SERS- and Electrochemically Active 3D Plasmonic Liquid Marbles for Molecular-Level Spectroelectrochemical Investigation of Microliter Reactions

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Abstract: Liquid marbles are emergent microreactors owing to their isolated environment and the flexibility of materials used. Plasmonic liquid marbles (PLMs) are demonstrated as the smallest spectroelectrochemical microliter-scale reactor for concurrent spectro- and electrochemical analyses. The three-dimensional Ag shell of PLMs are exploited as a bifunctional surface-enhanced Raman scattering (SERS) platform and working electrode for redox process modulation. The combination of SERS and electrochemistry (EC) capabilities enables in situ molecular read-out of transient electrochemical species, and elucidate the potential-dependent and multi-step reaction dynamics. The 3D configuration of our PLM-based EC-SERS system exhibits 2-fold and 10-fold superior electrochemical and SERS performance than conventional 2D platforms. The rich molecular-level electrochemical insights and excellent EC-SERS capabilities offered by our 3D spectroelectrochemical system are pertinent in charge transfer processes.

Liquid marbles are emerging lab-on-a-droplet platforms that efficiently isolate a liquid reaction medium within a three-dimensional (3D) particle shell to impart robustness and overcome rapid evaporation typical in small-volume processes. These versatile microreactors can be further imbued with function-specific attributes, such as photothermal and catalytic properties, via the selection of active encapsulating solids, and have diverse (bio)chemical applications. For instance, the plasmonic liquid marble (PLM) constructed using metallic nanoparticles has enabled the elucidation of critical reaction mechanism and dynamics using surface-enhanced Raman scattering (SERS) spectroscopy.

Despite the vast potential offered by liquid marbles, these reactors are limited to spontaneous chemical reactions driven intrinsically by the presence of multiple reactive reagents. Electrochemical (EC) reactions form the basis of important energy- and environment-related applications, such as energy storage mechanisms in chemical batteries and charge transfers in biochemical processes. The fusion of liquid marbles with electrochemistry is hence tantalizing to expand its application for electrochemical activation of small-volume inert reactions. The spherical shell of the liquid marble also provides a unique geometry as a working electrode. However, it remains a challenge to perform EC in liquid marbles owing to the dearth of in-depth understanding of charge-transfer properties across liquid marbles and the technical difficulties in incorporating commercial three-electrode system in a microliter droplet. Notably, owing to its inability in giving structural and adsorption data, EC is often coupled with molecular-specific spectroscopy, such as SERS, to provide unequivocal identification of species for a comprehensive understanding of complicated redox systems. A typical method to fabricate SERS-active electrodes is by metal oxidation–reduction cycles, which often leads to inconsistent surface roughness and suffers from reproducibility issues. While methods are developed to prepare electrodes with tunable metallic nanostructured surfaces, for example, metal film over nanospheres and sphere segment void nanostructured films, these designs often require multiple-step methods and it is difficult for the electrodes to be miniaturized down to the microliter scale.

Herein we demonstrate the PLM as an ideal microscale test-bed for spectroelectrochemical analysis capable of circumventing the aforementioned limitations, leveraging on the plasmonic and electrically conductive metallic shell as a bifunctional SERS- and EC-active platform. Exploiting the Ag shell as the first miniaturized 3D spherical working electrode, we first highlight the ability of the PLM soft metallic shell to perform EC reactions at precise voltages, and demonstrate the excellent reproducibility of our improvised three-electrode microsystem over an extended analysis period. PLM is then showcased as the smallest spectroelectrochemical cell capable of offering potential-dependent read-out of molecular vibrational fingerprints and electrothermodynamics simultaneously, using the colorless rutheniumhexammine(III) chloride (Ru(NH$_3$)$_6$Cl$_3$) and environmental toxin methylene blue (MB) as model compounds. Remarkably, the synergistic electrochemical and SERS capability of the PLM enables the elucidation of critical insights on EC reaction mechanism, molecular structural changes, and quantitative temporal concentration for kinetic dynamics that cannot be accomplished with EC or
We then evaluate the PLM as a conductive 3D working electrode (WE) by performing cyclic voltammetry (CV) using electroactive Ru(NH₃)₆Cl₃ as our model probe dissolved in the enclosed aqueous microdroplet (Figure 1B, Figure 2). A three-electrode system is improvised to allow precise regulation of applied voltage. A Pt electrode and a Ag/AgCl wire serve as a counter and quasi-reference electrode, respectively (Figure 1C; Supporting Information, Figures S7, S8). In a forward scan, we observe the emergence of a cathodic peak at −0.28 V as the applied potential is regulated systematically from −0.05 to −0.5 V (Figure 2B). An anodic peak subsequently evolved at −0.19 V as the applied potential is reversed from −0.5 V back to the initial −0.05 V. The peak separation is determined at 0.09 V, which is comparable with previous reports using similar droplet cell setup. These findings clearly reflect a one-electron redox process involving the electroreduction of Ru^3+ to Ru^2+. Furthermore, we note the similar peak currents of cathodic and anodic electrochemical responses quantified at 27 and 30 µA, respectively, which can be attributed to the excellent reversibility of Ru(NH₃)₆Cl₃ electroreduction. Such stable and well-defined redox peaks of our redox probe when using PLM as electroactive microreactor indicate the efficient electron transfer between the electrode and electroactive species. PLM as a working electrode is again affirmed on comparison with a control insulating silica liquid marble, which reveals featureless and negligible EC responses, signifying no redox SERS enhancement arising from the intense localized electromagnetic fields at the sharp edges[10] and also a 1.2-fold higher electrical conductivity compared to as-synthesized, polystyrene-capped Ag nanocubes (Supporting Information, Figures S2, S3). Upon the addition of a 40 µL aqueous droplet, the Ag nanocubes suspended in decane spontaneously assemble on the microdroplet surface to form a 3D encapsulating shell (Figure 1A) driven by the large interfacial energy between the aqueous and organic decane phases.[11] The Ag nanocube shell exhibits the appearance of bulk silver arising from the uniform and compact array at optimized Ag-to-water ratio of 7.5 µg mL⁻¹ (shell thickness ca. 500 nm; Supporting Information, Figure S4). An x−y hyperspectral SERS evaluation of PLM demonstrates its intense SERS activity with an analytical enhancement factor (AEF) of 2.6 × 10⁸ and excellent homogeneity (<10% relative standard deviation (%RSD)) across a large evaluation area (165 µm × 65 µm, >1300 spectra; Supporting Information, Figures S5, S6). The intense and homogeneous SERS responses directly affirm the compactness of the plasmonic shell, where plasmonic coupling of adjacent Ag nanoparticles in the x, y, and z spatial directions create dense and strong electromagnetic hot-spots evenly distributed around PLM. Hereafter, all experiments are conducted in a 40 µL droplet using the optimal Ag-to-water ratio of 7.5 µg mL⁻¹ to ensure electrical continuity and high SERS readout.
reaction under identical CV conditions. The 3D metallic shell also demonstrates a 1.5-fold higher peak current (ca. 27 \( \mu \)A) relative to the planar WE (ca. 18 \( \mu \)A) formed by dropcasting Ag nanocubes onto copper tape, which directly denotes the superior electrochemical performance of 3D PLM electrodes over the conventional planar counterpart. This is due to the efficient utilization of electrode materials with higher specific electrode surface area in a 3D configuration (Supporting Information, Text S1). Furthermore, the PLM shell is highly durable, as evident from both the excellent consistency of the measured current over 100 cycles of CV (%RSD \(< 3\)%) and preservation of Ag nanocube morphology (Supporting Information, Figure S9).

The similar symmetric shape of the CV compared to bulk EC also indicates minimal distortions arising from the high current density of the small CE area used (Supporting Information, Figure S10). Our results therefore collectively emphasize the immense potential of PLM as a robust and efficient microsystem for practical EC applications.

PLM as a microliter-scale spectroelectrochemical cell is further demonstrated by in situ reaction monitoring using SERS, which further provides both molecular-level identification and quantification of the entire electron-transfer processes occurring at the electrode surface. At the initial potential of \(-0.05\) V, we observe one SERS band centered at 495 cm\(^{-1}\) (Figure 2.C.D). The SERS intensity of the 495 cm\(^{-1}\) band remains relatively constant even as potential decreases to \(-0.15\) V. As the potential reaches \(-0.25\) V, the SERS intensity of 495 cm\(^{-1}\) band begins to decrease with the concomitant emergence of a new vibrational feature at a more red-shifted region of 455 cm\(^{-1}\). Further regulation of applied potential to \(-0.5\) V leads to a 4-fold decrease of 495 cm\(^{-1}\) SERS intensity, whereas the band at 455 cm\(^{-1}\) increases 3-fold (Figure 2.C.D). The SERS spectral changes are indicative of a single-step reaction occurring at the electrode surface. The band at 495 cm\(^{-1}\) can be assigned to the Ru\(^{3+}\)–N stretching while the 455 cm\(^{-1}\) band arises owing to the Ru\(^{2+}\)–N stretching.[7b,8a] This agrees with the fact that as the charge on Ru decreases, the polar interaction between amine ligand and Ru\(^{2+}\) metal center is reduced. Consequently, Ru–N bond strength decreases (lower vibrational force constant) and causes the bond to vibrate at lower frequency compared to Ru\(^{3+}\). The lower intensity of Ru\(^{2+}\)–N stretching could be due to the decrease in polarizability of the bond arising from the reduction of Ru\(^{3+}\) to Ru\(^{2+}\). Our SERS observations therefore clearly correlate to the CV cathodic peak at \(-0.28\) V where Ru\(^{3+}\) is reduced to Ru\(^{2+}\). This is further supported by the appearance of a broad NH\(_3\) rocking mode (585 cm\(^{-1}\)) at \(-0.25\) V,[9] which gradually experiences an increase in its intensity at more negative potential. Such phenomenon could be due to electric field-induced adsorption reorientations of the complex, whereby the closer proximity of ammine ligands to the Ag surface enhances their SERS responses.[14] It is also noteworthy that changes in these spectral features are reverted as the applied potential is tuned from \(-0.5\) to the initial \(-0.05\) V, a clear indication on the reversibility of the redox reaction. The SERS intensity of the Ru\(^{2+}\)–N mode at 495 cm\(^{-1}\) increases back to its maximal intensity, while the Ru\(^{2+}\)–N vibrational mode diminishes to negligible intensity (< 3 counts). Collectively, SERS- and electrically active PLM is important to track both molecular and concentration changes during EC reaction at the electrode surface, notably using just 40 \( \mu \)L of the reaction solution.

Heterogeneous electroreduction of methylene blue (MB\(^+\)) toxin is also performed as a second electrochemical model to demonstrate the versatility of our system in analyzing multi-electron redox systems (Figure 3; Supporting Information, Figure S11). As the potential is cycled between \(-0.1\) and \(-1\) V, MB\(^+\) exhibits two cathodic peaks at \(-0.42\) and \(-0.56\) V (peak 1 and 2) and two anodic peaks at \(-0.47\) and \(-0.31\) V (peak 3 and 4) during the forward (\(-0.1\) to \(-1\) V) and reverse scans, respectively (Figure 3B). Peaks 1–4 possess complementary peak currents of 8.7, 14.0, 6.5, and 13.0 \( \mu \)A, respectively. These peaks and their matching currents jointly reflect the two one-electron transfers from the applied current to MB\(^+\), resulting in the final reduced product, leucomethylene blue (LMB; Figure 3A). MB\(^-\) first receives an electron to form radical MB\(^-\) (peak 1; MB\(^-\)–MB\(^+\)). This is followed by protonation of MB\(^-\) to yield radical cation (HMB\(^{2+}\)), which is then reduced by receiving another electron to produce LMB (peak 2; HMB\(^{2+}\)–LMB). Correspondingly, the reversed reactions of LMB oxidation to HMB\(^{2+}\), and to

![Figure 3](image-url)
the initial MB⁺ are assigned to peak 3 (LMB → HMB⁺) and peak 4 (MB⁻ → MB⁺), respectively.[15] We highlight the importance of the Ag shell of PLM as an active electron relay center to resolve multi-step electron transfers by comparing the electrochemical responses with control experiment involving naked reaction microdroplet placed on top of the Cu tape (Figure 3B). The control CV reveals only one anodic peak and overall sluggish electron transfer, reflecting an irreversible process during the oxidation of LMB back to MB in the absence of Ag shell. This evidently emphasizes the importance of Ag nanocube shell as an efficient electron transfer modulator between the externally-applied current to electroactive species, which enables a more efficient reduction and its subsequent reversible oxidation process, resulting in enhanced degradation and representative EC investigations.[26]

Next, we quantitatively track the structural changes of MB⁺ at the Ag nanocube shell using in situ SERS, and relate the molecular-level information with the EC insights from the CV (Figure 3C; Supporting Information, Figure S12). In the reduction pathway, the nitrogen atom (yellow) of MB⁺ gains a proton (H⁺) and results in a structural change of the molecule where the C=N bond is reduced to C—N—H in LMB (Figure 3A). Hence, the sharp and intense C=N stretching mode at 1639 cm⁻¹ is selected to represent the presence of MB⁺ (and also MB⁺) during the potential-dependent SERS investigation.[6] In the forward scan from −0.1 to −0.5 V, the SERS intensities remain constant (ca. 347 counts, <3% change) because C=N bond remains present even as MB⁺ is reduced to MB⁻ at −0.42 V. This is followed by the onset of SERS intensity to a minimum of 213 counts at −0.8 V after applied potential is reversed at −0.9 V. On the reversal of the applied potential, the SERS intensity increases and eventually reaching a plateau of about 393 counts after −0.3 V, corresponding to the oxidation peak of MB⁺ to MB⁻ in the CV. Such observations can be explained using the Nernst equation, where the ratio reactant/product is dependent on the applied potential (Supporting Information, Figure S12). Hence, upon reversal of potential to less negative potentials, the reduction of MB to LMB still occurs but the ratio of [LMB]/[MB] decreases. Thus is also in close agreement with previous spectroelectrochemical investigations.[6c, 16] Owing to the surface-sensitive nature of SERS, these intensities are directly proportional to MB⁺ concentration at the Ag shell,[17] and the spectral changes reflect the potential-dependent molecular changes during the MB⁺ redox, congruent to the EC insights derived from CV. Quantitative examination using SERS thus holds an advantage over UV/Vis absorption spectroscopy, where the latter usually reveals bulk concentration changes that are not representative of the actual concentration profile at the electrode interface, especially for diffusion-limited analysis.[18] More importantly, structural changes, which cannot be obtained from UV/Vis spectroscopy, can be identified from the molecular fingerprints changes during the electroreduction of MB⁺ to LMB. For example, the C=N—C ring stretching (1410 cm⁻¹) that is only present in MB⁺ attenuates by 3-fold while the weak C=N—C stretching mode (1524 cm⁻¹) belonging to LMB red-shift by 6 cm⁻¹ and increases 2-fold owing to the formation of LMB (Supporting Information, Figure S13). We exclude any potential EC and SERS interference from the Ag shell, electrolyte, or systematic signal fluctuations from the consistent and featureless spectra in the control (Supporting Information, Figure S14).

Furthermore, PLM also offers ultrasensitive SERS activities for the determination of potential-dependent reaction kinetics (Supporting Information, Figure S15). Therefore, the onsite read-out of potential-induced molecular transformation clearly demonstrates the immense advantages of PLM to definitively identify EC species in its native reaction environment.

Finally, we demonstrate superior SERS and EC capability of the PLM over controls involving conventional 2D micro-platform and non-plasmonic active silica liquid marble (SLM; Figure 4A–C). Notably, the SERS intensity using PLM is more than 10-fold higher than its 2D counterpart (Figure 4D,E), prepared by the pre-deposition of Ag nanocubes onto the copper tape, while SLM exhibits negligible SERS response. These two control micro-platforms therefore emphasize the importance of 3D Ag nanocube shell in PLM for SERS enhancements. The better SERS performance in PLM over other systems stems from the direct sensing of the analyte at the top of the Ag nanocube shell of PLM, which enables a more accessible pathway for the excitation laser to reach the SERS platform and effective collection of Raman
scattered lights back to the detector. On the contrary, the laser and Raman scattered light are required to travel through the aqueous solution to reach the control 2D SERS platform at the bottom and detector, respectively, which could be both attenuated by the light-absorbing dye solution and impede the successful recording of vital molecular signatures. The remarkable SERS capability of PLM is again highlighted via its circa 5-fold higher sensitivity (115 counts/0.1 V) compared to the conventional 2D control (24 counts/0.1 V; Figure 4F), allowing the accurate distinction of minute concentration changes. This is especially critical in temporal SERS measurements, where any low signal intensity and its associated high error (average %RSD = 63%) of the SERS measurements could severely hinder the representative elucidation of temporal information and reaction kinetics (Figure 4G). Collectively, these comparisons therefore clearly reflect the ultrasensitivity of the PLM-based spectroelectrochemical microreactor necessary for efficient EC-SERS characterization, especially for EC processes with low species concentrations and/or weak Raman scattering.

In conclusion, we utilize the highly versatile PLM as an isolated and smallest 3D miniature reactor for simultaneous molecular-level analysis of EC processes. Together with its dual functionalities as SERS-active platform and working electrode, the Ag shell of PLM also facilitates electron relay and demonstrates immense potential for real-time molecular-level identification of transient species, using the electro-degradation of a toxin as a model. These insights are crucial for elucidation of tandem EC reactions dynamics, which is not possible by standalone EC methods. More importantly, our novel 3D electrode exhibits 2-fold and 10-fold better electrochemical- and SERS-activities than their conventional 2D counterparts. Despite the potential window limit of Ag, the rich information and excellent performance offered by PLM still serves as an important proof of concept, which can be easily extended to vast nanomaterials of various materials or shape to better cater for specific experiment conditions. This finding is valuable to impact future design of 3D electrode system for enhanced electrochemical performance relevant in the broad applications in environmental conservation, energy harvesting, and biochemical charge transfer.

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Conflict of interest

The authors declare no conflict of interest.

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