ABSTRACT: We report for the first time kinetic studies on chromium(III) detection in aqueous solution using citrate-capped silver nanoparticles (AgNPs) and the surface-enhanced Raman spectroscopy (SERS) technique. Moreover, we have shown an important effect of adding ethylenediaminetetraacetic acid (EDTA) on the enhancement and the stability of the Raman signal. The origin of the SERS signal was attributed to the coordination of Cr(III) by citrate/EDTA molecules and the formation of hot spots on aggregated AgNPs. Depending on the mixing method of Cr(III) and EDTA with AgNPs, the temporal SERS spectral features reveal a Prout–Tompkins or a Langmuir kinetic detection model. The UV–visible data, the temporal response of the Raman signal, and the scanning electron microscopy analysis have allowed us to elucidate the mechanism of Cr(III) detection. We observed that mixing simultaneously Cr(III), AgNPs, and EDTA leads to the most stable and intense time-dependent SERS signal. The obtained results should open the way to perform kinetic studies on different host-guest interactions in solution using the SERS technique.

1. INTRODUCTION
Surface-enhanced Raman spectroscopy (SERS) is an ultra-sensitive method that allows a high detection of Raman scattering by molecules once adsorbed onto metal aggregations, for example, on silver and gold nanoparticles (NPs).1–5 The highly sensitive detection of various analytes is possible through the mixing of aggregated metal NPs and probe material in the field of nano-bioanalysis.5,6 The SERS technique is based on the enhancement of the inelastic scattering from molecules in proximity to the nanostructured metallic surfaces when the latter exhibit a surface plasmon resonance (SPR) frequency close the excitation wavelength. One of the important optical properties of silver nanoparticles (AgNPs) is light absorption in the visible wavelength range, obtained by SPR. Indeed, SPR represents the collective oscillation of electrons in the conduction band during the excitation of the metal by a wavelength that corresponds to the SPR frequency. SPR of silver nanostructures depends strongly on the shape, size, and the environment of the nanostructure which opens the way for the use of silver nanostructures as nanosensors to study chemical modification, aggregation, and kinetics that encounter their nanometric surfaces.7 In recent years, an increasing number of various applications that use the SERS technique have been developed, mainly in the fields of environmental monitoring, bio-detection such as viruses, diagnostics, and bioimaging.8–10 For example, the SERS-based sensor is one of the most powerful nano-optical-based detection techniques for various synthetic analytes and harmful metal ions in water at very low concentrations.11

Chromium (Cr) is among the metals that are abundant in nature and classified as one of the toxic cations that could affect our health and environment. Chromium could be mainly found in three valence states: metallic chromium (Cr0), trivalent chromium (Cr3+), and hexavalent chromium (Cr6+).12 On the other hand, there are various industrial applications for chromium such as production of water-cooling towers, tanning, electroplating, metallurgy, and dyestuffs factories.13 Another source of chromium in water comes from the fact that the hexavalent chromium Cr(VI) has been used as an efficient corrosion inhibitor of water pipes.14 The toxicity and physicochemical properties of chromium depends entirely on the oxidation state of the metal. Trivalent chromium [Cr(III)] is known as a fundamental trace element for normal physiological function; in contrast, the hexavalent chromium [Cr(VI)] is extremely carcinogenic and mutagenic, about 100 times more toxic than Cr(III).15 In vitro studies have demonstrated that soluble Cr(III) is a potential toxin because it can behave as a competitive inhibitor in many cellular mechanisms.16 For this reason, a reliable and accurate method for the detection of Cr3+ will be always of great importance. Detection of trivalent...
chromium in water has been achieved using various techniques such as atomic absorption spectrometry,\textsuperscript{17} fluorescence imaging,\textsuperscript{18} chemiluminescence,\textsuperscript{19} electrolysis,\textsuperscript{20} and so forth. Although most of these methods are effective in the measurement of chromium, there is still a need for new accurate, ultrasensitive, and selective methods to be developed. SERS could be considered an alternative method to respond to sensitivity and specificity parameters with the aim to develop a sophisticated sensor. Ye et al. have developed a sensitive and selective SERS technique for chromium (III) detection using citrate-attached gold NPs.\textsuperscript{21} Additionally, the detection method of Cr(III) with a complex of ethylenediaminetetraacetic acid (EDTA) and subsequent adsorption on citrate-capped AgNPs have been investigated by means of resonance Raman spectroscopy.\textsuperscript{22} The method was shown to be selective for the detection of Cr(III), and a detection limit of 500 nM could be achieved on the basis of SERS measurements. The small organic molecule EDTA is a powerful complexing agent with a high affinity constant to form metal–EDTA complexes with divalent and trivalent cations.\textsuperscript{23} The complexation of Cr(III) by EDTA has several important advantages in the thermodynamic and kinetic studies. First, the stoichiometry between EDTA and Cr(III) is known to be 1:1. Second, the complexation can be driven to completion, and it is irreversible and relatively slow at ambient temperatures.\textsuperscript{24} Finally, as a complex of several ionic species, when EDTA binds with metal, it exhibits different vibrational modes assigned because of the metal-N and O stretching bands.\textsuperscript{25} On the other hand, the aggregation behavior of AgNPs which is a key factor in the SERS technique is time-dependent and very sensitive toward many factors, including solution chemistry (e.g., ionic strength, pH, and electrolyte composition) and NP coating layer.\textsuperscript{26} For example, the aggregation of citrate-capped AgNPs in the presence of Cr(III) is reported to be mainly due to the chelation of the citrate groups that are coating the NPs.\textsuperscript{27} Shang et al. reported that the addition of Ni\textsuperscript{2+} leads to AgNP aggregations because of the strong coordination bonds formed between Ni\textsuperscript{2+} ions and the carboxyl groups of citrate on the surface of the NPs.\textsuperscript{28} Thus, in order to build a powerful sensor, it is crucial to examine and understand the kinetic parameters that are accompanying the detection process. Indeed, the capability to probe faster and control the surface functionality is crucial for the development of spectrally tunable NPs. For example, DeVetter et al. reported the development of a SERS-based method to figure out the kinetics of gold–thiolate bond formation on colloidal gold NPs.\textsuperscript{29} Although the detection of Cr(III) using EDTA and SERS techniques on AgNPs has been reported, no kinetic studies on metal detection using the SERS technique are found in the literature to the best of our knowledge. In this work, we investigate the kinetic studies of Cr(III) detection in solution on colloidal AgNPs with the objective of finding the optimal conditions necessary for a successful preparation of optical nanoprobes and enhance the detection signal using SERS technique. Various mixing strategies of the different system’s components will be examined by measuring SERS intensity as a function of time. The kinetic of coordination is monitored by first mixing Cr\textsuperscript{3+} and AgNPs and then by adding EDTA on the former. We also mixed simultaneously the three components, and finally, studied the SERS raised from adding [EDTA–Cr\textsuperscript{3+}]– on AgNPs. The rate constant for all the kinetics features is compared and analyzed by using Prout–Tompkins and Langmuir equations.

2. EXPERIMENTAL SECTION

2.1. Reagents and Solutions. All the used chemicals, including silver nitrate (AgNO\textsubscript{3}), chromium nitrate non-a
dehydrate (Cr(NO\textsubscript{3})\textsubscript{3}·9H\textsubscript{2}O), disodium ethylenediaminetetraacetate (EDTA), sodium borohydride (NaBH\textsubscript{4}), trisodium citrate (Na\textsubscript{3}C\textsubscript{6}H\textsubscript{5}O\textsubscript{7}), and borax buffer solution (0.1 M pH = 8.0), were purchased from Sigma-Aldrich. SERS measurements were performed using either only a solution of Cr(III), the mixture EDTA + Cr(III) in 1:1 mol ratio, or the complex [Cr(EDTA)]\textsuperscript{3–}. In the latter case, the pink complex [Cr(EDTA)]\textsuperscript{3–} was prepared in an equal cation to ligand mole ratio, at a concentration of 10\textsuperscript{−5} M, 24 h prior to the experiment. This stock solution was diluted to the requisite concentrations for the corresponding SERS measurements. Fresh water for solution preparations was obtained by distillation and purification using a Milli-Q system (Millipore, Milford, MA).

2.2. Preparation of Citrate-Capped AgNPs. All the used glassware was thoroughly washed with water and air-dried. The AgNP dispersion was prepared according to a reported work with slight modifications.\textsuperscript{30} Briefly, 1 mL of 44 mM trisodium citrate is added to ~50 mL of 1.1 mM boiling solution of silver nitrate stirring on a hotplate. This was followed by the dropwise injection of ice cold freshly prepared aqueous solution of NaBH\textsubscript{4} (1 mL, 0.03 M) using a syringe, and it was kept under stirring for 30 min at room temperature. A pale-yellow color marked the end of the reaction and confirmed the formation of NPs. The nonreactant species were removed by centrifugation at 21,000 rpm for 60 min. Then, the collected NPs were dispersed in deionized water and stored in the dark at 4 °C until it is used for the measurement. The pH of the colloidal solution was measured and was found to be 8.1.

2.3. Scanning Electron Microscopy. Typically, two drops of the NPs were deposited on a pure copper metal substrate disc and kept for water evaporation (as shown in Figure S7) before performing the scanning electron microscopy (SEM) measurements. A JEOL, JSM 7000 series scanning electron microscope was used to obtain the morphological micrographs of AgNPs at 15.0 kV scan voltage.

2.4. UV−Visible Spectroscopy. The different samples (as-synthesized AgNPs, mixture of AgNPs and Cr(III), mixture of AgNPs and Cr(III) + EDTA, and finally mixture of AgNPs with the complex [Cr(EDTA)]\textsuperscript{3–}) were characterized by UV–visible spectroscopy. First, we have tried to follow the kinetic of the plasmon shift using UV−vis spectroscopy, but it was impossible in our conditions because the process was very fast. To the best of our knowledge, no studies have been reported on the kinetics of AgNP aggregations in the presence of analytes using UV−vis spectroscopy. For this reason, all the mixtures with AgNPs were prepared 1 h before measuring the UV−visible absorption. The concentration was reduced by five compared with that used for SERS measurements, as the AgNPs exhibited high absorbance in UV. The UV-2600 spectrophotometer (Shimadzu, Tokyo, Japan) was used to record the spectra ranging from 200 to 800 nm. Water was used as a blank, and the measured spectra were plotted using Origin software.

2.5. SERS Measurements. Fresh solutions of the Cr(III), EDTA + Cr(III), or [Cr(EDTA)]\textsuperscript{3–} complex prepared in borax buffer pH = 8.0 were mixed just before the SERS measurements with the citrate-capped AgNPs (in the ratio 4:1; v/v; AgNPs/analyte). EDTA and Cr(III) were used in equal mole ratio, and the final concentrations of Cr(III), EDTA + Cr(III), or the complex [Cr(EDTA)]\textsuperscript{3–} after mixing with AgNPs were equal to S
The chosen concentration $5 \times 10^{-4}$ M allowed us to have a strong and stable SERS signal and helped to understand the occurring interactions in the system during the detection process. The mixing time of the analyte with AgNPs was considered as the starting point for the kinetic studies in all the SERS measurements. The cleaned quartz cell was completely filled with the mixture, sealed, and put under the Raman laser to start the SERS measurements. The Raman spectra were collected using a LabRAM HR evolution spectrometer (HORIBA Scientific) in a backscattering geometry at ambient temperature. A He–Ne laser of $\lambda = 632.8$ nm and a power level of 2 mW were employed. An objective of 10× with a numerical aperture of N.A. 0.25 was used. The latest offers the capability of a large excitation of a high number of molecules in the solution. The Raman spectra were collected from sample solutions placed in the quartz cuvette that can let the light being transmitted into the solution without any considerable loss of intensity. In this setup, the laser focus was fixed in order to avoid variations in the excitation volume which could generate fluctuations in the Raman signal intensity.

### 3. RESULTS AND DISCUSSIONS

According to the SEM analysis, the synthesized AgNPs showed a size distribution between 2 and 50 nm, with 14 nm being the particle size of the highest percentage of appearance (Figure 1a,b). On the other hand, we have performed a UV–visible spectrophotometric analysis of the citrate-capped AgNPs in the presence of the different analytes. Figure 1c shows the UV–vis absorption spectrum of AgNPs with a peak centered at around 390 nm. In general, AgNPs without any analytes will be present in the form of single particles and dimer aggregates with a large gap distance, which explains the position of the maximum absorption at 390 nm that corresponds to the dipolar mode (Figure 1c, black spectrum). Upon adding chromium cations to the AgNPs, we immediately observed with the naked eye that the color of the mixture changed into red-orange, and a plasmon shift to higher wavelength was observed in the UV–vis absorption spectra (Figure 1c, green spectrum). This shift is attributed to the double effects of metal cation adsorption onto the surfaces, the aggregates, and the aggregation of AgNPs. Indeed, the addition of Cr$^{3+}$ leads to more aggregations of AgNPs, and consequently, the particles become closer to each other, which explains the presence of two resonances at around 380 and 520 nm, which is attributed to the quadruple and dipole mode, respectively. The blue shift for the dipole mode becomes more pronounced than in the case of chromium alone once we add EDTA or the complex [Cr(EDTA)]$^{-}$ (Figure 1c, pink and blue spectra). This important additional shift is originated from the effect of a very small gap distance between the NPs. Moreover, Figure 1c shows that the band located around 580 nm in the absorption spectrum of AgNPs with the complex [Cr(EDTA)]$^{-}$ is more flattened than the band of AgNPs in the presence of the EDTA + Cr(III) mixture. This difference could be attributed to Mie scattering originated from
the large size particles or agglomeration.\textsuperscript{34} Additionally, this result indicates different levels of NP aggregations depending on the added analytes. The UV−visible spectrophotometric study has allowed us to select the wavelength 632.8 nm as a suitable excitation wavelength in order to perform the SERS measurements.

The SERS experiments were performed in solution in order to figure out the different interactions that can be present between the components of the system. Initially, the time-dependent SERS measurements were done to study the interactions between Cr(III) and citrate-coated AgNPs (Figures S1 and 2a). The results indicate an increase in the Raman intensity of the vibrational mode (centered around 570 cm\textsuperscript{−1}) with time to reach a maximum after 1000 s (Figure 2a). This peak could be attributed to the Raman chromium−oxygen stretching band with the citrate on the surface of AgNPs. This vibrational mode cannot be followed by UV−visible spectroscopy in the presence of AgNPs and in our experimental conditions. Thus, the SERS technique offers the opportunity to conduct a kinetic study on Cr(III) monitoring in solution. It is worth noting that at pH around 8, the three carboxylic groups of the citric acid are deprotonated, as the dissociation constants of citric acid are $pK_a^1 = 3.13$, $pK_a^2 = 4.74$, and $pK_a^3 = 6.40$.\textsuperscript{35} The mixed modes of vibrations related to the coordination of Cr(III) with different carboxylic groups gradually cause the broadness in the Raman peak. The broadness of Raman peaks during a coordination process is already reported in literature. For example, DeVetter et al., in their work on measuring kinetics of thiolated molecules with gold NPs in solution using the SERS technique, have shown that the full width at half-maximum (fwhm) grows and saturates within the first hour of introducing from 50 to 115 cm\textsuperscript{−1} (for a mixed mode $\nu_{C-C}$ and $\nu_{C-H}$) and 32 to 48 cm\textsuperscript{−1} (for a fundamental mode $\nu_{C=O}$).\textsuperscript{36} In our spectra, the large band around 1000 cm\textsuperscript{−1} could be attributed to the C−H in the plane bending of citrate and EDTA. Although this band increases versus time because of the formation of hot spots, it is not connected with the Cr(III) signal during the detection process.\textsuperscript{37} After 1000 s, the peak intensity starts to decrease gradually because of the agglomeration of AgNPs and thus the loss of the SERS signal (Figure 2a). On the other hand, we have examined carefully the Raman peak shifts and the fwhm variation versus time. Interestingly, a Raman shift (from 570 to $590$ cm\textsuperscript{−1}) and an increase in fwhm were observed between 0 and 500 s and stabilized after that (Figure S2a,b). We must...
highlight that the peak intensity, the peak shift, and its fwhm were extracted by fitting all the Raman spectra by using a Gaussian function (see Figure S4). This observation indicates that the occurring interaction during this period of time corresponds mainly to the coordination between the citrate and the chromium. Based on these results, we can suggest that the observed SERS signal from 0 to 500 s is attributed mainly to the chemisorption enhancement as a result of coordination between the citrate and Cr(III) through the carboxylic groups. However, after 500 s, the SERS is mainly related to the electromagnetic enhancement due to the aggregation of NPs that lead to the generation of an increased number of hot spots for SERS enhancement signal. Our assumption is based on the fact that in all the reported SERS adsorption studies, the electromagnetic and chemical effects (if exist) are both involved in the SERS enhancement. Chemical enhancement has less effect than the electromagnetic one, and it is generated from different sources such as the resonances between the metal—molecule complex, deformation of the molecular polarizability, and electron charge transfer between the metal—molecule interface. However, the electromagnetic enhancement effect is based on the excitation of localized surface plasmons in the metallic nanostructures and is known to be more significant than the chemical enhancement, which is in agreement with our observation.

An examination of the temporal response between 0 and 700 s of the SERS spectral features taken from the fitting of all the maximum intensity of spectra located around 570 cm$^{-1}$ by a Gaussian function (Figure 2b) reveals a Prout–Tompkins variation model. Generally, autocatalytic reactions can be modeled with the Prout–Tompkins equation that is usually used in solid-state kinetics. This is a strong indication of a nucleation site buildup model where the coordination between citrate and chromium leads to the formation of aggregates. It has been reported that the aggregation of citrate-capped silver AgNPs in the presence of Cr(III) is mainly due to the chelation of the citrate molecules that are coating the NPs. The trivalent chromium could form a stable Cr(III)—citrate complex in a 1:2 binding stoichiometry (metal: ligand), leading to the NP aggregations as speculated in Figure 3. According to the Lewis acid–base theory, Cr(III) favors coordination with oxygen-donating chelates. Additionally, it is worth noting that Cr(III) can neutralize the surface charge of citrate-coated AgNPs through the coordination with the negatively charged carboxyl groups of the adsorbed citrate molecules. The charge neutralization in a first step will facilitate the approximation of NPs from each other and lead to further aggregation in a second step.

The rate equation of a first-order autocatalyzed reaction is given by eq 1

$$\frac{d\theta}{dt} = k_b(1 - \theta)$$  \hspace{1cm} (1)

where $\theta$ is the conversion fraction, $t$ is the time of reaction, and $k_b$ is the conversion fraction-dependent rate constant. In autocatalyzed reactions, $k_b$ is assumed to be a linear function of $\theta$, therefore, $k_b = k\theta$, where $k$ is the conversion fraction-independent rate constant.

The rate of eq 1 can be rewritten as in eq 2

$$\frac{d\theta}{dt} = k\theta(1 - \theta)$$  \hspace{1cm} (2)

Integrating and rearranging eq 2 will lead to eq 3 as follows

$$\theta = (1 + e^{-k(t-t_0)})^{-1}$$  \hspace{1cm} (3)

where $t_0$ is an integration constant. By adjusting the temporal curve of the Raman feature at the maximum intensities as per eq 3, the rate constant of this SERS kinetic process was calculated and was equal to 0.36 min$^{-1}$.

To the previous mixture, a solution of EDTA was added in the cuvette in equal mole ratio to Cr(III). The experiment was conducted by adding a volume of EDTA solution to the colloidal mixture AgNPs–Cr(III) that was used to measure the SERS in Figure S1. Thus, EDTA was added after 700 s from mixing AgNPs and Cr(III). The time zero for the SERS in Figure 4 was the time of adding EDTA to the mixture. The idea from this experiment was to examine the effect of adding EDTA on the SERS enhancement besides studying the kinetic of the detection process. Indeed, an enhancement more than 8 times was obtained when EDTA was added to AgNPs–Cr(III) (the SERS increased from 300 to around 2500 au).

This increase in the SERS signal could be attributed to Raman chromium—oxygen and chromium—nitrogen stretching bands with the EDTA molecules that can also contribute to the formation and stabilization of further aggregates. It is worth noting that EDTA comprises four carboxylic acid groups and thus has an overall negative charge at pH = 8. Its structure is flexible when it is unbound and adopts a rigid conformation, while interacting with cations. In general, the complexation between Cr(III) and EDTA is characterized by a low rate of coordination. The slow complexation and the molecular flexibility would allow us to propose that the organic chelator
EDTA coordinates with the surfaces of AgNPs and gradually with Cr(III). The chromium cation can complete its hexagonal coordination sphere with the citrate molecules that are exchanged with EDTA on the surface of the NPs. This proposed construction should lead to the proximity between the AgNPs which is confirmed in UV−visible by a higher plasmon resonance centered at 580 nm (compared with 519 nm in the absence of EDTA as shown in Figure 1c). The negative charge that is regained by the system would increase the stability of the system and avoid the agglomeration of large particles, which explains the stability of the SERS signals (Figure 4a,b).

Interestingly, we can observe in this case that the increase in the Raman intensity, the Raman peak shift (from 590 to 594 cm\(^{-1}\)), and the fwhm broadness occur all in the same range of time which is roughly between 0 and 450 s, while no significant variations are observed after this time (Figures 4b and S3). This important observation indicates that chemical SERS enhancement (due to the coordination between EDTA and chromium) and the electromagnetic SERS enhancement (due to the aggregation of NPs) occur at the same time.

Based on the Raman shift and fwhm variations, we can define the temporal regions in which chemical or electromagnetic enhancements are more predominant. The fitting using the Prout–Tompkins model was perfectly done on the combination of the two modes of enhancement, and this could be explained by the following: (1) the chemical and electromagnetic enhancement can start simultaneously from 0 until 700 s with the predominance of one type on the other and they cannot be separated. To the best of our knowledge, no studies have been reported on the separation of the two factors. However, when the fwhm and the Raman shift variations stop after 700 s, the electromagnetic enhancement becomes the only factor that causes the enhancement. (2) The electromagnetic enhancement depends on the chemical process, as we previously mentioned that the coordination with Cr(III) is considered as the key factor of the aggregation and thus the formation of hot spots leading to the electromagnetic enhancement. (3) The electromagnetic enhancement is several orders of magnitude stronger than the chemical enhancement. Thus, by considering the autocatalytic model, the rate constant was calculated using the Prout–Tompkins equation and found to be equal to 0.48 min\(^{-1}\). This result indicates an increase of the rate constant of the SERS kinetic detection compared with the detection of Cr(III) alone by citrate-capped AgNPs. Our data showed that the chemical enhancement always occurs as a first step in the mechanism of SERS detection and more rapidly than the electromagnetic enhancement. Thus, the aggregation of the NPs will be considered the rate-determining step of the mechanism, as it is the slowest step in the mechanism. The dominance of first-order kinetics has been reported in aggregation reaction of metal NPs. Dutta et al. have reported the kinetics of the gold NP aggregation process following the removal of the tri-sodium citrate stabilizer by dialysis. Detailed kinetic analysis showed that the aggregation process was reaction-limited, demonstrating a first-order kinetics. The data show that the aggregation step appeared to be the rate-limiting step, and thus, the overall order of the process is a first-order reaction. In order to further examine the role of EDTA in this detection process, the experiment has been performed by adding directly the mixture of EDTA + Cr(III) before complex formation to AgNPs, and the SERS signal versus time is displayed in Figure 5a. Again, the broadness of the SERS peak is due to the mixed vibration mode arising from the coordination between Cr(III) and citrate/EDTA molecules. Both ligands contain carboxylic groups that could be involved in

![Figure 5](https://dx.doi.org/10.1021/acsomega.0c04844)
the coordination of Cr(III). For us, it is impossible to separate between the two coordination processes in SERS, as the signals appear in the same region of the wavenumber (Figure S1, in the absence of EDTA and Figure 5a in the presence of EDTA). EDTA would offer a greater number of coordination bonds with Cr(III), which is explained by the important SERS enhancement compared with citrate only.

This time, the Raman shift and the fwhm variation are observed only in the time range of 0 to 800 s roughly. However, the SERS signal continues to increase until 2200 s and stabilizes after that (Figure 5a – d). The increase in SERS is more displayed in the time range where there is no change in the fwhm and the Raman shift. This result indicates that the mechanism is composed of a first step of coordination between the chromium and citrate and EDTA on the surface of the AgNPs, followed by a slower step of AgNPs aggregation leading to the formation of bigger particles as shown by the SEM image (Figure S5). Although, the electromagnetic enhancement is the major player of the SERS enhancement, the chemical one should also contribute, especially when chemical binding occurs before or even during aggregation.38 Thus, the fitting of the data was performed again using the Prout–Tomkins equation where the rate constant of the overall process was equal to 0.18 min⁻¹ (Figure Sb). It is worthwhile to mention that we have tried to fit the SERS temporal intensity in Figure Sb for the first step of the process (0–600 s), the second one (600–3500 s), and finally the whole data together. We have observed that the rate constant extracted for the whole points (k = 0.18 min⁻¹) is very close to that obtained by fitting the second step (separately, k ~ 0.18 min⁻¹). The results confirm that the second step of the mechanism is the rate-determining step, where mainly the electromagnetic enhancement dominates. Our results also show that the kinetic of detection is very dependent on the experimental conditions. The obtained data reveal that the detection process when adding EDTA to a system composed of AgNPs + Cr(III) was faster than in the case of adding a mixture of EDTA + Cr(III) to AgNPs (k = 0.49 min⁻¹ compared to k = 0.19 min⁻¹). These results indicate that adsorption of Cr(III) on citrate-capped AgNPs is a first step in this mechanism of detection. In the experiment of adding EDTA to AgNPs + Cr(III), the coordination was already established between citrate and Cr(III) when the EDTA was added. This explains the difference in kinetics between the two strategies. In addition, the formation of aggregations in this combination leads to a very important optical enhancement of the SERS signal. This important enhancement indicates that the combination between Cr(III) and EDTA before complex formation in the mixture with AgNPs is more stable and favorable for hot spot formation and thus a more enhanced Raman signal. The enhancement correlates with the higher absorption around 580 nm observed by the UV–vis spectrum shown in Figure 1c. Additionally, an interesting observation in this combination is the stability of the colloidal system that can last more than 12 h, while precipitation of AgNPs was observed by bare eyes after 2 h when the medium contains Cr(III) in the absence of EDTA (Figure S8). Finally, we have examined the effect of adding the complex [Cr(EDTA)³−] to AgNPs on the SERS signal. Indeed, the purple-colored complex [Cr(EDTA)³−] has been prepared 24 h prior to the Raman measurements in an equal mole ratio and then added to the citrate-capped AgNPs just before the SERS measurements. Regarding the charge of the formed complex, it is worth noting that EDTA is an hexaprotic system: four protons exist on four carboxylic groups, while the remaining two can be located on two nitrogen atoms when solutions are strongly acidic. At pH 8, the complex would be negatively charged and represented by [Cr(EDTA)³−]. The temporal SERS response is displayed in Figure S6, where we can observe the increase in Raman intensity with time and signal stabilization after 2000 s. As expected, no significant Raman shift was observed during this experiment (Figure S6) because the strong coordination between Cr(III) and EDTA was already established prior to the mixing with AgNPs. Moreover, one can see that the slight fluctuation of fwhm occurs mainly during the first 200 s which could be explained by the lower signal-to-noise ratio due to the weak SERS response in the beginning. This fluctuation persists during the whole mechanism because of the physisorption interactions between the citrate and the complex [Cr(EDTA)³−], where the latter could return to the solution after its adsorption. Additionally, the experiment was performed in the absence of free Cr(III) in order to compare the result with the previous experiments. For this reason, EDTA was used in a slight excess compared to Cr(III) in order to ensure that no Cr(III) is free to interact with the citrate molecules. Additionally, the complex [Cr(EDTA)³−] was prepared 24 h in advance to ensure complete formation of the complex that was verified by UV–visible spectroscopy. The complex EDTA–Cr(III) possesses a high stability constant (log K = 23.4) which is several orders of magnitude greater than the complex citrate–Cr(III) (log K = 6.95).49 For these reasons, Cr(III) cannot be exchanged between the two ligands once existing in solution at the same time. Based on these data, the only vibration modes that could arise in this range are related to the coordination of Cr(III) with EDTA. Thus, only one step in the mechanism of this approach is obtained, and it could be attributed to the adsorption of the [Cr(EDTA)³−] onto the surface of AgNPs, leading to the formation of hot spots and thus the increase of the SERS signal. Fitting the data versus time reveals a Langmuir-type curve.29,50 Indeed, it is likely that when a complex collides with the NPs, a direct adsorption on the surface could occur or it will return to the aqueous phase, indicating a first-order chemical kinetics. The SERS peak growth that follows a time-dependent Langmuir kinetics is described by the following equation29,50

\[ \theta = \theta_{sat} \times 1 - \exp(-k_{obs}t) \]  

(4)

where \( \theta \) represents the surface coverage at time \( t \), \( \theta_{sat} \) represents the saturation coverage which is dependent on the association or dissociation constants of the adsorption reaction, and \( k_{obs} \) is the adsorption rate constant.

The negative charges of the [Cr(EDTA)³−] complex and the surface of AgNPs due to the citrate capping generate an electrostatic repulsion between the two entities which can explain the lowest rate constant of adsorption (k = 0.072 min⁻¹). Additionally, the SERS enhancement was 17 times less displayed than during the previous way of mixing, indicating that this method generates a smaller number of hot spots that are essential for SERS signal (Figure 6).

In our work, the vibration mode we are discussing arises from the coordination between Cr(III) and citrate groups/EDTA molecules. Thus, considering the number of coordination that EDTA can afford in the complex formation with Cr(III), the SERS signal would be greater. This can be clearly seen in all the experiments where EDTA was used in the presence of Cr(III) and AgNPs. Regarding the difference in SERS between adding EDTA to Ag–Cr(III) or adding EDTA + Cr(III) to AgNPs, it seems that the process is very sensitive to the way the analyte is present in the solution. In the latter case, the electromagnetic
Figure 6. Plotting and fitting the maximum of Raman intensity vs time of \([\text{Cr(EDTA)}]^-\). The experimental part was fitted by using the Langmuir equation. Adj. \(R^2\) 0.954.

enhancement was more expressed, allowing us to suggest that this strategy was more convenient for aggregations and hot spot formation.

4. CONCLUSIONS

In this work, we have studied the kinetic of chromium detection in aqueous solution using the SERS technique on AgNPs. EDTA chelator appears to have an important effect on the mechanism and kinetic of Cr(III) detection as well as on stability and the enhancement of SERS signal. In the absence of EDTA, chromium(III) is initially coordinated with the citrate-capped molecules of AgNPs, leading to further aggregation of the NPs and loss of SERS signal after a short time. However, adding EDTA to this combination will mainly replace the citrate in the coordination of chromium(III) and cause an enhancement and a stability of the SERS signal.

An important difference has been observed in the kinetic mechanism of chromium(III) detection depending on the presence of EDTA in the medium. As a free chelator that will in situ coordinate with Cr(III) or as a formed complex \([\text{Cr-} (\text{EDTA})]^-\), the kinetic that arose from the temporal SERS signal was different. When the EDTA was added as a free ligand to the medium in the presence of chromium(III) and citrate-capped AgNPs, the mechanism of Cr(III) detection comprises two steps, which are EDTA–Cr coordination followed by AgNP aggregation, leading to an important enhanced and stable SERS signal. To the best of our knowledge, this work represents the first kinetic study in solution using the SERS technique on AgNPs to figure out the cation-ligand coordination. This result will pave the road toward the kinetic studies of other host–guest interactions in solution using the SERS technique.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04844.

Time-dependent Raman spectrum of AgNPs in the presence of Cr(III), Raman shift and fwhm versus time’s curve of Cr(III) in the presence of AgNPs, Raman shift and fwhm versus time’s curve of Cr(III) in the presence of AgNPs after adding EDTA, deconvolution of the Raman band near 590 cm\(^{-1}\) in the Raman intensity versus wavenumber spectrum, SEM image of AgNPs, Cr, and EDTA complexes, time-dependent Raman spectrum of \([\text{Cr(EDTA)}]^-\) in the presence of AgNPs, Raman shift versus time of \([\text{Cr(EDTA)}]^-\) in the presence of AgNPs, fwhm of the Raman peak versus time of \([\text{Cr(EDTA)}]^-\) in the presence of AgNPs, deposition of the samples on the copper metal substrate disc, and dispersion of AgNPs photos in the presence of Cr(III) + EDTA or only with Cr(III) (PDF).

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Braun, G.; Pavel, I.; Morrill, A. R.; Seferos, D. S.; Bazan, G. C.; Reich, N. O.; Moskovits, M. Chemically Patterned Microspheres for Controlled Nanoparticle Assembly in the Construction of SERS Hot Spots. J. Am. Chem. Soc. 2007, 129, 7760–7761.

(2) Yang, L.-b.; Chen, G.-y.; Wang, H.; Wang, T.-t.; Li, M.-q.; Liu, J.-h. Sunlight-Induced Formation of Silver-Gold Bimetallic Nanostructures on DNA Template for Highly Active Surface Enhanced Raman Scattering Substrates and Application in TNT/Tumor Marker Detection. J. Mater. Chem. 2009, 19, 6849.

(3) Yang, L.; Ma, L.; Chen, G.; Liu, J.; Tian, Z. Q. Ultrasensitive SERS Detection of TNT by Imprinting Molecular Recognition Using a New Type of Stable Substrate. Chem.—Eur. J. 2010, 16, 12683–12693.

(4) Yang, L.; Liu, H.; Wang, J.; Zhou, F.; Tian, Z.; Liu, J. Metastable State Nanoparticle-Enhanced Raman Spectroscopy for Highly Sensitive Detection. Chem. Commun. 2011, 47, 3583.

(5) Jana, N. R.; Pal, T. Anisotropic Metal Nanoparticles for Use as Surface-Enhanced Raman Substrates. Adv. Mater. 2007, 19, 1761–1765.

(6) Jiang, B.; Bosnick, K.; Maillard, M.; Brus, L. Single Molecule Raman Spectroscopy at the Junctions of Large Ag Nanocrystals. J. Phys. Chem. B 2003, 107, 9964–9972.

(7) Kruczewski, J.; Koloś, J.; Przepiorka, A. Plasmonic Nanoparticles in Chemical Analysis. RSC Adv. 2017, 7, 17559–17576.

(8) Bai, Y.; Feng, F.; Zhao, L.; Wang, C.; Wang, H.; Tian, M.; Qin, J.; Duan, Y.; He, X. Aptamer/Thrombin/Aptamer-AuNPs Sandwich Enhanced Surface Plasmon Resonance Sensor for the Detection of Subnanomolar Thrombin. Biosens. Bioelectron. 2013, 47, 265–270.

(9) Kukushkin, V. I.; Ivanov, N. M.; Novoseltseva, A. A.; Gambaryan, A. S.; Yaminsky, I. V.; Kopylov, A. M.; Zavyalova, E. G. Highly Sensitive Detection of Influenza Virus with SERS Aptasensor. PLoS One 2019, 14, No. e0216247.
91. and Its Compounds. ACS Omega http://pubs.acs.org/journal/acsodf

Alkyne-Coded SERS Test Kit. 2019

Thermodynamics and the Structure of the Binary and the Ternary Raman Sensor of Chromium(III) in Seawater Samples. Probe for Trivalent Chromium Detection Using Citrate Attached Gold Chromium(III) Nutritional Supplements. F. F.; Ramalho, T. C. Use of Ethylenediaminetetraacetic Acid as a Studies. S. S.; Das, D. 9-Acridone-4-Carboxylic Acid as an Efficient Cr(III)

Structure of the Chromium(III)-EDTA Complex. ChemTexts 2015, 1, 6.

Kendig, M. W.; Buchheit, R. G. Corrosion Inhibition of Aluminum and Aluminum Alloys by Soluble Chromates, Chromate Coatings, and Chromate-Free Coatings. Corrosion 2003, 59, 379–400.

Costa, M. Toxicity and Carcinogenicity of Cr(VI) in Aqueous Solutions by Ion Chromatography and 442.−86, 3391−3395.

Measuring Binding Kinetics of Aromatic Thiolated Molecules with Nickel Ion Using N-Acetyl-l-Cysteine-Functionalized Silver Nano-

Anal. Methods 2015, 5, 3711−3713.

Badawy, A. M. E.; Luxton, T. P.; Silva, R. G.; Sheckel, K. G.; Suidan, M. T.; Toloymat, T. M. Impact of Environmental Conditions (PH, Ionic Strength, and Electrolyte Type) on the Surface Charge and Aggregation of Silver Nanoparticles Suspensions. Environ. Sci. Technol. 2010, 44, 1260−1266.

Elavarasi, M.; Rajeshwari, A.; Alex, S. A.; Nanda Kumar, D.; Chandrasekaran, N.; Mukherjee, A. Simple Colorimetric Sensor for Cr(III) and Cr(vi) Speciation Using Silver Nanoparticles as a Probe. Anal. Methods 2014, 6, 5161.

Shang, Y.; Wu, F.; Qi, L. Highly Selective Colorimetric Assay for Nickel Ion Using N-Acetyl-l-Cysteine-Functionalized Silver Nanoparticles. J. Nanopart. Res. 2012, 14, 1169.

DeVetter, B. M.; Mukherjee, P.; Murphy, C. J.; Bhargava, R. Measuring Binding Kinetics of Aromatic Thiolated Molecules with Nanoparticles via Surface-Enhanced Raman Spectroscopy. Nanoscale 2015, 7, 8766.

Lee, P. C.; Meisel, D. Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Solids. J. Phys. Chem. 1982, 86, 3391−3395.

Liu, Y.; Liu, C.-y.; Chen, L.-b.; Zhang, Z.-y. Adsorption of Cations onto the Surfaces of Silver Nanoparticles. J. Colloid Interface Sci. 2003, 257, 188−194.

Jiang, M.-M.; Chen, H.-Y.; Li, B.-H.; Liu, K.-W.; Shan, C.-X.; Shen, D.-Z. Hybrid Quadrupolar Resonances Stimulated at Short Wavelengths Using Coupled Plasmonic Silver Nanoparticle Aggregation. J. Mater. Chem. C 2014, 2, 56−63.

Cortijo-Campos, S.; Ramirez-Jimenez, R.; Climent-Pascual, E.; Aguilar-Pujol, M.; Jimenez-Villacorta, F.; Martinez, L.; Jimenez-Ribóo, R.; Prieto, C.; de Andrés, A. Raman Amplification in the Ultra-Small Limit of Ag Nanoparticles on SiO2 and Graphene: Size and Inter-Particle Distance Effects. Mater. Des. 2020, 192, 108702.

Fan, X.; Zheng, W.; Singh, D. J. Light Scattering and Surface Plasmons on Small Spherical Particles. Light: Sci. Appl. 2014, 3, e179.

Deal, J. A. Lange’s Handbook of Chemistry, 15th ed.; McGraw-Hill, 1999.

DeVetter, B. M.; Mukherjee, P.; Murphy, C. J.; Bhargava, R. Measuring Binding Kinetics of Aromatic Thiolated Molecules with Nanoparticles via Surface-Enhanced Raman Spectroscopy. Nanoscale 2015, 7, 8766−8775.

Chowdhury, J.; Ghosh, M. Concentration-Dependent Surface-Enhanced Raman Scattering of 2-Benzoylpyridine Adsorbed on Colloidal Silver Particles. J. Colloid Interface Sci. 2004, 277, 121−127.

Valley, N.; Greenheltch, N.; Van Duyne, R. P.; Schatz, G. C. A Look at the Origin and Magnitude of the Chemical Contribution to the Enhancement Mechanism of Surface-Enhanced Raman Spectroscopy (SERS): Theory and Experiment. J. Phys. Chem. 1992, 4, 2599.

Brown, M. E. The Prout-Tompkins Rate Equation in Solid-State Kinetics. Thermochim. Acta 1997, 300, 93.

Gabriel, C.; Raptopoulou, C. P.; Terzis, A.; Tanguoulis, V.; Mateescu, C.; Salioglou, A. PH-Specific Synthesis, and Spectroscopic, Structural, and Magnetic Studies of a Chromium(III)−Citrate Species. Aqueous Solution Speciation of the Binary Chromium(III)−Citrate System. Inorg. Chem. 2007, 46, 2998−3009.

Huyinh, K. A.; Chen, K. L. Aggregation Kinetics of Citrate and Polyvinylpyrrolidone Coated Silver Nanoparticles in Monovalent and Divalent Electrolyte Solutions. Environ. Sci. Technol. 2011, 45, 5564−5571.

Shrivas, K.; Sahu, S.; Patra, G. K.; Jaiswal, N. K.; Shankar, R. Localized Surface Plasmon Resonance of Silver Nanoparticles for Sensitive Colorimetric Detection of Chromium in Surface Water, Industrial Waste Water and Vegetable Samples. Anal. Methods 2016, 8, 2088−2096.

Tripathi, A.; Emmons, E. D.; Christensen, S. D.; Fountain, A. W.; Guichetoua, J. A. Kinetics and Reaction Mechanisms of Thiophenol Adsorption on Gold Studied by Surface-Enhanced Raman Spectroscopy. J. Phys. Chem. C 2013, 117, 22834−22842.

Lopata, A.; Jójtárt, B.; Surányi, E. V.; Takács, E.; Beatrix, L.; Leveles, I.; Bendes, A. A.; Viskolcs, B.; Vértessy, G.; Tóth, B. Beyond Chelation: EDTA Tightly Binds Taq DNA Polymerase, MutT and DUTPase and Directly Inhibits DUTPase Activity. Biomolecules 2019, 9, 621.

Hedrick, C. E. Formation of the Chromium-EDTA Complex: An Undergraduate Kinetics Experiment. J. Chem. Educ. 1965, 42, 479.

Dutta, A.; Paul, A.; Chattopadhyay, A. The Effect of Temperature on the Aggregation Kinetics of Partially Bare Gold Nanoparticles. RSC Adv. 2016, 6, 82138.

Cerar, J. Reaction between Chromium(III) and EDTA Ions: An Overlooked Mechanism of Case Study Reaction of Chemical Kinetics. J. Chem. Educ. 2015, 6, 538−545.

Wacker, M.; Seubert, A. Determination of Stability Constants of Ligand Complexes Using Anion or Cation Exchange Chromatography and Atomic Spectrometry Detection. J. Anal. At. Spectrom. 2014, 29, 707.
(50) Karpovich, D. S.; Blanchard, G. J. Direct Measurement of the Adsorption Kinetics of Alkanethiolate Self-Assembled Monolayers on a Microcrystalline Gold Surface. *Langmuir* 1994, *10*, 3315−3322.