.e., \( \text{via} \) electrostatic interactions, hydrophobic interactions, \( \pi-\pi \) stacking, hydrogen bonding, etc.\textsuperscript{28-29} Hence, it may be hypothesized that GO provides a viable – if not superior - alternative to graphene in SEIRA applications. In the present study, a well-dispersed GO suspension in water was directly used to decorate an ATR waveguide surface \( \text{via} \) a simple drop-casting method,\textsuperscript{30} thereby eliminating potential interferences from surfactants or processing residues common to hydrophobic graphene. The SEIRA effect of GO was verified \( \text{via} \) pronouncedly enhanced IR fingerprints of selected target molecules in aqueous solution. By comparison of the amplification performance toward for a variety of deliberately selected molecules, potential mechanisms of the newly proposed GO-based SEIRA (a.k.a., GOEIRA) enhancement were derived.

Results and discussion

The general morphology and atomic structure of GO were investigated \( \text{via} \) aberration-corrected HRTEM (AC-HRTEM). GO sheets have extremely thin edges and large lateral dimensions covering the entire TEM grid, as shown in Figure S1a. The atomic structure of the thin edge of GO sheets is evident in the AC-HRTEM image (Figure S1b), where pores with lateral dimension <4 nm can be observed. The corresponding fast-Fourier-transform (FFT) pattern in Figure S1b indicates that GO sheets are predominantly AB-stacked bilayer structures (Figure S1c).\textsuperscript{31-32} The FT-IR spectrum of GO dried at the ATR waveguide surface is shown in Figure S2a, whereby the bands at around 3386, 1412, and 1080 cm\(^{-1} \) were ascribed to the C=O groups. The peaks at 1723 and 1226 cm\(^{-1} \) reveal the presence of C=O and O=C–O. The bands at 1051 and 1271 cm\(^{-1} \) are attributed to the stretching vibration of epoxide groups. The strong band located at 1622 cm\(^{-1} \) corresponds to the conjugated C=C ring stretching mode.
The surface composition and chemical states of GO were further confirmed by XPS studies. Figure 2b shows the C 1s XPS spectra of GO, whereby the four deconvoluted peaks centered at 284.5, 286.2, 286.9, and 289.5 eV were assigned to C=C/C–C in aromatic rings, C–O from epoxide/alkoxy, C=O, and O=C–O, respectively.33

Given the unique structural properties of GO, it has been reported as an efficient adsorbent for removing organic dyes in aqueous media.34 Consequently, the SEIRA effect of GO in the present study was first tested using several exemplary dye molecules dissolved in water at uniform concentrations (0.5 mg/mL), and at the same experimental/spectroscopic conditions. As shown in Figure 1, the IR signals of crystal violet, fuchsine, rhodamine 6G, and rhodamine B are dramatically enhanced via the presence of GO, as compared to spectra obtained at the pristine ATR waveguide surface. These spectra were then normalized and are displayed in a comparison in Figure S3, which further confirms that the presence of GO does not change the IR characteristics (i.e., band position/shape, baseline, etc.) of the analyte, yet only proportionally magnifies the absorbance strength of characteristic bands. To illustrate the IR signal variation, the experimental enhancement factor (eEF), i.e., the ratio of the peak intensity of the characteristic bands collected at the GO-decorated vs. the bare ATR waveguide surface was calculated and listed in Table S1 along with the band assignment.34 It is evident that the eEF values of different peaks of a specific dye molecule remain rather similar. Taking crystal violet as an example, the eEFs of the peaks at 1587, 1367, and 1171 cm⁻¹ (i.e., associated with the stretching modes of the aromatic ring and the C–N moiety) are 8.5, 8.8, and 9.2, respectively. Moreover, the eEFs of the four tested dye molecules are all within the same range of 8.5–16.9, thereby indicating the generic effectiveness of GO as an IR signal-enhancing material.
Figure 1. Comparison of IR signatures of dye molecules collected at the bare and the GO-decorated ATR waveguide surface. (a) crystal violet, (b) fuchsine, (c) rhodamine 6G, (d) rhodamine B. The insets are the chemical structures of the investigated dye molecules.

To further exploit the potential of GOEIRA, purines – one of the most widely distributed and relevant N-containing heterocyclic compounds in nature \(^{35}\) – were analyzed at a concentration of 0.2 mg/mL using the developed strategy. It should be noted that all prepared solutions of purine and purine derivatives were at neutral pH except for guanine and uric acid. Since guanine is only soluble in dilute acids and alkaline solutions, \(^{36}\) the solution pH was adjusted to 1.3 and 13.2 via diluted HCl and NaOH. Similarly, uric acid solutions were forced to pH 12.8, as the solubility in water increases with increasing pH. \(^{37}\) Apart from the solution pH, all purines were tested and compared at identical spectroscopic conditions. Figure 2 depicts the IR signatures of purine and several purine derivatives obtained at the bare and the GO-decorated ATR waveguide surface; Figure S4 shows the corresponding normalized spectra. The detailed band assignments and calculated eEFs of the characteristic peaks associated with the purines are summarized in Table S1. Contrasting the results, it is evident that GO has indeed enhanced the IR absorption of most purines, while again retaining their primary IR characteristics. However, it is also evident that the signal amplification via GO is not uniform across the range of investigated molecules. Specifically, the GOEIRA signals (i.e., the overall IR absorbance) of purine, adenine, and guanine at pH
1.3 appear distinctly higher compared to those obtained for other purine derivatives. Hypoxanthine, guanine at pH 13.1, caffeine, and adenosine show moderate enhancement, whereas uric acid at pH 12.4 and adenosine 5′-monophosphate disodium salt (AMP) reveal the lowest signal benefit. For example, the characteristic band around 1600 cm⁻¹ originating from the stretching mode of the substituted ring and/or C=N can be observed in all IR spectra shown in Figure 2. Nevertheless, the corresponding ΔEFs range from 1.1 (AMP) to 40.2 (purine). This discrepancy in GOEIRA effect among the purines implies that there are apparently different modes of interaction between GO and the purine derivatives.

![Figure 2](image)

**Figure 2.** Comparison of IR fingerprints of purine and purine derivatives collected at the bare and the GO-decorated ATR waveguide surface. (a) purine, (b) adenine, (c) hypoxanthine, (d) guanine at pH 1.3, (e) guanine at pH 13.2, (f) caffeine, (g) uric acid at pH 12.4, (h) adenosine, (i) adenosine 5′-monophosphate disodium salt (AMP). The insets represent the chemical structures of the investigated analytes.

In addition to organic dyes and purine derivatives, an additional set of deliberately selected analytes (for comparability also at a concentration of 0.2 mg/mL) was analyzed at the same conditions for mapping out potential mechanisms of the GO-induced IR signal enhancement.

Similar to purine, the IR absorbance of two aromatic N-heterocycles – imidazole and 1,10-phenanthroline – were noticeably enhanced via GO (Figure 3a, b), whereas their
IR bands are hardly recognizable using the bare ATR waveguide. This results in overall eEFs of 44.7 and 48.2, respectively (i.e., by averaging the eEFs of their characteristic bands listed in Table S1). Likewise, significant enhancements have been observed in dopamine hydrochloride and aniline solutions providing averaged eEFs of 17.0 and 44.4, respectively (Table S1). It should be noted that analytes with significantly enhanced IR features have aromatic moieties. Conversely, this is apparently a beneficial yet not a necessary condition for GOEIRA. This may be derived from the nearly unenhanced IR signatures of AMP (Figure 2i), and phenol (Figure 3e), and the indeed pronounced IR enhancement of the hexylamine fingerprint (Figure 3f; eEFs average at 12.1), as determined in the presence of GO. The normalized IR spectra in Figure S5 manifest that despite some spectral overlap within the GOEIRA spectra, the peak positions remain in accordance with the normalized results obtained at the pristine ATR waveguide surface, which confirms the reliability of the developed GOEIRA platform for capitalizing on MIR signatures across the entire spectral window.

**Figure 3.** Comparison of conventional IR vs. GOEIRA spectra for (a) imidazole, (b) 1,10-phenanthroline, (c) dopamine hydrochloride, (d) aniline, (e) phenol, and (f) hexylamine collected at the bare and the GO-decorated ATR waveguide surface. The insets reveal the chemical structures of the investigated analytes.

Figure 4a-h illustrate the time evolution of the IR signal for selected exemplary analytes after adding the sample solution onto the GO-decorated ATR waveguide surface. Figure 4i exhibits the corresponding peak intensities of selected bands as a function of time. It is evident that the GOEIRA signals have reached an equilibrium approx. 2 min after sample addition suggesting rapid interactions between GO and the molecules. Moreover, the nearly unchanged GOEIRA signatures after obtaining equilibrium signals indicate the stability of GO at the ATR waveguide surface, even though GO was not covalently attached to the surface. It should be noted that the GOEIRA spectra presented in Figure 1–3 were all recorded after 5 min of sample
addition ensuring comparable experimental conditions and equilibrated signals.

Figure 4. Time evolution of GOEIRA signals of (a) rhodamine 6G, (b) purine, (c) adenine, (d) guanine at pH 1.3, (e) guanine at pH 13.2, (f) pheanaurolin, (g) dopamine, and (h) hexylamine after addition of the sample solution onto the GO-decorated ATR waveguide surface. (i) IR peak intensities of selected features of the corresponding analytes vs. time after sample addition.

The HRTEM characterization has revealed that the GO sheets used in the present study are indeed defective (i.e., with many pores) and are decorated with a variety of oxygen-containing moieties. Thus, the electrical conductivity of GO is inherently poor on account of the disrupted sp² bonding network. Consequently, surface plasmon excitation (i.e., electromagnetic enhancement) mechanisms appear unlikely. Furthermore, GO has evidently simultaneously enhanced the absorption of molecular vibrations across the entire MIR band without affecting other IR features. Therefore, the observed GOEIRA effect is most likely attributable to chemical enhancement mechanisms dominated by an enrichment process at the GO surface and the specific interactions with the target analytes.

As previously introduced, GO is capable of adsorbing molecules via a diversity of non-covalent interactions, thereby enriching analytes within the penetration depth of the evanescent field. If the nature of interaction between GO and different molecules varies, it is hypothesized that the resulting GOEIRA activity will be affected.
Specifically, the organic dyes investigated herein are of anionic nature in aqueous media, and guanine at pH 1.3 (pK_a = 3.3 and 9.2) as well as 1,10-phenanthroline (sample pH 3.7; pK_a = 4.8) are present in their monoprotonated form. Hence, they have a pronounced affinity for the negatively charged surface of GO sheets. In addition, their conjugated aromatic structure allows binding to sp^2 carbon domains within GO via π–π interactions (Figure 5). At the conditions of the experiments herein, purine (sample pH 7.2; pK_a = 2.5, 8.9), adenine (sample pH 6.5; pK_a = 4.2, 9.8), hypoxanthine (sample pH 6.2; pK_a = 2.0, 8.9), caffeine (sample pH 6.8; pK_a = 0.7, 10.4), adenosine (sample pH 6.4; pK_a = 3.9, 12.4), and imidazole (sample pH 8.2; pK_a = 6.9, 14.4) are present as electroneutral/non-ionized molecules. Hence, electrostatic interactions with GO may be readily ruled out, leaving π–π stacking between their aromatic moieties and the sp^2 networks of GO the most likely option. However, the strength of π–π interactions depends on the aromaticity (i.e., size/shape of the cyclic π-conjugated system), and the substituents of the molecules. The resulting differences in adsorption efficiency may give rise to differences in enhancement effects. Guanine at pH 13.2 is deprotonated, thereby prohibiting its accumulation at the negatively charged GO surface via π–π stacking; hence, decreased GOEIRA signals compared to protonated guanine are anticipated and experimentally evident. Uric acid (at pH 12.8; pK_a = 5.4, 9.8) and AMP are most likely present in their diatomic form, which largely shields GO interactions due to the dominant electrostatic repulsion between negative charges, as confirmed by almost absent IR signal enhancement. Dopamine hydrochloride, aminole, and hexylamine (sample pH 11.6; pK_a = 10.2) are positively charged at the measurement conditions, thus prompting electrostatic adsorption on GO. The fact that neutral phenol (sample pH 5.5; pK_a = 9.9) does not appear to show any enhancement may be attributed to weak π–π stacking at GO. Hence, it is hypothesized that electrostatic adsorption more efficiently supports GOEIRA effects vs. π–π stacking interactions. Accordingly, it is derived from the present study that GO most selectively and significantly enhances the IR absorption of molecules that carry positive charges and/or possess extended π-conjugated moieties.

Figure 5. Schematic of the potential interactions between GO and analyte molecules.
In conclusion, a universal strategy taking advantage of graphene oxide serving as IR signal-enhancing material in IR-ATR spectroscopy has been developed. GO is rich in negatively charged sites and sp² domains, which may selectively adsorb a wide variety of analytes, yet, based on different interaction mechanisms including predominantly electrostatic interactions and/or π-π stacking. Thereby, pronounced chemical enhancement of the associated molecular IR signatures across the entire mid-infrared spectral band have been observed with equilibrated absorption signals obtained almost in real time in aqueous solutions. In future it is anticipated that other target molecules may capitalize on alternative non-covalent interactions with GO, e.g., via hydrogen bonding, hydrophobic interaction, etc. such that its signal-enhancing properties may be further explored. Last but not least, this study has shown that deliberate modulation of the investigated molecules and functional moieties enables mapping out the underlying chemical enhancement mechanisms in SEIRA spectroscopies. In summary, it appears that graphene oxide provides a more efficient, chemically more versatile, and in aqueous solutions more suitable SEIRA substrate material vs. pure graphene, which may give rise to further utilizing a wide variety of chemical signal enhancement strategies in GOEIRA spectroscopy and sensing scenarios.

Methods

Reagents and materials. All reagents were at least of analytical grade and used as-received. Deionized water (DIW) was applied throughout the experiments. Unless otherwise stated, the tested sample solutions were prepared by directly dissolving the corresponding chemical in DIW without pH adjustment. Commercial graphene oxide (GO) solid powder was purchased from Shenzhen JW-Innova Graphene Technology Co., LTD (Shenzhen, China). GO powder was dispersed in DIW at a concentration of 1.0 mg/mL by ultrasonic vibration before characterization and usage in this work.

Instrumentation. High resolution transmission electron microscopy (HRTEM) images of GO were obtained from an image side Cs-corrected Thermo Fisher Titan 80–300 TEM (USA) operated at 80 kV. To prepare the TEM samples, the GO suspension was dropped and dried on lacey carbon TEM grids. Surface characterization of GO was carried out on an X-ray photoelectron spectroscopy (XPS, Kratos XSAM 800, UK) equipped with an Al Kα monochromatic radiation. IR measurements were performed on a Bruker Vertex 70 FT-IR spectrometer operated with a liquid nitrogen cooled mercury-cadmium-telluride (MCT) detector (Bruker Optics, Germany), and along with an ATR accessory (ConcentratIR2, Harrick Scientific, USA) comprising a diamond ATR waveguide (10 internal reflections). IR spectra (4000–600 cm⁻¹) were recorded at a resolution of 2 cm⁻¹ averaging of 100 spectral scans, and processed utilizing the OPUS 7.2 software package (Bruker Optics, Germany).
**Experimental procedures.** A 3 μL droplet of the GO suspension was deposited at the ATR waveguide surface, and then completely dried on site (i.e. drop-casted) followed by adding 20 μL of pure water and immediately recording a background spectrum that would be automatically ratioed against each sample spectrum. To investigate the IR signal enhancing effect and selectivity of GO towards chemically diverse molecules, 20 μL of sample solution were studied at identical GO-decorated ATR waveguides following the same procedure. For comparison, pure samples were analyzed at identical conditions, yet, in absence of GO. The fluidic cell was thoroughly cleaned using ethanol/water after each measurement.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website.

Figure S1, aberration-corrected high-resolution transmission electron microscopy (AC-HRTEM) images of GO. Figure S2, FT-IR spectrum of GO dried on the ATR prism, and C 1s XPS spectrum of GO. Figure S3–S5, comparison of normalized IR spectra of different analytes measured on bare and GO-deposited ATR waveguide. Table S1, assignments and the calculated experimental enhancement factors (eEF) of characteristic bands of analytes measured with the proposed GO-SEIRA strategy (PDF).

**Acknowledgments**

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**References**


5110–5145.


(20) Farmer, D. B.; Avouris, P.; Li, Y.; Heinz, T. F.; Han, S.-J., Ultrasensitive


of uric acid and monosodium urate. Medical & Biological Engineering 1972, 10, 522-
531.
(38) Xiao, J.; Lv, W.; Xie, Z.; Tan, Y.; Song, Y.; Zheng, Q., Environmentally friendly 
reduced graphene oxide as a broad-spectrum adsorbent for anionic and cationic dyes 
(39) Ren, H.; Kulkarni, D. D.; Kodiyath, R.; Xu, W.; Choi, I.; Tsukruk, V. V., 
Competitive adsorption of dopamine and rhodamine 6G on the surface of graphene 
(40) Yang, T.; Kong, Q.; Li, Q.; Wang, X.; Chen, L.; Jiao, K., Highly sensitive and 
synergistic detection of guanine and adenine based on poly(xanthurenic acid)-reduced 
(41) Lin, D.; Xing, B., Adsorption of Phenolic Compounds by Carbon Nanotubes: Role 
of Aromaticity and Substitution of Hydroxyl Groups. Environmental Science & 
S., pKa Values of Guanine in Water: Density Functional Theory Calculations 
Combined with Poisson–Boltzmann Continuum–Solvation Model. The Journal of 
Physical Chemistry B 2003, 107, 344-357.
(43) Chen, J. L.; Yan, X. P.; Meng, K.; Wang, S. F., Graphene oxide based 
photoinduced charge transfer label-free near-infrared fluorescent biosensor for 
Electronic Supporting information

Selective chemical enhancement via graphene oxide in infrared attenuated total reflection spectroscopy

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1. Characterization of GO used in this work

**Figure S1.** Aberration-corrected high-resolution transmission electron microscopy (AC-HRTEM) images of GO. (a) Low magnification TEM image of GO showing the general morphology. (b) High magnification AC-HRTEM image of the framed area in (a) showing the atomic structure of GO. Red arrows indicate the pores existed in the GO sheets. (c) The corresponding Fast Fourier transform (FFT) pattern of (b).

**Figure S2.** (a) FT-IR spectrum of GO dried on the ATR prism. (b) C 1s XPS spectrum of GO.
2. Normalized spectra of dye solutions measured on GO-decorated and bare ATR waveguide

**Figure S3.** Comparison of normalized IR spectra of (a) crystal violet, (b) fuchsin, (c) rhodamine 6G, and (d) rhodamine B measured on bare and GO-decorated ATR waveguide.
3. Assignments and the corresponding experimental enhancement factors (eEF) of the molecular characteristic bands

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Tabel S1. Assignment and eEF of characteristic bands of molecules measured with the proposed GO-SEIRA strategy.
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<th>v(ring), 1,2,3,4,5-substituted aromatic ring</th>
<th>v(C=N), R–CO–NH–C</th>
<th>v(C=O), ring ketone</th>
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129
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*\( \nu = \text{stretching}, \delta = \text{bending / deformation.} *
4. Normalized spectra of purine and purine derivatives measured on GO-deposited and bare ATR prism

**Figure S4.** Comparison of normalized IR spectra of purine and purine derivatives recorded at the bare and GO-decorated ATR prism. (a) purine, (b) adenine, (c) hypoxanthine, (d) guanine at pH 1.3, (e) guanine at pH 13.2, (f) caffeine, (g) uric acid at pH 12.8, (h) adenosine, (i) adenosine 5’-monophosphate disodium salt (AMP).
5. Normalized spectra of other molecules collected on GO-deposited and bare ATR prism

**Figure S5.** Comparison of normalized IR spectra of molecules collected at the bare and GO-decorated ATR prism. (a) imidazole, (b) 1,10-phenanthroline, (c) dopamine hydrochloride, (d) aniline, (e) phenol, (f) hexylamine.
Acknowledgments

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Curriculum Vitae

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The content has been removed for data privacy protection
Declaration of Authorship

I hereby declare that this thesis is my own unaided work. All direct or indirect sources are acknowledged as references. The used literature is specified in the list of references.

Eidesstattliche Erklärung


______________________________  ______________________________
Ort, Datum                                  Unterschrift