PREPARATION OF THIN FILMS OF BIOMOLECULES BY THE ELECTROSPRAY METHOD*

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Introduction

The preparation of thin uniform films of biomolecules has been a frequently encountered problem in chemical analysis techniques such as charged particle activation analysis, photoelectron spectroscopy, and with the recently developed technique of ^{252}Cf plasma desorption mass spectrometry. Techniques such as vacuum evaporation from a heated filament and electrodeposition (1) which have been used to prepare thin films (<10 $\mu g/cm^2$) of inorganic compounds are generally not applicable for use with biomolecules (e.g., peptides, nucleotides, steroids, vitamins). The major problems are related to the thermal instability of these molecules and the requirement that any technique used to prepare a thin film must not alter the chemical identity of the molecule. Gentle methods such as deposition of a solution on a solid surface by solvent evaporation produce films that are aggregated and non-uniform. A method has been reported for the preparation of thin films using a specially designed nebulizer to produce a condensable finely dispersed mist of the solute without decomposition (2). However, films prepared by this method are also non-uniform, thick (10-1000 $\mu g/cm^2$) and require relatively large amounts of material.

With the development of the Cf-252 plasma desorption mass spectrometer (3), a better method was required for the rapid preparation of thin (<4 $\mu g/cm^2$), uniform targets of small quantities of fragile biomolecules over an area less than 0.75 cm^2. The requirements of the method were that it had to retain the chemical identity of the sample, be non-fractionating when used with mixtures, non-contaminating, reproducible, and generally applicable to a wide range of molecules.

Carswell and Milstead developed a method which is called electrospraying for preparing thin radioactive sources (4), utilizing a phenomenon first observed by Zeleny (5). The method was modified (6,7,8) and has been used by Nawab and Mason to prepare highly uniform emulsions of water and oil (9). The purpose of this paper is to report our results on the adaptation of this method for preparing thin uniform films of biomolecules and to show that this method satisfactorily meets the criteria we had established as necessary for preparing samples for ^{252}Cf plasma desorption mass spectrometry.

Experimental

The basis of the electrospray method is the generation of a fine, electrostatically charged mist occurring when a positive potential is applied to a small capillary containing a solution of the sample and collection of the droplets onto a collection plate positioned a short distance below the tip of the capillary. Electrical contact between the solution and the capillary can be made by threading a small wire through it, slightly smaller than the bore size. This also reduces the cross-sectional area, so that the solution is held within the capillary until the potential is applied. More reproducible targets can be easily obtained by using a stainless steel hypodermic needle with a small bore (I.D. = 0.8 mm) and applying the voltage directly to the needle. Nawab and Mason (9) reported that generation of the spray occurred at lower voltages when the needle was substituted for the glass capillary. In their experiments, the beveled tip of the needle was ground to a flat end; however, Verdingh and Lauer (8) found they could better contain the solution in the needle if the

beveled tip was further sharpened. Our experiments support this conclusion.

The collection plate consists of the target backing, a conducting foil, usually 5.3×10^{-4} cm aluminized Mylar or 1.0×10^{-4} cm Ni, secured by a ring on a threaded cylinder. The cylinder screws into an aluminum disk to insure electrical contact to ground and also prevents any movement so that exactly the same orientation is obtained for every target. The aluminum disc rests on a ring which is attached through three legs to a laboratory jack. The jack provides vertical adjustment only so that the foil can be raised or lowered with respect to the needle while maintaining axial alignment of the target. The neck of the needle fits tightly into a brass holder (0.32 cm thick); electrical contact is made via the brass holder to the needle (Fig. 1). Any non-conducting material near the field region can build up charge which may perturb the electric field lines. For this reason only metal surfaces were used in the field region and non-conducting materials were well shielded from the electric field region. The surface of the foil is slightly higher than any other conducting surface and the brass platform is sufficiently far away from the tip of the needle so that the stronger field lines are between the capillary and sample. The entire apparatus is placed in a glove box to minimize air currents and dust particles which also cause perturbations in the dispersion of the spray.

The sample to be electrosprayed is dissolved in an organic polar solvent, concentrations used may vary but are typically 1 μg/μℓ. The tip of the needle is dipped into the solution which is allowed to flow

into the needle by capillary action. For a needle, I.D. = 0.8 mm, approximately 6-7 μℓ of solution enters the capillary. The needle is gently withdrawn, making sure that it does not touch the wall of the vial which would cause the solution to flow out of the needle. Once the capillary is filled care is taken to prevent loss of solution by mechanical shock. If this occurs a smaller needle is substituted. The foil is positioned and raised to a height 5 cm from the tip of the needle. Slightly shorter or longer distances may be used depending on the desired size of the deposit. A heat lamp placed nearby is required to evaporate the solvent to prevent coalescence of the drops when they are dispersed on the foil. It appears the solvent is completely or nearly completely evaporated during the flight to the foil by use of the heat lamp. The positive voltage is applied extremely slowly; at very low voltages (~1 kv) no spray is formed and as it is gradually increased to about 3 kv the spray is generated and becomes more continuous and dispersed. In the 1 kv region the current fluctuates indicating a discontinuous spray but as the voltage is gradually increased to 3 kv the current remains stable over the region of 0.14-0.24 μamps. In the region of this plateau a continuous well dispersed spray is formed and the spot size can be varied slightly by fine tuning of the voltage. If the voltage is further increased the current begins to fluctuate sharply again and the spray may become so dense that the droplets coalesce on the foil resulting in a non-uniform deposit. In the plateau region, the current remains stable until the volume of liquid drops to a level at which the surface tension becomes too great to allow generation of the spray. At that

point, the current falls to zero. Approximately 2-4 can be deposited in 1-2 min with a 10% deviation between samples prepared using precisely the same configuration.

The solvent used to dissolve the compound must necessarily have high volatility. However, Neubauer and Vonnegut (10) had some success electrospraying water, NaCl and HCl solutions, and Eastwood has utilized acid solutions (1). The surface tension of the solvent is also an important consideration. It must be low to allow the spray to form but sufficiently high to prevent the solution from excaping from the needle prior to electrospraying. It has been shown that the dielectric constant also is an important factor (6,10). Many biomolecules are not soluble or only slightly soluble in organic solvents. In these cases a small amount of water can be added to the organic solvent to achieve dissolution of the compound. Brunnix and Rudstam found that 70:30 ethanol:water was the highest ratio allowable. This has been supported in our experiments and is in almost all cases a sufficient amount of water to dissolve biomolecules. Typical solvents that have been used are ethanol, methanol, isopropanol, and pyridine. Brunnix and Rudstam also have used acetone and ethyl ether. Nawab and Mason report that there is no limitation in the choice of solvents and have used solvents such as carbon tetrachloride to form emulsions by electrospray.

Radioisotope dilution allows one to indirectly determine the weight of an extremely small amount of material with a high degree of accuracy. A minute quantity of a radioactive material is added to the sample solution and a specified volume is evaporated to dryness

on a foil. Knowing the amount of activity/μℓ of solution and the concentration of sample in the solution, the amount of sample material deposited by electrospray can be accurately determined by measuring the counting rate of the electrosprayed sample and comparing it to the counting rate of the evaporated aliquot. In our experiments, $1.0\text{-}4.0 \times 10^{-3}$ Ci of ^{14}C-labeled valine is added to the solution. The quantity of ^{14}C-valine is exceedingly small so that it does not contribute significantly to the sample weight and is not detectible in the mass spectrometer. The β-counting rates are easily measureable for this amount of activity even for 100 ng samples. Typical weights of the targets are 1-3 μg. This can be increased or decreased by varying the concentration of the solutions or increased by electrospraying the same area several times.

Results and Conclusions

We have demonstrated that the electrospray method can be used to prepare thin, uniform films of fragile biomolecules without any alteration in the molecular structure. In our experiments, it is also necessary that the PDMS mass spectra are reproducible for a specified amount of the sample and that the quasi-molecular ion yield is proportional to the amount of material deposited. For films of the same thickness (±10%) the quasi-molecular ion yield for both the positive and negative ion differ only slightly. For example, a yield study was made using valine targets of varying thickness. When films of valine ranging from 0.1-2.0 μg were investigated it was found that the negative quasi-molecular ion yield was higher when valine was deposited on aluminized Mylar than when it was deposited on a nickel foil. The

The valine quasi-molecular ion $(M - 1)^-$ yield is proportional to the quantity deposited on aluminized Mylar from 0.1-1.0 μg and from 0.5-1.0 μg if it is deposited on a nickel foil; beyond 1.0 μg there is very little increase in the $(M - 1)^-$ intensity if the amount of material on either foil is further increased (Fig. 2). This effect was explainable after electron micrographs were taken of the valine surface of samples with weights representative of the proportional and saturated range of the graph (Fig. 3). When small amounts of valine (0.75 μg) were electrosprayed onto a nickel foil a non-uniform film results in which there are substantial areas that are not covered. A lower magnification (600 X) this appears as a very speckled distribution. However, when 0.4 μg valine are electrosprayed onto the Mylar a uniform deposit is obtainable and it appears that the entire surface is evenly coated. For thick films of valine (4.0 μg) the two micrographs are very similar and crystals are beginning to form on the surface. This indicates that the electrospray method completely saturates the surface at 1.0 μg and beyond this point the layers become so dense that crystallization is observed. This evidence is supported by the fact that the PDMS appears to be a surface phenomenon and therefore once the surface is entirely covered there cannot be any substantial improvement in the molecular ion yield if more material is deposited.

Summary

The electrospray method is a general technique for the production of a monodisperse spray and has been shown that it can be useful in many, varied applications. In this paper we have demonstrated that it can successfully be used to prepare thin films of biomolecules for

analytical purposes with a high degree of uniformity, sensitivity, reproducibility and ease.

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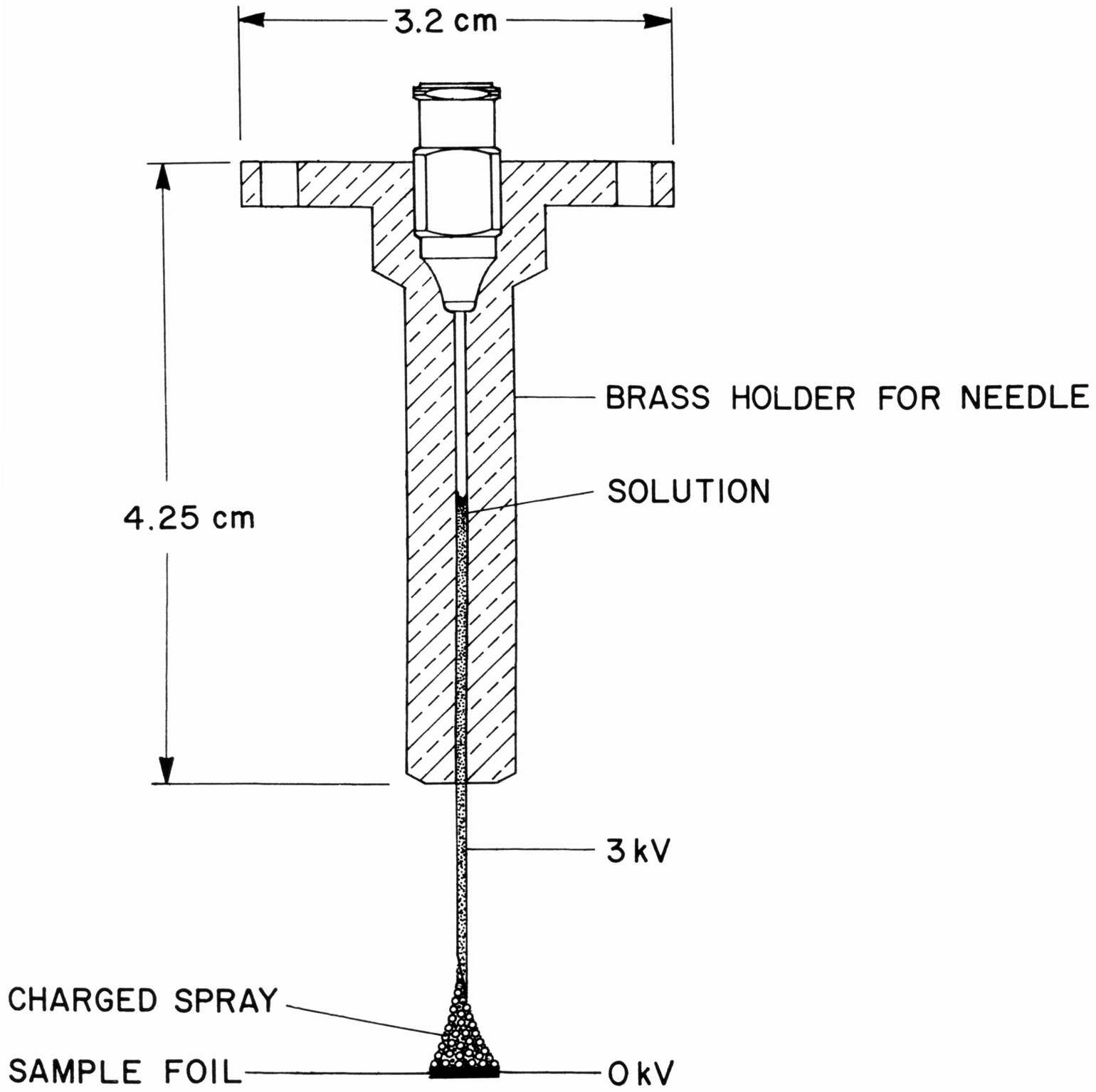


Fig. 1 Electrospray apparatus.

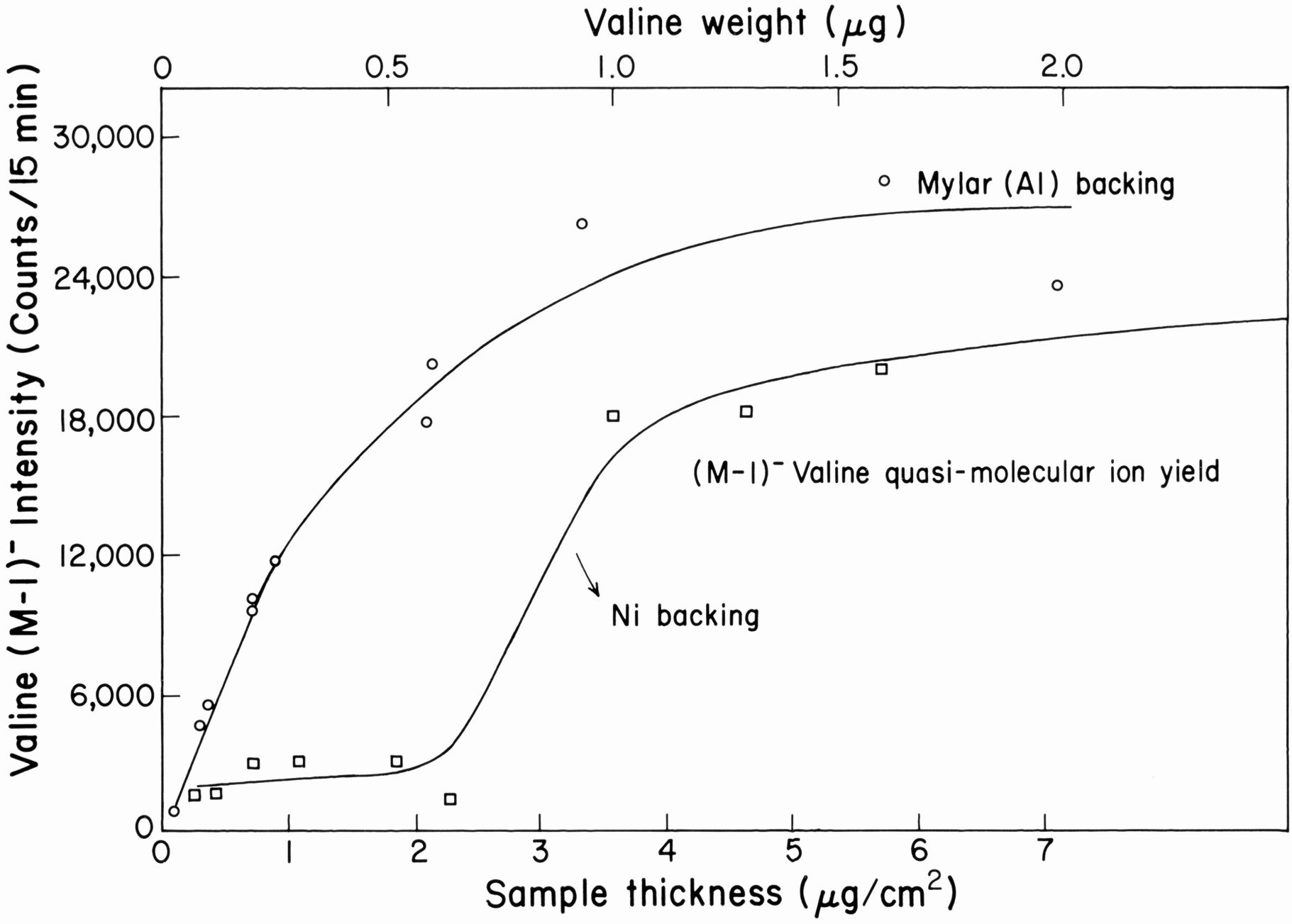
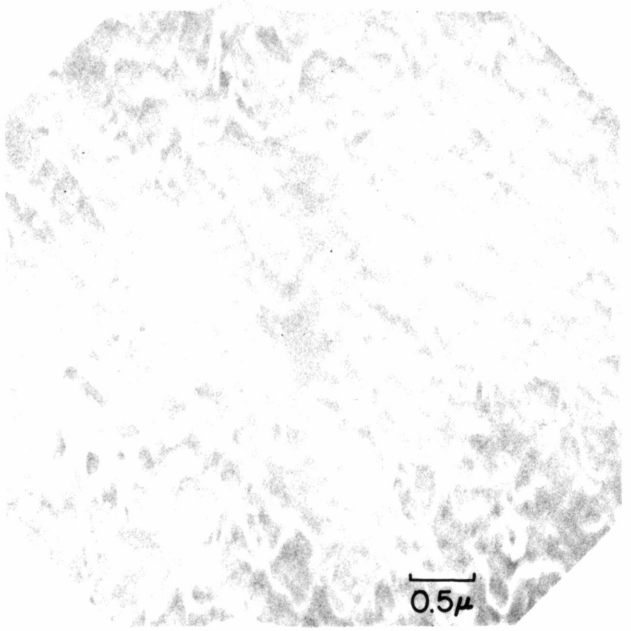
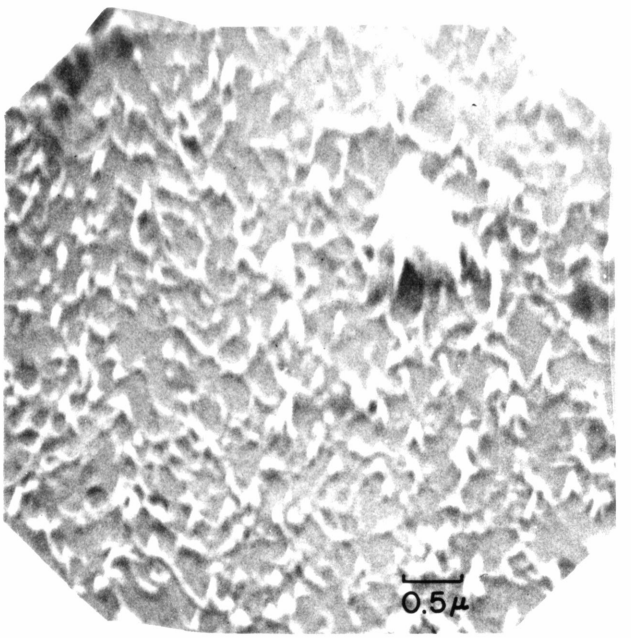


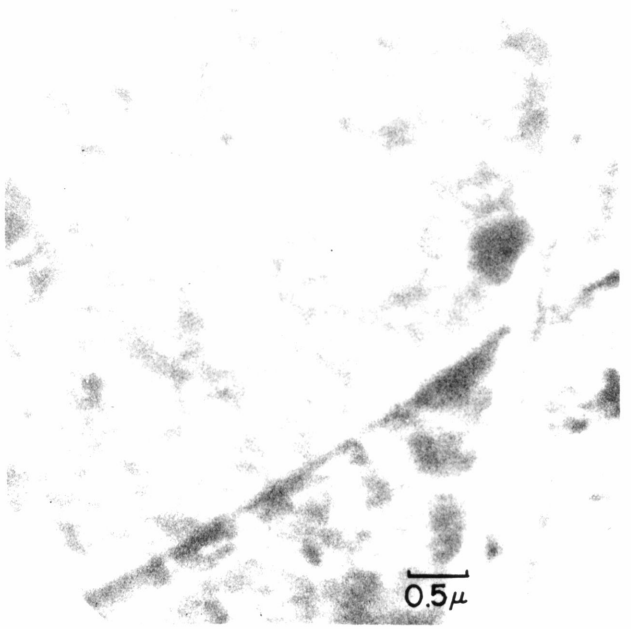
Fig. 2 $(M-1)^-$ yield as a function of sample thickness.



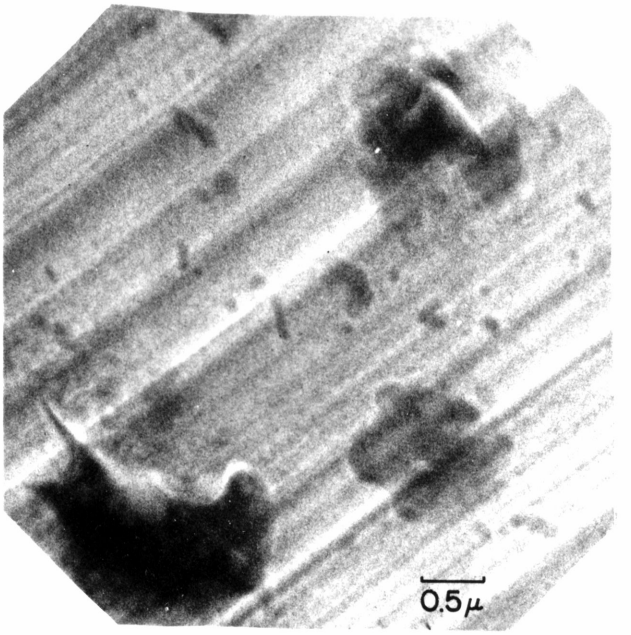
4.0 μg on Nickel



4.0 μg on Aluminzed Mylar



0.4 μg on Aluminzed Mylar



0.4 μg on Nickel

Fig. 3 Electron micrographs (18,000 x) of valine surface