fractions detected by the flame ionization detector. The sampling port is maintained at 300 °C so that the sample is released from the polymer into the first part of a glass capillary column cooled by liquid nitrogen in order to avoid spreading of chromatographic fractions due to non-instantaneous desorption of sample. Cooling is stopped after 5 min and the column then programmed from 35 °C to 210 °C.

Profiles of volatiles present in the room in different circumstances are compared in the Figure. First, a blank from the polymer and the 'background' of the room were recorded (A). In this chromatogram, about 100 peaks can be seen which are presumably due to trace volatiles (paint constituents, plasticizers, etc.) from the furniture, books and other objects in the room. The fractions marked as 'C' are caused by the volatiles from the concentration column which cannot be removed by conditioning over a long period of time. The procedure was repeated after an experimental marihuana cigarette[14] had been smoked in the room by means of a syringe (35 ml puffs). A typical 'fingerprint' of marijuana smoke can be seen in (B) as compared to the different profile from a standard tobacco cigarette[15] in (C). Similar chromatograms were obtained with different commercial tobacco cigarettes. Δ^9-tetrahydrocannabinol, cannabinol and cannabidiol in marijuana smoke and nicotine in tobacco smoke were tentatively identified from their retention times. It is evident that many fractions are common to marijuana and tobacco. However, several distinctive peaks may be diagnostic for marijuana smoke in addition to the usual cannabinoids. Additional studies by combined gas chromatography and mass spectrometry will be necessary to reveal the structures of these compounds.

Further experiments have shown that marijuana smoke can be safely recognized in the presence of tobacco smoke even at much smaller concentrations than are generated from single cigarettes, and detection in much larger rooms should be possible.

Reproducibility of the described experiments is remarkable. It is very likely that this coupling of a concentration technique with high-resolution capillary gas chromatography will find wider use in the solution of a number of problems associated with the trace analysis of complex volatile mixtures at concentrations well below ppb levels.

Zusammenfassung. Mit einer Analysenmethode gelingt es, Marijuanarauch von einer Zigarette oder weniger durch Kombination von Anreicherungsverfahren mit hochauflösender Gaschromatographie in Zimmerluft nachzuweisen. Charakteristische Profile von 200 bis 300 Komponenten (darunter Cannabinoide), die sich deutlich von denen von Tabakrauch unterscheiden, wurden mit Hilfe von Glaskapillarsäulen gewonnen.

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[1] V. Claussen and F. Korte, Tetrahedron Lett. *22*, 207 (1967).
[2] V. Claussen and F. Korte, Justus Liebigs Annln Chem. *713*, 162 (1968).
[3] F. J. Vieira, M. B. Aguiar, J. W. Alencar, A. P. Seabra, B. M. Tursch and J. Leclercq, Psychopharmacologia *10*, 361 (1967).
[4] E. B. Truitt, Pharmac. Rev. *23*, 273 (1971).
[5] A. P. Seabra, Revta bras. Farm. *49*, 13 (1968).
[6] J. A. Voellmin, I. Omura, J. Seibl, K. Grob and W. Simon, Helv. chim. Acta *49*, 1768 (1966).
[7] K. D. Bartle, L. Bergstedt, M. Novotny and G. Widmark, J. Chromat. *45*, 256 (1969).
[8] K. Grob and J. A. Voellmin, J. Chromat. Sci. *8*, 218 (1970).
[9] M. L. Lee and M. Novotny, to be published.
[10] A. Zlatkis, H. A. Lichtenstein, A. Tishbee, W. Bertsch and H. M. Liebich, Abstracts of the 164th National Meeting of American Chemical Society, New York 1972.
[11] M. Novotny, M. L. Lee, R. Farlow and M. McConnell, to be published.
[12] M. Novotny and A. Zlatkis, Chromat. Rev. *14*, 1 (1971).
[13] R. van Wijk, J. Chromat. Sci. *8*, 418 (1970).
[14] Experimental marijuana cigarettes containing 2.5% of Δ^9-tetrahydrocannabinol, supplied by the National Institute of Mental Health, Rockville, Maryland.
[15] Standard reference cigarettes, obtained from Tobacco-Health Research Institute, University of Kentucky, Lexington, Kentucky.

A New Method of Estimating Micropipette Tip Diameter

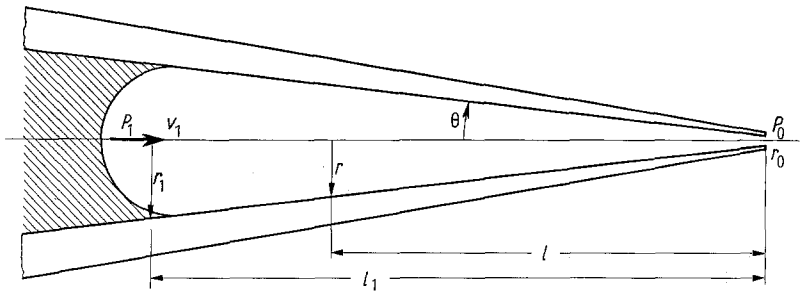
The difficulties involved in estimating the diameters of the tips of very fine electrolyte-filled micropipettes used in electrophysiology are well known. For many purposes it is desirable that the external diameter of the tip should be about 0.2μ (200 nm) or less, and this is well beyond the resolution of the light microscope. Some workers have used electron microscopy in special studies, but for most routine purposes the electrical resistance of the probe is measured to give an indication of tip diameter[1,2].

Estimates of tip diameter from resistance measurements are unreliable for a number of reasons. A group of apparently identical micropipettes frequently vary widely in resistance. This may be due to partial blockage of the tip by small particles flushed into it during filling. The micropipette resistance is non-Ohmic and depends on the direction, amplitude and duration of current flow. It is also strongly dependent on the taper angle at the tip and the resistivities of the filling and bathing media.

This short communication describes a simple and sensitive method for estimating tip diameter which appears to avoid the above unsatisfactory features. It is based on the method of filling very fine micropipettes described by Mullins and Noda[3] and used routinely in this laboratory. The micropipette is held in the air and 3 M KCl is injected into the barrel as close as possible to the tip, using a microsyringe. Capillary action then slowly ejects the remaining air in the micropipette through the tip. Motion of the solution meniscus towards the tip while it is some distance from it can be readily observed under an optical microscope of medium power, and the tip diameter can be calculated from the mensicus speed as shown below.

Consider the probe in the Figure which has an internal tip radius r_0. The solution meniscus is advancing towards the tip at speed v_1 when at a distance l_1 from the tip, where the radius of the pipette is r_1. Due to surface tension forces, the air pressure adjacent to the meniscus is $P_1 = P_0 + 2T/r_1$, where T is the surface tension at the interface and P_0 is atmospheric pressure.

[1] K. Frank and M. C. Becker, in *Physical Techniques in Biological Research* (Ed. W. L. Nasluk; Academic Press, New York 1964).
[2] L. A. Geddes, *Electrodes and the measurement of bioelectric events* (Wiley Interscience, New York 1972).
[3] L. J. Mullins and K. Noda, J. gen. Physiol. *47*, 117 (1963).



Illustrating the solution meniscus advancing towards the tip. The diagram is not to scale.

For non-turbulent flow of air along the tapered tube, the volume dV crossing the section of radius r in time dt is given by Poiseuille's relationship,

$$\frac{dV}{dt} = \frac{\pi r^4}{8\eta}\frac{dP}{dl}$$

where dP/dl is the rate at which pressure is changing with distance along the tube, and η is the viscosity of air.

In steady state flow at constant temperature, pdV is independent of l; that is

$$P\frac{dV}{dt} = P_1\pi {r_1}^2 v_1 = \frac{\pi r^4}{8\eta}P\frac{dP}{dl}$$

Integrating between the ends of the air plug,

$$\int_{P_0}^{P_1} P\,dP = \frac{{P_1}^2 - {P_0}^2}{2} = (8\eta P_1 {r_1}^2 v_1)\int_0^{l_1}\frac{dl}{r^4}$$

If $r_1 > 10\mu$, $\frac{2T_1}{r_1} << P_0$, and ${P_1}^2 - {P_0}^2 \simeq 4TP_1/r_1$

Thus $v_1 \simeq \frac{T}{4{r_1}^3\eta}\left(\int_0^{l_1}\frac{dl}{r^4}\right)^{-1}$

The integral in the above expression is determined by the inner profile of the micropipette. Because of the strong dependence on r, only the region in the immediate vicinity of the tip contributes significantly to the integral. If in this region the tip approximates to a truncated cone of half-angle Θ,

$$r = r_0 + l\tan\Theta$$

and $\int_0^{l_1}\frac{dl}{r^4} = \frac{1}{3\tan\Theta}\left[\frac{1}{{r_0}^3} - \frac{1}{(r_0 + l_1\tan\Theta)^3}\right] \simeq \frac{1}{3{r_0}^3\tan\Theta}$

if $l_1\tan\Theta >> r_0$

Hence $r_0 = r_1\left(\frac{4v_1\eta}{3\,T\tan\Theta}\right)^{1/3}$

The corresponding expression for the tip radius in terms of the electrical resistance R of a tapered micropipette filled with an electrolyte of resistivity ϱ is readily shown to be

$$r_0 = \varrho/(\pi R\tan\Theta)$$

Comparison of these two shows that resistance estimates of tip size are more dependent on taper angle than capillary flow estimates and are therefore more liable to error because of the difficulty of measuring this angle close to the tip. Furthermore, the external medium contributes to the measured resistance and a correction term should be subtracted. The magnitude of the correction is greatly dependent on the resistivity of the medium in the critical region very close to the tip where the solutions inside and outside the probe mix.

The capillary flow method is a very sensitive indicator of tip size since $v_1 \propto {r_0}^3$. Pipettes of unsuitable dimensions can be discarded before filling is completed, following a quick estimation of filling speed. It is generally found that a particular puller setting produces pipettes whose tip dimensions estimated by this method are reproducible to within 10%.

Substitution of $\eta = 1.81\times10^{-5}$ Kg m^{-1}s^{-1} for air at 20 °C and $T = 7.7\times10^{-2}$ N m^{-1} for 3 M KCl gives

$$r_0 = 6.9\times10^{-4}\, r_1\left(\frac{v_1}{\tan\Theta}\right)^{1/3}$$

where v is measured in microns per sec.

For example, if the filling speed is 2.5μ s^{-1} measured at a point where the inner radius is 20 μm, then $r_0 \simeq 0.06\mu$m for a taper angle $2\Theta = 4°$. BILS and LAVALLÉE[4] have shown in electron microscope studies that the ratio of inner and outer diameters at the tip is 0.5 if the pipette is drawn from glass having this ratio of diameters. The outer tip diameter is therefore about 0.24 μm for $r_0 = 0.06$ μm. A micropipette of these dimensions fills in about 5 min if solution is introduced from a microsyringe of external diameter 50 μm to a point about 1 mm from the tip. Solution flows through the tip only at the completion of filling, greatly reducing the likelihood of blockage. The filling technique is successful in about 90% of cases of very fine tips and is almost 100% successful for tips of outside diameter 1μ or larger.

Zusammenfassung. Eine neue und einfache Methode zur Bestimmung des Durchmessers von Mikropipetten wird beschrieben.

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[4] R. F. BILS and M. LAVALÉE, Experientia *20*, 231 (1964).

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