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POLYMER TRACK MEMBRANES FOR ATMOSPHERIC PRESSURE FIELD EXTRACTION OF IONS FROM LIQUID SOLUTIONS

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Abstract

An approach to the generation of gas phase ions by field extraction from liquid solutions has been investigated. The method uses a polymer membrane with nano-size channels as an interface between the liquid and the atmospheric pressure gas. Ions are produced by dissociation in the polar solvent and secondary ion-molecular reactions in the solution, which fills the channels of the membrane. Field extraction of the ions from the channels is stimulated by pulses of the electric discharge between the membrane and an adjacent electrode in the gas. The gas-phase ions are removed from the extraction zone by air flow and are detected by mass spectrometry. Possibilities of the membrane interface for generation of gas phase ions have been demonstrated from mass spectral investigation carried out for angiotensin II, gramicidin S and cytochrome C solutions. The current kinetics of the membrane ion source has been investigated to elucidate the mechanism of the ion extraction.

Keywords

track membrane; field extraction of ions; atmospheric membrane ion source

1. INTRODUCTION

The development of new methods of generating gas phase ions and ion beams is important in various fields of science and engineering. For example, mass spectrometry relies upon the production of ions in the gas phase as a necessary step in any analysis. In particular, biological mass spectrometry needs nondestructive methods for generation of gas phase ions of relatively large, nonvolatile and thermally unstable bioorganic molecules. One widely used method for generating the ions of such molecules from liquid solutions is electrospray ionization (ESI) [1]. In the ESI ion source, the ions are produced by spraying solutions from the tip of a micron-size capillary in the electric field. Using atmospheric pressure conditions, the electrostatic spraying results in generation of gas phase ions that are formed by several consecutive processes. Firstly, multi-charged droplets of micron size are generated. Evaporation of solvent

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molecules leads to increasing electrostatic repulsion between the charges in each droplet and, eventually, fission of a droplet into two separate droplets. After several such fissions, the electrostatic repulsion between ions on the surface of the remaining small charged droplets results in high electric fields near the liquid-vacuum interface, providing conditions suitable for field evaporation of ions from the liquid. Thus field assisted evaporation of cluster ions from the stable multi-charged droplets is the last stage in the generation of the gas phase ions in the ESI method. For water solutions, ion evaporation conditions are established at a droplet diameter of several tens of nanometers [2].

Another approach to the generation of ions by their extraction from liquids was proposed by Yakovlev et al. [3]. To avoid spraying the liquid, a polymer track membrane with nano-size channels was used for stabilization of the liquid in the strong electric fields. This stabilization required the electric forces acting on the liquid interface to be counterbalanced by surface tension [4]. The method can provide field evaporation of cluster ions and their direct introduction from glycerol/water solutions into the vacuum chamber of a mass spectrometer (MS) without loss of ions. The main principles of ion generation in this membrane ion source have been reported [4]. As with ESI, this method is described as “soft ionization” as no ion fragmentation occurs.

The possibility of applying such membranes for generating ions at atmospheric pressure was demonstrated in [5] for water and water/alcohol mixtures. Although the new atmospheric pressure technique was promising as it achieved high efficiency of transfer of liquid phase ions into the gas phase, long-term instability in the ion yield from the liquid was observed, despite the relatively high stability of the current maintained through the membrane.

It seems that the main difference between membrane ion extraction at atmospheric pressure compared with direct extraction into a vacuum is the limitation in the electric field strength imposed by the electric breakdown within the gas. In this case, pulsing of the electrical discharge between the membrane and the extracting electrode leads to corresponding pulses in the yield of ions from the liquid within the channels of the membrane.

As yet the specific mechanism of ion extraction from the liquid in the atmospheric pressure membrane ion source is not clear and its elucidation requires further investigation. Nevertheless, the application of multi-channel membranes may initiate a new direction in the generation of the gas phase ions of bioorganic substances. Importantly, the membrane interface may provide an alternative to the widely used ESI method in biological HPLC/MS. From this perspective, it is necessary to understand some properties of the interface related to its ability to carry out the analysis under a variety of conditions. In particular, it is highly desirable that the ions extracted from a multi-component solution of several compounds accurately reflect the ion composition of the sample. For identification of the various components by HPLC/MS, it is necessary to provide stable operation of the interface during the period of chromatographic separation. Ideally it should also be compatible with the various solvents typically used for optimal HPLC.

The aim of the present study was to monitor the properties of a membrane interface with the particular objective of evaluating its use in tandem methods such as HPLC/MS. Reducing the energy of the breakdown pulses was considered as a way of overcoming the long-term instability in the ion yield from the liquid. A possible design of the ion source with low energy discharge pulses is suggested. In addition, an experimental investigation of the ion current was carried out as a first step towards determining the mechanism of ion production in the atmospheric membrane ion source. It is anticipated that this will contribute to achieving long term stability in the operation of the membrane ion source.

2. EXPERIMENTAL

The experimental set-up used for identification of ions extracted from the membrane at atmospheric pressure is shown in fig.1. This is very similar to the design used in [5] but there is an additional possibility to provide a flow of the liquid sample so that a small amount of sample comprising a few μL fills the shallow cavity behind the membrane. The flow of the liquid sample was 200 $\mu\text{L}/\text{h}$.

To provide a more suitable integration with the membrane ion source, a short metal capillary of 5 mm in length and ID=0.29 mm, OD=0.72 mm was added to the atmospheric inlet of the MS. This capillary also served as the extracting electrode. The distance between the membrane and the inlet capillary was about 0.3 mm. A voltage of up to 3 kV was applied between the metal mounting of the ion source and the inlet capillary through a 1 GOhm resistor. A series of breakdown pulses stimulated the yield of ions from the membrane channels containing the sample solution. A typical value of the total current in the discharge chain was about 10÷100 nA.

Extracted ions together with the air flow passed through the inlet capillary of a commercial o-TOF mass spectrometer (Applied Biosystems Mariner) to be recorded as a mass selected ion current.

Another design of membrane interface was used to investigate the kinetics of the discharge current after the breakdown of the gas between the membrane and the metal extractor (see fig. 2). To decrease the energy of the discharge, the electric capacity of the gas-filled electrode gap was minimized by using a sharp needle electrode-extractor located close to the membrane surface. In this case, the carrier air flow removing the ions from extraction zone was directed parallel to the membrane surface.

A voltage of up to 3 kV was applied to the liquid electrode through a 2.5 GOhm resistor. The ion current was recorded on an oscilloscope by monitoring the voltage developed across a 100 Ohm resistor connected to the extracting electrode.

The poly(ethylene terephthalate) membrane was about 10 μm thick. The channel density was from 10^7 to 10^9 cm^{-2} and the diameter of the channels estimated from the airflow through the dry membrane was in the range 300÷700 Å.

3. RESULTS AND DISCUSSION

3.1. Analysis of extracted ions

Peptide analysis—As reported previously, peptides ionized with the membrane source exhibit multiply protonated ions similar to those typically observed in ESI.

Fig.3 shows a spectrum obtained by track membrane ionization of a mixture of gramicidin S and angiotensin II, each at a concentration of 5 μM in 40% acetonitrile acidified with 0.1% formic acid, i.e. solvent conditions that are typical of ESI. The cyclic peptide gramicidin S is widely used in ESI as a mass calibrant and is well known to show a weak singly charged molecular ion at m/z 1141.72 and a strong doubly charged ion at m/z 571.36 but no ions of higher charge states. By contrast angiotensin II with two basic residues and a free amino terminus gives singly, doubly and triply charged ions at 1046.54, 523.77 and 349.51 respectively in ESI. All of these ions are observed in the spectrum shown in fig.3 and the isotope spacing confirms the charge state in each case.

Decreasing the average current of the ion source in comparison with that used in [5] gave enhanced long-term stability of the ion current. As seen in fig 4, the time available for

uninterrupted work with the ion source can be more than 30 minutes, and when used with a fixed small volume of liquid, is similar to nanospray.

Analysis of proteins—The largest molecule previously reported to have been ionized with the membrane source was bovine ubiquitin with a molecular mass of 8.6 kDa. In the current study we established that larger proteins can be ionized as easily as by ESI. Furthermore it was also found that very different spectra could be obtained, depending upon the solution conditions, and the membrane technique can be used to monitor biophysical aspects of proteins with significant advantages over ESI. Fig. 5 shows membrane spectra of cytochrome C analyzed at a concentration of 80 μ M whereas the previous report used 1 mM solutions for ubiquitin and insulin.

The spectrum in Fig. 5a was obtained with solvent conditions typical of ESI, i.e. in 20% acetonitrile, 0.2% formic acid. Under these strongly denaturing conditions we observed a distribution of highly charged ions ranging from 10+ to 20+ with a maximum at 17+. By contrast the same sample ionized from pure water gave the spectrum in 5b, showing much lower charge states in the range 4+ to 11+ with a maximum at 6+. Clearly the protein ionized from pure water was in its native state whereas the acidified organic-containing solvent had unfolded the protein, exposing multiple sites for protonation. Nevertheless, whether the protein was in its native state in water or denatured in water/acetonitrile/formic acid, deconvolution of the spectra gave an identical molecular mass. A very encouraging aspect of this result is that membrane ionization from pure water proved to be very efficient, whereas ESI from pure water is generally inefficient.

It should be mentioned that although the overall current through the source was significantly lower than the 30 nA used in [5], irreversible changes in the polymer surface of the membrane could be observed by visual inspection. It appears that such physical changes are the factor that limits the long-term operation of this source. It is probable that decreasing the energy of the discharge pulses would reduce this membrane damage and would thereby improve the overall operation of the atmospheric membrane interface.

On the other hand, it is unlikely that optimizing the stimulating pulses and the design of the membrane interface will be possible without an understanding of the ion production mechanisms under the influence of the discharge pulses at atmospheric pressure. As a first step, possible models of an operating atmospheric pressure membrane ion source should be considered qualitatively.

3.2. Stimulating pulses

As was shown in [5], the ion current from a membrane source is a series of peaks initiated by pulsed breakdown of the gas. To provide more reproducible results, a sharp extracting electrode was introduced to decrease the energy of the breakdown pulses (fig.2). Considering the gas-filled electrode gap as an electrical capacitor, the maximum energy in the pulse can be evaluated as

$$W = C_g U_b^2 / 2 \quad (1)$$

where C_g is the electrical capacity of the gas between the membrane and the extracting electrode and U_b is the breakdown voltage. Using the new design, the discharge energy was reduced to several nJ, contrasting with the earlier experiments where it was in the order of several μ J. The reducing the energy was connected mainly with decreasing C_g due to the change of the geometry of the extracting electrode. Decreasing the distance between the membrane and the

extractor also leads, in accordance with (1), to a reduction in the energy as it is quadratic in U_b .

On the other hand, the energy of the discharge pulse can be calculated directly from the measurement of the discharge current and the breakdown voltage of the gas as

$$W_{\text{exp}} = Q U_b / 2, \quad (2)$$

where Q is a charge equal to the discharge current integrated over the time of the pulse. So, from (1) for the energy in the discharge pulse was examined experimentally.

To study more specific features of the breakdown in the liquid/membrane/gas system, the kinetics of the discharge current was compared with the kinetics of the current after replacing the membrane by a flat metallic electrode of the same geometry. Fig.6 shows the kinetics of the discharge on the metal electrode and on the membrane with 10^{-2} M potassium iodide solution in 50/50 water/glycerol mixture. Under these conditions, the kinetics of the current proved to be reproducible over a long time for both the membrane and the flat metal electrode. Moreover, no visible changes were observed on the membrane surface after extended operation.

A short pulse of current lasting about 200 ns was observed for the metal electrode (fig.6A). In this case, the gas capacitor was discharged completely by a breakdown and the electric field strength in the gas subsequent to the discharge was close to zero.

After replacing the metal electrode by the membrane, the amplitude of the current was lowered by a factor 10. The integrated current for the discharge on the membrane was less than that for the metal electrode by a factor of about 3. This suggests that, in the case of the membrane, the electrical gas capacitor is not completely discharged after the breakdown and an electric field exists in the gas even after the discharge pulse. Probably, this is due to the discharge current being limited by the high electrical resistance of the membrane.

Compared with the metal electrode, a unique feature of the breakdown on the membrane is an additional slow component with a specific fast decay at the end (fig.6B). An increase in the electrode separation leads to an increase in the breakdown voltage together with a delay in the occurrence of the specific fast decay of the current.

There are two possibilities for the explanation of the observed current kinetics. The first one can be connected with the charging of the dielectric membrane during the discharge. The charging leads to decreasing the discharge current and observed changes in its kinetics. In this case, the electric field strength in the gas gap just after the discharge pulse can be evaluated from the comparison of the integrals of the current for discharge on the metal electrode and on the membrane as

$$E = E_b (Q_{\text{met}} - Q) / Q_{\text{met}} \approx 20 \text{ kV/cm}, \quad (3)$$

where E_b is the breakdown electric field strength of about 30 kV/cm, Q_{met} is the charge equal to the discharge current integrated over the time of the pulse for the discharge on the metal electrode.

The second way for explanation of the slow component of the observed current can be associated with yield of positive ions from the liquid in the channels of the membrane. In this

case, the time of the specific fast decay of the current corresponds to the time of flight of the extracted ions in the gas

$$\tau_d = d_g / \mu_i E, \quad (4)$$

where d_g is the electrode distance and μ_i is the mobility of the ion. Taking $d_g \approx 0.3$ mm, $\tau_d = 1.5$ μ s and $\mu_i \approx 1$ cm²/Vs, the electric field strength in the gas after the breakdown corresponds to

$$E = d_g / \tau_d \mu_i \approx 20 \text{ kV/cm}, \quad (5)$$

which is slightly lower than the well known breakdown electric field strength. This is consistent with our comparison of the current for the breakdown on the metal electrode and the membrane filled by the liquid solution and the observation that, in the case of the membrane, the electrical gas capacitor does not discharge completely during the breakdown.

The strong electric field in the gas reduces the likelihood of removing the extracted ions from the inter-electrode gap by air flow. To remove the ions more effectively, the air flow velocity in the electrode gap should be comparable with the drift velocity of ions in the electric field.

$$v_{\text{flow}} \approx v_{\text{drift}} \approx \mu_i E \quad (6)$$

By using E from (3), equation (6) gives $v_{\text{drift}} \approx 0.2$ km/s. This velocity is very close to the velocity of sound in air at atmospheric pressure: $v_{\text{sound}} \approx 0.33$ km/s. because the velocity of the air flow in our set-up is significantly lower than v_{sound} , no influence of the air flow on the discharge current was observed experimentally. Thus we conclude that, in our experiments, the majority of the extracted ions are not removed by the gas flow but are lost by striking the electrode.

3.3. Extracting mechanism

It is likely that the field extraction of ions from the channels of the membrane can be considered as a possible way for the ion yield from the liquid. A strong electric field on the surface of the liquid, which is necessary for ion extraction, can be formed by negative charge carriers moving to the polymer membrane and charging it. This mechanism of extracting field formation is very similar to that suggested for the membrane ion source working in vacuum [4,6,7], the main difference being the origin of the particles that charge the membrane surface. The surface charge increases the electric field strength on the surface of the liquid within the membrane channels and can result in local extracting electric field strengths of more than 1 MV/cm, even when the potential difference between the surface of the membrane and the liquid U_m is only 10 V [4,6].

Ignoring the surface conductivity of the polymer, the maximum potential difference between the liquid and the surface of the membrane just after the one breakdown pulse can be evaluated as

$$U_m \approx U_b d_m / d_g \cdot \varepsilon, \quad (7)$$

where $\varepsilon \approx 3$ is the permittivity of the membrane.

For the conditions used, $U_m \approx 10$ V, a value that can be applied as an average in the area of the breakdown. The local potential difference can be significantly higher because the charging

current is not homogeneous. On the other hand the real membrane/liquid potential difference may be lower due to the fact that the electrical gas capacitor does not discharge completely after the breakdown. Based on these considerations, it is likely that U_m lies in the range 3÷30 V. Taking into account that the surface charge of the membrane can be accumulated during a time of several breakdown pulses, the potential difference between the membrane surface and the liquid sample can be even significantly higher. So, the potential difference between the liquid and the surface of the membrane considered here can be in the appropriate range.

Two processes lead to generation of gas phase ions in such strong electric fields. The first is direct field evaporation of ions from the liquid, i.e. the cluster ion yield from the liquid. The second is the spraying of the liquid from the channels of the membrane followed by transformation of charged droplets into ions by evaporation of the solvent in air, i.e. electrospray. Results obtained previously for membranes similar to those we used showed that a transition takes place from direct ion evaporation to electrospray at $U_m > 50$ V [4,6]. Taking account of the uncertainty in our estimation of U_m for our experimental conditions, it is not possible to separate these processes. So, in the current model, electrical breakdown plays the role of generator of charge carriers (electrons and ions), which are capable of charging the membrane to the necessary potential.

Although mass spectrometry is essential to characterize the ions produced in the membrane source, it does not reveal the mechanism of their formation. Therefore new approaches are necessary for determination of the actual mechanism of ion generation in the atmospheric pressure membrane ion source.

CONCLUSIONS

Mass spectra obtained with the atmospheric pressure membrane ion source demonstrate the potential applicability of this interface for the extraction of ions from liquid solution without their fragmentation. The membrane is easy to handle, and can be made at lower cost than the high-quality pulled glass nano-electrospray capillaries. The technique is of potential importance for the mass spectrometry of biomolecules such as polypeptides and proteins. It can deal with different solvents, for example, ionization from water solution has proved to be very efficient whereas ESI from pure water is problematical. Nevertheless challenges remain, particularly establishing conditions that provide long term stability of operation.

It is important to undertake a more comprehensive investigation of the mechanism of atmospheric pressure ion extraction from the membrane. Investigation of the kinetics of the current of the ion source initiated by the breakdown pulses should yield further insights into the mechanism of ion production from liquid solutions. It is clear that both ion evaporation and electrospray from separate channels of the membrane can be considered as possible mechanisms of ion extraction within the atmospheric pressure membrane interface.

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Figure 1. The membrane ion source used for the mass spectral investigation of ions extracted from liquid solutions at atmospheric pressure.

Figure 2.
Schematic diagram of the membrane interface used to investigate the discharge current kinetics in the system liquid/membrane/gas.

Figure 3. Mass spectrum recorded with the membrane interface for a mixture of gramicidin S and angiotensin II. Solvent conditions were typical of ESI.

Figure 4.

The total current of ions detected by the mass spectrum versus time after switching on the voltage on the ion source for 10^{-4} M angiotensin II solution in water. The maximum number of ions in the mass spectrum was about $5 \cdot 10^5$. The applied voltage was 2 kV.

Figure 5. Mass spectra of cytochrome C ions: a) under strongly denaturing conditions and b) using native solvent conditions. Although the different solvent conditions gave different charge states, both spectra can be deconvoluted to give 12228.8 Da as the molecular mass.

Figure 6. Oscilloscope traces of the discharge current for A: a metallic electrode, and B: the membrane as the positive electrode. The breakdown voltage was 1 kV and the time between breakdown pulses was in the range 1.6÷1.8 ms.