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Properties of an electrospray emitter coated with material of low surface energy

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Abstract

This paper describes the properties of a recently developed electrospray emitter coated with a fluorinated polymer of low surface energy. The emitters can produce stable electrospray from solvents of various surface tensions including distilled water with a high surface tension at a flow rate range of micro- to nanoliters without the aid of any nebulizing gas. The electrically non-conductive nature of the tips virtually eliminates electrical discharge and allows stable electrospray in the negative ion mode. The emitters are suitable for hyphenating HPLC to mass spectrometry in both positive and negative ion modes at low flow rates.

Keywords: Electrospray emitter; Instrumentation; Mass spectrometry; Proteins; Prostaglandins; Lipids

1. Inroduction

Electrospray ionization (ESI) mass spectrometry (MS) is now widely used for analyses of biomolecules, pharmaceutical compounds, synthetic polymers and so on [1]. The need to enhanced sensitivity and better connectivity for capillary high-performance liquid chromatography (HPLC), especially in proteomics and metabolomics, have led to ESI operation in the low flow rate range of less than 1 μl/min. This was achieved using tapered capillary emitters with a very small outlet orifice, usually made of glass and fused silica with or without either metal or conducting-polymer coating, and of stainless steel [2–7]. Practice with these emitters have, however, revealed problems such as easy clogging at their exit orifices and instability of spraying water-rich solution of a high surface tension at an early stage of gradient elution on reversed-phase HPLC being frequently used for high-throughput proteomics and metabolomics.

Both problems are mutually related and arise primarily from the high surface energy of both aqueous solutions and materials the emitters are made of. Electrospray initiates from expansion of a liquid into a dynamic cone with the charged surface (Taylor cone) at the capillary exit by the applied high electric field [for review, see ref. [1] and references cited therein]. When the force of the applied field on and Coulombic repulsion of the surface charges surpass the surface tension of solution (γ_L), droplets with excess charge of a given sign detach from the cone. Large surface tensions of the tip material ($\gamma_S \sim 100$ mN/m and γ_{SL}) and of aqueous solutions ($\gamma_L \sim 73$ mN/m) produce a cone supported by the base of a larger diameter on the outer wall of the tip, where γ denotes the surface tension, and the subscripts S, SL, and L glass/air, glass/water, and water/air interfaces, respectively (Fig. 1). This leads to a lower charge density on the cone surface, because the rate of excess charge production at the electric contact site of the emitter is rather constant [1]. This causes difficulty in spraying, and eventually a need to extremely sharp emitter tips. To circumvent these problems, I recently developed the emitters coated with a fluorinated polymer (FP) of low surface energy (FortisTip). FP coating of the emitter tip greatly decreases γ_S and γ_{SL}, favoring stable spraying from water-rich solutions, and their properties are reported here.

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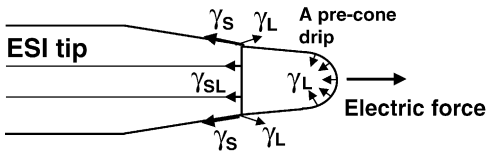

Fig. 1. Large surface tension on a fused-silica tip prevents from spraying water-rich solvents. The symbol γ indicates the surface tension, and the subscripts S, L, and SL the fused silica/air, water/air, and fused silica/water interfaces, respectively.

2. Experimental

2.1. Materials

Angiotensin II, *N*-lignoceroyl-4-sphingenine (S), and *N*-lignoceroyl-sphinganine (Sa) were obtained from Sigma. *N*-Stearoyl-4-hydroxy-sphinganine (P) was purchased from Avanti Polar Lipids. Prostaglandins D_2 (PGD_2) and J_2 (PGJ_2) and 15-deoxy-$\Delta^{12,14}$-PGJ_2 were purchased from Cayman Chemicals. FortisTips of 20-μm inner (i.d.) and 150-μm outer (o.d.) diameters and of 50-μm i.d. and 150-μm o.d. were supplied by OmniSeparo-TJ (Hyogo, Japan) that will commercialize these tips. A document describing the fabrication of FortisTips has been submitted to the Patent Cooperation Treaty of the World Intellectual Property Organization (Tojo, H. "Electrospray emitter coated with material of low surface energy").

2.2. HPLC

A Magic 2002 HPLC system (Michrom BioResources, Inc.) was operated in the capillary HPLC mode on a Magic C18 separation column (50 mm × 0.2 mm, Michrom BioResources) and a C_{18} trap column (5 mm × 0.3 mm, Optimize). For peptide separation the column was pre-equilibrated with solvent A (2% acetonitrile and 0.1% formate in water), and developed with a linear concentration gradient of solvent B (90% acetonitrile and 0.1% formate): from 2 to 60% in 20 min at the flow rate of ∼1 μl/min. The total flow rate of Magic2002 pumps was set at 50 μl/min, and the flow rate across the capillary column was regulated with a Magic variable splitter. A 20 μm i.d. × 150 μm o.d. FortisTip was directly connected to an outlet stainless fitting of the column to minimize a peak broadening by diffusion. For lipid separation the same column was pre-equilibrated with 15% solvent B and developed with a linear concentration gradient of solvent B from 15 to 30 in 1 min and from 30 to 45% in 20 min at the flow rate of ∼1.5 μl/min.

2.3. Mass spectrometry

An ion-trap mass spectrometer LCQ (ThermoElectron, CA) was used for testing the performance of the emitters because of its good connectivity for HPLC. A FortisTip was placed at an appropriate distance, typically 3–5 mm in front of a heated capillary inlet of LCQ with a xyz stage (AMR, Tokyo). Input of a contact signal from HPLC to LCQ started applying an ESI voltage (e.g., 1.7 kV) to the column outlet fitting. The mass spectrometer was operated in either positive or negative ion mode and in the alternate polarity-switching mode with the automatic gain control on. The temperature of the heated capillary was 130–150 °C. For MS^2, MS^3, and MS^4 scans, relative collision energies of 32–40% were used and in some experiments "dependent-scan" mode was used.

3. Results and discussion

3.1. General properties of FortisTips

The emitter bodies are made of fused-silica capillary, and in contrast to conventional pulled capillaries, the emitter tips were tapered only on the outer surface with the lumen intact, which is important to reduce the risk of particle clogging especially during a high-throughput work. The outer wall of the tip and the entire inner wall were coated with FP, making the tip surface highly unwettable (the contact angle of 122 °C, a measure of unwettability). FP coatings are colorless and transparent, which makes the solvent flow in the emitter tip visible.

3.2. Performance of the emitter tips with water-rich solvent

At the initial stage of gradient elution on reversed-phase HPLC being frequently used for high-throughput proteomics and metabolomics, emitters should produce a stable jet [8] from water-rich solution, e.g., 2% acetonitrile and 0.1% formate in water, immediately after the onset of a high voltage. To test whether FortisTips meet this requirement, their efficiency of spraying distilled water was examined by visual inspection and stability of total ion current (TIC) with LCQ. Distilled water was delivered to a FortisTip of 20-μm i.d./150-μm o.d. with a syringe pump at the flow rate of 0.8 μl/min being suitable for HPLC on a capillary column of 0.1–0.2 mm in i.d. The counter electrode voltage of 1.8 kV was applied and total ion current (TIC) of *m*/*z* 150–2000 was recorded for 20 min; optimal voltages depended on dimensions of the emitters and the surface tension values of sample solutions, typically in the range of 1.2–2.0 kV. Using these setups the FortisTip can stably spray distilled water as shown in Fig. 2A.

It is desirable to keep the high voltage off while either washing column between runs or injecting a sample to avoid contamination, although the use of capillary HPLC allows MS to tolerate such a contamination more than that of conventional HPLC (5). With the voltage off, droplets in various sizes grows at the tip of emitter, which often prevents conventional, uncoated or metal-coated pulled emitters from spraying after the high voltage is applied again on the next run. To mimic those unfavorable conditions encountered on

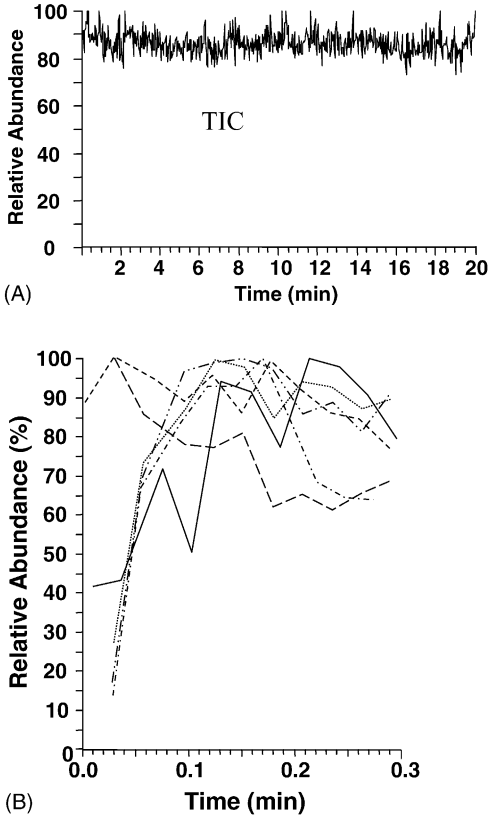

Fig. 2. Performance of FortisTips with distilled water. (A) Stability of spraying distilled water from the emitter. (B) Rapid onset of electrospray from distilled water, a high surface tension solvent. The total ion current traces (m/z = 150 ∼ 2000) from six experiments using the indicated time lags between starting a pump and the onset of a high voltage applied to the emitter are superimposed.

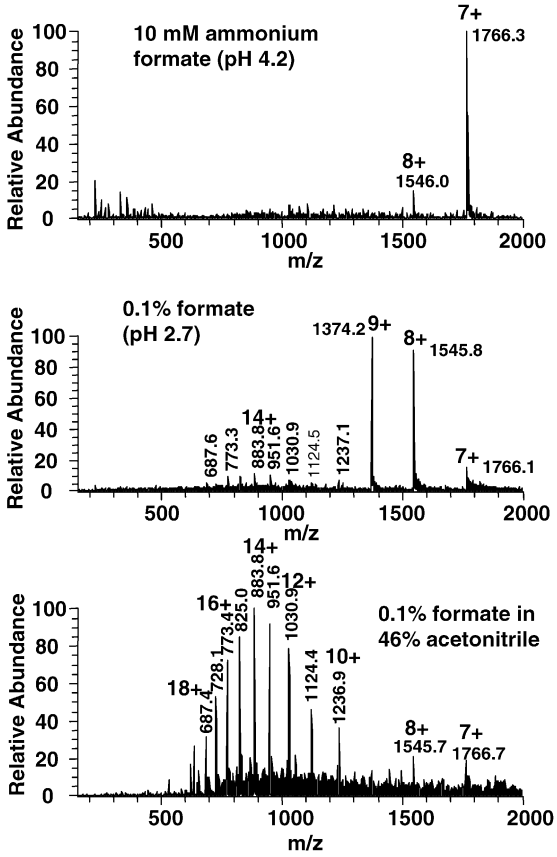

Fig. 3. Positive ion mass spectra of cytochrome *c* (1 μM) in distilled water containing the indicated additives. The spectra were accumulated for 20 scans. A FortisTip of 20 μm i.d. × 150 μm o.d. was used.

HPLC–MS, we first started delivering distilled water to a FortisTip through a syringe pump and then applied a high voltage after some lag times during which water droplets varying in size form at the emitter tip. All TIC traces of m/z 150–2000 in the positive ion mode from experiments using 6 different lag times (0, 0.5, 1, 1.5, 2, and 2.5 min from the onset of charging) shows rapid onset of stable electrospray from distilled water in the cone-jet mode within ∼0.15 min (Fig. 2B). These results demonstrate that FortisTips are compatible with high-throughput HPLC–MS analyses using an autosampler and water-rich solvent for column initialization as well as direct infusion analyses of samples in aqueous solution. FortisTips were so robust to be usable for more than 600 injections of tryptic digests of proteins prepared from clinical sources such as biopsies and surgically removed tissues on a reversed-phase HPLC–ion-trap MS (personal communication, Professor Toshihide Nishimura and Dr. Kiyonaga Fujii of Clinical Proteome Center, Tokyo Medical University).

We next applied FortisTips to examine the effects of organic solvent concentrations on ESI mass spectra of proteins (Fig. 3). The envelopes of peaks of multiply charged positive ions of horse heart cytochrome *c* (1 μM) shifted towards lower m/z values with their maximal intensities at 1766.3, 1374.2, and 883.8 mass units (corresponding to the charges +7, +9, and +14, respectively) with an increase in denaturing power of solvents used: water containing 10 mM ammonium formate (pH 4.2) < water containing 0.1% formic acid (pH 2.73) < 46% acetonitrile containing 0.1% formic acid, respectively. This is consistent with the results of previous studies, and those shifts to lower m/z was attributed to the degrees of exposure of ionizable side chains to solvent by protein conformational changes [9]. FortisTips would therefore help study conformations, unfolding/folding, and interactions of proteins in gas-phase as a function of their conformation in solution including native conformation in purely aqueous solutions. Moreover, the recent development of ultra-high resolution Fourier transform ion cyclotron resonance MS are drawing much attention to analyses of whole proteins by MS including MS/MS without protease digestion [10]. Organic solvents are usually included in sample solutions to assist ESI by conventional emitters, but are prone to cause uncontrollable denaturation and aggregation of proteins. The present emitters compatible with MS analyses in their absence will be helpful in studying whole proteins.

It was reported that a microsyringe with a tapered stainless steel needle tip was suitable for spraying water by direct infusion [9]. It is difficult to use this setup for hyphenation of

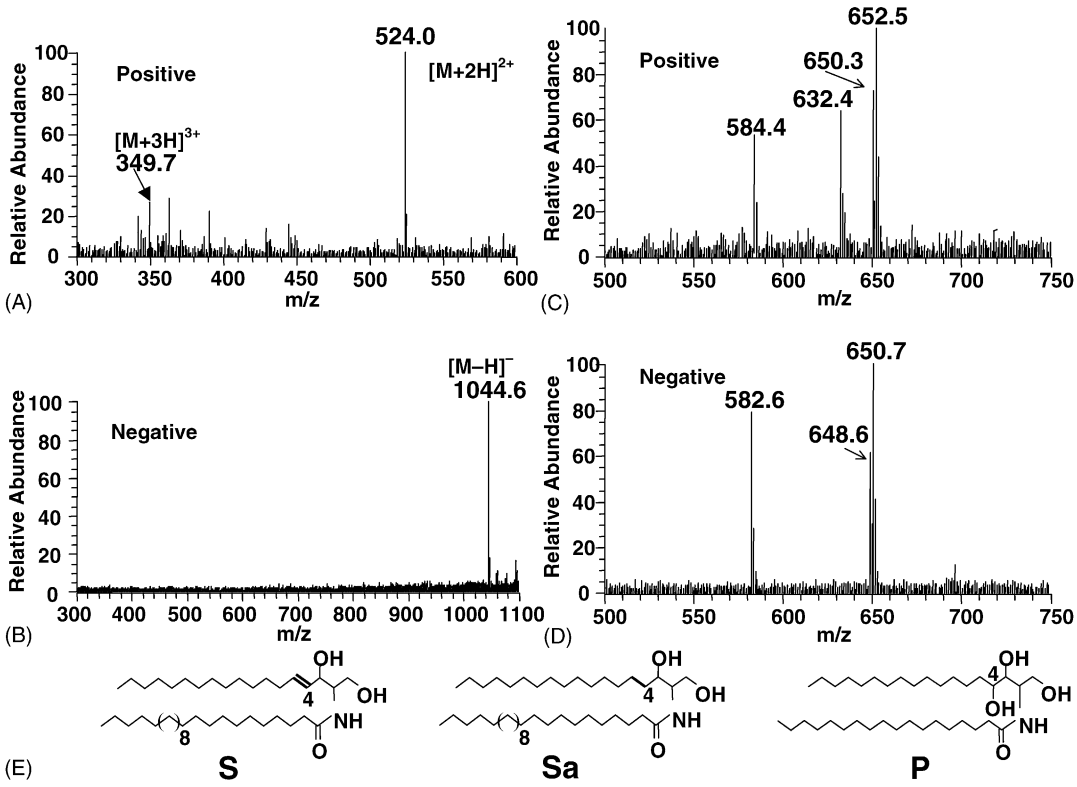

Fig. 4. Positive and negative ion mass spectra of angiotensin II and ceramides (0.1 μM each). Angiotensin II (A and B) and ceramide mixtures containing *N*-lignoceroyl-4-sphingenine, *N*-lignoceroyl-sphinganine, and *N*-stearoyl-4-hydroxy-sphinganine (C and D). The structures of the ceramides are in E. Experimental setups are described in Section 2. Ceramide spectra were acquired in the alternate positive and negative ion switching mode.

HPLC with MS. The use of stainless steel emitter in the previous tip would be prone to electric discharge in the negative ion mode as described below.

3.3. Capability of the emitter for negative ion MS

There is an increased risk of corona discharge or arcing in the negative ion mode versus in the positive ion mode [1]. In particular, conventional metal-coated emitters are prone to experience corona discharge that damages a coated tip. This prevents a stable spraying of negative ions, thereby hindering their coupling to HPLC or other separation methods that require a stable operation of ESI for a longer time period. Nanoelectrospray emitters coated with polyaniline, a conductive polymer, were very recently developed and they are resistant to electrical discharge and durable for at least an hour in the negative ion mode [11]. The tips of FortisTips are electrically non-conductive. This virtually eliminates electrical discharge and allows stable electrospray in the negative ion mode as well as in the positive ion mode. This is exemplified by mass spectra of peptides, ceramides and prostaglandins as follows.

Angiotensin II (0.1 μM) dissolved in seven volumes of solvent A and three volumes of solvent B was continuously infused through a 20 μm i.d. × 150 μm o.d. FortisTip at the flow rate of 0.4 μl/min. The onset of electrospray was initiated by applying a voltage of 1.8 kV. The mass spectrometer was separately tuned and spectra were individually acquired for the positive and negative ion scans. Angiotensin II contains an acidic and two basic residues with a calculated pI of 7.81, and is positively charged in the presence of 0.1% formate (pH 2.73), unfavorable for the formation of negative ions in solution. The negative ion spectrum, however, exclusively contained abundant, singly charged $[M - H]^-$ ions at *m*/*z* 1044.6, compared to the positive ion spectrum with $[M + 2H]^{2+}$ ions of *m*/*z* 524 predominant (Fig. 4A and B). The signal intensity of the base peak in the positive ion spectrum were 8.4 times that of the corresponding peak in the negative ion spectrum, but their signal-to-noise (S/N) ratios were similar to each other, consistent with lower levels of chemical noises and contaminant peaks in the negative ion mode. These results indicate a potential importance of applying negative ion MS measurements with FortisTips to proteomics studies.

Another example using less polar solvent mixtures is a comparison of the positive and negative ion spectra for three ceramide species (Sa, S, and P) that were acquired simultaneously by an alternate polarity switching [12,13]. These ceramides (0.1 μM each) were dissolved in hexane–2-propanol–ethanol (5:4:1, v/v/v) containing 5 mM ammonium formate. The mixtures were sprayed through a 50 μm i.d. × and 150 μm o.d. FortisTip at the flow rate of ∼2 μl/min with

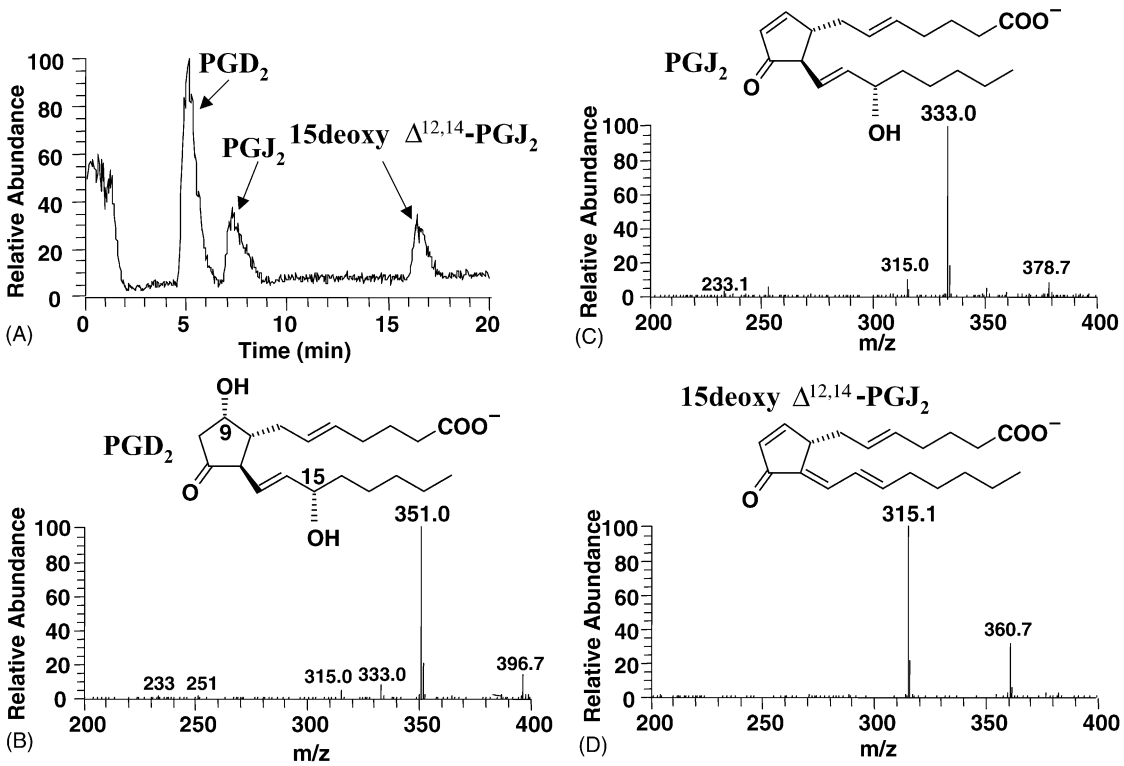

Fig. 5. Analysis of prostaglandins by capillary HPLC–ion-trap MS in the negative-ion modes using aqueous acetonitrile. A ForisTip of 20 μm i.d. × 150 μm o.d. and an ESI voltage of 1.7 kV were used. An 1 μl aliquot of mixtures of PGD_2, PGJ_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (0.5 pmol each) was gently drawn into a microsyringe containing 10 μl solvent A, and then injected onto a trap column fitted to a 10-port Valco valve. The columns were developed as in Section 2. (A) Base peak chromatogram (*m/z* 200-450); (B) PGD_2; (C) PGJ_2; (D) 15-deoxy-$\Delta^{12,14}$-PGJ_2.

an applied voltage of 1.4 kV. The low surface tension of the solvent system used and the emitter tips led to stable electrospray in the positive and negative ion modes with the alternate polarity switching controlled by the Xcalibur software. The signal intensity of a base peak in the positive ion spectrum was ~20 times that of the corresponding peak in the negative ion spectrum, but the S/N ratio was somewhat better in the latter. $[M + H]^+$ ions of Sa, S, and P at *m/z* 652.5, 650.3, and 584.4, respectively, and the corresponding $[M - H]^-$ ions were predominantly observed (Fig. 4C and D). A fragment at *m/z* 584.4 arises from loss of H_2O from S, characteristic of 4-sphingenine containing ceramides. The solvent system contained 5 mM formate to enhance the ionization efficiency of ceramides, which was prone to form formate adduct ions in the negative ion mode. Applying an in-source low voltage (ca. 20 V) prevented forming these adducts, and helped generate structurally informative $[M - H]^-$ ions of ceramides (Fig. 4D).

To further examine the utility of FortisTips in the negative ion mode using aqueous solvents, we analyzed the mixtures of PGD_2 and its bioactive metabolites PGJ_2 and 15-deoxy-$\Delta^{12,14}$-PGJ_2 by capillary reversed-phase HPLC on a Magic C_{18} column (50 mm × 0.2 mm). PGJ_2 and 15-deoxy-$\Delta^{12,14}$-PGJ_2 are formed from PGD_2 by the elimination of one and two water molecules, respectively. Stable electrospray immediately occurred from water-rich, 15% solvent B and last until the end of gradient (Fig. 5A). Full-scan spectra of these prostaglandins (0.5 pmol each) contain abundant deprotonated ions and minor formate-adduct ions. No signal derived from coating material was observed in the ESI mass spectra over the entire gradient range. The MS^2 product ion spectra of $[M–H]^-$ ions of PGD_2 and PGJ_2 contain abundant fragment ions generated by the loss of one and two water molecules (*m/z* 333, and 315) and loss of water and hexanal (*m/z* 233) (Fig. 6A and B). Similar spectra were reported in a previous study with fast atom bombardment MS [14]. The neutral loss of CO_2 from the $[M - H]^-$ ions of 15-deoxy-$\Delta^{12,14}$-PGJ_2 yielded abundant *m/z* 271 ions [15], which underwent extensive fragmentation of the charge remote type on MS^3 scans with *m/z* 315, and 271 ions isolated sequentially for collision-induced dissociation. MS^4 scans, with *m/z* 351, 315, and 271 ions isolated sequentially as precursor ions, gave a similar pattern, confirming the fragmentation path of the carboxylate anions of PGD_2.

In conclusion, FortisTips can electrospray water stably, compatible with high-throughput reversed-phase HPLC using water-rich solvent in the early phase of gradient elution and with experiments using intact proteins in purely aqueous solutions. They can also produce stable electrospray plume in the negative ion mode, useful for ESI-MS application to metabolomic [13] and pharmacological profiling.

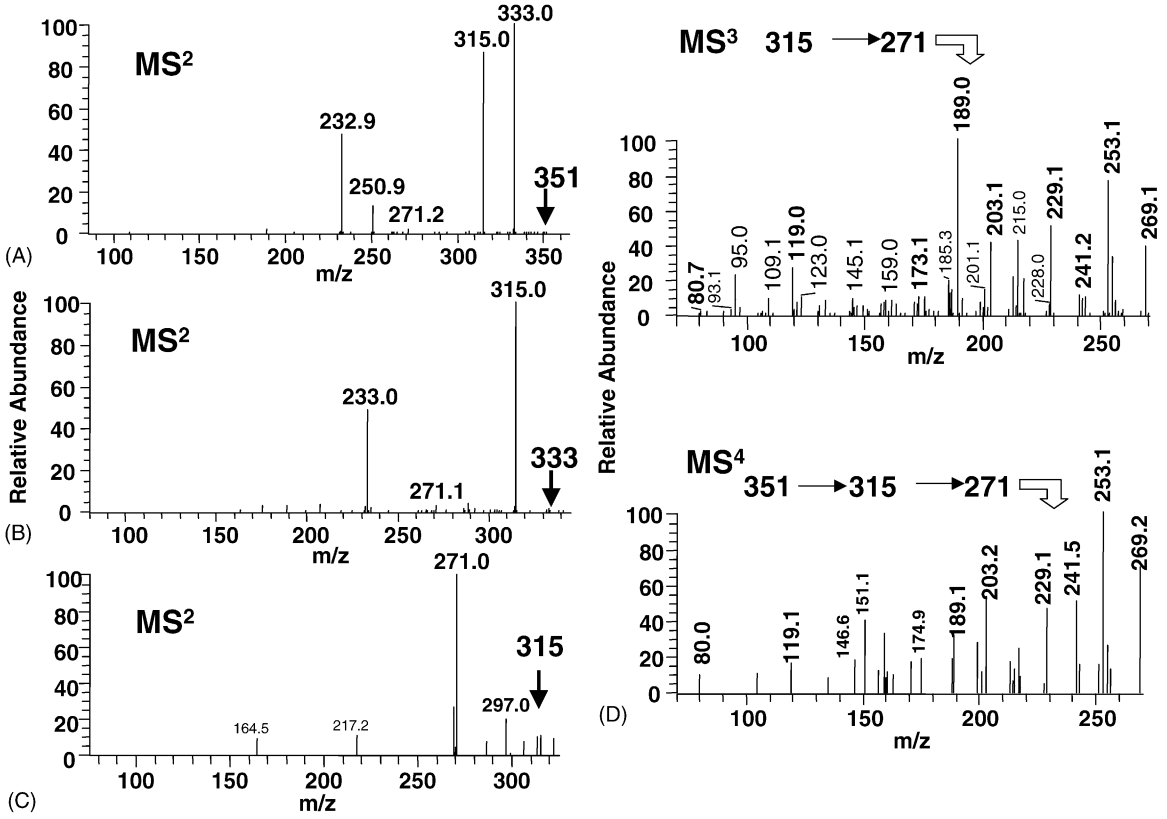

Fig. 6. On-line MS^2, MS^3, and MS^4 spectra of prostaglandins. (A) PGD_2; (B) PGJ_2; (C) 15-deoxy-$\Delta^{12,14}$-PGJ_2; (D) The *m/z* 271 ions in C were fragmented on MS^3 scans (upper), and for MS^4 scans of PGD_2 carboxylate anions the *m/z* 351, 315, and 217 ions were sequentially selected (lower).

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