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Gold nanoparticles bridging infra-red spectroscopy and laser desorption/ionization mass spectrometry for direct analysis of over-the-counter drug and botanical medicines

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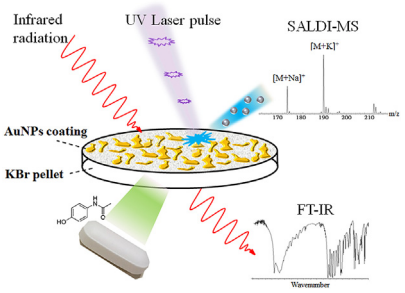
HIGHLIGHTS

- UV-absorbing AuNPs coating enables direct LDI-MS analysis for OTC Drugs and CCM granules.
- IR-transparent AuNPs coating allows standard FT-IR screening of major drug ingredients.
- Quantification of phytochemical marker compounds in CCM granules was achieved, with LOD down to nanogram level.

GRAPHICAL ABSTRACT



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ABSTRACT

With a coating of gold nanoparticles (AuNPs), over-the-counter (OTC) drugs and Chinese herbal medicine granules in KBr pellets could be analyzed by Fourier Transform Infra-red (FT-IR) spectroscopy and Surface-assisted Laser Desorption/Ionization mass spectrometry (SALDI-MS). FT-IR spectroscopy allows fast detection of major active ingredient (e.g., acetaminophen) in OTC drugs in KBr pellets. Upon coating a thin layer of AuNPs on the KBr pellet, minor active ingredients (e.g., noscapine and loratadine) in OTC drugs, which were not revealed by FT-IR, could be detected unambiguously using AuNPs-assisted LDI-MS. Moreover, phytochemical markers of Coptidis Rhizoma (i.e. berberine, palmatine and coptisine) could be quantified in the concentrated Chinese medicine (CCM) granules by the SALDI-MS using standard addition method. The quantitative results matched with those determined by high-performance liquid chromatography with ultraviolet detection. Being strongly absorbing in UV yet transparent to IR, AuNPs successfully bridged FT-IR and SALDI-MS for direct analysis of active ingredients in the same solid sample. FT-IR allowed the fast analysis of major active ingredient in drugs, while SALDI-MS allowed the detection of minor active ingredient in the presence of excipient, and also quantitation of phytochemicals in herbal granules.

1. Introduction

The distinctive photo absorption/emission properties of metal nanoparticles (NPs) have long been raising the interests of analytical scientists in developing sensitive and specific molecular

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sensing agents for chemical and biochemical analysis [1–5], including its application for *in vitro* cell imaging and *in vivo* animal tissue imaging for drug screening and disease diagnosis. Indeed, when metal decreases from bulk-size to nano-size, the degeneration of the continuum electronic levels to discrete levels would introduce outstanding quantum effect which may alter the photo-absorption energy bands and enhance the photo-absorption efficiency [6,7]. By combining several properties of metal NPs, their analytical applications can be further expanded. For instance, noble metal NPs (e.g. those of Au, Ag and Pt) [8–11] having efficient photo-absorption and rapid laser-induced heating properties, have been employed as the laser-absorbing substrate for Surface-assisted Laser Desorption/Ionization Mass Spectrometry (SALDI-MS) analysis of metabolites [9,12–18]. Moreover, our group had previously combined the surface plasmonic property and laser desorption/ionization property of AuNPs for bridging optical and mass spectrometry imaging of latent fingerprints [19]. It is believed that NPs could be adopted in different analytical fields, enhancing and bridging various analytical techniques for tackling challenging analytical problems.

Western Medicine and Traditional Chinese Medicines (TCM) are two major components of the medicinal system all over the world. In Western medicine, pharmaceutical products usually contain single active compound with excipients, while TCM employs remedies composed of a number of medicinal herbs, each contains a wide variety of phytochemicals. Nevertheless, quality control of pharmaceutical products is important to both western medicine and TCM, in order to ensure the safety and efficacy of the treatment.

The quality of these products is usually monitored by assaying the active ingredient (western drug) or phytochemical markers (medicinal herbs) using High-Performance Liquid Chromatography with UV–Vis detection (HPLC/UV) or other techniques according to national/regional pharmacopoeia [20,21]. However, HPLC analysis often requires extensive sample preparation for solution extract, followed by time-consuming elution and washing.

Fourier Transform Infrared (FT-IR) spectroscopy has been widely adopted for the qualitative analysis of active pharmaceutical compounds in the solid state, as specified in various national and regional pharmacopoeias. Nevertheless, identification based on FT-IR spectroscopy would be interfered by the presence of impurities or excipients. Although advanced FT-IR technique such as 2D FT-IR has been developed with increased specificity, the analysis may not be able to provide a full chemical profile and the huge data analysis time also remains a challenge [22–24]. Quantitative analysis of active pharmaceutical compounds in the presence of excipient matrix has also been reported, but it usually requires careful preparation of a series of matrix-matched spike samples as the calibrant [25]. Recently, mass spectrometry has been developed for direct sample analysis with the advancement of new ionization methods. In particular, Laser Desorption/Ionization Mass Spectrometry (LDI-MS) has been applied in different analytical aspects [26–33]. Using noble metal NPs as the laser-absorbing substrate, the conventional masking effect in the low m/z region ($<m/z$ 800) due to organic matrix (e.g. CHCA or SA) could be eliminated, which allows the detection of various small molecule drugs and phytochemicals. Here, we adopted AuNPs as the substrate to coat on a KBr sample pellet containing either over-the-counter drugs or medicinal herbal granules, for the SALDI-MS analysis of active ingredients and phytochemical markers. Since the inorganic AuNPs substrates do not possess IR-active functional groups, the KBr pellet could be analyzed by FT-IR before and after AuNPs coating. SALDI-MS would either confirm the FT-IR detection, or reveal the presence of active pharmaceutical compounds when FT-IR detection was masked by strongly IR absorbing excipients. Furthermore, the SALDI-MS could also perform quantitative analysis of phytochemical markers in herbal extract using standard addition method.

2. Experimental

2.1. Materials and chemicals

Potassium bromide (>99%, IR grade) used for making FT-IR sample pellets was purchased from Fisher Scientific (Pittsburgh, PA). The reference standards of phytochemical markers of Coptidis Rhizoma, including berberine chloride (≥98%), and coptisine hydrochloride (≥98%) were purchased from Sichuan Weikeqi Biological Technology (Sichuan, China). Palmatine chloride (≥98%) was purchased from Chengdu Herbpurify (Sichuan, China). Three OTCs, namely **OTC-1**: Panadol® Joint Extend, contains 665 mg of acetaminophen per tablet (710 mg each), GlaxoSmithKline (Australia); **OTC-2**: AP® Noscapine, contains 25 mg of noscapine per tablet (410 mg each), Advance Pharmaceutical (Hong Kong); **OTC-3**: Fortune NT-ALERGI®, contains 10 mg of loratadine per tablet (250 mg each), Fortune Pharmacal (Hong Kong). Concentrated Chinese Medicine granules of Coptidis Rhizoma were from three different manufacturers, namely **CCM-1**: Sheng Chang Pharmaceutical Co. Ltd.; **CCM-2**: Sheng Foong Co. Ltd. (Taiwan); **CCM-3**: Hong Kong Haitian concentrated Chinese medicine Co., Ltd. (Hong Kong). All OTCs and concentrated Chinese Medicine granules were purchased from local pharmacy and herbal medicine stores in Hong Kong.

2.2. FT-IR analysis

The FT-IR experiments were performed using an IRAffinity-1S FT-IR spectrophotometer (Shimadzu, Kyoto, Japan). Fine sample powder (1–2 mg) was evenly mixed with KBr (120 mg) using a mortar and pestle. The sample pellet for FT-IR experiments was prepared using a manual pelletizer. The IR absorption measurement range was 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. Each IR spectrum combined 16 numbers of scans. Pure KBr was analyzed as the blank and air background spectrum subtraction was performed for all IR spectra.

2.3. Coating of AuNPs by argon ion sputtering

The KBr sample pellets were held on a clean glass and then coated with AuNPs by argon ion sputtering. The sputter coater (SCD 005; Bal-Tec AG, Liechtenstein) was operated using ultra high purity argon gas (99.999%) and a high purity gold target (99.99%; Ted Pella Inc., Redding, CA). The sputtering conditions were as follows, distance between the gold target and sample: 80 mm, sputtering current: 30 mA, chamber pressure during sputtering: 0.04–0.06 mbar, and sputtering time: 25 s.

2.4. SALDI-TOF MS analysis

Ultraflex II MALDI-TOF/TOF MS with a 355 nm solid state laser (Bruker Daltonics, Bremen, Germany) was employed for performing the mass spectrometry experiments. The AuNPs-coated KBr sample pellets were mounted on a modified stainless steel MALDI sample plate using electrically conductive tapes (9713 XYZ-Axis; 3M, St. Paul, MN) and the plate was then introduced into the mass spectrometer. The conductive tapes and AuNPs coating on the sample surface can eliminate the electrical conductivity problem between the KBr sample and the MALDI plate. The modified MALDI plate with indentation (117 × 71 mm^2 in area and 0.2 mm in depth) was used in order to partly compensate the thickness (0.4 mm) of the KBr sample. For SALDI–TOF MS analysis of OTC samples, the mass spectrometer was operated in positive reflectron mode. The