

## Article

# Consequences of Surface Composition and Aggregation Conditions of Ag Nanoparticles on Surface-Enhanced Raman Scattering (SERS) of Pesticides

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**Abstract:** Surface-enhanced Raman scattering (SERS) is highly dependent on the adsorption of target molecules onto metallic surfaces, such as colloidal metallic nanoparticles. The selection of suitable substrates is crucial for optimizing SERS performance. Herein, we investigated the dependence of two pesticide SERS signals, thiabendazole (TBZ) and carbendazim (MBC), on both Ag nanoparticles (reduced by hydroxylamine—AgH or citrate—AgCT) and the aggregation conditions induced by adding different salts (NaCl, KCl or KNO<sub>3</sub>). In addition to SERS experiments, in order to assess the induced aggregation of the Ag nanoparticles, UV-Vis absorption spectroscopy, dynamic light scattering (DLS) and zeta potential were employed. For AgH, the use of salts did not yield the greatest effect in the presence of TBZ, as only with the pesticide was it possible to achieve the highest aggregation and greater intensity of the SERS signal. In contrast, with the MBC pesticide, the KNO<sub>3</sub> salt promoted the greatest aggregation state and was crucial for obtaining the most amplified SERS signal. The thicker coating layer of AgCT prevented the adsorption of both pesticides on the surface of the nanoparticles, which was achievable using salts containing Cl<sup>−</sup> ions. Additionally, to obtain the SERS signal of MBC with AgCT, besides the presence of chlorinated salts, other adjustments were necessary, such as changing both the pH of the medium (from pH 5.8 to pH 8, for which MBC is in its neutral form) and the laser lines (from 785 to 514.5 nm). These findings demonstrated that although the pesticide molecules belong to the same chemical functional group, their detection was strongly influenced by the surface of the silver nanoparticles and the salts added. This highlights the specific nuances in detection depending on the method of Ag synthesis and the nature of the aggregating agents used.

**Keywords:** SERS; Ag nanoparticles; nanoparticles aggregation; pesticides detection



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## 1. Introduction

Pesticides are extensively applied in several crops, and their persistence in residues of food and ground water has become a risk to health and the environment. These pesticides are applied frequently, becoming saturated in soil and crops. Their frequent application leads to accumulation in soil and crops. Due to their high toxicity and slow degradation [1],

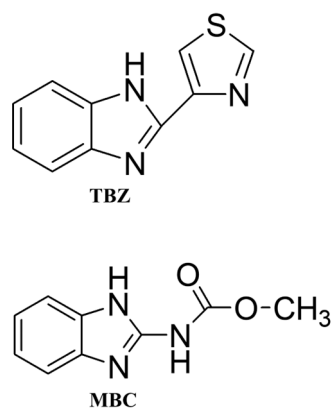
it is crucial to monitor and determine the presence of these compounds in soil, food and water samples [2]. Among a wide range of products, the fungicides belonging to benzimidazole groups, such as thiabendazole (TBZ) and carbendazim (MBC), present high-efficiency, broad-spectrum and systemic properties, i.e., they are absorbed and transported to all parts of the plant [3]. Surface-enhanced Raman scattering (SERS) is a technique that conciliates the ability to recognize compounds with sensibility in low concentrations, up to the limit of single molecule detection [4,5]. Signal amplification in SERS primarily arises from both electromagnetic and chemical mechanisms. In the electromagnetic mechanism, the analyte benefits from the near-electrical field of the nanostructure, while the chemical mechanism entails a charge transfer between the analyte and the nanostructure [6]. Among the array of SERS substrates available, colloidal nanoparticles hold prominence, owing to their ease of synthesis, cost-effectiveness and versatility in terms of functionalization and morphology tuning. Further, colloidal AgNPs were selected for this work due to their well-known advantages as SERS substrates, including their strong plasmonic activity, ease of preparation and tunable size and shape [7]. These properties make AgNPs particularly suitable for enhancing Raman signals of analytes like pesticides, which often require high sensitivity for detection at trace levels. Additionally, AgNPs exhibit versatility in interacting with analytes through both electromagnetic and chemical enhancement mechanisms, allowing us to study adsorption phenomena and analyte–surface interactions in detail. Their use also aligns with the goal of optimizing SERS conditions for real-world applications, where scalability and reproducibility are essential.

The efficacy of SERS experiments depends on proximity and affinity between analyte and nanoparticle, emphasizing the critical significance of the chemical properties of the nanoparticle surface. In this regard, a variety of synthesis routes have been developed, resulting in distinct chemical surface properties [8]. Although variations in the growth of colloidal nanoparticles can be observed with different anionic precursors of silver (such as  $\text{Ag}_2\text{SO}_4$ ,  $\text{AgClO}_4$  or  $\text{AgNO}_3$ ) [9], relevant differences can be accessed from the choice of reduction agent. For instance, hydroxylamine hydrochloride, trisodium citrate dihydrate and sodium borohydride have been employed as reduction agents, resulting in differing chemical surface properties of the nanoparticles [10]. The adsorption process is a key point in achieving the SERS effect involving colloidal nanoparticles, as it necessitates the interaction of the analyte with nanoparticles that exhibit an affinity for the chemical surface. This interaction can be facilitated by electrostatic forces or chemical bonds [11,12]. In SERS experiments using colloidal nanoparticles, the use of salts to promote the colloidal aggregation is common. This practice enhances the formation of regions with maximal electric field enhancement, often referred to as “hot-spots”, increasing the SERS signal. Several salts are employed for this purpose, including monovalent, divalent and trivalent cations [13,14]. It has been observed that divalent and trivalent salts induce nanoparticle aggregation at lower concentrations compared to monovalent salts. Notably, Khrushchev et al. [15] report a synergistic effect between divalent cations in the presence of chloride ions to produce higher SERS intensity. Their study demonstrates that chloride ions facilitate the adsorption of malachite green onto the nanoparticle surface, while divalent salts promote more effective colloidal aggregation. In fact, chloride ions are frequently utilized in SERS experiments involving colloidal nanoparticles due to their capacity to “activate” the colloid [10]. However, there is not a consensus regarding the optimal methodology for achieving substantial signal amplification through the combined use of reduction agents (impacting nanoparticle chemical surface) and salts (influencing nanoparticle aggregation). In this study, we investigate the influence of nanoparticle chemical surface properties and the use of the salts NaCl, KCl and  $\text{KNO}_3$  in SERS experiments involving two pesticides, thiabendazole (TBZ) and carbendazim (MBC). To this end, we have selected

two commonly used spherical silver nanoparticles: those reduced using hydroxylamine (referred to as AgH) and those reduced using citrate (referred to as AgCT). Our experiments encompass Raman scattering and UV-Vis extinction spectroscopy techniques, as well as complementary assessments involving dynamic light scattering (DLS) and zeta potential measurements.

## 2. Materials and Methods

The chemicals TBZ and MBC (Figure 1), hydrochloride hydroxylamine, sodium hydroxide, trisodium citrate trihydrate, NaCl, KCl and KNO<sub>3</sub> were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA). The Milli-Q system provided ultrapure water (18 MΩ.cm resistivity and pH 5.6). TBZ and MBC were dissolved in ethanol stock solutions ( $1.0 \times 10^{-3}$  mol/L). All experiments with the obtained colloids were performed in an aqueous medium. The stock solutions were prepared in ethanol due to the low solubility of the pesticides in water (less than  $10^{-3}$  mol/L). During measurements, only 1% ethanol was used in the colloids.



**Figure 1.** Molecular structures of the pesticides TBZ and MBC.

### 2.1. AgH and AgCT Synthesis

The protocol for the AgH synthesis was similar to that described by Leopold and Lendl [16]. Briefly, it was prepared as a solution containing 15.4 mg of silver nitrate in 10 mL of ultrapure water. Using an Erlenmeyer flask, 90 mL of ultrapure water, 300 µL of 1.0 mol/L NaOH solution and 10.4 mg of hydroxylamine hydrochloride were added and stirred. A volume of 10 mL of silver nitrate solution ( $1.0 \times 10^{-2}$  mol/L) was added drop-wise to the solution. After the silver nitrate addition, the solution changed the color from transparent to a green-grey aspect, confirming the formation of nanostructures.

The AgCT was prepared using the method described by Adianez Garcia-Leis et al. [17] based on the previously described Lee and Meisel method [18] and here summarized. An amount of 8.5 mg of silver nitrate was added into 50 mL of ultrapure water and placed in a heating device (under reflux). After the boiling process, 1 mL of citrate solution (1% *w/v*) was added to the nanoparticle formation and maintained for 1 h.

### 2.2. SERS, UV-Vis, DLS and Zeta Potential Measurements

SERS measurements were performed in a micro-Raman spectrograph Renishaw in-Via model, equipped with a CCD camera. The spectra were recorded using the laser line at 785 nm and diffraction grating with 1200 L/mm or laser line at 514.5 nm and diffraction grating with 1800 L/mm. In both setups, 10 s of integration time and 1 accumulation were applied. The laser power was adjusted accordingly to avoid saturation, and offset correction was applied in the spectra. SERS measurements were performed as follows: 960 µL of the different colloids (AgH or AgCT) was added in a cuvette, and afterwards

40  $\mu\text{L}$  of the salt solution ( $\text{NaCl}$ ,  $\text{KCl}$  or  $\text{KNO}_3$ , 0.5 mol/L) was added and shaken. The Raman spectra of AgH and AgCT were obtained and are shown in Figure S1. Furthermore, a qualitative identification of the pesticides TBZ and MBC was performed by acquiring Raman spectra of the pesticide powders (Figure S2A,B). Then, 10  $\mu\text{L}$  of the mixture was removed, and then 10  $\mu\text{L}$  of TBZ or MBC in ethanol solution ( $1.0 \times 10^{-3}$  mol/L) was added. This resulted in 1000  $\mu\text{L}$  of suspension, with a final concentration of  $1.0 \times 10^{-5}$  mol/L for TBZ or MBC and  $2.0 \times 10^{-2}$  mol/L of salt ( $\text{NaCl}$ ,  $\text{KCl}$  or  $\text{KNO}_3$ ). The Raman Enhancement Factor (EF) [19] used to quantify the increase in Raman signal intensity on an enhanced surface was determined following the Equation (1)

$$EF = \frac{\frac{I_{\text{SERS}}}{M_{\text{ads}}}}{\frac{I_{\text{Raman}}}{M_{\text{bulk}}}} \quad (1)$$

where  $I_{\text{SERS}}$  is the Raman intensity measured under SERS conditions,  $M_{\text{ads}}$  is the number of molecules on the SERS surface contributing to the signal,  $I_{\text{Raman}}$  is the Raman intensity measured under standard conditions and  $M_{\text{bulk}}$  is the number of molecules in the sample contributing to the Raman signal. For this purpose, calculations were performed by comparing the SERS intensity divided by the number of molecules present in the SERS sample, with the Raman intensity (for  $10^{-3}$  mol/L ethanol solutions of pesticides) divided by the number of molecules present in the Raman sample. The measurements were performed under the same experimental conditions (laser power and accumulation) or by applying the necessary conversions. Equation (1) illustrates how this calculation was performed.

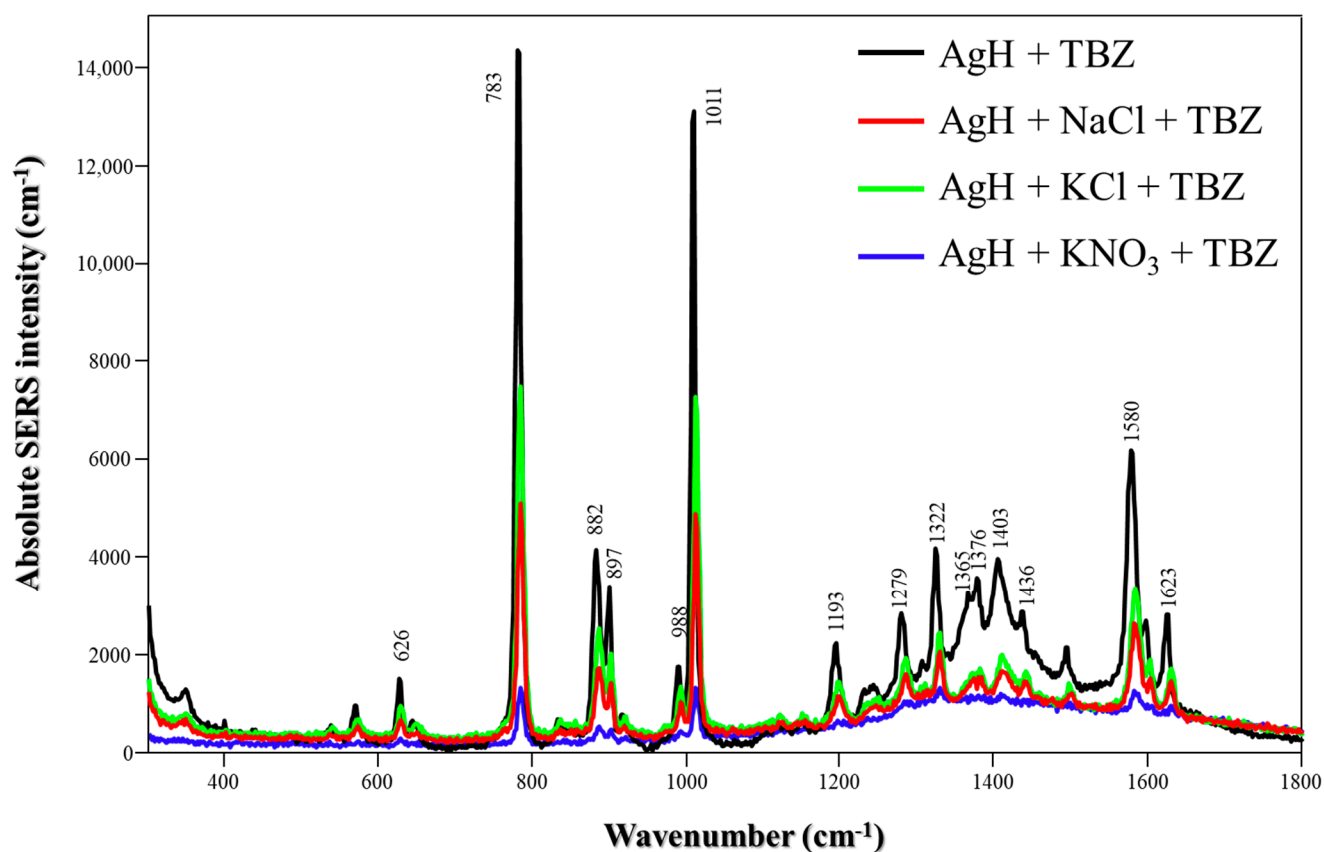
For UV-Vis, DLS and zeta potential measurements, 1 mL of the sample described above was diluted in 2 mL of ultrapure water. The extinction spectra were carried out in a Varian UV-Vis spectrophotometer, model Cary50, in a spectral range between 190 and 1100 nm. The colloidal dispersions were further characterized by DLS and zeta potential at 23 °C using a Malvern Zetasizer Nano ZS90 (Malvern, Worcestershire, UK). Size measurements were performed using a scattered angle of 90°, and the data were reported as Z-average (per intensity and volume). The voltage for zeta potential was adjusted automatically by the software.

### 3. Results and Discussion

#### 3.1. SERS of TBZ and MBC Using AgH and Salts

Figure 2 illustrates the absolute SERS spectra of TBZ in the presence of either neat AgH NPs or AgH colloids and salts. The profile spectra are similar to each other and consist of two main peaks at 783 and 1011  $\text{cm}^{-1}$ , which are assigned to C-S stretching and C-N stretching, respectively [20]. Also, the peaks at 882 and 897  $\text{cm}^{-1}$  are thiazole features, including the C-S stretching mode and in-plane deformation of the ring [21] at 1580 and 1623  $\text{cm}^{-1}$ , assigned to C-C stretching of the benzimidazole moiety [22].

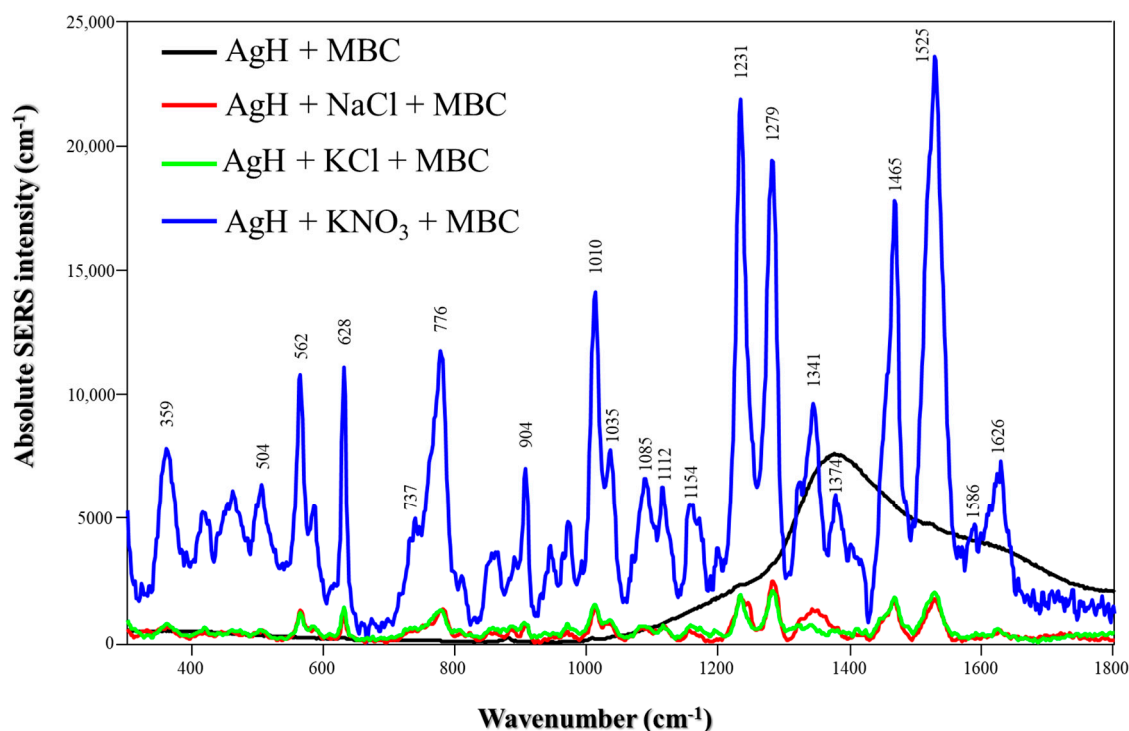
The significant differences were evident in the intensities of the spectra. The highest enhanced signals were achieved using AgH NPs without salts. With the addition of salts, comparable signal intensities could be obtained in the AgH + TBZ + NaCl/KCl systems. Conversely, incorporating  $\text{KNO}_3$  resulted in the lowest SERS intensity. Overall, the enhancement was strongly correlated with the nanoparticle aggregation level. Although the amplification signal varied depending on the type of salt used (due to its aggregation tendency), no new peaks or changes in the relative peak intensities were observed. Thus, for AgH synthesis, the presence of salts does not significantly affect the adsorption of TBZ onto the nanoparticle surface, in which the S atom of TBZ plays a crucial role in interacting with the Ag surface, as previously demonstrated by Oliveira et al. [23].



**Figure 2.** Absolute TBZ SERS spectra with different salts (NaCl, KCl or KNO<sub>3</sub>) using AgH NPs at pH 5.8. Laser: 785 nm.

The absolute MBC SERS spectra obtained using AgH and different salts are presented in Figure 3. In contrast to TBZ, MBC does not exhibit characteristic Raman peaks on AgH NPs in the absence of salts. This is probably due to its lower affinity for the NP surface. Using KNO<sub>3</sub>, the most intense peaks appearing at 1231, 1279, 1465 and 1525 cm<sup>−1</sup> are assigned to N-H bending (amine moiety), N-H bending (imidazole moiety), N-H bending (amine moiety) + C-H bending (methyl) and N-H bending (amine moiety), respectively [24]. Also, the less intense peaks at 562, 628 and 1010 cm<sup>−1</sup> are attributed to N-H bending (imidazole moiety) + C-H bending (benzene ring), C-C bending (benzene ring) and C-N bending (imidazole moiety) + C-C bending (benzene ring) + C-O-CH<sub>3</sub> stretching [24], which is approximately four times higher compared to nanoparticles in the presence of NaCl or KCl. This indicates that KNO<sub>3</sub> is the most suitable salt for designing SERS sensors for MBC detection. The influence of salts on the detection of TBZ and MBC was also analyzed using EF calculations (Table S1), which confirmed the same amplification trends observed in the Raman spectra.

The influence of salts on Ag nanoparticles was also investigated. Raman spectra of AgH were recorded both with and without NaCl, KCl and KNO<sub>3</sub>, as shown in Figure S3A. The peak at 240 cm<sup>−1</sup>, corresponding to Ag-Cl [25–27] stretching, was observed in both the neat AgH spectra (due to the chloride ions present from synthesis) and in the presence of all the salts. Furthermore, in the SERS spectra of both TBZ and MBC using AgH (Figure S3B,C), no peak shifts associated with Ag-Cl were observed, regardless of the salt used.



**Figure 3.** Absolute MBC SERS spectra with different salts (NaCl, KCl or  $\text{KNO}_3$ ) using AgH NPs at pH 5.8. Laser: 785 nm.

### 3.2. AgH Aggregation in the Presence of Salts, TBZ and MBC

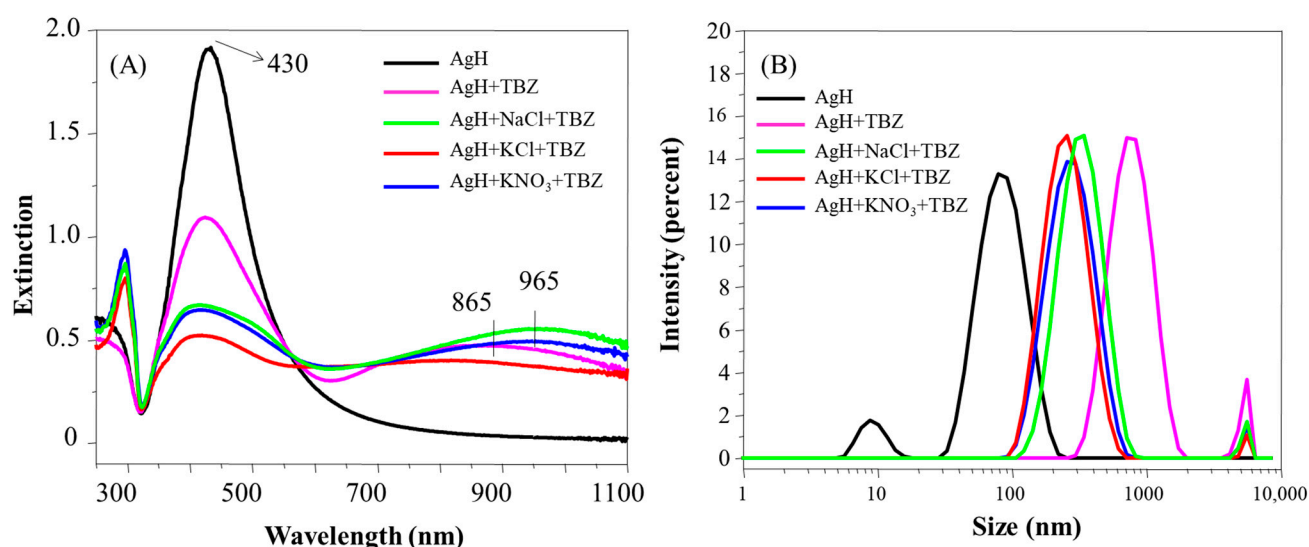
Figure S4A presents the UV-Vis spectra of AgH nanoparticles added to TBZ in the absence and presence of the salts NaCl, KCl and  $\text{KNO}_3$ . The localized surface plasmon (LSP) peak intensity was centered at 430 nm, typical of the spectra found in the literature [16], corresponding to isolated Ag nanoparticles. With the addition of NaCl, KCl or  $\text{KNO}_3$  to AgH dispersion, the LSP peak decreased, and the extinction intensity at higher wavelengths slightly increased (700–1100 nm), independent of the salt added. Such an effect indicates the formation of small aggregates induced by the salt, without precipitation. The instability of the Ag nanoparticles and precipitation due to colloidal coagulation (bigger aggregates) was observed by Futamata et al. [28] only after the addition of concentrations higher than 50 mM of salt. Under our conditions, the colloid aggregation indicated by the UV-Vis extinction was caused by the addition of NaCl, KCl or  $\text{KNO}_3$  salts (20 mM) maintaining the colloidal stability.

Complementarily, the formation of aggregates was studied by DLS and zeta potential techniques. AgH dispersion presents two populations with intensities around 10 and 70 nm (Figure S4B), and, according to volume measurements (Figure S5), the hydrodynamic diameter is predominantly around 10 nm. Also, the polydispersity index was 0.301 (Table 1), which is typical from this synthesis, with predominantly spherical nanoparticles [29]. Likewise, verified by UV-Vis spectra, the size distribution confirmed that the presence of the salts does not promote pronounced aggregation (especially in comparison with the aggregation induced by TBZ and MBC pesticides, presented in Figures 2 and 3), with the two populations remaining at 10 and 90 nm. Only slight changes were observed, such as a decrease in the intensity, displacement and enlargement of the distribution band, marked by arrows in Figure S4B. Also, the zeta potential values were not disturbed in the presence of salts, maintaining values between  $-36$  and  $-38$  mV (Table 1).

**Table 1.** Polydispersity index and zeta potential of AgH samples at pH 5.8.

| Sample                       | PDI   | Zeta Potential (mV) |
|------------------------------|-------|---------------------|
| AgH                          | 0.301 | $-36 \pm 1$         |
| AgH + KNO <sub>3</sub>       | 0.410 | $-36 \pm 2$         |
| AgH + KCl                    | 0.388 | $-38 \pm 2$         |
| AgH + NaCl                   | 0.387 | $-37 \pm 2$         |
| AgH+ TBZ                     | 0.368 | $13 \pm 1$          |
| AgH + KNO <sub>3</sub> + TBZ | 0.301 | $18 \pm 0.5$        |
| AgH + KCl + TBZ              | 0.336 | $28 \pm 1$          |
| AgH + NaCl + TBZ             | 0.322 | $13 \pm 2$          |
| AgH + MBC                    | 0.378 | $-18 \pm 1$         |
| AgH + KNO <sub>3</sub> + MBC | 0.268 | $-5 \pm 2$          |
| AgH + KCl + MBC              | 0.495 | $-32 \pm 1$         |
| AgH + NaCl + MBC             | 0.331 | $-31.0 \pm 0.3$     |

Figure 4A presents the UV-Vis extinction spectra of AgH nanoparticles added to TBZ in the absence and presence of the salts NaCl, KCl and KNO<sub>3</sub>. The LSP intensity decreased and shifted from 430 nm to 424 nm. Additionally, the joint addition (TBZ + salts) further shifted the LSP band to smaller wavelengths (424 nm). A new band appeared at higher wavelengths (700–1100 nm), attributed to the higher affinity of TBZ to the nanoparticle surface [23], forming coupled LSP of AgH. The formation of AgH + TBZ aggregates was more sensitive to the presence of KCl compared to NaCl and KNO<sub>3</sub>, following the aggregation tendency of the Hofmeister series. It is also important to stress that, in the presence of the salts, a band emerges at 295 nm, coincident with the band of the neat TBZ solution [30], associated with the TBZ species not adsorbed onto the nanoparticle surface.

**Figure 4.** Extinction spectra (A) and hydrodynamic diameter distribution (B) of AgH with TBZ in the presence and in the absence of the salts NaCl, KCl or KNO<sub>3</sub>. pH 5.8.

The size distribution of AgH in the presence of either TBZ or TBZ + salts is given in Figure 4B. TBZ was responsible for the disappearance of the population of nanoparticles at 10 nm, indicating the absence of isolated nanoparticles in the AgH + TBZ and AgH + TBZ + salts systems. The larger aggregation was induced after TBZ addition, in either the absence or presence of the salts, which, in some way, seems to modulate the colloidal aggregation. When only TBZ was present, large aggregates were formed (diameter ~1000 nm), while with salts (NaCl, KCl or KNO<sub>3</sub>), the most were around 300 nm (Figure S4B). The decrease of aggregate size promoted by the presence of salt

on the dispersion may be a result of the ion solvation by the dipoles of the TBZ molecules and the reason for the UV band at 295 nm to have emerged (with free TBZ not adsorbed onto the nanoparticle surface). The efficient aggregation of nanoparticles in the presence of TBZ leads to a zeta potential increase from  $-36$  mV of AgH to  $13$  mV of AgH + TBZ (Table 1). The positive zeta potential values in the presence of TBZ are also maintained when the salts are added. The colloidal stability depends on nanoparticle size and zeta potential, being stable at  $<-20$  mV or  $>20$  mV [31,32]. In our case, the adsorption of TBZ increases the zeta potential, inducing the aggregation.

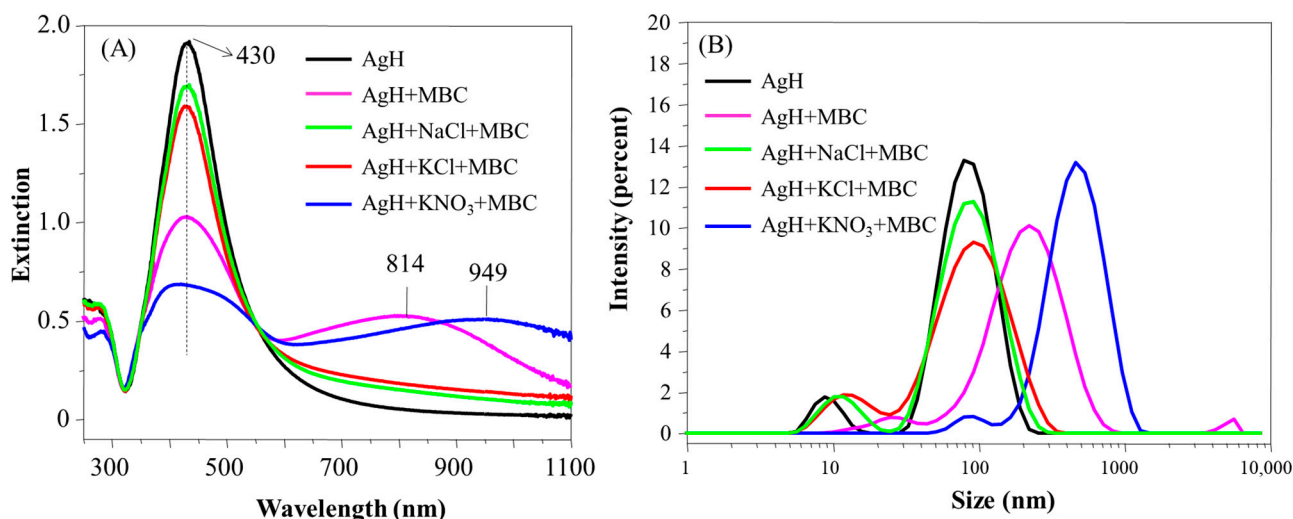
The changes to the AgH nanoparticle plasmons vary depending on the type of salts added. The KCl salt was responsible for the higher decrease in AgH aggregation, followed by NaCl and  $\text{KNO}_3$ , with the spectra practically overlapping. The different effects can be discussed according to the relation between the Hofmeister series and colloidal particle aggregation [33–35]. Basically, the Hofmeister series is a sequence of ions used to explain various scientific phenomena occurring in the presence of salts. In this context, charged particles remain stable or aggregated depending on the salt present in the medium. The typical sequence for colloidal stability [34] is  $\text{CO}_3^{2-} < \text{SO}_4^{2-} < \text{H}_2\text{PO}_4^- < \text{F}^- < \text{Cl}^- < \text{Br}^- < \text{I}^- < \text{NO}_3^- < \text{ClO}_4^- < \text{SCN}^-$  for anions and  $\text{N}(\text{CH}_3)_4^+ < \text{NH}_4^+ < \text{Cs}^+ < \text{Rb}^+ < \text{K}^+ < \text{Na}^+ < \text{H}^+ < \text{Ca}^{2+} < \text{Mg}^{2+}$  for cations. Ordinarily, the negatively charged particles follow the Hofmeister series directly [33]. Further, the cations (counterions) are relevant for negatively charged particles, whereas the anions are relevant for positively charged particles [36]. Therefore, considering the ions used here, it is expected that the KCl salt causes the higher instability of the Ag colloid, promoting the more effective aggregation in the colloids, probably by the influence of  $\text{K}^+$  ions in the negatively charged AgH.

The presence of MBC on AgH colloidal dispersion induced similar TBZ effects in the UV-Vis spectra (Figure 5A), with the decrease of the LSP band and appearance of a new band at 700–1100 nm being more expressive in the AgH + MBC +  $\text{KNO}_3$  system, which was able to promote the appearance of the LSP band at 405 nm (in addition to 430 nm). Indeed, even the spectral alterations on AgH + MBC (without salt) were more pronounced than when either NaCl or KCl were added to the colloid. The band below 300 nm related to the MBC not adsorbed was not observed [37,38], indicating the poor solvation capability of MBC molecules. By excluding the ionic species studied (as a result of the absence of effects with NaCl and KCl), it can be inferred that the ion  $\text{NO}_3^-$ , in conjunction with MBC, promoted the greatest nanoparticle aggregation. López-León et al. [34] studied the colloidal stability of negative and positive nanoparticles of several ions, including  $\text{Cl}^-$  and  $\text{NO}_3^-$ . The authors inferred that the  $\text{NO}_3^-$  ions can accumulate more efficiently onto the negative nanoparticle surface than the  $\text{Cl}^-$  ions, attributing such phenomena to the action of dispersion forces at the particle–water interface. In our case, with the accumulation of the  $\text{NO}_3^-$  anions near the negative AgH surface, the charge density also increases, which would provide a greater adsorption of the MBC dipoles on the surface of the nanoparticles. The AgH + MBC +  $\text{KNO}_3$  system favored the formation of the higher aggregates, around 800 nm (Figure 5B), and was also accompanied by a zeta potential increase related to the MBC adsorption onto the nanoparticle surface and increased instability (Table 1).

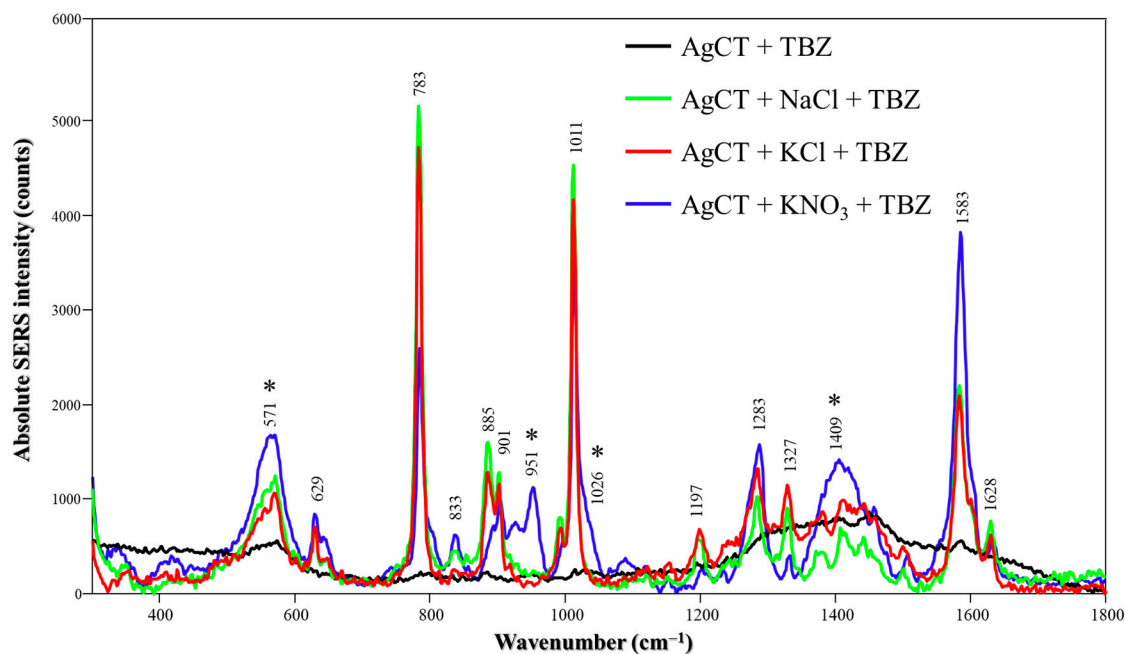
### 3.3. SERS of TBZ and MBC Using AgCT and Salts

The SERS spectra of TBZ on AgCT NPs are shown in Figure 6. A SERS signal was observed only in the presence of salts. The SERS spectrum displayed a similar spectral profile and peak attributions as observed with AgH; however, some variations were noted depending on the specific salt used. While the peaks seen for TBZ in AgCT/NaCl or KCl are similar to those seen on AgH nanoparticles, the TBZ SERS spectrum under AgCT/ $\text{KNO}_3$  conditions displays features at 571, 952, 1026 and  $1409\text{ cm}^{-1}$  (marked with asterisks) and

also presents the spectra containing only AgCT in presence of  $\text{KNO}_3$  (Figure S6). The bands are assigned to citrate ions [39] and may indicate that both TBZ and citrate ions adsorb onto the nanoparticle surface when  $\text{KNO}_3$  is used to activate the system. In contrast, the citrate signal is entirely absent with NaCl or KCl, suggesting that chloride ions are able to replace citrate from the surface. Additionally, the SERS intensity, and consequently the signal-to-noise ratio, using NaCl and KCl salts was higher than with  $\text{KNO}_3$  as a consequence of the replacement of citrate by chloride ions and the promotion of ionic interactions with positively charged groups in TBZ.



**Figure 5.** Extinction spectra (A) and hydrodynamic diameter distribution (B) of AgH with MBC in the presence and in the absence of the salts NaCl, KCl or  $\text{KNO}_3$ . pH 5.8.

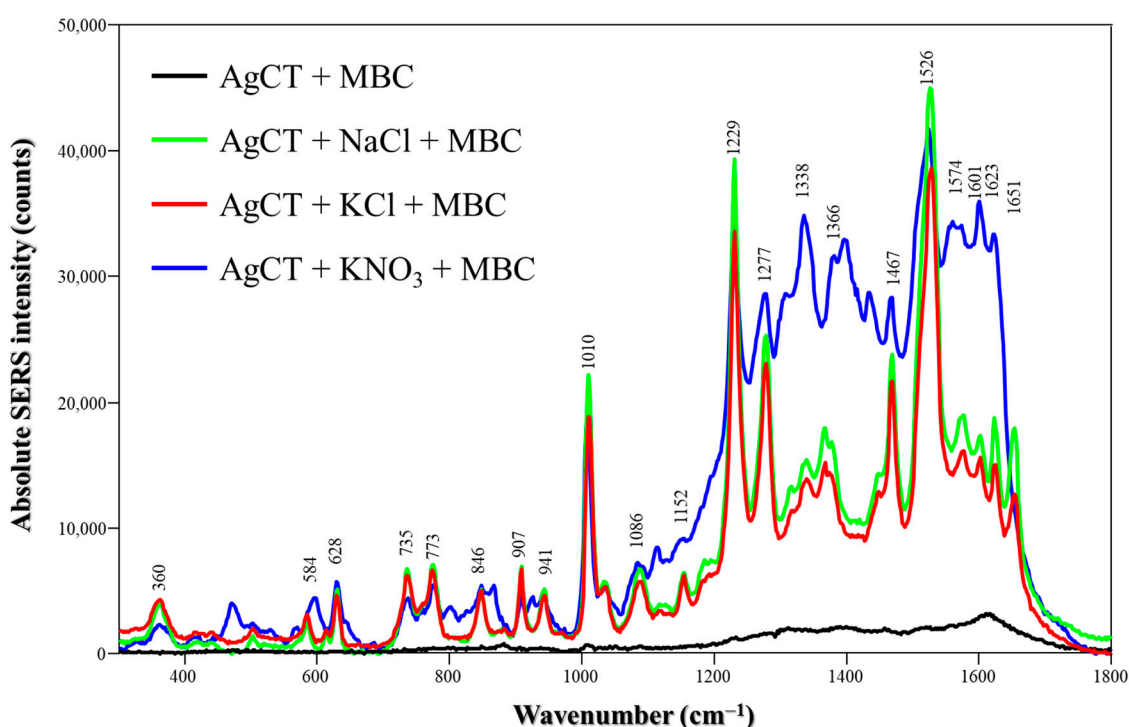


**Figure 6.** Absolute TBZ SERS spectra in the absence and presence of different salts (NaCl, KCl or  $\text{KNO}_3$ ) using AgCT NPs at pH 5.8. Laser: 785 nm. The asterisks indicate peaks that coincide with the AgCT spectrum exclusively in the presence of  $\text{KNO}_3$ .

For MBC detection using AgCT, the presence of salts in the colloid was not sufficient to obtain a suitable SERS signal (Figure S7), indicating a low affinity of MBC for the AgCT nanoparticle surface. To explore whether other experimental parameters can induce the

detection of MBC using AgCT, the laser line at 514.5 nm was employed. In addition, taking advantage our prior findings on the study of the MBC SERS pH range [24], which indicate that the SERS species correspond to the neutral MBC, experiments at pH 8 (neutral form of MBC) were conducted. The change in laser line from 785 to 514.5 nm itself had no impact on the appearance of the SERS signal from the MBC, as demonstrated in Figure S8. The peaks observed from the 1250 to 1750  $\text{cm}^{-1}$  region of the spectra are attributed to by-products or an oxide layer formed during the synthesis [40].

Lastly, MBC SERS spectra using AgCT were obtained by changing the medium to pH 8 and using the laser line at 514.5 nm (Figure 7). The spectra are dominated by vibrations from amidic groups (N-H deformation at 1229, 1467 and 1526  $\text{cm}^{-1}$ ) or imidazolic groups (N-H deformation at 1277  $\text{cm}^{-1}$  and C-N deformation at 1010  $\text{cm}^{-1}$ ); MBC presents  $\text{pK}_{\text{a}1} = 4.5$  and  $\text{pK}_{\text{a}2} = 10.6$ , and the molecules are at neutral form at pH 8 [38]. We previously published findings on the affinity of MBC for silver surfaces using AgH as the SERS substrate, focusing on its behavior in different pH environments [24]. Our results indicated that neutral and negatively charged molecules produce a significant SERS signal, with the spectra predominantly reflecting neutral molecules adsorbed onto the nanoparticle surface through the imidazole moiety. It is plausible to hypothesize that in a higher pH medium, molecular solubility increases, promoting adsorption. For the synthesis of AgCT, the EF calculations (Table S1) demonstrated that the effect of salts on the detection of TBZ and MBC corresponded with the amplification observed in the Raman spectra.



**Figure 7.** Absolute MBC SERS spectra in the absence and presence of different salts (NaCl, KCl or  $\text{KNO}_3$ ) using AgCT NPs at pH 8. Laser: 514.5 nm.

In the low-frequency region of the AgCT spectra, as shown in Figure S9A, no peaks were observed in the absence of salts. However, in the presence of NaCl or KCl, peaks at 240 and 234  $\text{cm}^{-1}$  appear, which are assigned to Ag-Cl stretching vibrations [25–27]. In contrast,  $\text{KNO}_3$  does not seem to displace the citrate ions from the nanoparticle surface, as evidenced by the appearance of a peak at 228  $\text{cm}^{-1}$  in the presence of the salt, which is attributed to Ag-O stretching from the citrate adsorbed layer [41]. Since this peak is absent in the AgCT spectra without salt, we suggest that the signal may arise from possible

nanoparticle aggregation in the presence of  $\text{KNO}_3$ . Additionally, the chloride ions from NaCl or KCl are adsorbed onto the nanoparticle surface, replacing the citrate ions previously adsorbed. This finding is consistent with the strong affinity between chloride ions and silver and further supported by XPS measurements [11,42], showing the replacement of previously adsorbed ions by chloride ions.

The addition of TBZ to AgCT nanoparticles in the presence of both NaCl and KCl did not alter the bands associated with Ag-Cl stretching vibrations. These results suggest that chloride ions remain on the surface even after TBZ adsorption. However, the introduction of  $\text{KNO}_3$  (Figure S9B) led to the appearance of a peak at  $220\text{ cm}^{-1}$ , attributed to Ag- $\text{NH}_4$  [43]. Moreover,  $\text{KNO}_3$  seems to influence the adsorption of TBZ onto the surface of AgCT nanoparticles. Although the interaction primarily occurs via the S atom, the benzimidazole group (containing the nitrogen group) remains in close proximity to the surface. This finding was further confirmed by the increased peak intensities at  $1283$  and  $1583\text{ cm}^{-1}$  in the presence of  $\text{KNO}_3$  (Figure 6). These peaks correspond to C-C stretching in the benzimidazole moiety of TBZ, also indicating closer proximity of the benzimidazole moiety to the nanoparticle surface.

For MBC using AgCT and chloride salts (Figure S9C), the peak appearing at  $223\text{ cm}^{-1}$  is assigned to AgCl-NH, being the Ag nanoparticle surface bonded to the imidazolic MBC group, as reported previously by our group [24]. In contrast, in the presence of  $\text{KNO}_3$ , the band that emerged at  $212\text{ cm}^{-1}$  is likely associated with the vibration of Ag-O [44], due to the higher proximity of the MBC amide group to the Ag nanoparticle surface. This can also be confirmed by the increased intensity of the band at  $1338\text{ cm}^{-1}$  and the appearance of a new band at  $1601\text{ cm}^{-1}$ , both attributed to the NH bending of the amide group.

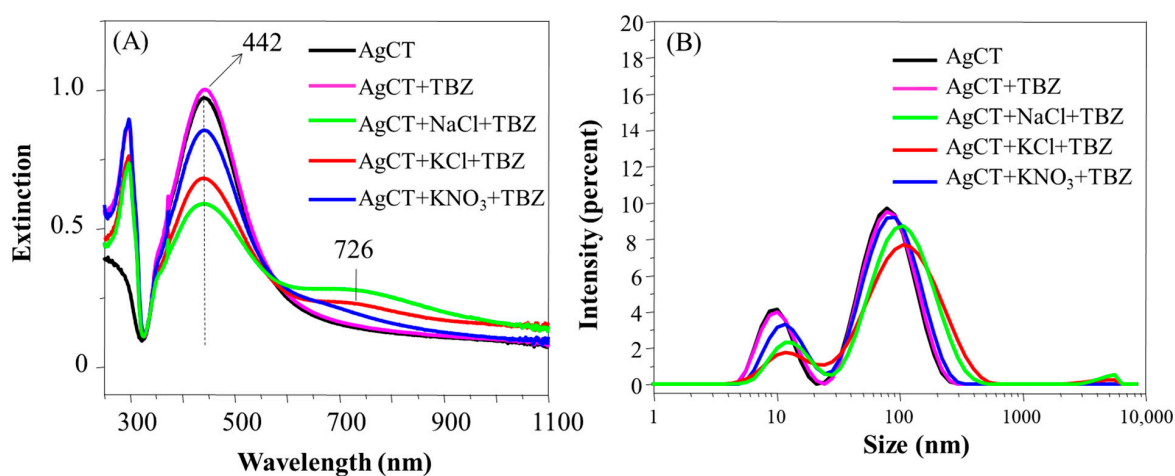
### 3.4. AgCT Aggregation in the Presence of Salts, TBZ and MBC

The aggregation behavior of the AgCT in the presence of the pesticides containing or not containing the salts NaCl, KCl or  $\text{KNO}_3$  was also studied via UV-Vis spectra, DLS and zeta potential. Figure S10A shows the UV-Vis extinction spectra of the AgCT nanoparticles, classical for the LSP band of citrate-silver nanoparticles in the range of 300–600 nm and with the maximum of the extinction band centered at 430 nm. The spectral changes caused by the addition of the salts in the AgCT were even smaller than those observed for the AgH colloid. Indeed, the citrate promotes a higher stabilization of the Ag colloid compared to other reductants [45]. The citrate ion layer on the nanoparticles is thicker, which makes it more repellent, impeding further aggregation [46] and, consequently, the achievement of the SERS signal for pesticides in the absence of salts. The minimal effects verified in the UV-Vis spectra of the AgCT nanoparticles in the presence of salts were not enough to cause any variation in both size distribution and zeta potential values of the nanoparticles (Figure S10B and Table 2, respectively). This makes it evident that the ion specificity (Hofmeister effects) towards AgCT does not play a determining role in aggregation.

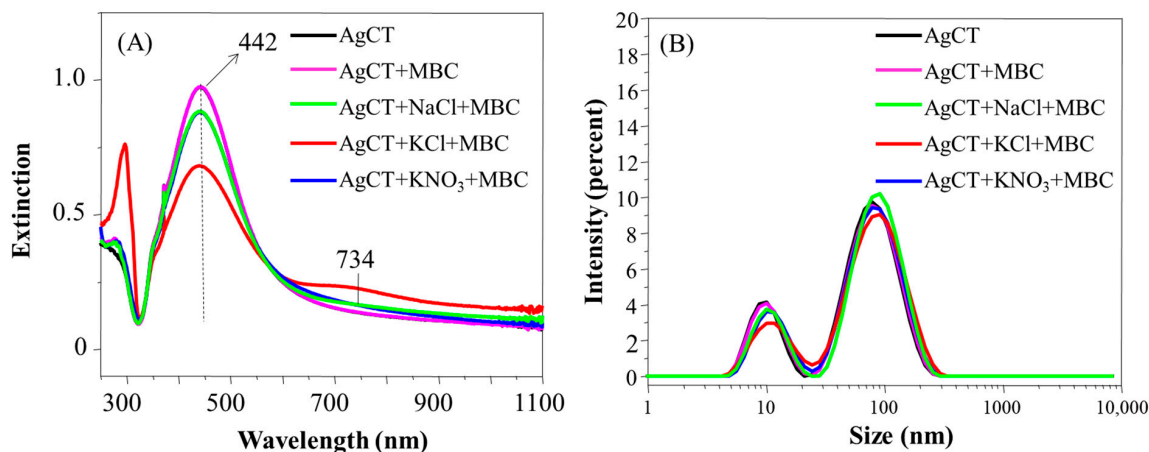
The presence of TBZ was also not capable of inducing the aggregation of AgCT nanoparticles (Figure 8A). The higher decrease of the LSP peak and the formation of a prominent extinction band at 726 nm in the presence of NaCl and KCl make evident the larger destabilizing effects of the  $\text{Cl}^-$  ion compared with  $\text{NO}_3^-$  in the nanoparticles covered with citrate ions. Such a tendency was also verified in the size distribution profile and the zeta potential (Figure 8B and Table 2). In systems containing NaCl and KCl salts, a subtle displacement of the size distribution peak occurred alongside a decrease in zeta potential values. Despite the differences observed in UV-Vis spectra according to the salt used, it was possible to observe in the spectra the band at 294 nm belonging to the TBZ not adsorbed onto the surface of the AgCT, which was also probably due the ion solvation by the TBZ molecules.

**Table 2.** Polydispersity index and zeta potential of AgCT samples at pH 5.8.

| Sample                        | PDI   | Zeta Potential (mV) |
|-------------------------------|-------|---------------------|
| AgCT                          | 0.591 | $-32 \pm 0.5$       |
| AgCT + KNO <sub>3</sub>       | 0.607 | $-28 \pm 2$         |
| AgCT + KCl                    | 0.567 | $-32 \pm 1$         |
| AgCT + NaCl                   | 0.589 | $-32 \pm 2$         |
| AgCT + TBZ                    | 0.591 | $-27 \pm 1$         |
| AgCT + KNO <sub>3</sub> + TBZ | 0.559 | $-30 \pm 1$         |
| AgCT + KCl + TBZ              | 0.529 | $-25 \pm 1$         |
| AgCT + NaCl + TBZ             | 0.524 | $-23 \pm 1$         |
| AgCT + MBC                    | 0.591 | $-28.0 \pm 0.2$     |
| AgCT + KNO <sub>3</sub> + MBC | 0.550 | $-28 \pm 1$         |
| AgCT + KCl + MBC              | 0.569 | $-32 \pm 1$         |
| AgCT + NaCl + MBC             | 0.586 | $-31 \pm 1$         |

**Figure 8.** Extinction spectra (A) and hydrodynamic diameter distribution (B) of AgCT with TBZ in the presence and in the absence of the salts NaCl, KCl, KNO<sub>3</sub>. pH 5.8.

The AgCT UV-Vis spectra in the presence of MBC and salts are presented in Figure 9A. The aggregation was modest compared to the AgCT/TBZ + salts system, as evidenced by the absence of variations in the DLS results (Figure 9B) and zeta potential values (Table 2). This reveals the high colloidal stability of AgCT, even in the presence of the pesticide MBC and salts. It is interesting to highlight the more prominent UV spectral change of the system AgCT + MBC containing KCl, following the tendency of the system with only AgCT.

**Figure 9.** Extinction spectra (A) and hydrodynamic diameter distribution (B) of AgCT with MBC in the presence and in the absence of the salts NaCl, KCl or KNO<sub>3</sub>. pH 5.8.

## 4. Conclusions

This study has demonstrated the fundamental role of both the type of Ag synthesis and salts in the aggregation of nanoparticles, with direct consequences for the SERS signal of pesticides and detection quality. The difference in the coatings of the AgH or AgCT synthesis and the salts used caused differences in the aggregation of nanoparticles and, consequently, in the adsorption of pesticides on the surfaces of nanoparticles and the detection of pesticides via SERS. For TBZ, due its high affinity with the AgH surface, the system without salts proved essential for achieving the highest aggregation and greater SERS signal intensity. In the case of the MBC pesticide, the KNO<sub>3</sub> salt played a pivotal role in promoting a more significant aggregation state, crucial for obtaining the most amplified SERS signal. The thicker coating of AgCT hindered the adsorption of both pesticides onto the nanoparticle surface. The process was facilitated only after the addition of chloride salts NaCl and KCl. The incorporation of salts in the AgCT was not enough to obtain the SERS signal of MBC; additional modifications, such as altering laser parameters and adjusting the medium's pH, were required. The salts did not interfere with the way TBZ and MBC adsorb on the surface of AgH nanoparticles. Conversely, in the case of AgCT, the KNO<sub>3</sub> salt played a significant role in altering the adsorption of both pesticides on the Ag NP surface. The UV-Vis absorption spectroscopy, DLS and zeta potential were used simultaneously to determine the aggregation features of each nanoparticle synthesis (AgH or AgCT) and the presence of the salts NaCl, KCl and KNO<sub>3</sub>. The difference in the spacing of the coatings of the AgH or AgCT synthesis caused differences in the aggregation of nanoparticles and, consequently, in the adsorption of pesticides on the surfaces of nanoparticles and the detection of pesticides via SERS. Regarding the salts used, for AgH, although KCl emerged as the most effective aggregator among the salts employed, such a pattern was not maintained in the presence of pesticides. In summary, our findings underscore that the effectiveness of SERS experiments is influenced not only by the synthesis of the nanoparticles and the inclusion of aggregating agents like salts but also by the intrinsic nature of the molecule being targeted for signal amplification.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/chemosensors13010013/s1>: Figure S1: Raman spectra of AgH and AgCT in absence of both salts and TBZ and MBC, using the lasers 514 and 785; Figure S2: Raman spectra of (A) TBZ and (B) MBC in powder, using the lasers 514 and 785; Figure S3: SERS spectra at low frequency of (A) only AgH, (B) AgH+ TBZ and (C) AgH + MBC in the presence and absence of salts. Laser: 785 nm. pH 5.8; Figure S4: Extinction spectra (A) and hydrodynamic diameter distribution (B) of AgH in the presence and in the absence of the salts NaCl, KCl or KNO<sub>3</sub>. pH 5.8; Figure S5: Hydrodynamic diameter distribution in volume of AgH with TBZ in the presence and in the absence of the salts NaCl, KCl or KNO<sub>3</sub>. pH 5.8; Figure S6: AgCT SERS spectra in the presence of KNO<sub>3</sub> using AgCT NPs at pH 5.8 Laser: 514.5 nm; Figure S7: Absolute MBC SERS spectra in the absence and presence of different salts (NaCl, KCl or KNO<sub>3</sub>) using AgCT NPs at pH 5.6. Laser: 785 nm; Figure S8: MBC SERS spectra using AgCT and different salts (NaCl, KCl or KNO<sub>3</sub>) at pH 5.8. Laser line 514.5 nm; Figure S9: SERS spectra at low frequency of (A) only AgCT, (B) AgCT + TBZ and (C) AgCT + MBC in the presence and absence of salts. pH 5.8; Figure S10: Extinction spectra (A) and hydrodynamic diameter distribution (B) of AgCT in the presence and in the absence of the salts NaCl, KCl or KNO<sub>3</sub>. pH 5.8; Table S1: Enhancement Factor of AgH and AgCT samples.

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