proteome may involve the analysis of complex mixtures of up to several thousand different proteins within a sample. Analysis of these complex mixtures requires a multi-dimensional separation of the components of the mixture in order to identify and quantify the levels of the specific proteins. The prior art demonstrates 2-dimensional ("2D") gel electrophoresis as the most common means of performing this first separation. Excising of protein spots from the 2D gel, proteolytically digesting the proteins in each spot followed by mass spectrometry/ mass spectrometry ("MS/MS") analysis is well demonstrated. This approach is limited by the ability to quantify and image the protein spots in the gel matrix.

[0035] Recently, 2D liquid chromatography ("LC") separations have been demonstrated for complex protein analysis using capillary LC columns. Combining the LC-LC separation with MS/MS results in a powerful, multidimensional separation of complex proteomic samples. The first LC separation phase is commonly based on ion exchange (strong cation exchange) or size exclusion separation modes. The second phase is most commonly based on a reversed phase separation mode. In the case of ion exchange, a complex protein sample may be separated using an increasing salt concentration in the elution buffer over time. By performing a salt gradient in a stepwise method, for example, fractionation of complex mixtures from the first phase being an ion exchange phase to a second phase being a reversed phase provides for a 2D separation of the sample. The prior art teaches this for use in a microcolumn. Further combining this with mass spectrometry/mass spectrometry provides for 2 additional dimensions of separation based on mass/charge ratio. The prior art teaches that by using LC/LC additional proteins are identified compared to 2D gel electrophoresis approaches.

[0036] The present invention is directed toward a novel utilization of these features to improve the sensitivity of prior disclosed microchip-based electrospray systems and integration of microchip-based separations and electrospray ionization within a single microfabricated device.

SUMMARY OF THE INVENTION

[0037] The present invention relates to an electrospray device for spraying a fluid which includes a substrate having an injection surface and an ejection surface opposing the injection surface. The substrate is preferably an integral monolith and has at least one spray unit. Each spray unit includes an entrance orifice on the injection surface; an exit orifice on the ejection surface; a channel extending through the substrate between the entrance orifice and the exit orifice; and a recess extending into the ejection surface and surrounding the exit orifice. A separation material is associated with the device at a location suitable to effect chromatographic separation of analytes passing through the device. The electrospray device also includes an electric field generating source positioned to define an electric field surrounding at least one exit orifice. In one embodiment, the electric field generating source includes a first electrode attached to the substrate to impart a first potential to the substrate and a second electrode to impart a second potential. The first and the second electrodes are positioned to define an electric field surrounding the exit orifice. This device can be operated to generate multiple electrospray plumes of fluid from each spray unit, to generate a single combined electrospray plume of fluid from a plurality of spray units, and to generate multiple electrospray plumes of fluid from a plurality of spray units. The device can also be used in conjunction with a system for processing an electrospray of fluid, a method of generating an electrospray of fluid, a method of mass spectrometric analysis, and a method of liquid chromatographic analysis.

[0038] According to another aspect of the invention, the electrospray device further includes a reservoir upstream of and in fluid communication with the entrance orifice, wherein the reservoir is filled with a separation material suitable to effect chromatographic separation of analytes passing through the electrospray device.

[0039] Another aspect of the present invention is directed to an electrospray system for generating multiple sprays from a single fluid stream. The system includes an array of a plurality of the above electrospray devices. The electrospray devices can be provided in the array at a device density exceeding about 5 devices/cm^2, about 16 devices/cm^2, about 30 devices/cm^2, or about 81 devices/cm^2. The electrospray devices can also be provided in the array at a device density of from about 30 devices/cm^2 to about 100 devices/cm^2.

[0040] Another aspect of the present invention is directed to an array of a plurality of the above electrospray devices for generating multiple sprays from a single fluid stream. The electrospray devices can be provided in an array wherein the spacing on the ejection surface between adjacent devices is about 9 mm or less, about 4.5 mm or less, about 2.2 mm or less, about 1.1 mm or less, about 0.56 mm or less, or about 0.28 mm or less, respectively.

[0041] Another aspect of the present invention is directed to a method of generating an electrospray wherein an electrospray device is provided for spraying a fluid. The electospray device includes a substrate having an injection surface and an ejection surface opposing the injection surface. The substrate is preferably an integral monolith and includes an entrance orifice on the injection surface; an exit orifice on the ejection surface; a channel extending through the substrate between the entrance orifice and the exit orifice; and a recess surrounding the exit orifice and positioned between the injection surface and the ejection surface. The method can be performed to generate multiple electrospray plumes of fluid from each spray unit, to generate a single combined electrospray plume of fluid from a plurality of spray units, and to generate multiple electrospray plumes of fluid from a plurality of spray units. The electrospray device also includes an electric field generating source positioned to define an electric field surrounding the exit orifice. In one embodiment, the electric field generating source includes a first electrode attached to the substrate to impart a first potential to the substrate and a second electrode to impart a second potential. The first and the second electrodes are positioned to define an electric field surrounding the exit orifice. Analyte from a fluid sample is deposited on the injection surface and then eluted with an eluting fluid. The eluting fluid containing analyte is passed into the entrance orifice through the channel and through the exit orifice. A first potential is applied to the first electrode and a second potential is applied to the fluid through the second electrode. The first and second potentials are selected such that fluid discharged from the exit orifice of each of the spray units forms an electrospray.