JOURNAL OF MASS SPECTROMETRY
*J. Mass Spectrom.* **33**, 1141–1147 (1998)

# Optimization of a Hydrophobic Solid-phase Extraction Interface for Matrix-Assisted Laser Desorption/Ionization

**Adam H. Brockman,*† Nina N. Shah‡ and Ron Orlando**
Complex Carbohydrate Research Center, Department of Biochemistry and Molecular Biology, and Department of Chemistry, University of Georgia, Athens, Georgia 30602-4712, USA

**Matrix-assisted laser desorption/ionization (MALDI) probe surfaces derivatized with octadecanethiol ($C_{18}$) can be used as hydrophobic solid-phase extraction devices to isolate and desalt biopolymers directly on the probe surface. Using quantitative MALDI, it was possible to determine the approximate amount of peptide that bound to $C_{18}$ surfaces and thus to calculate a surface density. It was determined that the amount of peptide bound at the probe surface was independent of the analyte concentration in the immersion solution (from high- to sub-ng $ml^{-1}$ concentrations), but rather was dependent on the immersion time of the surface as it was exposed to the analyte. The capacity of $C_{18}$-derivatized probes to bind biopolymers in fixed amounts frees the analyst from the necessity for adjusting analyte concentration through multiple step procedures such as serial dilution or vacuum drying. This time savings result in an overall increase in the efficiency of the MALDI technique. © 1998 John Wiley & Sons, Ltd.**

KEYWORDS: matrix-assisted laser desorption/ionization mass spectrometry; solid-phase extraction; coupled methods

## INTRODUCTION

Matrix-assisted laser desorption/ionization (MALDI) has evolved to revolutionize the scope with which mass spectrometry can be applied to large non-volatile biological molecules.[1] The method is suitable over a large mass range, is extremely sensitive and can be applied quantitatively. However, MALDI is vulnerable to limitations arising from commonly encountered interferences, and the necessity for preparing samples in a suitable analyte-to-matrix ratio can prohibit rapid analysis by the method.

Several mass spectrometric interferences are common to the types of samples that interest the pharmaceutical and biotechnology industries. These interferences with mass spectrometric analysis can result from charge competition in diverse mixtures, salts, chaotropes or surfactants. Signal suppression caused by interferences occurs through inhibition of analyte ionization in the ion source. The primary cause of this inhibition is thought to occur in two ways, by the contaminant preventing the co-crystallization of the analyte with the matrix and by charge competition in the laser-induced plasma plume at the surface. Additional loss of the analyte signal can be observed on account of fragmentation and adduct formation. An ideal solution to these challenges would be an on-line process that removes the interferences from the analyte.

On-line, column-based separations could be used to eliminate interferences from samples prior to MALDI analysis. However, MALDI is most commonly performed by drying the analyte on the probe surface in a mixture with a light-absorbing matrix, and is therefore a solid-phase ionization method. The solid-phase nature of the MALDI preparation makes on-line liquid-phase separation interfacing very challenging, although some progress has been made in this area.[2]

An alternative approach to coupled MALDI separations is the use of on-probe separations. In an on-probe separation, the probe is derivatized with separation media that would selectively bind the analyte of interest but allow the contaminants to be removed by washing with a solvent. Such an approach offers a great deal of sensitivity, as no elution step is necessary preceding the detection event of the separation. In addition, probe tips modified in this fashion facilitate automation of MALDI, as issues involving concentration and analyte-to-matrix ratio become less critical if surfaces with capacities in the proper range are used. We argue that the approach herein is appropriately referred to as 'solid-phase extraction mass spectrometry' (SPE/MS). This broadly applicable term could be used to describe

* *Correspondence to*: A. H. Brockman, Covance Laboratories, Department of Pharmaceutical Chemistry, 3301 Kinsman Boulevard, Madison, Wisconsin 53704, USA.
E-mail: adam.brockman@covance.com
† Present address: Covance Laboratories, Inc., Department of Pharmaceutical Chemistry, 3301 Kinsman Boulevard, Madison, Wisconsin 53704, USA.
‡ Present address: Department of Chemistry, Oglethorpe University, 4484 Peachtree Road NE, Atlanta, Georgia 30319-2791, USA.
Contract/grant sponsor: National Institutes of Health; Contract/grant number: T32 AI07322.
Contract/grant sponsor: National Science Foundation; Contract/grant number: 9626835.

CCC 1076–5174/98/111141–07 $17.50


*Received 28 May 1998*
*Accepted 24 August 1998*

any surface-based separation used in conjunction with a surface-based ionization method in mass spectrometry. SPE/MS methods will be especially valuable to the biological and pharmaceutical science communities as MALDI continues to grow in popularity and quantitative MALDI methods become more refined.

Several investigators have participated in the development of on-probe preparative procedures for MALDI/MS. Preparative methods involving affinity capture have been developed utilizing protein-derivatized beads affixed to the probe surface,[3,4] or by using derivatized self-assembled monolayers (SAMs).[5–7] In addition to affinity capture methods, methods employing matrix crystal inclusion,[8] hydrophobic membranes,[9] thick matrix underlayers[10] and binary matrices have also been explored.[11] However, these methods contribute in some instances to field distortion in the source.

SAMs are especially attractive as a separation medium. SAMs are a covalently attached medium that generate minimal surface distortion. They are also relatively easy to construct,[12] are well characterized[13,14] and are relatively resistant to solvents and buffers once formed. The first on-probe separations methods using SAMs were directed towards affinity separations.[3–7] Recently, the commercial availability of reagents to compose a variety of SAMs which are well suited to general separation approaches has encouraged work using on-probe reversed-phase separations.[15] These separations not only permit interference elimination directly on the probe tip, but also appear to allow the isolation of peptides within a suitable analyte-to-matrix concentration range in a single step. However, since the surface density of a peptide-saturated SAM-derivatized probe surface has never been ascertained, it is difficult to predict with certainty what the optimal conditions for a SAM method might be.

A recent contribution produced data on the nature of non-specific protein adsorption on methyl-terminated SAMs.[16] This work was carried out using an acoustic plate mode device to measure the degree of interaction of fibrinogen samples ranging in concentration from 3 to 280 $\mu g\ ml^{-1}$. Solutions lower in concentration than 3 $\mu g\ ml^{-1}$ were not studied owing to a lack of measurable adsorption. It was found that the rate of increase in binding over time was very low for solutions of 3 $\mu g\ ml^{-1}$. It was proposed that this difference in the adsorption capacity at the surface could be attributed to the unfolding of the protein to a larger radiance of occupancy on the surface. However, a lack of sensitivity on the part of the acoustic plate mode device could also have contributed to the lack of measurable adsorption at low concentrations. It is likely that the sensitivity of quantitative MALDI, which has already been established by other workers,[17–21] would be useful for characterizing the peptide adsorption process at lower concentrations.

Bioanalytically interesting concentrations of peptide are relatively low (low $\mu g\ ml^{-1}$ to $pg\ ml^{-1}$). The correspondingly small amounts of peptide adsorbed on $C_{18}$ surfaces must therefore be established using a very sensitive surface analytical technique. For this reason, we explored the use of the already established method of quantitative MALDI to determine the nature of non-specific adsorption of peptide on C18 surfaces. Although quantitative MALDI methods are not yet robust enough to be applied on a routine basis owing to shot-to-shot variability and variability in the matrix-to-analyte ratio from spot to spot, it is one of the few methods by which the amount of peptide adsorbed can be readily determined with adequate sensitivity. This is an important parameter to consider in optimization issues surrounding the SPE/MS interface, and should provide a useful benchmark for future investigations.

There are two optimization issues of primary concern in the use of C18 SPE/MS. The first of these is the concentration of the peptide analyte. In our earlier work it appeared that an upper threshold exists on the binding capacity such that the analyte-to-matrix ratio never became too high.[15] However, this was not confirmed in the previous work, so a study of peptide concentration was performed here to demonstrate that the amount of immobilized peptide does not increase significantly when the probes are immersed in a range of immersion solutions containing various concentrations of peptide.

Another major issue is binding time. In our original work, the probes were immersed in the peptide solution for an excess period of time (overnight) in order to allow maximal binding to occur. This was based on our observation that a momentary deposition of a microliter volume of peptide solution on the surface followed by washing and analysis yielded sporadic signals in comparison with a very robust signal obtained after extended incubations. The use of the extended binding time to maximize binding also contributed to our hypothesis that the derived surfaces had an upper limit on the amount of peptide adsorbed, since no amount of time seemed to yield an inhibitory amount of bound peptide. We studied this phenomenon in more detail here by correlating the MALDI response with immersion time.

A final consideration examined here is the determination of a benchmark describing the capacity of derivatized probes. The elucidation of such a benchmark should provide an important reference by which the capacity of subsequent derivatization chemistries can be compared. In this fashion, derivatization chemistries complementary to the current C18 chemistry can be designed for various situations as necessary. Surface density was calculated for this benchmark because the parameter is independent of probe surface area and has proven to be a useful consideration for similar surfaces used in different scenarios.[22]

## EXPERIMENTAL

Our goals in this work were to optimize methods further for the use of C18 SAMs in the MALDI analysis of peptides using quantitative MALDI. To demonstrate the effectiveness of the method, the determination of rennin substrate in phosphate-buffered saline (PBS) was performed for 1 $\mu g\ ml^{-1}$ and 1 $ng\ ml^{-1}$ solutions using the SPE/MS method. Additionally, the utility of the method in the analysis of mixtures was demonstrated by the acquisition of spectra from a sample containing trypsin-digested horse heart cytochrome *c* (Sigma, St

 

Louis, MO, USA) peptides in 20 mM phosphate buffer (pH 2.0) which had been prepared in a conventional fashion.[23]

A reference curve for conventionally deposited rennin substrate tetradecapeptide (Sigma) was constructed using 1 mM sinapinic acid (Aldrich, Milwaukee, WI, USA) with 1.5 μg $ml^{-1}$ human angiotensin (Sigma) added as an internal standard. This reference curve was used to demonstrate the linear response of the rennin substrate/angiotensin integrated intensity ratio to the amount of rennin on the probe, so that an estimate of the surface density of SPE/MS-isolated peptide on the probes could be made. Finally, a range of analyte concentrations and binding times were explored to compare the behavior of our probes with respect to the surfaces studied in the APM measurements.[16]

MALDI data were collected using an HP LDI1700 XP linear time-of-flight mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA). The repeller was operated at 30 kV and the intermediate extraction lens was operated at 9 kV. A mass gate was employed to deflect ions below $m/z$ 800. The matrix was a 2 mM solution of sinapinic acid in volatile buffer B, i.e. 80% aqueous acetonitrile (ACN) (J. T. Baker, Phillipsburg, NJ, USA) with 0.05% trifluoroacetic acid (TFA) (Aldrich).

'Conventional' MALDI refers to typical MALDI preparations in which the analyst mixes a volume of matrix and a volume of sample and then dries the resulting mixture on the probe surface. To prepare the reference curve on the C18-derivatized tips, each serial dilution of rennin substrate was mixed 1:1 with a matrix–internal standard mixture, and 1 μl of the mixture was deposited on the surface in this conventional fashion. A new C18 SPE/MS tip was used in each experiment to avoid losses in binding capacity between experiments. The strategy, as with most external standard calibrations, is to compare the analyte/internal standard peak area ratio generated using conventional MALDI with the ratio generated using SPE/MS. The analyte-to-internal standard ratio was determined as an average of several shots.

C18 surfaces were prepared as described elsewhere.[15] The C18 monolayer is hydrophobic and serves to adsorb peptides selectively from solution while allowing interferences to be washed away. The probe surfaces used consisted of a ring of eight raised surfaces referred to as 'mesas.' It is from the derivatized tops of these mesas that spectra were obtained.

Solutions of 1 μg $ml^{-1}$ and 1 ng $ml^{-1}$ to rennin substrate (Sigma) were made in PBS (10 mM phosphate, 150 mM NaCl, pH 7) in order to demonstrate the utility of the tips. The tips were incubated overnight in a 1 ml aliquot of each solution and then analyzed after washing with 0.06% TFA.

A reference curve was constructed for the MALDI response as typically described in the literature.[16] Human angiotensin with an $m/z$ of 1297 $[M + H]^+$ was chosen as an internal standard as it has a similar p$I$ and does not have any peaks overlapping with those of rennin substrate ($m/z$ 1760 $[M + H]^+$. The standard matrix was prepared as described above, with a concentration of 2 mM sinapinic acid and 3 μg $ml^{-1}$ human angiotensin such that a 1:1 mixture with the analyte made a final solution of 1 mM sinapinic acid and 1.2 pmol $\mu l^{-1}$ angiotensin. The matrix and internal standard were delivered by droplet deposition as opposed to electrospray deposition with the intention of allowing bound peptide to be evenly distributed with matrix and better included in matrix crystals as they were formed from solution. A serial dilution of rennin substrate was then performed from 200 μM to 200 pM so that deposited amounts after mixing with matrix would range from 100 pmol to 100 amol in intervals of 10 amol. Such a large dynamic range is not thought to be practical for high-precision quantitative MALDI,[18] but our purpose was to obtain a rough idea of the amount of peptide adsorbed over as large a range as possible. A sufficiently linear reference curve for the conventionally deposited rennin substrate can be correlated with SPE/MS-isolated rennin substrate. A linear regression was used to perform this correlation using the equation $y = mx + b$, where $m$ is the slope, $x$ is the area ratio (response) and $b$ is the $y$ intercept. Two-tailed $t$-tests were employed to demonstrate the statistical significance of the slope.

Time curves were established by immersing the tips for various time intervals in an excess volume of a 1 μg $ml^{-1}$ solution of rennin substrate. The tips were removed after each time interval and rinsed and matrix with internal standard diluted 1:1 with buffer B was then applied. The peak-area ratio was obtained in triplicate for each time interval and fitted by linear regression to determine if the response could be correlated significantly as a function of the incubation time of the tips.

A serial dilution was performed with rennin substrate to study the effects of high peptide concentration on the binding. The dilution covered the range 300–3 μg $ml^{-1}$, similar to previous work.[16] The tips were then incubated in each solution overnight, the tips were removed for each immersion solution concentration, rinsed and matrix with internal standard diluted 1:1 with buffer B was then applied. The peak-area ratio was obtained in triplicate for each concentration interval and fitted by linear regression to determine if the response could be correlated significantly as a function of concentration. The tips previously appeared to prevent the accumulation of surface densities so high as to create a poor analyte-to-matrix ratio, but this was not confirmed. Therefore, the peak-area ratio response was correlated with peptide concentration to see if the surface density increased significantly with concentration of analyte in solution during binding.

## RESULTS AND DISCUSSION

The results of a typical C18 SPE/MS analysis of a salty solution of 1 ng $ml^{-1}$ and 1 pg $ml^{-1}$ of rennin substrate are shown in Fig. 1. The signal-to-noise ratios do not change dramatically when using SPE/MS even though the concentration of the immersion solution is changed by as much as three orders of magnitude. In addition, clean spectra were acquired despite the presence of salts. We never encountered non-binding or competitively binding peptides.

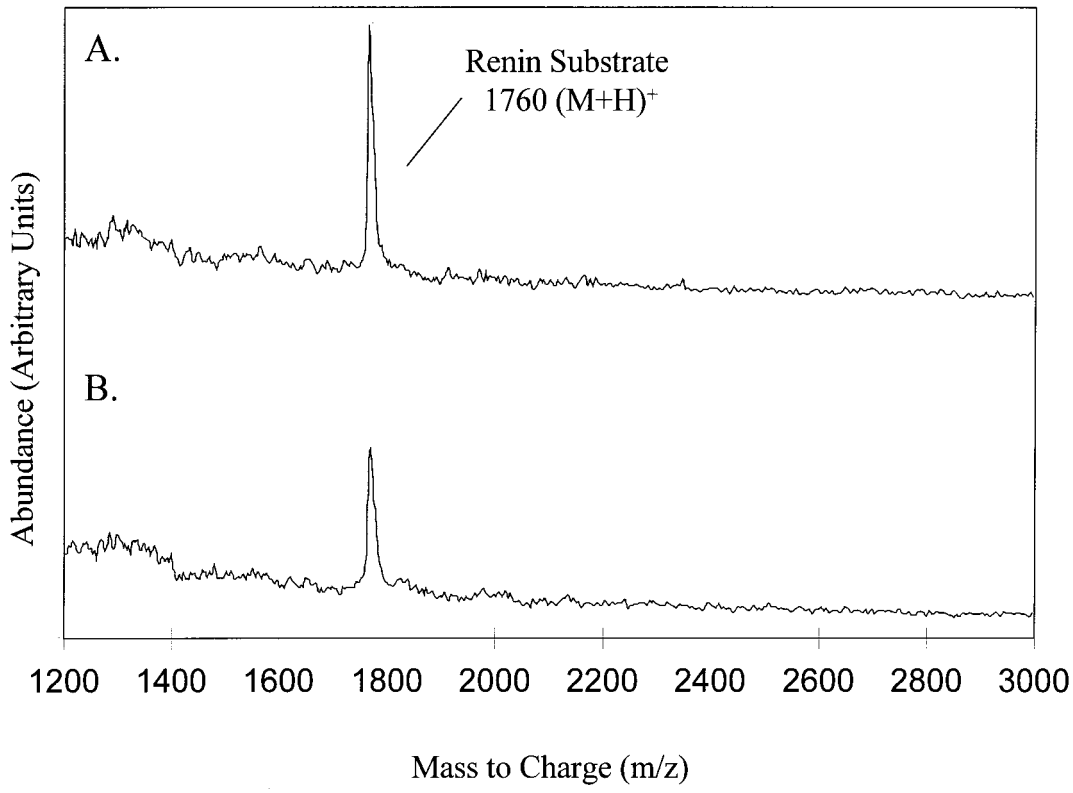


**Figure 1.** SPE/MS analysis of (A) 1 μg $ml^{-1}$ and (B) 1 ng $ml^{-1}$ renin substrate in PBS.

A mixture of tryptic cytochrome *c* peptides was analyzed by conventional MALDI and by SPE/MS to demonstrate the utility of concentration-dependent binding (Fig. 2). Components of peptide mixtures appear when using SPE/MS [Fig. 2(b)] which do not appear when using conventional MALDI [Fig. 2(a)]. These results are typical, and represent the reasoning behind our hypothesis that there is an upper limit to the capacity of these probes and non-competitive binding kinetics at low concentrations. Since this capacity is

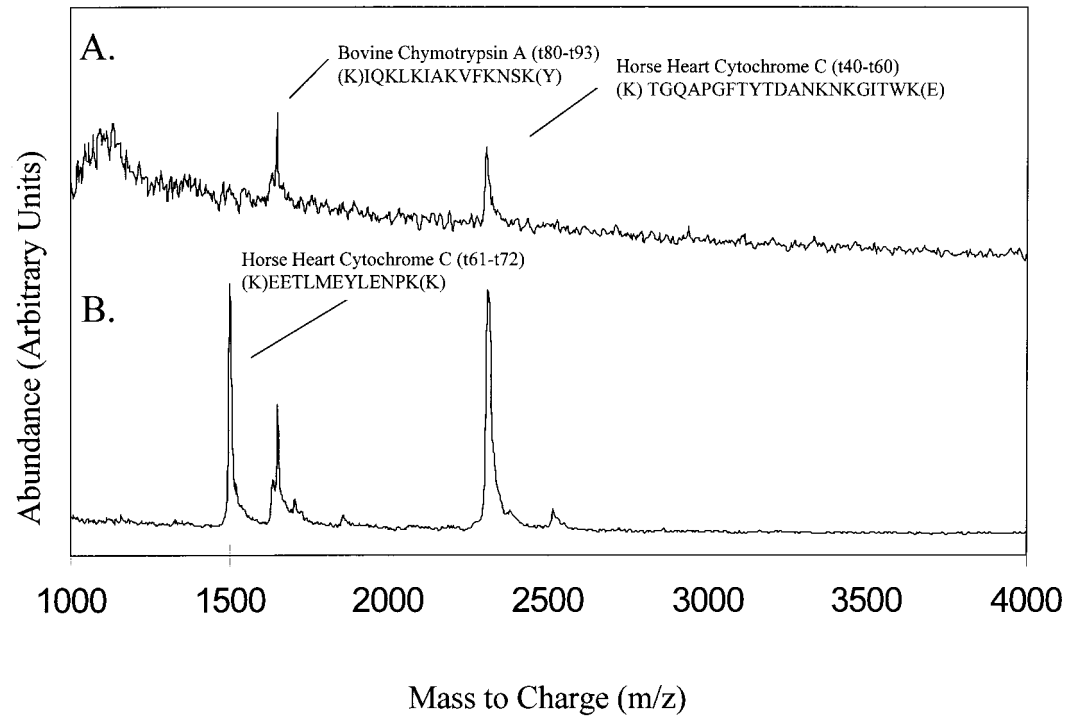


**Figure 2.** (A) Conventional MALDI analysis of a peptide mixture resulting from a tryptic digest of cytochrome *c*; (B) SPE/MS analysis of the same peptide mixture.

**Table 1. Degrees of freedom ($n-1$), slope ($m$), $y$-intercept ($b$), $t$-value and $t_{\alpha/2}/(t_{\alpha/2})$ values tabulated for comparison of the significance of response for three different relationships[a]**

| Parameter | $m$[b] | $b$ | $t$ | $t_{\alpha/2}$ | $n-1$ |
|---|---|---|---|---|---|
| Amount deposited | $2.2 \times 10^{-7}$ | 2.01 | 5.18 | 2.78 | 26 |
| Time | $7.31 \times 10^{-4}$ | 0.365 | 4.28 | 2.81 | 23 |
| Concentration | $-1.37 \times 10^{-3}$ | 0.737 | −1.14 | −2.82 | 22 |

[a] The correlations were performed for integrated analyte-to-internal standard ratio (response) *vs*. amount deposited, time and concentration.
[b] Slope comparisons are not indicative of rate differences as the ranges of immersion times, immersion solution concentrations and amounts are in different units for each correlation. Slopes were in the following units: amount, amol/response; time, min/response; and concentration, mg $ml^{-1}$/response.

independent of the concentration of the analyte in the immersion solution, it effectively dictates an acceptable analyte-to-matrix ratio for all analytes in solution. In order to confirm these results, however, correlations between MALDI response and the variables of interest (amount of peptide on the surface, immersion time and immersion solution concentration) are necessary.

Angiotensin is not an optimal internal standard for the quantitation of rennin substrate when compared with some of the standard–analyte pairs presented in the literature.[19] However, since our purpose was to evaluate response as a function of variables of interest as opposed to quantitation, careful matching of rennin substrate with an internal standard was not explored exhaustively. The results can be explained, however, since rennin substrate has a very different hydropathy to angiotensin. Rennin substrate has a free energy of surface transfer ($\Delta F$) of $-7.70$ kcal $mol^{-1}$ (1 kcal = 4.184 kJ) and an HPLC index of 81.5 whereas angiotensin has a $\Delta F$ value of $-4.29$ kcal $mol^{-1}$ and an HPLC index of 56.2.[24,25] If the physio-chemical properties of the analyte and internal standard are matched carefully enough, differing rates of matrix crystal inclusion and charge affinity are matched, and the precision of the method increased.[18] Overall, the use of angiotensin and renin substrate was sufficient for our purposes as no difference was observed between conventional MALDI and SPE/MS using this system.

The relationship between the MALDI response (rennin substrate/angiotensin integrated intensity ratio) and the variables of interest (amount deposited, time of immersion and concentration of immersion solution) are outlined in the regression summary shown in Table 1. A two-tailed $t$-test was performed to demonstrate whether or not the slope was significantly greater than zero so that the statistical significance of the correlation could be established at the 99% confidence level. The linear regression results illustrate a significant relationship between response and each variable if $t$ is greater than $t_{\alpha/2}$, or $-t$ is less than $-t_{\alpha/2}$.

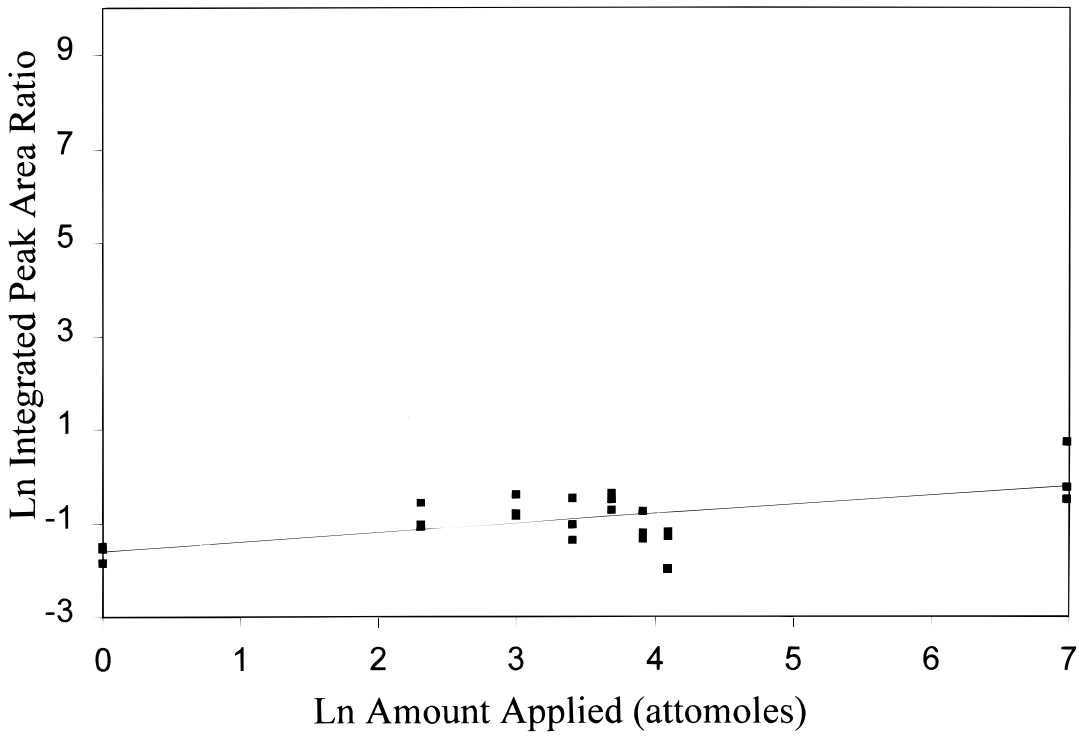


**Figure 3.** Response of integrated peak-area ratio (analyte-to-internal standard), correlated with the concentration of the immersion solution.

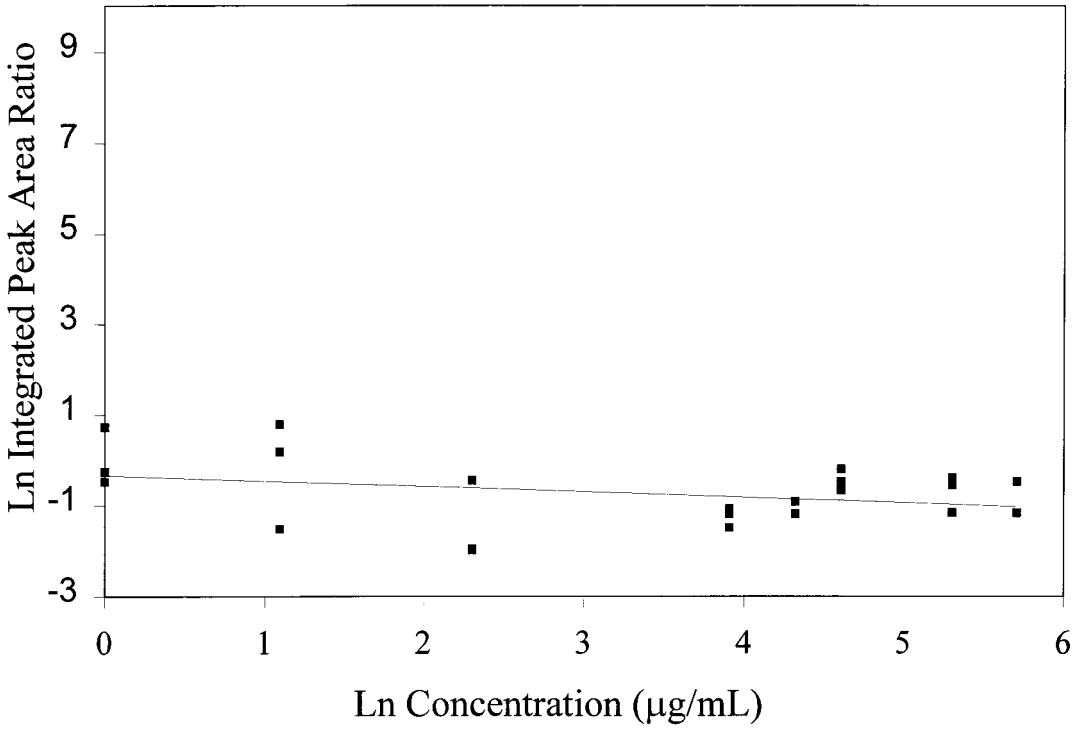


**Figure 4.** Response of integrated peak-area ratio (analyte-to-internal standard), correlated with the concentration of the immersion solution. The slope is not statistically greater than zero.

The relationship between immersion time and MALDI response was significant although the slope was very shallow (Fig. 3). Since Whitesides and co-workers have shown the kinetics of peptide adsorption on methyl terminated SAMs to be first order and slow for concentrations below 3 μg $ml^{-1}$ using an acoustic plate mode device,[16] this result is to be expected. The result indicates that an overnight binding time is excessive for solutions in the 1 μg/$ml^{-1}$ range, and confirms Whitesides and co-workers' conclusions with regard to the slow kinetics of low-to-sub-microgram solutions. The result also indicates that the amounts of each peptide adsorbed from a mixture should be similar (non-competitive) provided that the amount of the peptides does not exceed 3 μg $ml^{-1}$. Therefore, the appearance of additional peaks in the SPE mass spectrum shown in Fig. 2(B) can be explained by the fact that analytes of very low concentration are equally represented on the surface with analytes of fairly high concentration within the low-sub-μg $ml^{-1}$ range of interest.

In Fig. 4 the relationship between immersion solution concentration and MALDI response is shown. Concentrations were allowed to proceed overnight so that an excess immersion time would be allowed for surface

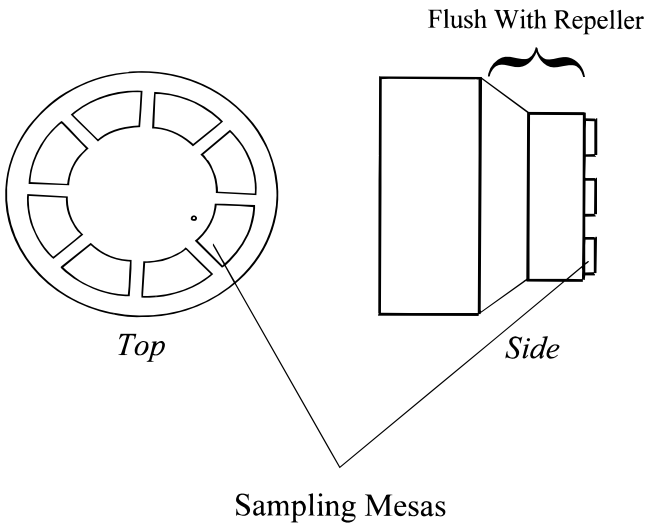


**Figure 5.** The geometry of our probe tips. Sample is applied to the eight raised mesas. Hewlett-Packard has manufactured a disposable ring of mesas that can be screwed on to the probe tip. We determined the surface volume to be $9.12 \times 10^{20}$ $Å^3$, the peptide volume to be 319 $Å^3$, the total theoretical molecular volume to be $4.75 \times 10^{-6}$ mol and the experimental surface density to be $(2.18 \pm 1.6) \times 10^3$ molecules $mm^{-3}$.

 

densities to reach the surface density maximum. The slope of the regression line is not statistically greater than zero, as shown in Table 1. This result confirms our suggestion that there is an upper limit of capacity for the surfaces.

The schematic diagram in Fig. 5 shows the basic geometry of our MALDI probe for which we calculate the surface density of adsorbed peptide. These calculations were based on values taken from the literature,[22] and rest on the underlying assumption that a gold-sputtered, etched stainless-steel surface is similar to an etched silver surface. The average predicted value from seven measurements of a 1 $\mu g\ ml^{-1}$ SPE/MS isolation that was allowed to proceed overnight was $3.30 \pm 2.47$ pmol per mesa. Since the surface volume of each mesa was calculated to be $9.12 \times 10^{20}$ Å$^3$, the surface density would be $(2.18 \pm 1.63) \times 10^3$ molecules $\mu m^{-3}$. Because the amount of peptide captured has been shown to be independent of concentration, the surface density value is also independent of analyte concentration. This suggests that surface chemistry could be optimized by altering the surface density such that an optimized matrix-to-analyte ratio is always obtained. The surface density provides a useful benchmark for the estimation of the capacity of the tips that is independent of probe surface and geometry.

## CONCLUSION

The results shown here indicate that the upper threshold of adsorbed peptide is not a function of immersion solution concentration. In addition, the adsorption of a peptide on the surface is confirmed to be a slow reaction which is described by first-order kinetics in the literature.[16] The results demonstrate that an overnight binding time is excessive for immersion solution concentrations in the region of 1 $\mu g\ ml^{-1}$ and that each component from sub-$\mu g\ ml^{-1}$ mixtures of peptides will be represented by similar amounts of adsorbed peptide on the probe.

The surface density of adsorbed peptide appears to have a relatively low limit on the SPE/MS probe surfaces. This offers the advantage of restricting the analyte-to-matrix ratio to within acceptable limits in an automated fashion. Since the total capacity of the surface is divided by the number of components in a mixture of analytes to describe the surface density of each component, a surface capacity that is too low could act as a limitation in the analysis of mixtures. However, since MALDI detection limits lie in the sub-femtomole range, the limit on the number of components imposed by the $C_{18}$-derivatized surface is more than a 1000 different peptides. This result suggests that other chemistries should be explored which offer different capacities for different situations. Such studies require a probe-type independent benchmark such as that offered by the surface density which is $(2.18 \pm 1.63) \times 10^3$ molecules $\mu m^{-3}$ for the C18-derivatized surface.

### Acknowledgements

The authors thank Dr Mark Farmer of the University of Georgia Department of Cellular Biology for continuing access to his gold-sputtering apparatus and Jennifer Brockman for critical review of the manuscript. We greatly appreciate financial support from NIH grant T32 AI07322 and NSF grant 9626835 which made this project possible.

## REFERENCES

1. F. Hillenkamp, M. Karas, R. C. Beavis and B. T. Chait, *Anal. Chem.* **63**, 1193A (1991).
2. M. D. Beeson, K. K. Murray and D. H. Russell, *Anal. Chem.* **67**, 1981 (1995).
3. T. W. Hutchins and T. Yip, *Rapid Commun. Mass Spectrom.* **7**, 576 (1993).
4. D. I. Papac, J. Hoyes and K. B. Tomer, *Anal. Chem.* **66**, 2609 (1994).
5. Schriemer and L. Li, *Anal. Chem.* **68**, 3382 (1996).
6. A. H. Brockman and R. Orlando, *Anal. Chem.* **67**, 4581 (1995).
7. A. H. Brockman and R. Orlando, *Rapid Commun. Mass Spectrom.* **10**, 1688 (1996).
8. F. Xiang and R. C. Beavis, *Rapid Commun. Mass Spectrom.* **8**, 199 (1994).
9. A. J. Alexander and J. A. Blackledge, *Anal. Chem.* **67**, 843 (1995).
10. O. Vorm, P. Roepstorff and M. Mann, *Anal. Chem.* **66**, 3281 (1994).
11. A. I. Gusev, W. R. Wilkinson, A. Proctor and D. M. Hercules, *Anal. Chem.* **67**, 1034 (1995).
12. L. H. Dubois, *Ann. Phys. Chem.* **43**, 437 (1992).
13. D. R. Jung and A. W. Czanderna, *Crit. Rev. Solid State Mater. Sci.* **19**, 1 (1994).
14. C. D. Bain, J. Evall and G. M. Whitesides, *J. Am. Chem. Soc.* **111**, 7155 (1989).
15. A. H. Brockman, B. S. Dodd and R. Orlando, *Anal. Chem.* **69**, 4716 (1997).
16. R. R. Seigel, P. Harder, R. Dahint, M. Grunze, F. Josse, M. Mrksich and G. M. Whitesides, *Anal. Chem.* **69**, 3321 (1997).
17. D. C. Muddiman, A. I. Gusev, L. B. Martin and D. M. Hercules, *Anal. Chem.* **354**, 103 (1996).
18. D. C. Muddiman, A. I. Gusev and D. M. Hercules, *Mass Spectrom. Rev.* **14**, 383 (1995).
19. J. Y. Wu, K. Chatman, K. Harris and G. Siuzdak, *Anal. Chem.* **69**, 3767 (1997).
20. S. R. Weinberger and K. O. Boernsen, paper presented at the Kyoto '92 International Conference on Biological Mass Spectrometry, September 20–24, 1992, Kyoto, Japan.
21. R. W. Nelson, M. A. McLean and T. W. Hutchens, *Anal. Chem.* **66**, 1408 (1994).
22. D. C. Muddiman, A. H. Brockman, A. Proctor, M. Houalla and D. M. Hercules, *J. Phys. Chem.* **98**, 11570 (1994).
23. J. A. McCloskey (Ed.), *Methods in Enzymology: Mass Spectrometry*. Academic Press, San Diego (1990).
24. B. H. Bull and K. Breese, *Arch. Biochem. Biophys.* **161**, 665 (1974).
25. C. A. Browne, H. P. J. Bennett and S. Solomon, *Anal. Biochem.* **124**, 201 (1982).