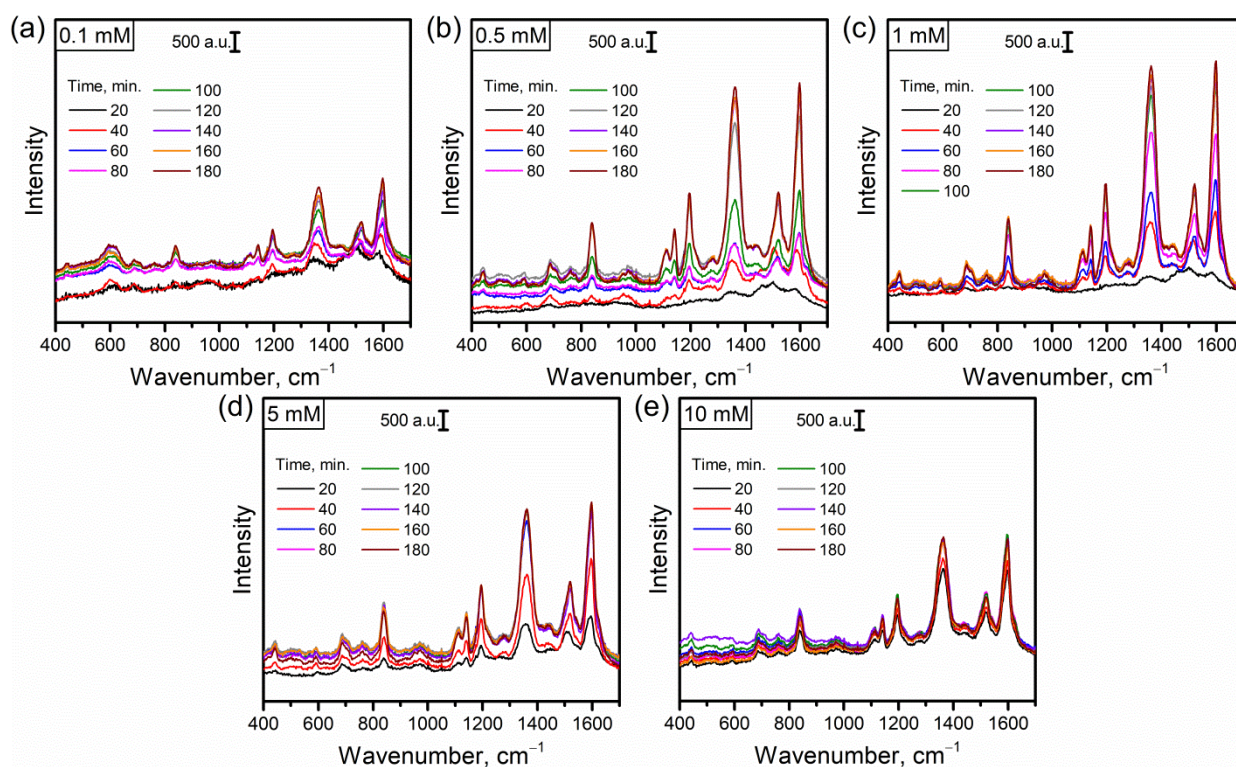
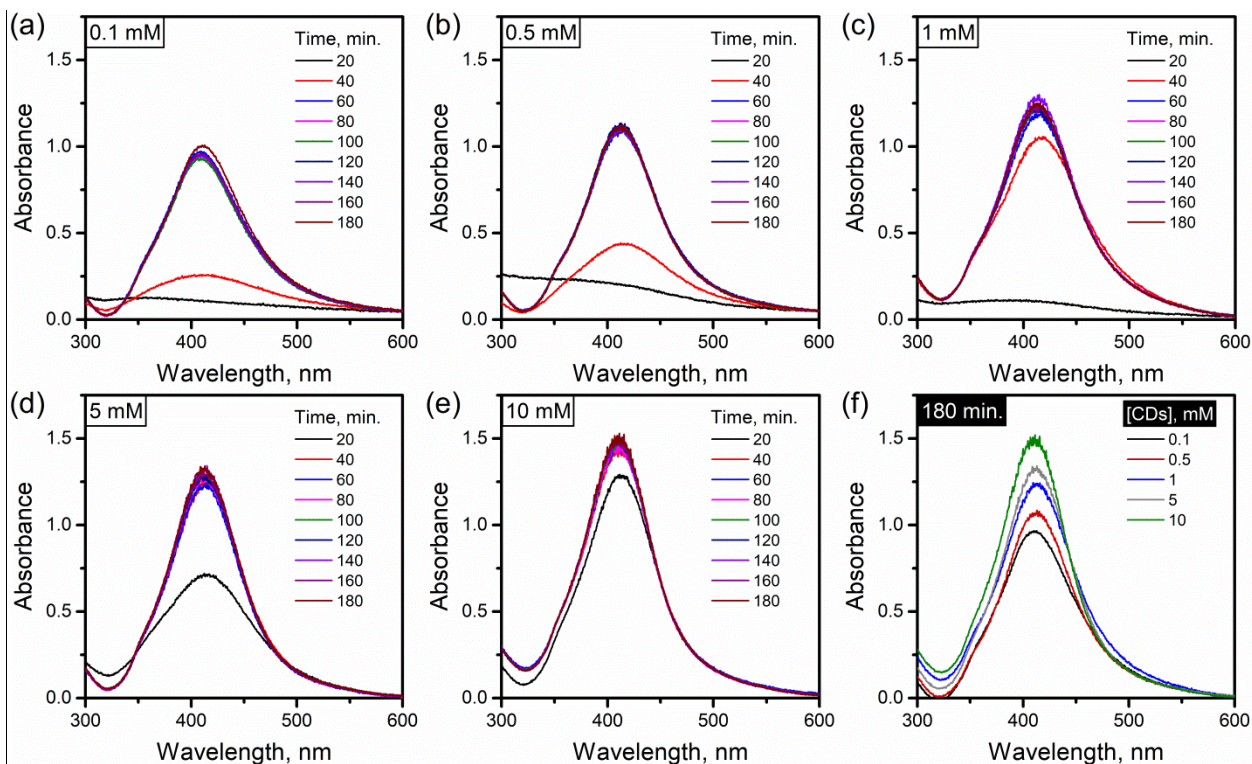


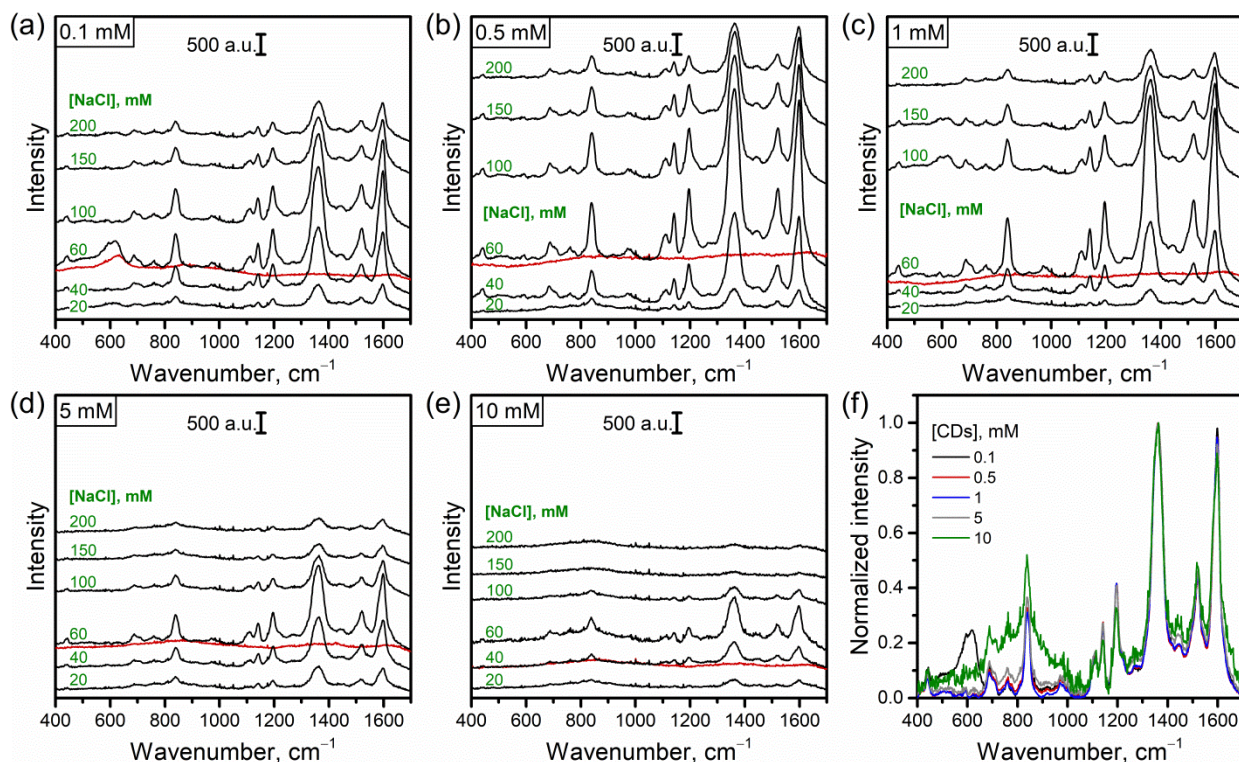
**Figure S1.** Influence of pH in acidic (a) and alkaline media (b) and NaCl concentration in a neutral medium (pH 6.5) (c) on the SERS spectra of MTX ( $0.1 \mu\text{g mL}^{-1}$ ) obtained using AgNPs without and with modification with CD molecules (control AgNPs and AgNP-CD, respectively). Concentration of CD used for modification was fixed at 1 mM.



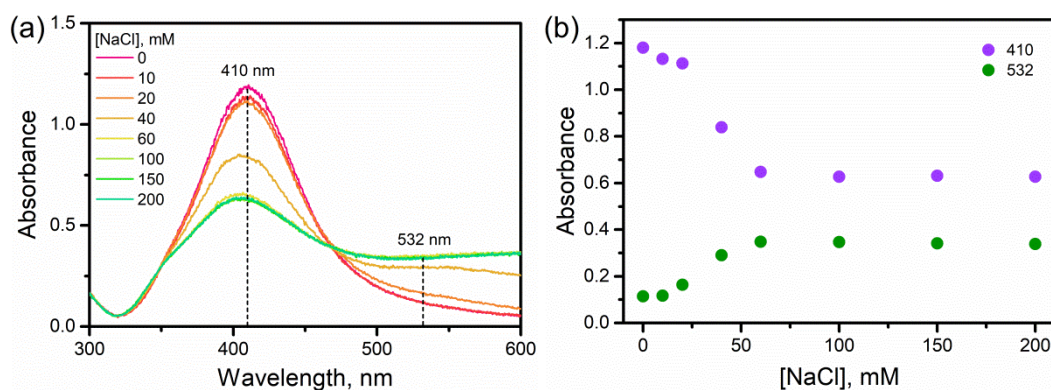
**Figure S2.** Dependence of the SERS signal of MTX on the reaction time and CD concentration (a – 0.1, b – 0.5, c – 1, d – 5, e – 10 mM) used for the synthesis of AgNP-CD. Conditions used to obtain the SERS signals:  $0.25 \mu\text{g mL}^{-1}$  of MTX, 60 mM of NaCl, pH 6.5.



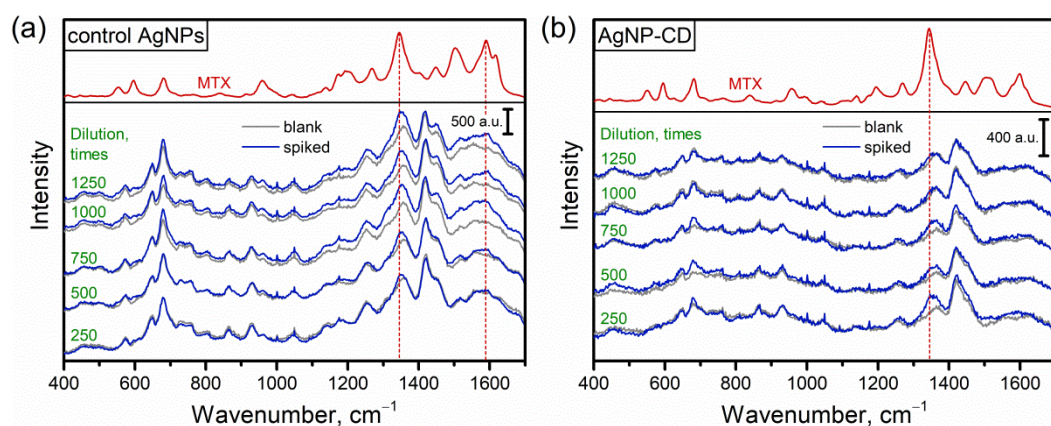
**Figure S3.** (a–e) Dependence of the absorbance spectra of AgNP-CD on the reaction time and CD concentration (a – 0.1, b – 0.5, c – 1, d – 5, e – 10 mM) used for the synthesis of AgNP-CD. (f) Absorbance spectra of AgNP-CD obtained using various CD concentrations and at fixed reaction time (180 min).



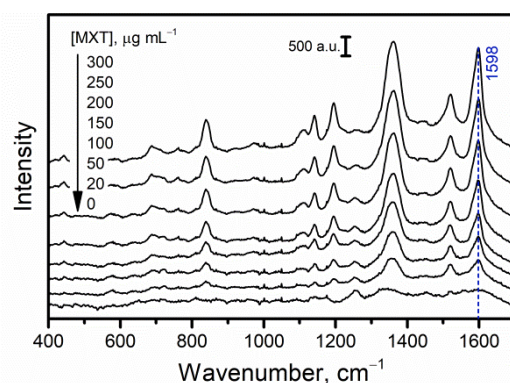
**Figure S4.** Dependence of the SERS signal of MTX ( $0.1 \mu\text{g mL}^{-1}$ ) on CD concentration used for the synthesis of AgNP-CD (a – 0.1, b – 0.5, c – 1, d – 5, e – 10 mM) and the concentration of NaCl used to increase the intensity of the SERS signal (20–200 mM, green numbers). The signals of AgNP-CD without analyte molecules (i.e., background signals) are shown by red lines (obtained using 60 mM of NaCl). (f) Normalized SERS spectra of MTX ( $0.1 \mu\text{g mL}^{-1}$ ) obtained using fixed NaCl concentration (60 mM) and various concentrations of CD molecules.



**Figure S5.** Influence of the NaCl concentration (10–200 mM) used to increase intensity of the SERS signal of MTX on (a) the extinction spectra of AgNP-CD and (b) extinction of AgNP-CD at fixed wavelengths. Wavelength at 410 nm corresponds to the position of the main SPR peak of AgNP-CD. Wavelength at 532 nm corresponds to the position of laser wavelength used for excitation of the SERS signal. Concentration of CD used for modification was fixed at 1 mM.



**Figure S6.** Effect of the degree of sample dilution on the SERS spectra of morning urine without (blank) and with (spiked) the addition of MTX ( $20 \mu\text{g mL}^{-1}$ ). The spectra were obtained using control AgNPs and CD-modified AgNPs and using correction of pH value till 13.25 with NaOH.



**Figure S7.** Averaged SERS spectra of urine samples spiked with MTX ( $20\text{--}300 \mu\text{g mL}^{-1}$ ) and diluted 1000 times. Six urine samples were used to obtain each spectrum using the assay with optimized measurement conditions. The peak at  $1598 \text{ cm}^{-1}$  was used to make a calibration plot (Figure 5b).