Analytical Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lanl20

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Published online: 11 Feb 2008.

To cite this article: Yu-Chih Chiu & Yu-Chie Chen (2008) Carboxylate-Functionalized Iron Oxide Nanoparticles in Surface-Assisted Laser Desorption/Ionization Mass Spectrometry for the Analysis of Small Biomolecules, Analytical Letters, 41:2, 260-267, DOI: 10.1080/00032710701792653

To link to this article: http://dx.doi.org/10.1080/00032710701792653

Analytical Letters, 41: 260–267, 2008

ISSN 0003-2719 print/1532-236X online
DOI: 10.1080/00032710701792653

Carboxylate-Functionalized Iron Oxide Nanoparticles in Surface-Assisted Laser Desorption/Ionization Mass Spectrometry for the Analysis of Small Biomolecules

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Abstract: Iron oxide particles have been demonstrated previously as an effective SALDI-assisted material in SALDI MS analysis. The addition of a proton source such as citrate salts is helpful for rendering ionization efficiency. To simplify the sample preparation steps, we immobilize polyacrylic acid (PAA) onto the surface of the iron oxide particles. The modified iron oxide particles can be used as the SALDI-assisted material without the addition of extra proton source. Small biomolecules including bradykinin, melittin, and insulin are used to examine the feasibility of this approach. The results show that the SALDI mass spectral results are improved by using polyacrylic acid ($MW_{average} = \sim 2$ kDa) coated iron oxide nanoparticles.

Keywords: Iron oxide, nanoparticles, SALDI, polyacrylic acid

INTRODUCTION

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) (Karas et al. 1985; Karas and Hillenkamp 1988) is suitable for analysis of

Received 3 September 2007; accepted 10 October 2007

We would like to thank National Science Council (NSC) of Taiwan for financially support this research. We also thank Cheng-Tai Chen for his assistance in preparing the figures.

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proteins, peptides, oligonucleotides, and polymers using aromatic organic acids as the matrix. Alternatively, inorganic materials such as graphite powder (Sunner et al. 1995; Dale et al. 1996, 1997), activated carbon powder (Chen et al. 1998; Chen 1999; Chen and Tsai 2000), metal particles (Tanaka et al. 1988; McLean et al. 2005; Huang and Chang 2006; Su and Tseng 2007), sol-gels (Lin and Chen 2002; Ho et al. 2003; Teng and Chen 2003; Lin et al. 2004), and metal oxide particles (Schürenberg et al. 1999; Chen and Chen 2003, 2004a, 2005) have been used as the assisted material for energy transfer in laser desorption/ionization mass spectrometry, which is generally called surface-assisted laser desorption/ionization (SALDI) mass spectrometry (Sunner et al. 1995). The surface structure and the enhanced surface area of the SALDI-assisted materials can affect the SALDI MS results (Alimpiev et al. 2001; Okuno et al. 2005). Furthermore, electric and thermal properties are critical to SALDI MS analysis.

Although the detectable upper mass limit in SALDI MS is lower compared to MALDI MS, there are several advantages offered by SALDI MS analysis. The most apparent features are low matrix background and homogeneous sample deposition. Therefore, many researchers have made efforts in exploring useful inorganic materials for SALDI MS analysis. For instance, we have previously demonstrated that iron oxide nanoparticles can be used as the SALDI-assisted material. The addition of a proton source is required to render the ionization efficiency for analytes. Conventionally, glycerol is added in the SALDI sample as the proton source. However, the presence of glycerol in the SALDI sample may affect the vacuum in mass spectrometers. Therefore, employing SALDI MS to high-throughput analysis is limited. Citrate salts are alternatively used as the proton source, and they reduce the problem on the maintenance of a high vacuum in the mass spectrometer (Chen and Chen 2004). We provide the reason for this that the addition of a proton source can be avoided if the inorganic materials have been bound with functional groups which are capable of providing protons. Carboxylate-metal oxide complexations have three modes including monodentate, bidentate chelating, and bidentate bridging (Kirwan et al. 2003). Polyacrylic acid (PAA) contains a number of carboxylates which are capable of chelating with iron oxide nanoparticles and providing protons for the protonation of analytes simultaneously. The modified nanoparticles can be used as the SALDI-assisted materials without the addition of extra protons.

EXPERIMENTAL SECTION

Reagents

Bradykinin (MW = 1060.22), citric acid monohydrate (≥98%), insulin (MW = 5733.55 Da), and methanol were purchased from Sigma (St. Louis,

MO). Poly(acrylic acid) (PAA, $MW_{average} = \sim 2$ kDa), and aqueous PAA ($MW_{average} = \sim 900$ kDa) were obtained from Aldrich (Steinheim, Germany), while ethanol was obtained from Showa (Tokyo, Japan). Iron(III) chloride hexahydrate was purchased from Riedelde Haën (Seelze, Germany), and hydrochloric acid (30%) was obtained from Merck (Darmstadt, Germany). Iron(II) chloride tetrahydrate ($\geq 99.0\%$) and melittin (MW = 2846.52 Da) were purchased from Fluka (Steinheim, Germany), and finally, aqueous PAA ($MW_{average} \sim 90000$ Da, 25 wt.%) was obtained from Acros (Belgium, New Jersey).

Preparation of the Fe_3O_4 Magnetic Nanoparticles

$FeCl_3$ (2 g) and $FeCl_2$ (5.4 g) were dissolved in hydrochloric acid (2 M, 25 ml) at room temperature under sonication. After the salts were completely dissolved in the acidic solution, the mixture was degassed using a pump. Aqueous ammonia (25%, 40 ml) was slowly added under nitrogen with stirring at room temperature, and the mixture was continually stirred for another 30 min. The iron oxide nanoparticles were rinsed with deionized water several times to remove unreacted chemicals, followed by re-suspension in ethanol. To immobilize PAA onto the surface of the iron oxide nanoparticles, the latter (3.5 mg) were sonicated with 1% (w/w) PAA aqueous solution (1 ml) for 15 min followed by vigorous vortex-mixing for another 1 h. After rinsing by deionized water, the PAA coated nanoparticles were resuspended in deionized water (0.7 ml).

Preparation of the SALDI MS Samples

The iron oxide nanoparticle suspension obtained above (0.5 μl) was mixed with the analytes (0.5 μl). The mixture (0.3 μl) was deposited onto a sample plate. After evaporation of the volatile solvent, the plate was introduced into a Bruker Biflex III mass spectrometer (Germany) for SALDI MS analysis.

RESULTS AND DISCUSSION

Figure 1a presents the SALDI mass spectrum of bradykinin by using unmodified iron oxide nanoparticles as the SALDI-assisted material. There are a number of peaks appearing around m/z 1060, corresponding to the sodium and potassium adductions of bradykinin. Figure 1b presents the SALDI mass spectrum of melittin using unmodified iron oxide nanoparticles as the SALDI-assisted materials. A broad peak appears at m/z ~2850 derived from melittin. The results indicate that the SALDI mass spectral quality is

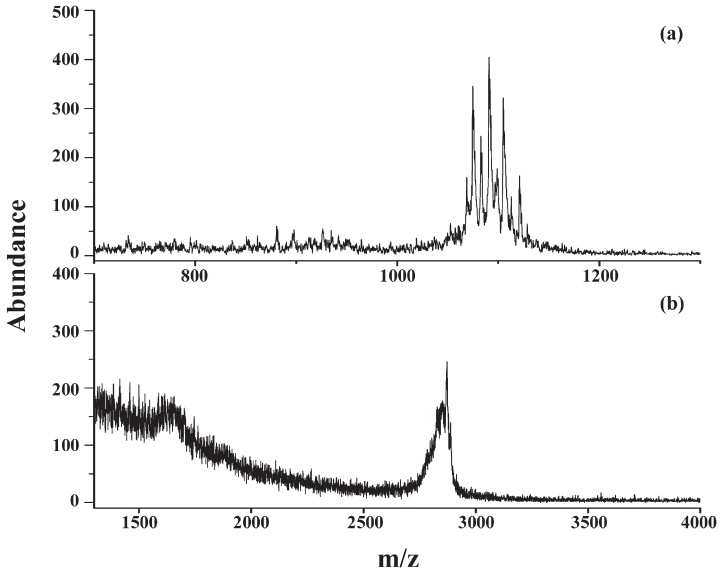
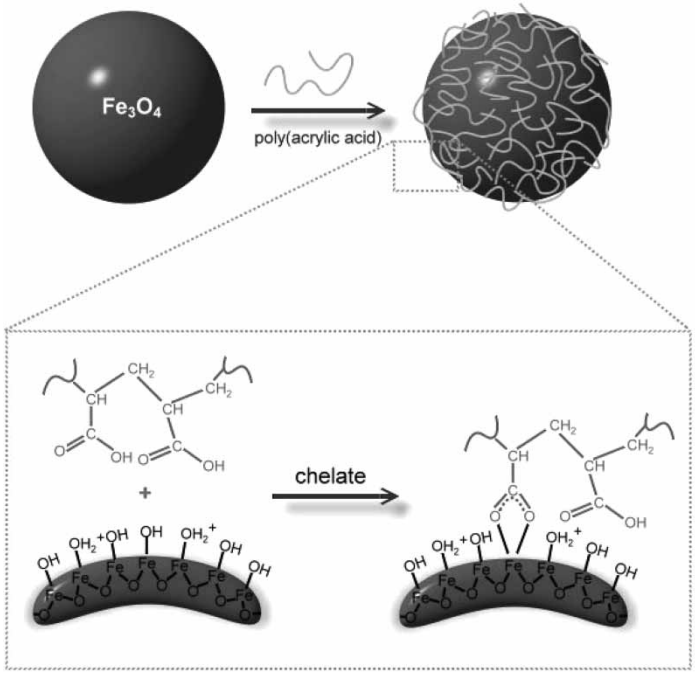


Figure 1. SALDI mass spectra of (a) bradykinin (14 pmol) and (b) melittin (26 pmol) using unmodified iron oxide nanoparticles as the SALDI-assisted material.

unsatisfactory using unmodified iron oxide nanoparticles as the SALDI matrix. The appearance of alkali adductions of analytes leads to low mass resolution. This may be due to the presence of abundant alkali metal ions and the absence of sufficient protons in the SALDI sample.

In order to improve the mass spectral results, we immobilized PAA ($MW_{average} = \sim 2$ kDa), which contains a number of carboxylates, onto the surface of iron oxide nanoparticles via the chelation of carboxylic acid. Scheme 1 shows the proposed design by immobilizing PAA onto the surface of the iron oxide nanoparticles via bibridging chelating. Some carboxylates chelate with the nanoparticles, and the rest of carboxylates in PAA provide protons for rendering the ionization of analytes. It is anticipated that carboxylates on the nanoparticles can compete in the binding with alkali metal ions to reduce the formation of alkali adductions of analytes. To estimate the binding amount of PAA on the surface of iron oxide nanoparticles, we employed Thermal Gravimetric Analysis (TGA) to determine the PAA amount on the nanoparticles. Figure 2 presents the TGA result by plotting the nanoparticle weight% as a function of elevated temperature. The weight dramatically decreased to 88% as the temperature changed from $250 \sim 350^{\circ}$C. Thus, we estimated 12 mg of PAA onto 100 mg of the PAA coated iron oxide nanoparticles based on the TGA result.

Figure 3a presents the SALDI mass spectrum of bradykinin using the iron oxide nanoparticles coated with PAA. A sharp peak appears at m/z 1061, which corresponds to the protonated pseudomolecular ions of bradykinin



Scheme 1. PAA immobilized iron oxide nanoparticles.

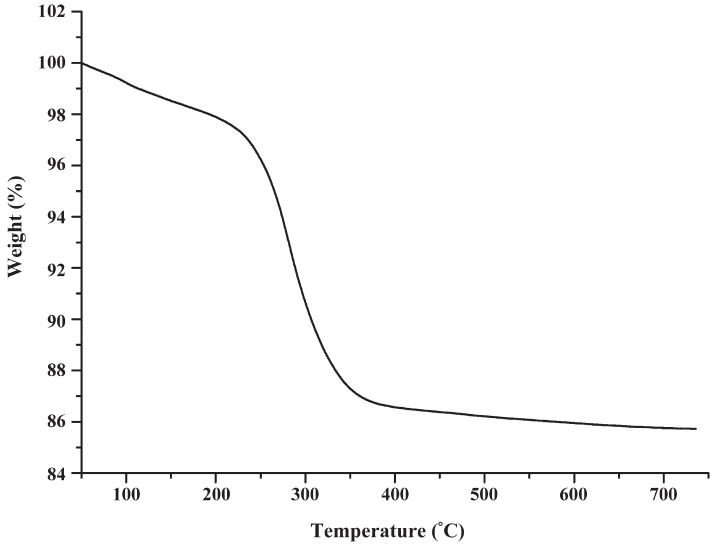


Figure 2. TGA analysis result of PAA ($MW_{average} = \sim 2$ kDa) immobilized iron oxide nanoparticles.

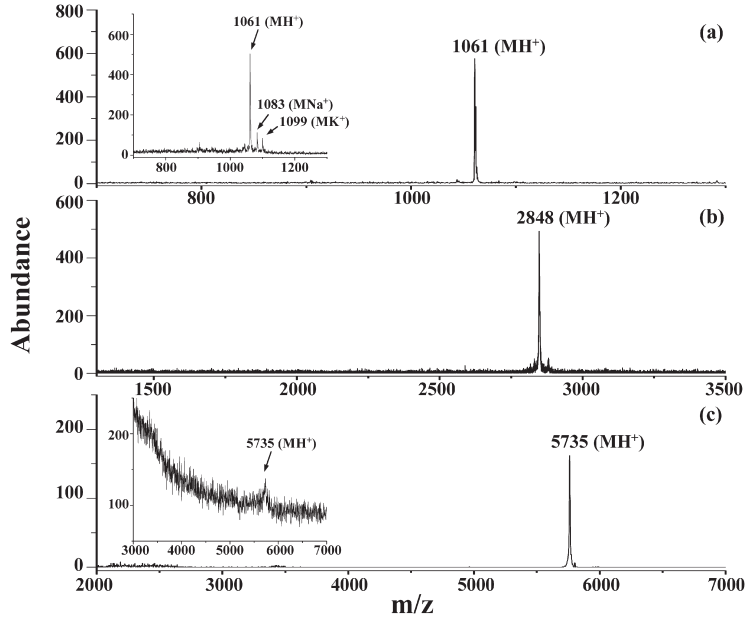


Figure 3. (a) SALDI mass spectra of bradykinin (14 pmol). The inset is the SALDI mass spectrum of bradykinin (708 fmol). (b) SALDI mass spectrum of melittin (26 pmol). (c) SALDI mass spectrum of insulin (9.1 pmol). The inset is the SALDI mass spectrum of insulin (2.3 pmol). PAA ($MW_{average} = \sim 2$ kDa) modified iron oxide nanoparticles were used as the SALDI-assisted material.

(MH^+). The inset SALDI mass spectrum was obtained by further lowering the bradykinin concentration. Besides the protonated pseudomolecular ion of bradykinin (MH^+), the sodium and potassium adductions of bradykinin with weak intensities also appear at m/z 1083 (MNa^+) and 1099 (MK^+), respectively. The mass spectral quality in Fig. 3a is much improved as compared to that shown in Fig. 1a. The SALDI mass spectra of melittin and insulin using iron oxide coated with PAA respectively are shown in Figs. 3b,c. The peak at m/z 2848, corresponding to the protonated pseudomolecular ions of melittin, appears in Fig. 3b, while the peak at m/z 5735, corresponding to the protonated pseudomolecular ions of insulin, is shown in Fig. 3c. The mass spectral results are greatly improved in terms of mass resolution. The results indicate that using PAA-immobilized iron oxide nanoparticles as the SALDI-assisted material is suitable for obtaining SALDI mass spectral results. The alkali adductions are also drastically reduced.

We contemplate whether immobilizing a larger PAA onto the surface of the iron oxide nanoparticles could make the modified nanoparticles become a better SALDI-assisted material and further improve the SALDI MS results. Thus, we alternatively immobilized PAA with an average molecular weight

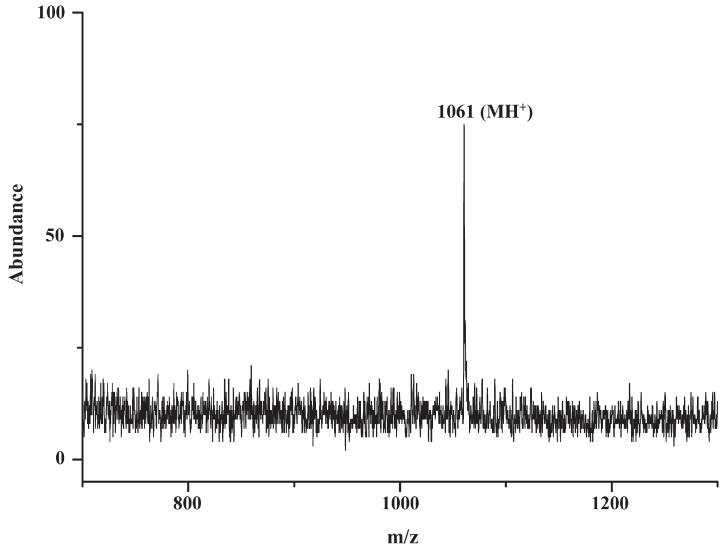


Figure 4. SALDI mass spectrum of bradykinin (2.3 pmol) using PAA ($MW_{average}$ = 900 kDa) coated iron oxide nanoparticles as the SALDI-assisted material.

~900 kDa onto the surfaces of the iron oxide nanoparticles. Figure 4 presents the SALDI mass spectrum of bradykinin (2.3 pmol) using PAA ($MW_{average}$ = 900 kDa) coated iron oxide nanoparticles. A sharp peak at m/z 1061, corresponding to the protonated pseudomolecular ions of bradykinin, still appears in the mass spectrum. However, the detection limit of using PAA 900 kDa coated iron oxide nanoparticles as the SALDI-assisted material is higher than that obtained using PAA 2 k-coated iron oxide nanoparticles. Presumably, the molecule size of PAA (900 kDa) is too large to effectively transfer energy from the iron oxide nanoparticles to the analytes.

CONCLUSIONS

We have demonstrated a straightforward approach to immobilize PAA onto the surfaces of iron oxide nanoparticles simply by using carboxylate-chelating interactions. The results show that the PAA-modified iron oxide nanoparticles are suitable SALDI-assisted materials. The SALDI mass spectral results using the PAA-modified iron oxide nanoparticles as the SALDI-assisted material are better than those obtained using unmodified iron oxide nanoparticles as the SALDI-assisted material. The protonated pseudomolecular ions of analytes generally dominate the PAA-modified iron oxide nanoparticles of the SALDI mass spectra and the alkali adductions of analytes are greatly reduced. The upper detectable mass limit is ~6 kDa. It might be

possible to further functionalize the PAA-coated nanoparticles with binding affinity for specific analytes since carboxylates can be easily derivatized.

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