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Manganese oxide nanoparticle-assisted laser desorption/ionization mass spectrometry for medical applications

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Abstract
We prepared and characterized manganese oxide magnetic nanoparticles ($d = 5.6$ nm) and developed nanoparticle-assited laser desorption/ionization (nano-PALDI) mass spectrometry. The nanoparticles had MnO_2 and Mn_2O_3 cores conjugated with hydroxyl and amino groups, and showed paramagnetism at room temperature. The nanoparticles worked as an ionization assisting reagent in mass spectroscopy. The mass spectra showed no background in the low m/z. The nanoparticles could ionize samples of peptide, drug and proteins (approx. 5000 Da) without using matrix, i.e., 2,5-dihydroxybenzoic acid (DHB), 4-hydroxy-α-cinnamic acid (CHCA) and liquid matrix, as conventional ionization assisting reagents. Post source decay spectra by nano-PALDI mass spectrometry will yield information of the chemical structure of analytes.

Keywords: metal oxide, transmission electron microscopy, ionization, mass spectrometry

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Recently, the use of nanoparticles in the fields of nanomachines [1, 2], imaging methods [3], biosensors [4], diagnostics [5] and drug-delivery systems [6–8] has been reported. Especially, magnetic nanoparticles are attracting much attention in the nanobioengineering, magnetic resonance imaging [5, 9, 10] and delivery systems [6, 11–13]. In a mass spectrometry field, it is well known that nanoparticles with metal oxide core can assist laser desorption/ionization (LDI) of analytes in presence of glycerol [14], although this method is complex, and analyte is diluted by the glycerol solution. Zinc oxide nanoparticles appeared highly useful for desorption/ionization of low molecular weight samples [15]. However, their shape was anisotropic, e.g. cubic and rectangular with vastly varying sizes in the range 20–200 nm. To the best of our knowledge, the use of uniform nanoparticles in direct mass spectrum analysis has not been described yet.

Among the various biomolecules, metabolites in terms of metabolic intermediates, hormones and other signaling molecules [16] are known to play important biological roles. We focused on the matrix-assisted laser desorption/ionization (MALDI) based on mass spectrometry because it could directly detect metabolites from biological samples [17] and tissues [18] as well as from purified samples. One of the major problems of MALDI-mass spectrometry for identification of biomolecules is usage of the conventional

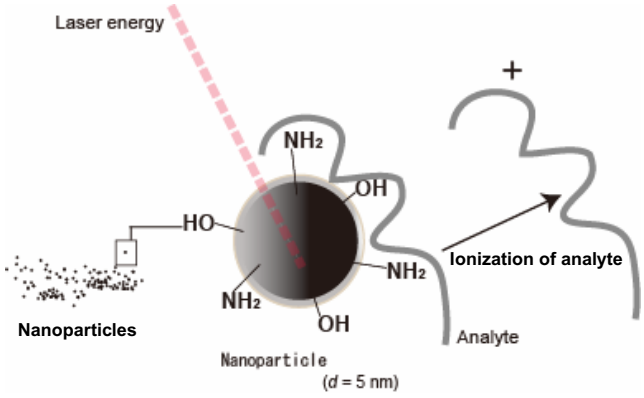

Figure 1. Schematic illustration of nano-PALDI mass spectrometry.

chemical ionization-assisting reagent (matrix) such as 2,5-dihydroxybenzoic acid (DHB) and 4-hydroxy-α-cinnamic acid (CHCA). The analyte has to be embedded within the matrix crystals. Matrix peaks and fragment peaks overlap in the lower mass region ($m/z \sim 800$). As an improvement, we prepared manganese oxide magnetic nanoparticles ($d =$ 5.6 nm), which are based on metal oxide core coated with functional silicate sheet. This functional silicate sheet drastically improved energy transfer between the metal oxide core and analyte; viz. analyte was ionized only by the nanoparticles. This paper shows for the first time, that manganese oxide magnetic nanoparticles can assist ionization in nanoparticle-assisted laser desorption/ionization (nano-PALDI) mass spectrometry without significant increase of background signals (figure 1). We also applied nano-PALDI mass spectrometry to chemical compounds and biological samples, such as reserpine, substance P and insulin, using manganese oxide magnetic nanoparticles with spherical structure.

2. Materials and methods

2.1. Preparation of manganese oxide based magnetic nanoparticles

Manganese oxide based magnetic nanoparticles were prepared by mixing aqueous solutions of $MnCl_2 \cdot 4H_2O$ (20 ml, 100 mM; WAKO Pure Chemicals Japan) and (3-aminopropyl)triethoxysilane (20 ml, γ-APTES; Shinetsu Kagaku Japan) (figure 2). After 1 h of stirring at room temperature, the resulting precipitates were washed several times with ultrapure water and dried at 80 °C in an incubator. The dried samples were crushed in a porcelain mortar. The morphology and diameter distribution of the nanoparticles were investigated with transmission electron microscopy (TEM; H-7100, Hitachi, Japan). The CuKα x-ray powder diffraction (XRD, MiniFlex II, Rigaku, Japan) patterns of the nanoparticles were recorded at room temperature to confirm particle structure. Optical absorption spectra were recorded with a UV spectrometer (V-530, JASCO Japan). The presence of the amino groups, hydroxyl groups and silicates was confirmed by Fourier transform infrared spectroscopy (FTIR; Spectrum One, Perkin Elmer, USA). To assess the surface electric charge, zeta-potential was measured by Zetasizer Nano ZS (Malvern, UK). Magnetization measurement was performed with a Quantum Design magnetic property measurement system, a superconducting quantum interference device (SQUID, MPMS, Quantum Design, Japan) magnetometer.

2.2. Nano-PALDI mass spectrometry

The usefulness of the nanoparticles as an assisting ionization material in mass spectrometry was confirmed by a MALDI-TOF-type instrument (TOF = time of flight; Voyager-DE-RP; Applied Biosystems, Germany) using N_2 laser emitting at 337 nm. The drug (reserpine) or sample peptide (substance P acetate hydrate) or protein (insulin from bovine pancreas) was chosen. The nanoparticles (10 mg) were dispersed in 1 ml of methanol. Each sample was independently dissolved in distilled water at a concentration of 10^{-4} mol l^{-1}. Each analyte solution (1 μl) was dropped on nanoparticles-coated target plates with a pipette. The external calibration peptides were deposited on the plate to minimize mass shift. The analyte surface was irradiated with 500 laser shots.

3. Results and discussion

3.1. Physical characterization of nanoparticles

Figure 2(a) shows TEM image of the nanoparticles. The nanoparticle diameter was deduced as 5.6 ± 0.2 nm (statistics: 100 measurements; figure 2(b)). The image reveals that the nanoparticles did not aggregate but disperse. The peaks at $2\theta = 19$, 37 and 35 degrees in the XRD pattern of the nanoparticles were assigned to the 111 and 121 reflections of MnO_2 and 110 reflection of Mn_2O_3, respectively (figure 2(c)). This result indicates that the crystal growth of the nanoparticles proceeds in the *a*-axial and *b*-axial directions. The nanoparticle was estimated as ~5 nm from the half-width of the XRD peaks, in good agreement with the TEM result.

UV absorption appeared around the 337 nm wavelength of the N_2 laser (figure 3(a)). This result indicated that the nanoparticles can be used as ionization assisting reagent of analyte for nano-PALDI mass spectrometry. We analyzed the nanoparticles by FTIR transmittance (figure 3(b)) to confirm chemical bonding between organic molecule and particles surface. Si–O (1000–800 cm^{-1}), C–N (1560–1480 cm^{-1}), C–H (2930–2830 cm^{-1}) and O–H (3680–3100 cm^{-1}) bonds are present at the nanoparticle surface, even after multiple washing of the samples to remove physisorbed organic molecules. The surface electric charge was analyzed with zeta-potential measurements (figure 3(c)). An isoelectric point was found for ~ pH 9.8. The nanoparticles showed positive charge in pH < 10 indicating that NH_2 groups transformed into NH_3^+ group on the nanoparticle surface. The zeta potential was more than +30 mV that was considered as the threshold value for electrostatic stabilization [19]. Magnetization results (figure 3(d)) indicated paramagnetic behavior at 300 K and antiferromagnetic behavior with sigmoid curve at 5 K [20,21]. Therefore, it is thought that our nanoparticles contain MnO_2 and Mn_2O_3 bearing hydroxyl and amino groups.

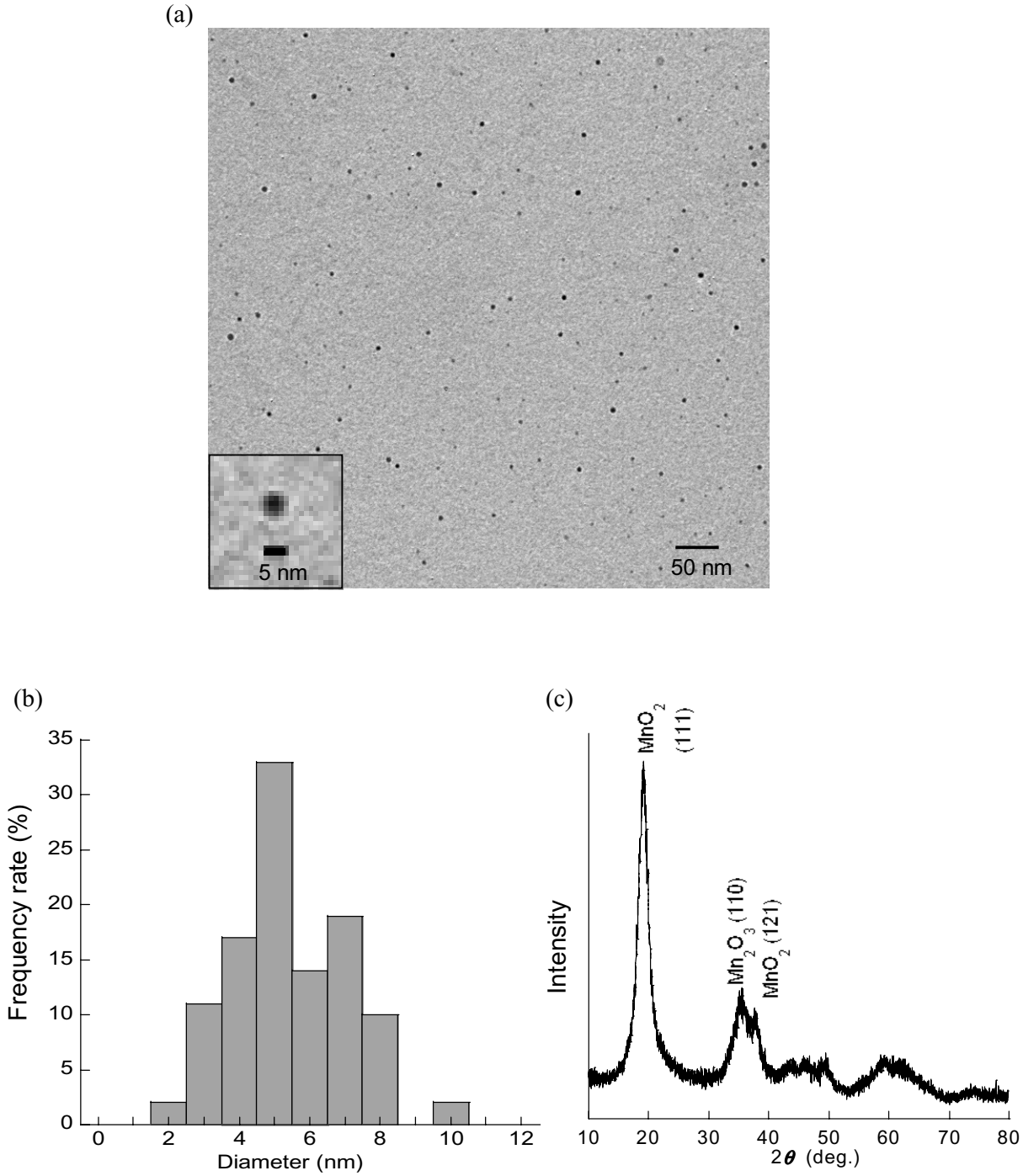

Figure 2. (a) TEM image of nanoparticles, (b) diameter distribution of nanoparticles and (c) XRD patterns of manganese oxide nanoparticles.

3.2. Ability of nanoparticles to assist ionization of pure sample analytes

We evaluated the usefulness of the nanoparticles as laser desorption/ionization material using reserpine (MW: 608.7) as a sample of low-molecular-weight drug, substance P acetate hydrate (MW: 1346.7) as a sample of peptide and insulin (MW: 5733) as a sample of protein.

Signal of the oxidative reserpine was observed at m/z 607.5 in the spectra of figure 4(a), indicating that oxidation of reserpine occurred due to air and ambient light [22]. Thus, the observed signal was oxidative reserpine called 12,13-dehaydroreserpine [23]. The reserpine sample alone or nanoparticles alone did not yield any signals (figures 4(b) and (c)). Using CHCA as a conventional matrix, the signals by themselves were observed at low molecule range (figure 4(d)). In case of substance P as peptide analyte (figure 4(e)) or insulin as protein analyte (figure 4(f)), the nanoparticles did facilitate ionization of

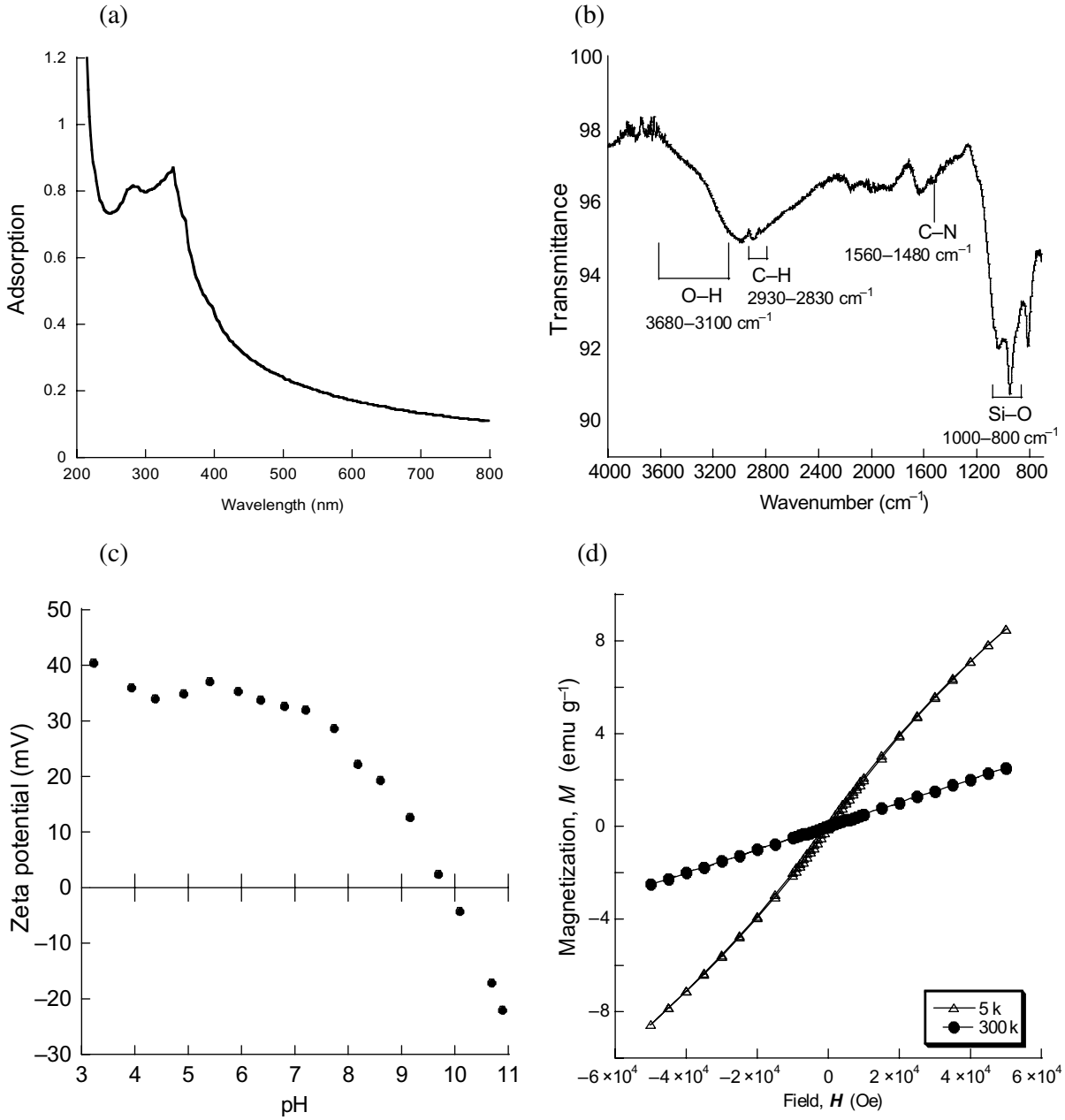

Figure 3. (a) Optical absorption spectrum of manganese oxide nanoparticles. (b) FTIR spectra of nanoparticles. (c) Zeta potential versus pH for the nanoparticles dispersed in water. (d) Magnetization measurements of nanoparticles at 300 K (●) and 5 K (□).

the analytes. This result indicated that nanoparticles can function as ionization-assisting reagents over a wide range of analytes. Interestingly, no nanoparticle-related background signals were observed in the *m*/*z* range 0–7000 that facilitates the analysis of the target molecules.

To perform post-source decay (PSD) mass spectrometry by nano-PALDI for structural analysis, we used reserpine as representative sample. The major intense ions were observed at m/z 395 (Y1 ion) and m/z 195 (Z1 ion) due to cleavage of ether bond (figures 5(a) and (b)). From these fragment ion signals, we could achieve structural analysis by nano-PALDI.

Regarding the mechanism of improved ionization by nanoparticles, we speculate that nanoparticles rapidly attain high temperature during laser irradiation. Under rapid heating condition, the analyte does not degrade, but is rapidly ionized by the heat from the nanoparticle [24]. This is attributed to the unique structure of nanoparticles, which acts as an efficient ionization assisting material. The proximity of the hydrophilic hydroxyl and amino groups at the particle surface improves the interaction between nanoparticle and the analyte. Previously, we have performed mass spectrometry for a substance P sample using commercial Au colloids [25] as reference samples. Au could also ionize the analyte with cluster-ion of itself in the low *m*/*z* region [6]. Therefore, magnesium nanoparticles are more suitable for the analysis of low molecular weight samples.

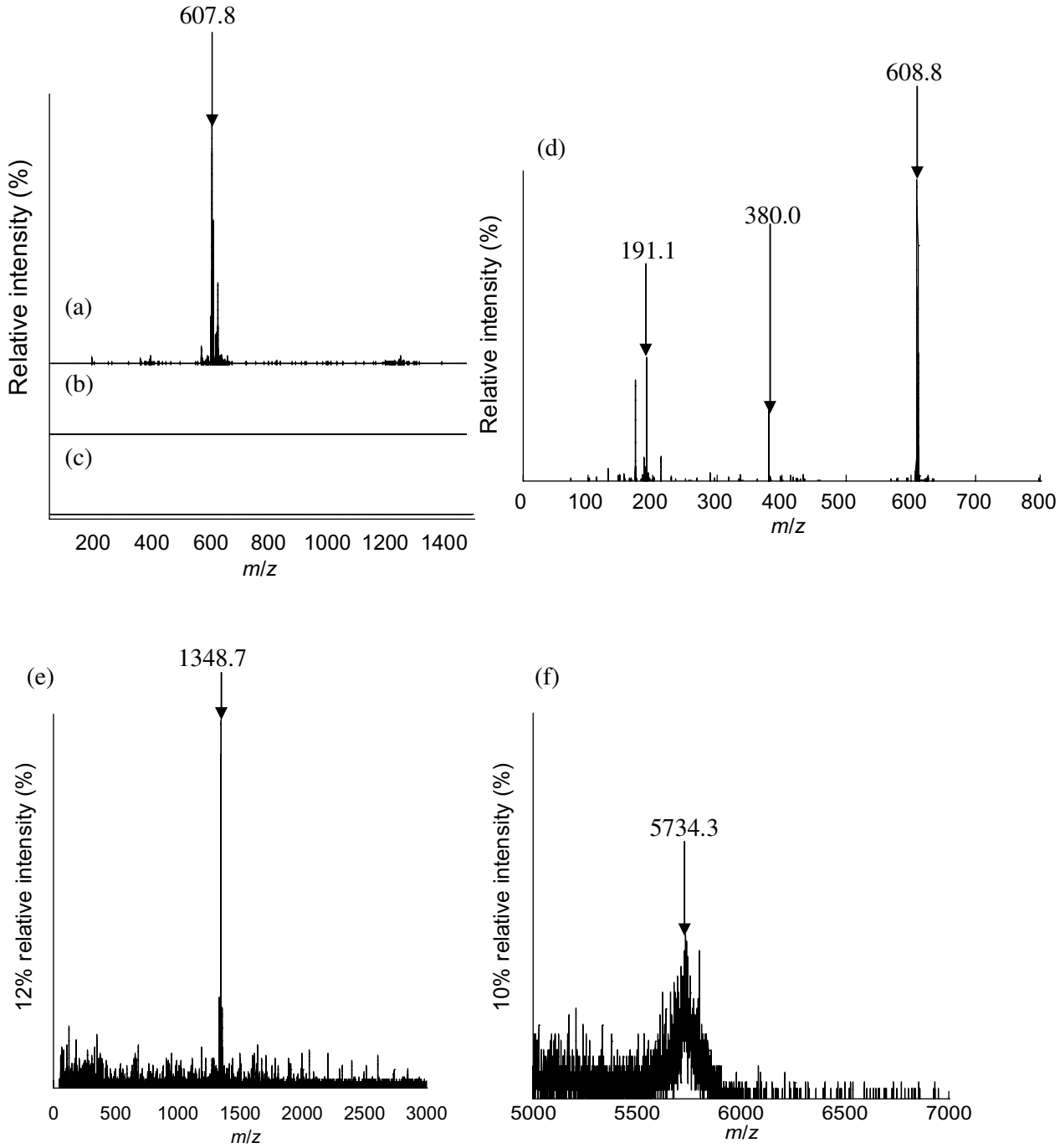

Figure 4. (a) Nano-PALDI mass spectra of reserpine (100 pmol) with nanoparticles, (b) nanoparticles alone and (c) reserpine alone; (d) mass spectra of reserpine with CHCA; (e) nano-PALDI mass spectra of substance P (100 pmol) and (f) insulin (100 pmol).

4. Conclusions

Nanoparticle can be used as a background-free ionization assisting reagent for a wide range of analytes such as chemical drugs and biomolecules. Nanoparticle themselves can ionize the analyte without combining with glycerol.

In previous study, we took advantage of localization of functionalized magnetic nanoparticles by external magnetic field [6] and the cell-specific delivery system of the particles using folic acid or amino acid [7]. Moreover, through a combination of our previous works and the reported here use of magnesium nanoparticles, we might be able to customize nanoparticles as an ionization assisting reagent with a specific delivery ability. In addition, the nanoparticles may be utilized in the detailed two-dimensional mass spectrometry analysis of biomedical tissues [3] and in cellular analysis [26]. The present nanoparticle-based approach allows simple and efficient identification of various biomolecules.

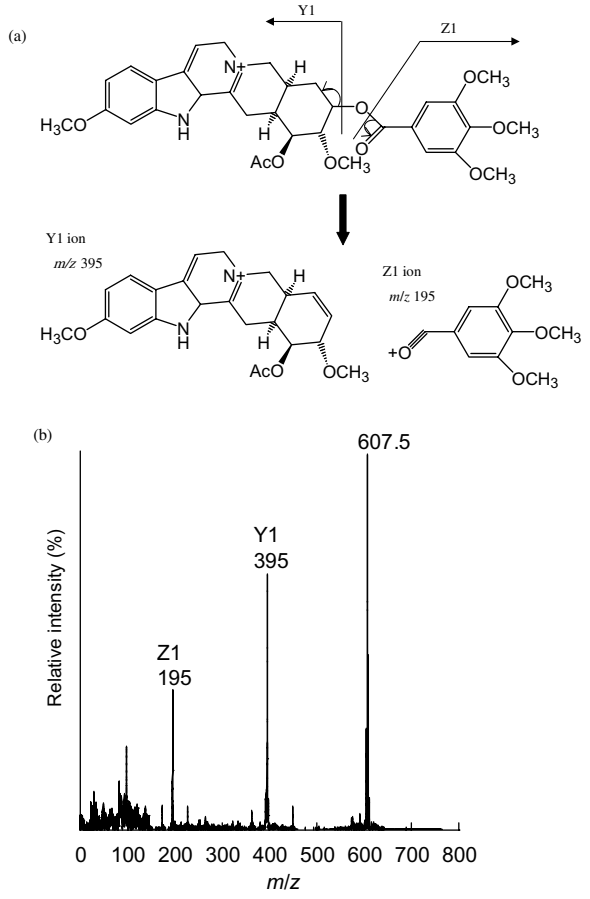

Figure 5. (a) Chemical structure of oxidative reserpine (MW 607.7). (b) Post-source decay nano-PALDI mass spectra of reserpine.

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