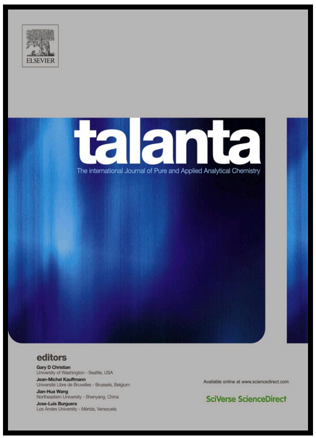

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Copper nanowire coated carbon fibers as efficient substrates for detecting designer drugs using SERS

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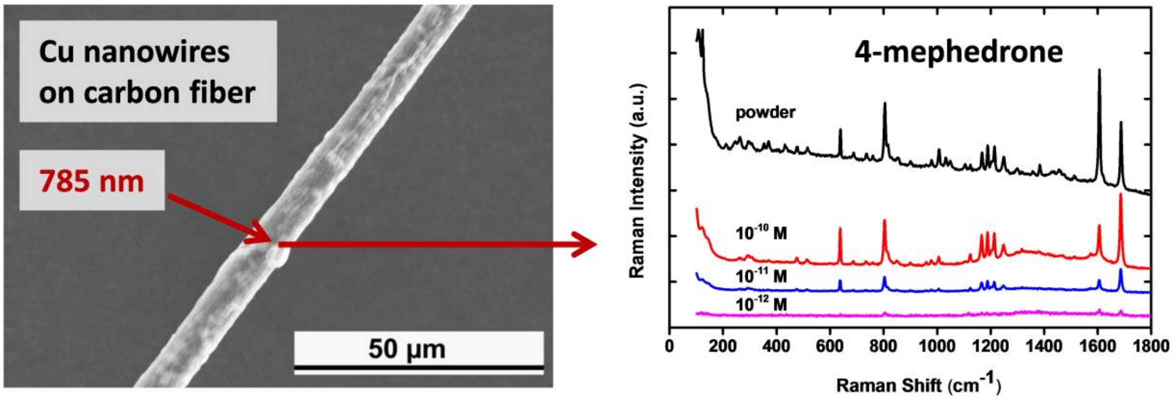
Abstract:

Miniature Surface Enhanced Raman Scattering (SERS) sensors were fabricated by coating the carbon fiber microelectrodes with copper nanowires. The coating procedure, based on anodizing the copper wire in ultrapure water followed by cathodic deposition of the anode-derived material onto carbon fiber electrodes, provides a "clean" copper nanowire network. The developed miniature (10 µm in diameter and 2 mm in length) and nanoscopically rough SERS substrates are applicable in drug sensing, as shown by the detection and resolving of a range of seized cathinone designer drugs in trace amounts (microliter volumes of 10^{-10}-10^{-12} M solutions). The copper nanowire modified carbon microfiber substrates could also find further applications in biomedical and environmental sensing.



1. Introduction

Fabrication of substrates for Surface Enhanced Raman spectroscopy (SERS) belongs to intensely researched topics as SERS is a promising, rapid and non-destructive technique, that allows for probing trace amounts of molecular species, in special cases even down to the single molecule level [1]. SERS active substrates contain nanoscopic metal material (predominantly silver, gold or copper [2-4]) with features size-matched to the wavelength of the exciting laser light. The target molecules, when brought into contact with SERS substrate, experience strong electromagnetic fields of the excited surface plasmons in which the probability of Raman transitions is increased. Very intense local-fields (SERS hotspots) occur if neighboring nano-features exist at distances in the order of 1–2nm [1].

Although a low-cost option, copper is less frequently employed as SERS active metal due to its tendency to surface oxidation. It was, however, shown that the intensity of the SERS spectrum of molecules adsorbed at copper surfaces can remain constant in time [5, 6] and that nanostructured copper can compete with the frequently used silver and gold, providing similar values of SERS enhancement factors (EF) [7]. Recently, Guo et al. [8] developed Cu-based SERS substrates employing electrochemical roughening of smooth copper sheet in 1M sulphuric acid by square wave potential pulses (0÷-1.4V vs. Ag/AgCl at 10 Hz for 5 sec) and registered the SERS spectrum of pyridine on this substrate immersed to 0.05 M aqueous pyridine solution. Irregular nanostructured features, fabricated onto copper sheets by ultrafast laser ablation in aqueous medium [9] provided SERS substrates capable to record spectra of 5-amino-3-nitro-1,2,4-triazole and 2,4,6-trinitrotoluene after evaporating 10 µL aliquots of 10^{-6} M solutions. Nanowires, thanks to their tendency to form bundles and networks, may provide increased number of hotspots located on crossings of individual nanowires and nanowire branches. Twinned copper nanowires prepared using polyoil synthesis were employed for SERS detection of 4-mercaptobenzoic acid (MBA) [10]. A suspension of Cu nanowires was allowed to dry on a silicon plate, the resulting SERS substrate was immersed into 10^{-4} M solution of MBA followed by SERS spectra acquisition after evaporating the solvent. The employed synthetic procedure, however, was tedious requiring heating the mixture of CuCl with oleylamine at 170°C for 60 hours. Xu et al demonstrated electrochemical preparation of Cu nanowires on quartz glass by electrochemical reduction of $Rb_4Cu_{16}Cl_{13}I_7$ ionic conductor and showed the resulting SERS substrate as capable to provide Raman spectrum of rhodamine 6G after evaporating 10 µL drop of its 10^{-11} M solution on the substrate [11].

In this work an innovative, facile and green procedure for coating copper nanowires over carbon fiber microelectrodes (CFME) is presented and the high efficiency of these microelectrodes acting as miniature (10 µm in diameter x 2 mm in length) SERS substrates is demonstrated. To our best knowledge, there is no report in the literature concerning the use of carbon fiber as a support for SERS active metal nanostructure immobilization. To assess the advantage of the developed SERS substrates we have decided to test their capability to provide SERS spectra of trace amounts of the designer drugs. Designer drugs are substances that are related to the structure of controlled drugs and cause similar effects, but are not listed among illegal substances [12-14]. Most often, their effects are similar to the effects of 3,4-methylenedioxymethamphetamine (Ecstasy), cocaine and/or substances of amphetamine group. Common designer drugs include 4-mephedrone (4-

methylmethcathinone, 4-MMC), methylone (3,4-methylendioxy-N-methylcathinone, bk-MDMA, MDMCAT, MDMC), butylone (β-keto-N-methylbenzodioxolyl-propylamine, bk-MBDB) and naphyrone (1-(naphthtalen-2-yl)-2-(pyrrolidin-1-yl)pentan-1-one). The aforementioned compounds have, however, been already included in the list of illegal substances. The still "legal" drugs are buphedrone, products containing 1-benzyl-4-methylpiperazine (MBZP), para-methoxyphenylpiperazine (MOPP) and 4'-Methoxy-α-pyrrolidinopropiophenone (MOPPP).

In drug analysis, liquid and also gas chromatography with mass spectrometry (LC-MS, GC-MS) are the routinely used techniques [15, 16]. Recent work by S. Bell et al. [17] demonstrates the SERS detection of four cathinone drugs (mephedrone, 4-methylmethcathinone, 4-chloromethcathinone and benzedrone) on hydroxyethylcellulose films with embedded silver nanoparticles. The sample loadings necessary to obtain high S/N SERS spectra (approx. 100 µg i.e. ~ $1x10^9$ molecules per $µm^2$ of the substrate) indicate that rather concentrated solutions of samples were needed, corresponding to 10 µL of ca. 10^{-2} - 10^{-1} M solutions dried on their SERS substrates. In agreement with [17], we demonstrate here that SERS is a technique capable to securely distinguish among various structurally similar drugs, thus representing an alternative to LC/GC-MS based analysis while offering improved detection limits. Such an application perfectly fits the high needs in designer drugs detection, often distributed in the form of powder, capsules, caplets or tablets which usually contain only one active ingredient with high purity exceeding 95%. Although the SERS assay specificity is not strictly required for designer drug samples, it is possible to analyse simple mixtures e.g. using spectral deconvolution software [18-20] or following our singular value decomposition (SVD) analysis-based protocol [21].

2. Materials and methods

2.1. Reagents and drug samples

Copper wire was purchased from Alfa Aesar (1 mm in diameter, 99.95% purity). Designer drugs samples (Table 1) were obtained from the Police of the Czech Republic. The identification of the samples was performed by LC-MS and GC-MS according to previously published protocols [22, 23].

Table 1: Tested drug samples

Sample	Physical form	Main compound	Alternative name(s)
P2	Powder	(RS)-2-methylamino-1-(4-methylfenyl)propan-1-on	4- mephedrone, 4-methylmethcathinone
P6	Capsule	2-(methylamino)-1-phenylbutan-1-one	Buphedrone
T9	Powder	1-benzyl-4-methylpiperazine	MBZP
T18	Capsule	1-(4-Methoxyphenyl)piperazine	MOPP
P5	Powder	1-(1,3-benzodioxol-5-yl)-2-(methylamino)butan-1-one	3,4-butylone

Working solutions of drug samples were prepared from powders by stepwise dilution in Millipore water and ethanol (Chromasolv, Sigma-Aldrich) mixture (50:50 v/v). In the case of tablets, the material was taken from their inner parts, which were finely filed and ca. 10 mg of the resulting powder suspended with 1 mL of ethanol. The mixture was filtered off and evaporated to dryness under stream of nitrogen. The post-evaporation residue was reconstituted with water-ethanol mixture (50:50 v/v).

2.2. Preparation of cylindrical carbon fiber microelectrode and its coating with copper nanostructure

A polyacrylonitrile-based carbon fiber (diameter 7 µm, Sigrafil C30) was obtained from a local distributor (GRM-systems, Czech Republic). The carbon fiber taken out from the bundle was glued using conductive silver epoxy (EC101, Polytec, Germany) onto a copper wire, the junction was then hardened at 130 °C for 10 min. The fiber with copper contact attached was fitted into a glass capillary and about 5 mm of the fiber was left protruding from its tapered end. Both ends of the capillary were sealed using low viscosity epoxy resin (L200, Havel Composites Inc., Czech Republic). The fiber end of the electrode was briefly sonicated in dichloromethane in order to clean the fiber. To prepare copper nanostructure onto CFME, a copper wire electrode (1 mm diameter, 2 cm length) was anodized in a two-electrode cell containing ultrapure water as the medium while the CFME was connected as a cathode, the interelectrode distance being 1 cm. Unless stated otherwise, the applied voltage was 30 V and deposition time 5 minutes. To ensure reproducibility of the coating procedure, each electrode was coated separately, after each coating the used water medium was discarded and the cell was refilled with ultrapure water. Cu coated CFMEs were dried in the air and used for SERS measurements within one week after the preparation.

2.3. Scanning electron microscopy and EDX spectroscopy

Scanning electron microscopy (SEM) images were obtained with a Vega 3 (Tescan, Brno, Czech Republic, SE image resolution 2 nm at 30 kV). The images were collected with a high voltage of 15 kV at a working distance ranging from 5 to 25 mm. The sample material (i.e. carbon fibers) was immobilized onto conductive carbon discs after cutting the fiber from the CFME. EDX spectra were collected using the Quantax EasyEDS module (Bruker) integrated into the SEM instrument. The analysis of EDX spectra was performed using EasySEM software with the One-Touch EDX tool.

2.4. Raman experiments

To acquire SERS spectra, copper nanostructure coated carbon fiber was cut-off from the microelectrode, placed onto a silicon plate and overlaid by a drop (5 µL) of the sample containing the tested drug. The droplet was spread to cover the fiber substrate using a micropipette tip. For standard samples, concentrations of 10^{-12}, 10^{-11} and 10^{-10} mol/L were analysed. After drying of the sample solution, Raman measurements were performed in backscattering configuration on a Renishaw inVia Reflex microscope using a diode pumped solid state laser (λ = 785 nm) as excitation source with maximum power 60 mW on the sample; to avoid heating of the tested molecules the power of the laser was attenuated to 1% of the maximum. The laser light was focused on the coated carbon fiber using a 50x, 0.75 NA objective of a Leica DMLM microscope. The 10 µm x 0.5 mm area of the copper coated carbon fiber substrate was first scanned with rapid (30 s, 10-50 sampling spots) acquisition to find best spots on the substrate, final spectra were then recorded from the spot exhibiting highest enhancement by accumulating five successive scans lasting for two min each. The frequency shifts were calibrated by an internal Si reference.

3. Results and discussion

3.1 Copper nanowire coated CFME preparation

Most of the SERS substrates published so far use silicon or glass (e.g. quartz or ITO glass) as a support for metal nanostructure immobilisation. The use of plastic plates or foils, which allow lightweight, flexible and mass producible SERS substrates is less frequent due to problems associated with interfering Raman signals of the polymers as well as local overheating of these substrates by excitation laser [24, 25]. Overcoming the substrate heating is crucial for miniaturization of the SERS substrates, needed for experiments in which the sample uptake has to be kept at a minimum e.g. in the analyses of artwork, where samples in the sub-µg to pg range need to be successfully analyzed [26]. Carbon fiber represents a unique material in terms of high stiffness, tensile strength, low weight, high chemical resistance, good thermal conductivity, high temperature tolerance and low thermal expansion. Coating of carbon fibers with metals by electrodeposition is a suitable way of metal microelectrode fabrication, since the work with cylindrical metal microwires is cumbersome due to their extreme fragility. Furthermore, electrochemical deposition can be employed to fabricate not only smooth, but also nanostructured metal layers.

The coating procedure used in this work is based on anodizing the copper wire in ultrapure water, the released anode-derived material is then deposited onto carbon fiber connected as a cathode. Anodic dissolution of copper in water is a subject of recent paper [27], in which deposit of Cu_2O is identified using *ex situ* Raman analysis on copper anode surface and Cu^{II} ions are found in the interelectrode space. By using *in situ* atomic emission spectroelectrochemistry the “simultaneous” mechanism described by the reaction sequence (eq. 1,2) was found valid at high current densities while “redeposition” mechanism (eq. 2,3) was consistent with experimental data at low current densities. The next considered “sequential” mechanism (eq. 1,4) was not confirmed.

$$2Cu + 2H_2O \rightarrow Cu_2O + 2e^- + 2H^+ \qquad (1)$$

$$Cu \rightarrow Cu^{II} + 2e^- \qquad (2)$$

$$Cu^{II} + Cu + H_2O \rightarrow Cu_2O + 2H^+ \qquad (3)$$

$$Cu_2O + 2H_2O \rightarrow Cu^{II} + 2e^- + H_2 + 2OH^- \qquad (4)$$

The low concentration of Cu^{II} ions available for electroreduction (expected maximum is 1.7×10^{-7} mol dm^{-3} Cu^{II} given by K_s ($Cu(OH)_2$) = $2.2x10^{-20}$) together with the absence of the supporting electrolyte during the deposition represent key factors in the formation of a high number of nuclei leading to formation of metal-nanostructures rather than deposits containing smooth or larger particles. Due to low conductivity of ultrapure water, the coating process requires higher applied potential (5-30 V) than values usually used in electrochemical metal deposition from supported solutions. Depending on potential used for the deposition, three types of morphologies of copper deposits can, however, be achieved (Fig. 1).

At low potential (10 V), isolated copper nanoparticles can be observed on CFME after 10 min. deposition (Fig. 1A). The potentials close to 30 V used for synthesis lead to modification of CFME by coatings with more pronounced nanowire character (Fig. 1C), compared to structure obtained at 0 V (Fig. 1B), which was previously applied by us as an amperometric sensor in flow injection analysis and

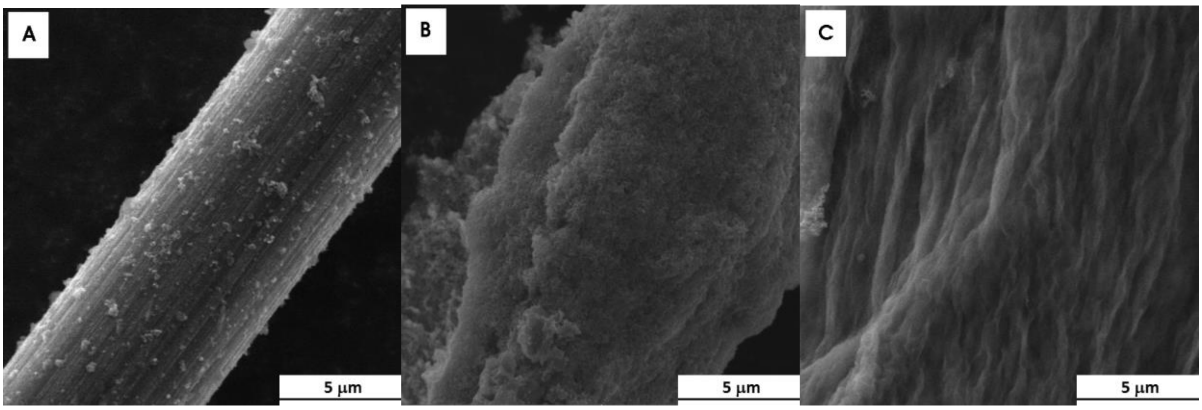


Fig. 1: Copper deposits on carbon fibers prepared at 10 V, 10 min. (A); 20 V, 10min. (B); 30 V, 10 min. (C).

HPLC [28]. At the origin of the obtained nananowire morphology is the proton reduction (accompanying the cathodic reduction of Cu^{II}) forming hydrogen gas bubbles observable by naked eye during the coating procedure. For the SERS study, the deposition was performed with the potential of 30 V applied for 5 min, whereby CFME was connected to negative pole and the copper wire to positive pole of the power supply. Shortened deposition time led to thinner deposits, as documented in Fig. 2, where SEM images of bare (Fig. 2A, 2B) and copper coated carbon fibers (Fig. 2C, 2D) in two different magnifications are shown, along with the EDX spectrum (Fig. 2E).

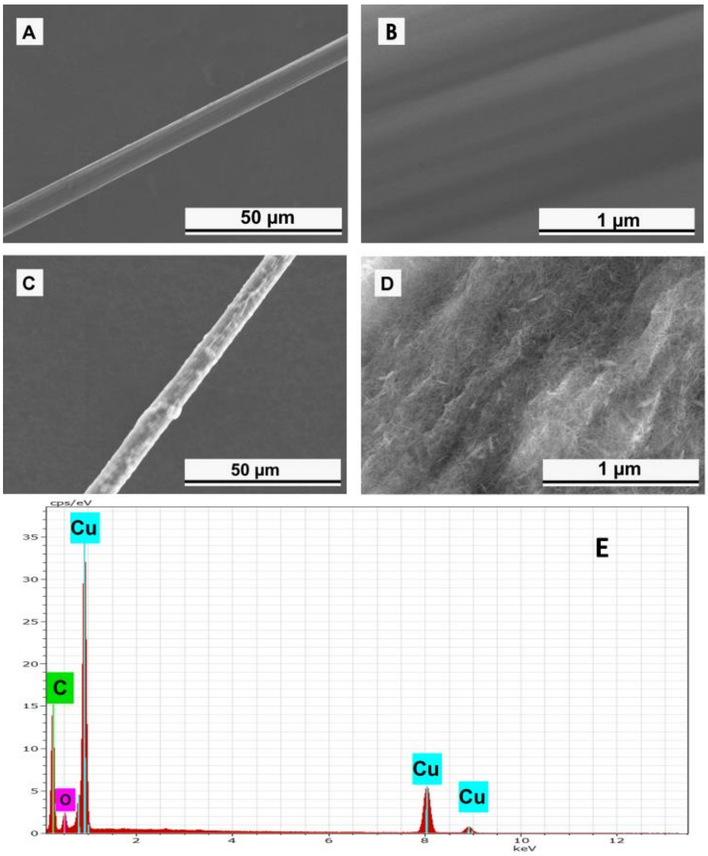


Fig. 2: Bare carbon fiber (A,B), global (C) and detailed (D) view on copper coated carbon fiber. EDX spectrum of copper layer (E).

The estimated dimensions of the nanowires in the final deposit are 8-10 nm in diameter and several hundreds of nanometers in length. EDX spectra present copper peaks as the most intense features, carbon peak originating from carbon fiber and also a minor peak of oxygen, evidencing a low degree of oxidation of the copper layer in air atmosphere. For application as a SERS substrate, the advantage of the process is the minimum risk of contamination since only copper metal and ultrapure water are involved in the coating procedure.

3.2 Surface enhanced Raman Spectroscopy of designer drugs

Raman experiments performed in this work were done by excitation at 785 nm. The above excitation wavelength matches quite well the Cu absorption and scattering optical properties [29] while, on top, tempers the intensity of the photoluminescence background signal of the designer drugs which is so strong that hinders the raman signal at high energy (e.g. 514 nm) excitation. Raman spectrum of carbon fiber material before and after modification with copper nanostructure is shown in Fig. 3A.

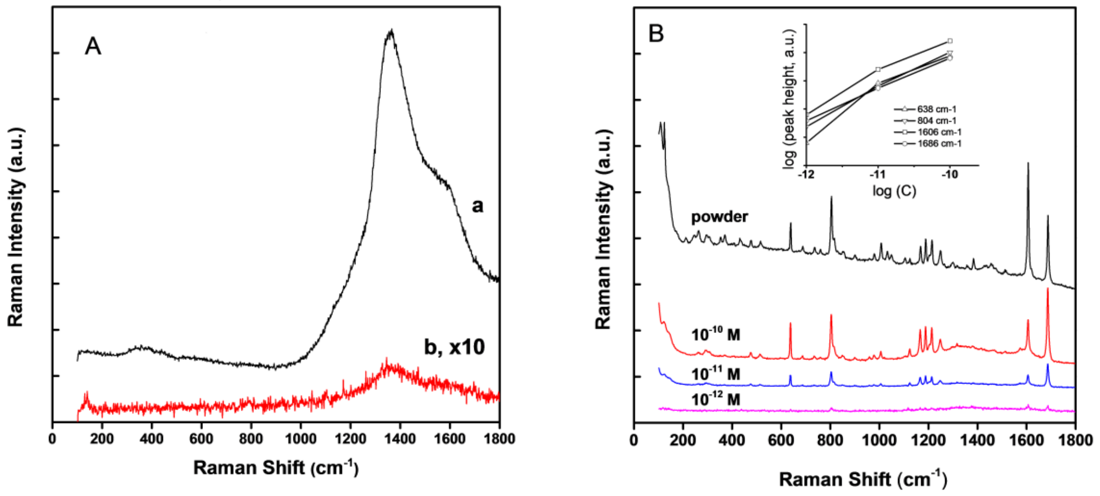


Fig. 3: (A) Raman spectrum of the carbon fiber before (a) and after (b) coating with copper nanostructured layer (the trace was magnified x10 on y-scale) (B) Raman spectra of 4-mephedrone in powder form as well as on copper coated carbon fiber. SERS spectra were recorded after evaporating 5 µL of 10^{-12} M, 10^{-11} M and 10^{-10} M 4-mephedrone solution. The positions of individual spectra were shifted for clarity of the presentation. Inset: concentration dependence of the main mephedrone Raman peaks intensity

The spectrum of bare carbon fiber is represented by a broad D line centred at 1362 cm^{-1} and overlapping partially the G band (ca. 1588 cm^{-1}) which is of lower intensity. The broadening and ratio between G and D peak intensities indicate that the used carbon fiber is a low temperature (<1400 °C) carbonized polyacrylonitrile [30]. After coating the fiber with copper, the carbon-like Raman spectrum is significantly attenuated. Surface enhanced Raman spectra of designer drugs were acquired at metal–solid interface by placing a drop (5µL) of aqueous-ethanolic solutions (50% v/v) of the studied drugs onto Cu-coated CFME substrate and evaporating the solvent. Fig. 3B shows the

Raman spectrum of 4-mephedrone powder and SERS spectra obtained from extremely diluted aqueous-ethanolic solutions (10^{-12} – 10^{-10} M) of 4-mephedrone. Taking into account the focal area of the attenuated laser, as described above, the calculated power density at sample was 0.5 mW/μm^2 for all the SERS spectra, which safely prevents damage of the samples. It is noteworthy to mention that SERS spectra were easily obtained after focusing the laser beam onto the nanostructured copper layer substrate while no Raman signals were obtained in the absence of the SERS substrate and the spectrum identical to that shown in Fig 3A (trace a) was obtained using the carbon fiber without Cu layer. Taking into an account the sample concentration, volume and the area over which the sample is spread, approx. 60, 6 and 0.6 target molecules/μm^2 are present in the probed area for 10^{-10}, 10^{-11} and 10^{-12} M 4-mephedrone solutions, respectively. It can be assumed that above values in the population of target molecules can be underestimated since in analogy to the coffee ring effect [31], the density of molecules on the Cu nanostructure coated fiber substrate can be enhanced due to accumulation of the sample molecules onto the coated fiber during the drying process. The log-log plot, shown in inset of Fig. 3B, indicates linear concentration dependence of the mephedrone 804 and 1686 cm^{-1} peaks while peaks at 638 and 1606 cm^{-1} deviate from linearity. In addition, powder Raman and Cu surface enhanced spectra of 4-mephedrone are very similar. Most Raman bands seen in the powder are apparent on the copper surface, differing by intensity variations only, of which the decrease in the intensity of C=C stretch peak at 1606 cm^{-1} and increased intensity of 1686 cm^{-1} C=O stretch peak are the most remarkable. The close similarity between powder Raman and Cu SERS spectra, in contrast to the result obtained on Ag and Au substrates, has been recently reported also for p–aminobenzenethiol [32].

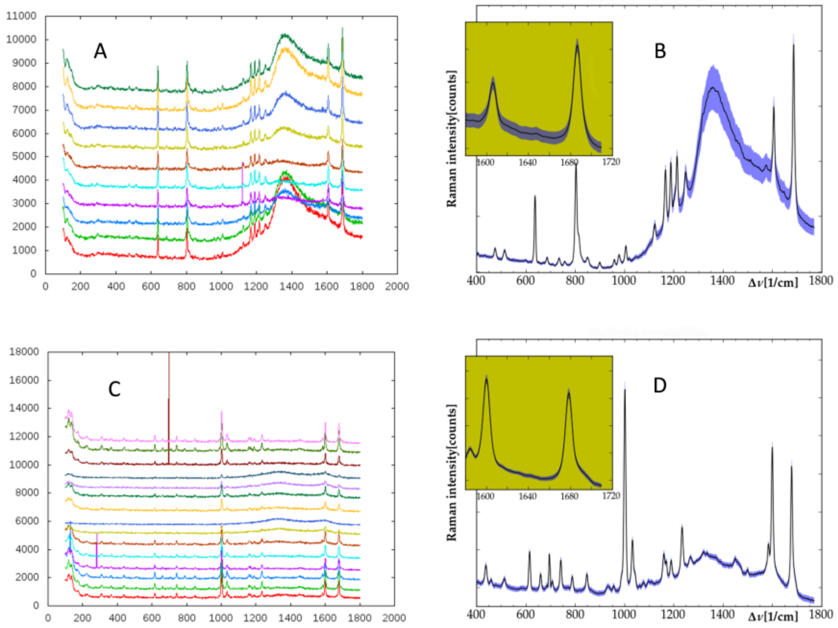


Fig. 4: Raman mapping of 4-mephedrone and buphedrone SERS. As-measured spectra of mephedrone (A), constituting line map (9 points per 60 μm); (B): average spectrum from the map (black) together with corresponding ±σ band (blue); inset shows detail of 1607 and 1687 cm^{-1} peaks used to evaluate SERS EF. (D): spectra from the line map of buphedrone (14 points per 75 μm) and corresponding statistical plot (E).

The uniformity of the Raman enhancement of 4-mephedrone is demonstrated in Fig. 4A,B, showing spectra from a line mapping performed diagonally along the Cu coated CFME substrate (integration time ??) along with an average spectrum (black) and its standard deviation (±σ band, blue). The inset shows detailed information for peaks used to evaluate SERS EF. Apart from the background signal of carbonaceous support, appearing in part of the mapped area, the variation in the peak heights across the map is below 10%. The appearance of the background signal may be attributed to imperfections of the coating or to its mechanical damage during manipulations such as cutting the coated fiber off the electrode body and placing it onto Si plate. Fig. 4C, 4D shows analogical Raman map of buphedrone, which is free of the above discussed undesired background signal.

Fig. 5 shows Raman spectra of powder samples of a series of designer drugs from Tab. 1 and their corresponding SERS spectra obtained from diluted solutions dried on the copper coated CFME substrate.

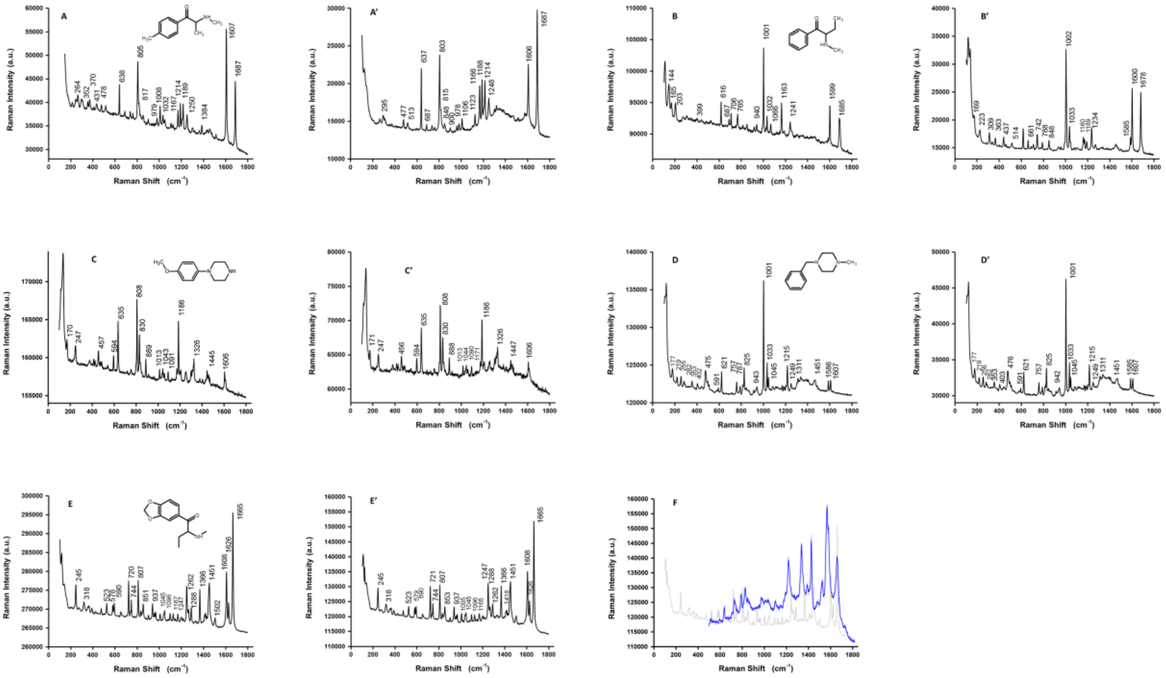


Fig. 5: Raman spectra of powdered drug samples (A-E) and accumulated surface enhanced Raman spectra obtained from diluted solutions (A'-E'), 10^{-10} M. A: P2 (contains 4-mephedrone), B: P6 (contains buphedrone), C: T18 (contains MOPP), D: T9 (contains MBZP), E: P5 (contains 3,4-butylone). SERS spectrum of 3,4-butylone, obtained on Silmeco Au substrate (F, blue trace) is shown alongwith the spectrum obtained on Cu coated CFME (grey trace).

For all studied drugs, the procedure carried out with 10^{-10} M solution gave Raman spectra with signal to noise ratios comparable to that obtained from solid powder samples and with high degree of similarity in peak positions and heights, allowing secure identification of the drug being analysed. The background signal was, however, 2.5-6 times lower for SERS, indicating an improved Raman to photoluminescence intensity ratio. This improvement can be attributed to luminescence quenching caused by the copper (nano)structure over the CFME. The reduction of the photoluminescence background level is crucial for recording high quality SERS spectra suitable for quantitative analyses [33]. Raman spectra of 4-mephedrone (5A), buphedrone (5B) and 3,4-butylone presented in Fig. 5 match well the spectra presented in the literature [34]. The spectra of all drug samples under study contain Raman bands of the benzene C=C stretching vibrations at 1600-1610 cm^{-1}. The C=O stretch at 1680-1690 cm^{-1} is present for drugs containing carboxyl group, i.e. 4-mephedrone (5A), buphedrone (5B) and 3,4-butylone (5E). The benzene in-plane ring deformation manifested as very strong peak between 1000-1015 cm^{-1} is prominent for buphedrone (5B) and MBZP (5D), due to monosubstituted benzene moiety in these molecules. This vibration mode is present also in compounds containing p-disubstituted benzene moiety (i.e., mephedrone (5A) and MOPP (5C)), but with lower intensity. The out of plane CH vibrations in the benzene rings at 800-810 cm^{-1} are most clear for the para-substitution of benzenes in 4-mephedrone (5A), MOPP (5c) and 3,4-butylone (5E). Strong peak at 825-830 cm^{-1} pertaining to ring breathing mode of piperazine [35] is observed in MBZP (5D) and MOPP (5C) drugs which contain this moiety.

To calculate the SERS enhancement, we have taken 100 s reference spectra from 0.1 M solutions of the samples in capillary (for 4-mephedrone, the concentration was 0.017 M due to solubility limit). Using 20x lens with 0.4 NA and full power of the 785 nm laser, the power density was 3.7 mW/μm^2 in the plane of focus and approximately 8.8x10^{10} molecules were contributing to Raman spectrum within the diffraction cone. By relating the intensity of the SERS (see Fig. 4) to the Raman signal of the drugs in solution and normalizing for the power density and concentration, the SERS enhancement factors have been estimated. Mean EF was in the range of 10^6 to 10^7 for all samples, eg. for the two main 3,4-butylone peaks, 1608 cm^{-1} and 1665 cm^{-1}, EF reached 2x10^6 and 8x10^6, respectively.

Finally, we employed commercially available Au SERS substrate (Silmeco) in order to compare the performances of our Cu coated CFMEs. We successfully obtained SERS spectra of the studied drugs only when using more concentrated (10^{-7} M) drug solutions. The best spectra, featuring the highest S/N ratio was recorded for 3,4-butylone and mean spectrum is shown in Fig. 5F (blue trace) along with the spectrum of 3,4-butylone obtained using Cu coated CFME (grey trace).

The miniature carbon fiber based SERS substrates will continue to be the subject of our further research. In addition to designer drugs detection, the copper coated microelectrodes could find a broad range of applications, including the determination of traces of biomolecules and emerging pollutants in water environments. It must be noticed that in addition to obvious possibility to analyse very small sample volumes, miniature SERS substrates emerge as prospective detectors in microfluidic devices and lab-on-the-chip systems [36]. In fact recently, electrochemical regeneration of SERS substrates fabricated by inkjet printing of silver nanostructure onto an ITO electrode was demonstrated, allowing multiple consecutive measurements in a continuous-flow system, circumventing common problems of signal carry-overs caused by irreversible adsorption of analytes (“memory effect“) [37]. In this regard, we are now investigating the integration of the fabricated carbon fiber microelectrodes into microfluidic channels.

4. Conclusions

Copper nanowire network was prepared onto carbon microfiber by cathodic deposition of the material obtained by anodizing the copper wire in ultrapure water. The developed miniature (10 µm in diameter and 2 mm in length) and nanoscopically rough SERS substrates were applied to detect trace amounts (microliter volumes of 10^{-10}-10^{-12} M solutions) of a range of seized cathinone designer drugs. Using our treatment, the SERS enhancement factors of the order 10^6 to 10^7 were readily obtained. The presented innovative approach to construct extremely sensitive SERS substrates based on copper nanowires prepared by electrosynthesis is characterized by high added value as it is able to provide easy, fast and cost efficient SERS substrate with low risk of contamination of the sensing layer, thanks to use of pure materials in synthesis and microelectrode fabrication.

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