Table I. Apparent (CP_a) and True (CP_t) C-Peptide Concentrations in Unchromatographed and Chromatographed Plasma Samples, Respectively, and Calculated Proinsulin (PI) Concentrations ($N = 72$, $\bar{x} \pm$ Standard Deviation)

PI infusion	CP_a, nmol/L	CP_t, nmol/L	PI, nmol/L
0	0.83 ± 0.17	0.82 ± 0.20	
37 nmol/(m^2h)	4.66 ± 0.77	0.65 ± 0.22	4.01 ± 0.89

C-peptide activity. Altogether, the complete procedure for proinsulin removal resulted in a final recovery of C-peptide of 44.1 ± 11.9% ($\bar{x}$ ± SD), an average estimated for 216 plasma samples. The intraassay recovery of C-peptide after radioimmunoassay in a homologous sample pool was established to have a relative standard deviation amounting to 5.7% ($n = 8$). In chromatographed samples with C-peptide concentrations in the range of 0–1.6 nmol/L, which were spiked with 8 nmol/L proinsulin, no difference was found in C-peptide concentrations measured by radioimmunoassay when compared to those of unspiked plasma samples ($R = 0.981$; $y = 0.19 + 0.92x$).

Apparent plasma human proinsulin concentration was determined with the radioimmunoassay for immunoreactive C-peptide by using human proinsulin as the standard. True human proinsulin concentrations were then calculated in molarities as the difference of apparent C-peptide and proinsulin and chromatographed plasma C-peptide concentration (Table I). With this approach, the lower limit for detection of human proinsulin was 50 pmol/L, while the relative standard deviation for the estimation of human proinsulin was <8%.

In summary, the HPLC pretreatment of plasma samples described in this study enables both the radioimmunological determination of C-peptide concentrations and the indirect estimation of human proinsulin concentrations in sample containing supraphysiological amounts of human proinsulin. The advantage of this approach is that no highly specific antisera, neither againt proinsulin nor against C-peptide, are required. The disadvantage of this method is its lower detection limit for proinsulin when compared with those of radioimmunological methods especially developed for estimation of this compound (*4–8*).

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Registry No. C-peptide, 59112-80-0; proinsulin, 9035-68-1.

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CORRESPONDENCE

Electrochemically Assisted Fast Atom Bombardment Mass Spectrometry

Sir: Fast atom bombardment (FAB) mass spectrometry (*1–3*) has proven to be an extremely useful tool in the analysis of samples that would otherwise be too intractable for conventional mass spectrometry, such as peptides, oligosaccharides, and oligonucleotides (*4–7*), due to their low volatility and ready thermal degradation. There are limits on its applicability, however, in that it works best with samples that are either already ionic, or predisposed to become so by simple proton transfer to or from the matrix (*8–10*). We are aware of only one report of nonpolar compounds (polycyclic aromatic hydrocarbons) being responsive to this technique (*11*). There have been many attempts to overcome this limitation, including use of a wide variety of matrix materials and ionization by specific chemical treatment (*12*). These methods have in general been highly specific in their applicability, which can be a useful property in analysis; nevertheless, a more universal method of rendering samples responsive to FAB mass spectrometry is desirable. We report here a method which we believe has the potential to become such a technique, while retaining some selectivity in sample ionization. We denote this new form of FAB as electrochemically assisted fast atom bombardment, or EFAB, since it involves activating the sample by electrolysis, carried out directly in the glycerol matrix.

The probe commonly used to support the FAB matrix in the atom beam is a solid rod, floated at the source potential. If the tip of such a probe is modified to include a second electrode, then a potential difference may be applied to the sample. Glycerol, the most commonly used liquid matrix for FAB work, has a relatively high dielectric constant of 42 (*13*), so that it will conduct current reasonably well. There are a few reports of electrochemistry in glycerol, but due to low ionic diffusion coefficients, the electrochemical possibilities of this solvent have not been extensively explored (*14*, *15*). Redox

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processes of analytes in glycerol under fast atom bombardment have been observed (*16*).

EXPERIMENTAL SECTION

The EFAB probe consists of an outer copper tube (the "ring" electrode), with the outside diameter that of the original FAB probe, ca. 7 mm diameter, and an inner brass rod (the "disk" electrode), separated from each other by a press-fit Teflon sleeve of ca. 0.1 mm thickness. The disk is slightly recessed from the tip of the ring to create a small well. This assembly fits into the source of a MS-50 mass spectrometer in place of the standard FAB probe. The ring electrode floats at the source potential (8 kV), while the voltage on the disk electrode can be varied by a battery-powered voltage divider–voltage follower circuit floated at the source potential, over a ±15-V range relative to the ring. The input to the voltage follower circuit is protected by diode clippers to prevent failure of the 741 op amp during voltage surges when turned on at the 8-kV source potential. The sample is dissolved in glycerol (ca. 1 to 10 mM) and a thin layer of this is placed on the probe tip so as to contact both electrodes of the cell. Spectra are obtained first at 0 V potential between the electrodes, to give a standard FAB mass spectrum, then at various oxidizing or reducing potentials to determine the effect of the electrochemistry on the sample. We adopt the convention of using the term V_e to refer to the potential of the ring electrode, relative to the disk, even though it is the disk potential that is actually varied by the electronics. This is because, as noted below, we believe that the ions that are observed in the mass spectrometer are primarily those formed at the ring electrode. No reference electrode is used in this work, and the junction potentials for a cell and solvent such as this will be quite large. Thus, a given ion will not necessarily be produced at the nominal voltage corresponding to its $E_{1/2}$ value. Both cations and anions are produced in the matrix at the same time: for voltages with the ring positive with respect to the disk, oxidation will occur at the ring and reduction at the disk. For negative voltages, the opposite will occur.

All samples were obtained commercially and used as received.

RESULTS AND DISCUSSION

When V_e is set to 0 V, we observe spectra typical of normal FAB MS: pure glycerol yields the (M + 1), (2M + 1), and (3M + 1) matrix ions in positive mode, and the corresponding (M − 1) ions in negative ion mode. In the absence of analyte in the glycerol, no other ions are seen as V_e is varied over ±12 V. The total ion current varies considerably, falling off by up to 2 orders of magnitude as V_e is varied over this range. This is probably due to perturbation of the electrical focusing field in the source by the presence of the voltage on the disk electrode, since if the instrument is tuned with V_e at some nonzero voltage, the maximum is now at that voltage, with a falloff in TIC as V_e is set to zero. Peak shape does not change as V_e is varied, but there is a mass shift of ca. 50 ppm at the voltage extremes.

When 1-bromohexadecane is added to the glycerol matrix, no ions indicative of it are observed above the noise level with V_e = 0 V. At V_e greater than 2 V in positive ion mode, a 1000-fold increase in the $303^+/305^+$ (M − 1) signal occurs, to where it becomes comparable in size to the base matrix peak. Other fragment peaks such as $(M - Br)^+$ and $C_7H_{13}^+$, prominent in the EI mass spectrum, are also seen, as shown in Figure 1. For $V_e < 0$ V, these ions are not observed. This indicates that we are only observing ions from oxidation at the ring and not those when the disk is the anode. This is consistent with the very low ionic diffusion coefficient for glycerol, plus the nature of the FAB beam sampling only ions at the surface of the matrix. The glycerol bead used is thinnest at the edge of the drop near the ring, so that ions produced there are near the surface at all times. Ions produced at the disk, however, are beneath a thicker layer of the liquid and are thus less likely to migrate to the surface and be sampled. A dependency on the thickness of the glycerol bead is observed for both relative ionic signal strength and total ion current

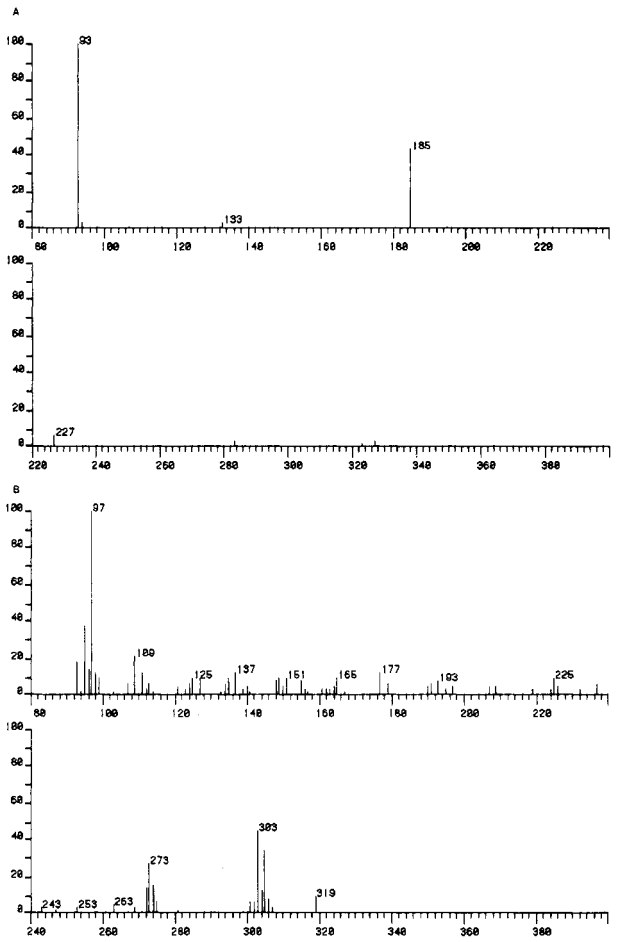

Figure 1. Positive ion mass spectrum of a 1 mM solution of 1-bromohexadecane in glycerol at (A) V_e = 0.0 V and (B) V_e = 3.0 V.

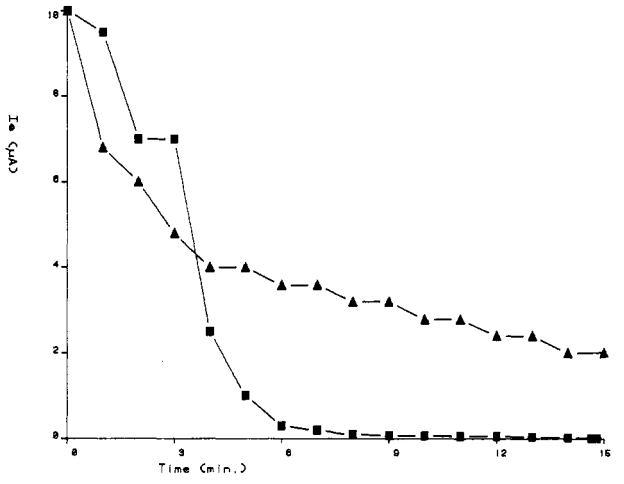

Figure 2. Intensity of 303^+ in arbitrary units (▲) and electrochemical current in microamperes (■) as a function of time. V_e = +8 V.

vs. voltage; best results are obtained by using as thin a layer of glycerol as possible. The structure of the electrolysis cell on the probe tip is being redesigned to reduce this effect. If a microammeter is used to measure the current drawn from the floating power supply for the EFAB probe, a value of about 1 μA/V is seen for pure glycerol. When bromohexadecane is added, this value increases by a factor of 2 or 3, slowly falling with time in parallel to the slow loss of the $303^+/305^+$ signal, until it reaches a value typical of the pure glycerol when the sample signal is gone (Figure 2).

Hexanophenone, $PhCOC_5H_{11}$, gives no ions under EFAB conditions at 0 V or more negative, but at +3 V or lower a small (M + 1) ion appears plus larger signals corresponding

to $PhCO^+$, $C_5H_{11}CO^+$, McLafferty rearrangement product, and various alkyl group losses from the side chain. At the most positive voltage, a $PhCHOH^+$ ion is also seen; this can be attributed to a product of reduction at the disk, followed by slow migration to the ring and the oxidation to the cation. Decanophenone behaves similarly.

Although anisole is electrochemically active in more conventional solvents (*17*), we observe no radical cation or dimer product for it at any voltage; 1,2-dimethoxybenzene behaves similarly. In contrast, 1,3,5-trimethoxybenzene shows a prominent radical cation signal from +3 to +12 V. A possible problem here is the mass range of the MS-50 in FAB mode; it was calibrated to examine data only down to ca. 90 amu. Products of electrochemical reactions of smaller mass were not observed therefore.

Nitrobenzene does not show any distinctive peaks in the negative ion mass spectrum at any EFAB potential, although there is a dependence of probe current on time similar to that described above for bromohexadecane. 1,2- and 1,3-Dinitrobenzene both give radical anion peaks as the base peaks in the spectrum at voltages from +3 to +12 V, with no evidence of higher multiples or fragmentation products.

A number of other compounds have been briefly examined. Naphthalene, anthracene, and phenanthrene are very poorly soluble in glycerol. EFAB spectra of these do not show large signals, but there are both (M + 1) and (M + 3) cations observed. The latter may be due to some process similar to the reduction/oxidation combination mentioned above for the phenones (*17*). Triolein, in negative ion mode and the ring reducing, does not show a radical anion (it is unlikely that the electron in such a species would be bound) but does give strong signals for the carboxylate anion $C_{17}H_{33}CO_2^-$.

The major problem with this technique at present is the limited solubility in the glycerol matrix of many of the samples tried. We have emphasized the use of less polar compounds so far, since these are the ones least successful with conventional FAB MS (*1-3, 11*). The high polarity of glycerol, which makes it useful for electrochemistry, also limits the solubility of nonpolar species. We are currently trying a number of other possible matrix materials that should favor the solubility of lipids and aromatic species but still support electrochemistry.

In conclusion, we believe that this is a technique that will expand the ability of FAB MS to handle a wide variety of compounds, using a relatively minor instrumental modification. We are continuing our investigations into the design and chemistry of this technique.

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AIDS FOR ANALYTICAL CHEMISTS

Modified Cell for Stripping Voltammetry Using the Static Mercury Drop Electrode

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Stripping analysis has become increasingly important for highly sensitive measurements of trace electroactive species (*1-3*). Conventional and adsorptive stripping schemes are now available for measuring low levels of numerous metals and organic compounds. The increasing interest in stripping analysis has been, in part, due to the introduction of the modern static mercury drop electrode (SMDE), the EG&G PAR Model 303, which allows virtually instantaneous automatic production and renewal of hanging mercury drop electrodes (HMDEs) (*4*). A number of similar devices aiming at automatic generation of HMDEs are now available commercially from different sources.

While the EG&G PAR Model 303 SMDE represents a major instrumental advance, its cell assembly suffers from a major drawback when stripping applications are concerned. Simply stated, mercury drops—dislodged from the capillary at the end of each stripping cycle—accumulate at the bottom of the cell. The consequence of the mercury presents at the bottom is a gradual decrease in the efficacy of the convective mass transport, employed to facilitate the deposition process (because of increasing resistance to the angular motion of the stirring bar). The small cell dimension (9 mm bottom radius) further enhances the severity of the problem, as only tiny stirring bars can be employed. As the deposition current at

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