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Mineral oil-, glycerol-, and Vaseline-coated plates as matrix-assisted laser desorption/ionization sample supports for high-throughput peptide analysis

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ABSTRACT

A novel protocol for rapid and high-quality sample preparation prior to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS) has been developed by coating bare stainless steel plates with one of three adhesives: mineral oil, glycerol, or Vaseline. The advantages of these three adhesive coats are that they take little time to both prepare and wipe away, hold the matrices to prevent them from flying from the support, reduce the background matrix, and affect neither the resolution of the peptide peaks nor the accuracy of their determined molecular masses. Consequently, the signal intensity, detection limit, and tolerance of the analytes to contaminants on the three adhesive-coated plates are improved. In the two strategies of on-plate desalting and concentration of the peptide mixture, all three adhesives reduced the loss of peptides, especially in the case of larger molecular mass peptides. The microscope and stereomicroscope images of the deposited droplets showed that after dropping onto the adhesive coats, the droplets formed a reduced spot size, were more homogeneous, and showed sticky crystallization. Therefore, this is an easy-to-use, reproducible, highly sensitive, tolerant (to salts), and high-throughput method of peptide sample preparation for MALDI–TOF MS analysis.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF MS)[1] has become a mainstream method for the identification and analysis of peptides and proteins because of its rapid analysis time, high sensitivity, easy signal accumulation, tolerance to salts and other contaminants, and minimal sample preparation requirements [1,2]. It enables the direct measurement of a variety of analytes in a wide range of biological samples, from lipids to peptides and intact proteins [3]. Moreover, it is well suited for the analysis of complex biological mixtures, such as brain sections [4], single cell peptides [5], and nerve signaling peptides [6], and so the technique can be applied in cancer research, neuroscience, and pharmaceutical development [7]. Although a number of approaches have been taken to improve mass accuracy and sensitivity, the preparation of high-quality cocrystallization of analyte and matrix is still a key factor in detection. The MALDI analysis of proteins and peptides is applied routinely using organic matrices such as sinapinic acid (SA), 2,5- dihydroxybenzoic acid (DHB), and α-cyano-4-hydroxycinnamic acid (CHCA), which is widely used for peptides. However, some problems also exist; for example, analyte signals may be found only from "sweet spots," the high-background signals of the matrix cluster formation in the low-mass region that is especially true for peptides and proteins in low abundance [8,9], and the matrix may fly off the target when subjected to a laser pulse. Moreover, the contamination of samples with high concentrations of salts, buffers, or detergents reduces the sensitivity of detection and suppresses MS signals. High concentrations of sodium and potassium salts can inhibit the formation of MALDI matrix crystals or can form alkali–ion adduct, which is a major cause of low detection limits and poor mass resolving power [10]. Thus, the introduction of purification and concentration steps prior to MALDI analysis is very useful. Previous methods to address the problems mentioned above consisted of developing different matrices for different analytes [11] such as porphyrins [12], inorganic material [13], and porous silicon dioxide [14]. More recently, three articles showed that carbon nanotubes would work for low-molecular-mass analytes mass analysis. [15–17], and ionic liquid matrices have been used successfully for phospholipid analysis [18]. Bogan and coworkers reported that control of the magnitude of the net charge during the period of time droplets were leviated, could be used to promote the CHCA/peptide cocrystallization [19]. To enhance detection,

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[1] *Abbreviations used:* MALDI–TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SA, sinapinic acid; DHB, 2,5- dihydroxybenzoic acid; CHCA, α-cyano-4-hydroxycinnamic acid; TFA, trifluoroacetic acid; CT, calcitonin; AP, *Aplysia* acidic peptide; INS, insulin; S/N, signal-to-noise; DBC, desalting before cocrystallization; DAC, desalting after cocrystallization; MCP, mineral oil-coated plates; GCP, glycerol-coated plates; VCP, Vaseline-coated plates.

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peptides were added directly to a sample of nitrocellulose dissolved in acetone, allowing them to interact in a solution phase organic solvent and leading up to 10-fold enhancement [20].

The protocols used also involve the removal of salts and other contaminants, and they commonly include (i) ZipTip C_{18} reversed-phase microcolumns, (ii) on-plate washing of samples with 1% trifluoroacetic acid (TFA) [21] or distilled water [8], and (iii) Teflon-based AnchorChip plates coated with Teflon containing an array of gold spots that provide hydrophilic anchors for the target.

In this article, the advantages of novel MALDI stainless steel plates coated with one of three adhesives—mineral oil, glycerol, or Vaseline—are reported. A comparison of the MS analysis of peptides, including calcitonin (CT), *Aplysia* acidic peptide (AP), insulin (INS), and a peptide mixture, on the uncoated stainless steel plate or the three different adhesive-coated plates, revealed that the introduced methods provided an obvious enhancement to both the signal intensity and the detection limit. The reproducibility and sensitivity of these methods were tested by extending the method to directly analyze peptides in human serum that had been preseparated using only a Sephadex LH-20 microcolumn [22], and as a result more peptide signals were detected, with a high sensitivity, from the coated plates. The combined techniques of microcolumn separation and MALDI–TOF MS with the adhesive-coated plates can also be used for the direct analysis of biological fluids. The tolerance of the adhesive-coated plates to salts was also tested using a series of sodium chloride and urea concentrations. Improved tolerance was demonstrated, especially for dilute samples. On-plate washing was used to desalt the peptide samples on both coated and uncoated plates. Because peptides were preferentially lost from the uncoated plates, washing the adhesive-coated plates appeared to be a superior method for desalting. Moreover, the intensity of MALDI–TOF MS was improved sharply, and this was very useful for larger molecular mass peptides. The mechanism of the phenomenon was tested to demonstrate the characteristics both of the cocrystallization of peptide and matrix and of the adhesives used to coat the plates. This study was undertaken to devise a robust, easy-to-use, inexpensive, reproducible, highly sensitive, tolerant (to salt), and high-throughput method of peptide sample preparation for MALDI–TOF MS analysis.

Materials and methods

Materials

The matrix, CHCA, was purchased from ICN Biomedicals (USA). All solvents used in our experiments, such as TFA and acetonitrile, were HPLC grade and purchased from Tedia (USA). Milli-Q water (Millipore, USA) was used throughout. Adhesives containing sodium alginate, chitin, polyvinyl alcohol PVA-124, polyethylene glycol, glycerol, mineral oil, and Vaseline were of analytical grade and used without further purification. Chitin (2 mg) was suspended in 1 ml of 1% formic acid/Milli-Q water (v/v). Sodium alginate, polyvinyl alcohol PVA-124, and polyethylene glycol were dissolved in Milli-Q water at a concentration of 0.02% (w/w). Vaseline (40 mg) was suspended in 1 ml of *n*-hexane.

Instrumentation

All analyses were performed using a Reflex III mass spectrometer (Bruker Daltonics, Germany), operated in the delayed extraction and linear mode for positive ion detection and equipped with a nitrogen laser ($\lambda = 337$ nm). The accelerating voltage was 20 kV. Delayed extraction was optimized for signal-to-noise (S/N) for the necessary mass range, and the medium delay was employed for the collection of all data. Laser power was attenuated to just above the threshold of ionization. Spectra were accumulated in multiples of 20 laser shots, with 400 shots in total unless otherwise mentioned. Stainless steel targets were employed for sample placement (MTP 384 target plate ground steel TF, Bruker Daltonics). The images of cocrystallization of peptide and matrix, with or without adhesive coats, were characterized using an Olympus microfluorescence and stereomicroscope imaging system.

Peptides preparation

AP, which was synthesized at the Biotechnology Center of the University of Illinois at Urbana–Champaign, and INS and CT, which were purchased from Sigma–Aldrich (USA), were prepared as stock solutions in Milli-Q water at concentrations of 1000, 1, and 60 pmol/L, respectively. A peptide calibration standard (cat. no. 206195, Bruker Daltonics) was used as the external standard for molecular weight calibration and, with 100-fold dilution using Milli-Q water, could be used as the peptide mixture analyte for MALDI analysis.

Mass spectra of human serum peptides were obtained before preseparation with Sephadex LH-20 microcolumn liquid chromatography (height 7 cm, diameter 2 cm). Serum (300 μl) diluted using the same volume of 0.1% aqueous TFA solution was suspended at 4 °C for 30 min and centrifuged for 10 min at 5000*g* before it was loaded onto the gel column. Aqueous TFA solution (0.1%) was used to equilibrate the column and to elute the sample at a flow rate of 0.5 ml/min. The 0.5-ml fractional sample, monitored at 280 nm, was collected every minute, and each sample was concentrated 10-fold (to 50 μl) and stored at 4 °C before being deposited onto a MALDI–TOF MS sample plate.

Preparation of adhesive-coated plates

The preparation of different adhesive-coated plates was distinct. In the case of the aqueous solutions of adhesives, 1 μl of solution was laid onto the stainless steel plate wells, with a smooth movement from the center to the edge, to form a homogeneous coat, and was dried in ambient air. In the case of mineral oil, a pipette tip was dipped into the oil and dotted into the plate wells, with one dipping being sufficient to coat four or six plate wells. In the case of glycerol, a 2-μl aliquot of solution was added to cover each plate well and left for 20 min before the excess liquid was absorbed using a cotton applicator. Finally, in the case of Vaseline or chitin, 0.5 μl of suspension of Vaseline in hexane or chitin in formic acid/Milli-Q water was spotted into each plate well and evaporated, forming a homogeneous solid surface. This process needed to be finished within a short time. Solid Vaseline could also be patterned onto the surface of the plate wells.

A saturated matrix solution was prepared by dissolving CHCA in 40% acetonitrile/0.1% aqueous TFA. The analyte (0.7 μl) for mass analysis and an equal amount of CHCA matrix solution were mixed, and 0.7 μl of the mixture was deposited onto the stainless steel plate wells or adhesive-coated plate wells and then dried in ambient air.

Desalting of peptide samples on adhesive-coated plates

A solution of CT was prepared in Milli-Q water; in a series of 0.1-, 0.25-, 0.5-, and 1-mol/L concentrations of NaCl; or in 1 mol/L urea solution to study the tolerance of the samples on the plates with and without adhesive coats to salts or denaturants.

To remove salts and denaturants from the peptide samples, two types of sample-on-plate desalting strategies were used on the plates with and without adhesive coats. The first strategy involved desalting before cocrystallization (DBC). The peptide solution was carefully dropped into the stainless steel plate wells or the adhesive-coated plate wells and was dried in ambient air. Following

this, a 2-μl aliquot of Milli-Q water at room temperature was placed on the sample spot for 5 s and immediately aspirated with a pipette tip to take off excess salts before the procedure was repeated once more. Finally, an equal volume of CHCA solution was deposited and mixed on-plate. The second procedure involved desalting after cocrystallization (DAC). The washing protocol was used for the cocrystallization of the sample and matrix mixed on-plate. It is critical that extreme care was taken at this washing/desalting step because an extended stay of Milli-Q water will result in the excessive loss of peptides from the washing surface.

Results and discussion

Detection of better adhesives for MALDI steel plates

The principal motivation for the method development described here was to identify a better method of preparing MALDI plates that could be used to promote efficient laser desorption of the samples, influence the energy spread of the ions at the detection point, and provide improved matrices for MALDI–TOF MS. It is believed that the parameters critical to plate preparation are (i) to produce no interference peaks [11], (ii) to ensure uniform crystallization of the matrix and favor MALDI analysis, (iii) to significantly improve the detection limit and mass resolving power, and (iv) to improve tolerance to contaminants such as salts and denaturants.

To test whether the adhesives selected were suitable for the preparation of plates, mass spectra were obtained from wells where only CHCA solution had been deposited onto the stainless steel plate wells and from adhesive-coated plate wells in the positive ion mode. The chitin-, polyethylene glycol-, and sodium alginate-coated plates showed significant background interference from matrix cluster formation. Observations were made to evaluate the influence of detection sensitivity caused by adhesives when depositing a CT/CHCA mixture onto the plate. Hardly any peptide signals were visible during analysis of sodium alginate-coated plates, and they were obviously suppressed in polyethylene glycol- and polyvinyl alcohol-coated plates. Moreover, the signals had a nearly identical intensity from the chitin-coated plate and the stainless steel plate. However, the absolute intensities detected in the mineral oil-coated plates (MCP), the glycerol-coated plates (GCP), and the Vaseline-coated plates (VCP) were comparable to those achieved with bare stainless steel plates at comparable analyte amounts and intensity. Enhancement was up to as much as 4- to 12-fold, and no interference peaks were observed from the three adhesives. Furthermore, the use of these three adhesives improved sample homogeneity, improved signal reproducibility, and reduced the time required to search for the sweet spots.

Further observations were made in testing the influence of the adhesive coats on the matrix ionization during mass analysis. CHCA generally give matrix cluster peaks at the mass region below $m/z \sim 500$. But many experiment factors can affect the extent of matrix cluster formation, including the matrix used, the salt content in sample, the laser power, and (perhaps) the adhesive coats on the support. Varying laser energies were used to examine the formation of matrix cluster and the tolerance of the matrix to laser power on different supports. Laser attenuation as a function of intensity and the relative laser power can be varied from 0 to 100%. In the experiment, values (the initial value was 90% and was reduced by 5% every time) were used to ionize the matrix on bare stainless steel and the three different adhesive-coated plates. Varying the relative laser power 90 to 75%, the background interference of matrix cluster formation from the CHCA on stainless steel plates was extensive, and the intensity of cluster peaks was greatly increased (up to the maximization at 75%) and was slightly reduced from 75 to 55%. Continuously increasing intensity, the signal of matrix became more and more slight and ultimately came out of the threshold at 45%. The phenomenon is probably due to the higher laser power partly destroying the matrix crystallization on the steel, and the process was displayed by the optical system with a video monitor. The background interference was significantly eliminated on the three adhesive-coated plates. CHCA was more stable to the laser power, even though the values varied between 90 and 45%, and came out of the signal threshold at 35%. A comparison of the matrix cluster formation from the CHCA on bare stainless steel with the three coated plates with the same intensity value showed that the background interference was nearly eliminated on the latter, especially on GCP, followed by VCP and then MCP. Moreover, relative laser power was fixed at 55% in the following experiment. Obviously, all three adhesive-coated plates have great potential for application in the detection of a variety of low-molecular-weight and low-abundance species. Also, the three adhesive coats can be prepared at different points on a plate, facilitating the experimental process.

Application of MCP, GCP, and VCP for peptide mass analysis

The use of the three adhesives ensured that ion signals were obtained over an extended period of time. Fig. 1 shows a direct comparison of detection intensity with and without an adhesive coat and indicates the absolute intensity of analyte ion signals from a particular and changeless position with MALDI analysis of equal amounts of CT. Each mass spectrum was obtained by applying 20 laser shots at a particular position in the plate well. Continuous mass spectra were collected for each position until a threshold intensity of 100 was reached. This procedure was applied to five positions, and the average of the five positions provided the result. As shown in Fig. 1, the MCP, GCP, and VCP produced higher absolute intensities in the first 15 shots and all three had a much higher intensity at the first shot, indicating that the presence of MCP, GCP, or VCP increased the time available for analysis at a particular desorption spot by minimizing the time-consuming search for the sweet spots. It should be mentioned that the three methods of sample preparation affected neither the resolution of the peptide peaks nor the accuracy of their molecular masses as determined by MALDI–TOF MS.

A comparison of the three adhesives showed that the intensity of VCP was the highest at first but was reduced sharply as the number of shots increased. The intensities of MCP and GCP increased by degrees at first but then were reduced again. The signals on the GCP, in particular, lasted longer than those on the stainless steel plate. Mass spectra were obtained from 20 random positions with 20 shots for each position, and the calculated absolute intensities are shown in Fig. 2A (CT), which reveals up to fourfold enhancement of the absolute intensities.

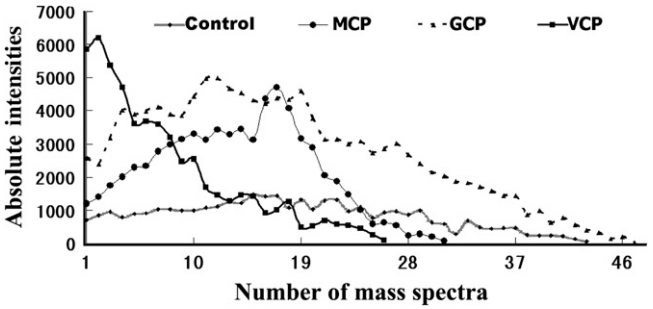

Fig. 1. Comparison of absolute intensities of continuous analyte ion signals in particular positions in the plate wells on stainless steel (control) plate, MCP, GCP, and VCP.

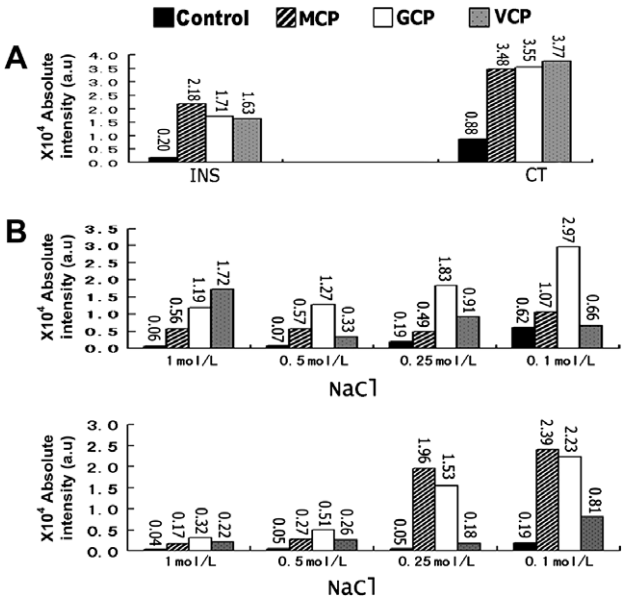

Fig. 2. Comparison of signal intensities of the analyte on stainless steel plate (control), MCP, GCP, and VCP using MALDI mass analysis. (A) INS and CT. (B) CT at 6 pmol/L (upper panel) and 150 fmol/L (lower panel) with a series of NaCl concentrations.

In the case of application of the three different supports to other classes of peptides, the same results were obtained when analyzing INS in Fig. 2A (INS) and AP in Fig. 3. The AP stock solution was diluted to a series of concentrations, but the advantages of MCP, GCP, and VCP were still quite obvious, as shown in Fig. 3. In particular, when the concentration of analyte was lower than 2 pmol/μl, hardly any analyte ion signal was detected on the stainless steel plate, but good peptide peaks with usable S/N were obtained on all three MALDI sample supports, with improvement in the detection limit and sensitivity of the peptide. The three plates—MCP, GCP, and VCP—provided as much as 12-, 10-, and 9-fold enhancement, respectively, in the detection of the peptide. Thus, the three methods have universal application in peptide MALDI mass analysis.

The stock solution of peptide mixture containing seven substances, and used as the peptide calibration standard, was diluted 100-fold and deposited onto the stainless steel plate or onto the three different sample supports for mass analysis. Absolute intensities of seven ion peaks are shown in Table 1 (“A” columns for control, MCP, GCP, and VCP), and these were reduced as the molecular weight increased, meaning that in the mixture the detection of substances with larger molecular masses was more difficult but

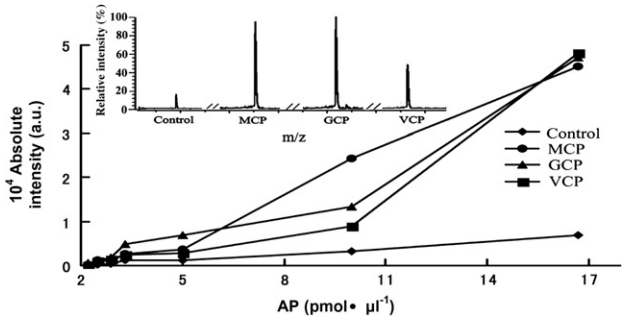

Fig. 3. Comparison of signal intensities of AP on stainless steel plate (control), MCP, GCP, and VCP using MALDI MS analysis. The main figure shows AP at a series of concentrations. The inset shows mass spectra of AP on a stainless steel plate, MCP, GCP, and VCP at 2.5 pmol/μl.

Table 1
Absolute intensities of a peptide mixture analyzed using MALDI–TOF MS on plates with or without adhesive coats

Peptides	Control		MCP		GCP		VCP	
	A	B	A	B	A	B	A	B
1	2683	2118	5687	5157	5751	2207	6186	2940
2	2346	600	7398	6830	6367	1832	7058	2968
3	2314	150	6468	1644	5994	590	6347	949
4	718	36	3016	2016	2414	148	2478	157
5	165	0	2898	219	1554	76	910	52
6	568	63	5547	105	2529	227	2883	357
7	57	0	1334	96	626	30	138	48

Note. Values in table are absolute intensities. Ion peaks 1 to 7 of the mixture are as follows: 1047.20, 1297.51, 1348.66, 1620.88, 2094.46, 2466.73, and 3149.61. Column A: diluted 100-fold peptide mixture analyzed on the plates with or without adhesive coats. Column B: on-plate desalting applied to the peptide mixture contaminated by 2 mol/L urea with or without adhesive coats.

more important. However, the problems were well solved with MCP, GCP, and VCP. For example, the absolute intensities of the peptide ion peaks at 2094.46 showed approximately 18-, 9-, and 6-fold improvement, respectively, with the three supports. Moreover, the production of peptide ion peaks with molecular masses greater than 2094.46 on the three supports is also comparable to that on the stainless steel plate, and the problem of intensities being cut down as the molecular weight increasing was well overcome.

Application of MCP, GCP, and VCP for serum peptide mass analysis

The three supports were used to analyze more complicated peptides from human serum preseparated with Sephadex LH-20 microcolumn liquid chromatography, and the spectrum is shown in the inset of Fig. 4A. The peptide mass spectra were obtained every 3 min, and Fig. 4 shows the results of peptides collected during 24 to 25 min and concentrated 20-fold before use. The signal intensities, as well as the detection sensitivity, of the peptides obviously increased on the three different supports (Fig. 4B–D). In each one, four ion peaks with high S/N can be identified unambiguously from the background. Other samples yielded abundant information but are not shown here.

Effect of salts on peptide detection on stainless-steel- and adhesive-treated plates

The presence of high levels of various salts hinders and/or complicates mass analysis and can inhibit the formation of cocrystallization, form adducts with analyte [23], and spread the signal of a single compound into multiple peaks in the mass spectrum, and this may reduce the qualitative sensitivity of analysis [3]. The effect of the salts on peptide detection was studied using a series of NaCl concentrations (0.1, 0.25, 0.5, 1 mol/L), as shown in Fig. 2(B). As the concentrations of NaCl ranged from 0.1 to 1 mol/L, absolute intensities of the CT on all supports were suppressed, but the cases went from bad to worse when analysis took place on the stainless steel plate. Despite concentrating the NaCl up to 1 mol/L, the signal intensities on the three different supports were still comparable to the average (control). The effect of the increasing concentrations of salts on the MCP, GCP, and VCP was inconspicuous, improving the tolerance of the matrix to the salts during MALDI analysis, but became more sensitive when the analyte was at low concentration (Fig. 2B, lower panel), causing the signal intensities to be sharply reduced (0.5 and 1 mol/L of NaCl). It is worth mentioning that the salt tolerance on MCP, GCP, and VCP was distinctive and that, of the three, the improvement seemed to be most significant on GCP.

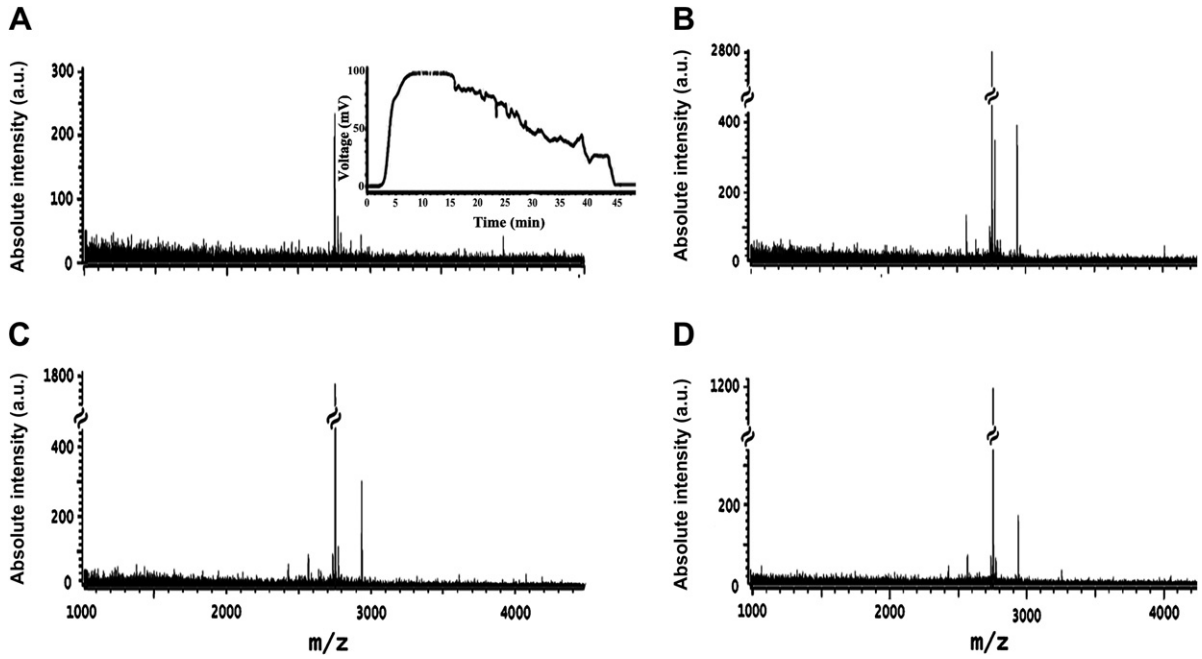

Fig. 4. Spectrum for serum preseparated with Sephadex LH-20 microcolumn liquid chromatography (inset of panel A) and peptides collected during 24 to 25 min of MALDI mass analysis on a stainless steel plate (A), MCP (B), GCP (C), and VCP (D).

Comparison between two methods of on-plate desalting

Because high salt content suppresses the peptide signals, the removal of salts from the peptides without peptide loss is a desirable goal. As stated above, the three adhesives not only improved the tolerance of the CHCA to salts but also reduced the influence of urea (see Fig. 5A). A comparison of the two protocols of on-plate desalting, DBC and DAC, showed that both methods desalted the peptide effectively and yielded a high S/N resulting from the masking of the peptide signals by alkali–ion adducts. In addition, the samples were immobilized on the three adhesive coats and so prevented the loss of peptides during the washing process. A comparison of the two methods showed that DBC may result in a greater loss of peptides as deduced from their reduced intensity and strength relative to the DAC method, but it avoided the effect of the washing solution on the matrix, which became crisper after more than four desaltings took place, and this could be useful in the case of analytes with contaminants that need more washing.

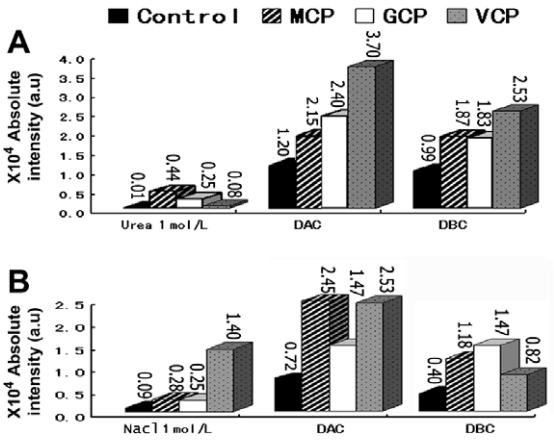

Fig. 5. Comparison of on-plate desalting on a stainless steel plate (control), MCP, GCP, and VCP. The analytes were contaminated by 1 mol/L of urea (A) or NaCl (B) and had on-plate desalting before cocrystallization (DBC) or after cocrystallization (DAC).

In investigating the on-plate desalting of a peptide mixture contaminated by 2 mol/L of urea, the ion peaks of seven substances in the mixture were suppressed and hidden in the background noise before desalting, not only on the stainless steel plate but also on MCP, GCP, and VCP. Using the DAC washing protocol, the products are shown in Table 1 ("B" columns). The mass spectra of peptides desalted and washed on stainless steel plates were consistently inferior due to the loss of peptides, especially when peptides of larger molecular mass were apparently lost. In the spectra of the peptide mixture on uncoated stainless steel plates, only five of the detected ion peaks were at an m/z identical to the peptide mixture, but ion peaks at m/z 2094.46 and 3149.61 had no signals. Furthermore, a mass cluster of peaks existed in the spectra, which always appeared in assemblies of several members, with one or two members in the center having the highest intensity. The existence of cluster peaks seriously interfered with the detection of peptides and hid the interesting peaks in a mass of background noise. The loss was partly made up by an enhancement to the detection limit, and more comprehensive information regarding the peptides was obtained on MCP, GCP, and VCP. A systematic comparison of the three methods of sample preparation, together with on-plate desalting, shows that MCP, GCP, and VCP compared favorably as methods of sample preparation prior to MALDI and were clearly superior to uncoated stainless steel plates.

Microscopy of the deposited droplets

To understand the reasons for the enhancement in signal intensity, increase in detection limit, tolerance to salts and denaturants, and stability during the desalting procedure, stereomicroscopy and microscopy were used to produce images of deposited droplets and the crystallization morphology on the bare stainless steel plates or MCP, GCP, and VCP. The crystal characteristics of CHCA on the three different coats were heterogeneous in comparison with CHCA on VCP in Fig. 6B (image g), on MCP in Fig. 7 (image b), on GCP in Fig. 7 (image g), and on bare stainless steel in Fig. 6B (image

b). The mineral oil and Vaseline coats on the stainless steel surface acted as hydrophobic sample supports during both the desalting and concentrating steps. When deposited onto the surfaces, the droplets were focused in the center of the probe well with a reduced spot size rather than diffused in an uncontrolled spot size. Small sample spots on the hydrophobic target are beneficial to MALDI analysis [24], and sample and matrix spots of a reduced spot size on the hydrophobic polymer surface result in dramatically enhanced sensitivity [25]. The properties of concentration and focus were obviously significant with the VCP. In Fig. 6, the crystal images of CHCA, the CHCA and analyte mixture, the mixture with 1 mol/L of NaCl, and the mixture after DBC and DAC washing protocols for the removal of NaCl were obtained by stereomicroscopy and microscopy on stainless steel plates (Fig. 6A, images a–e, and Fig. 6B, images a–e) and on VCP (Fig. 6A images f–j, and Fig. 6B, images f–j). A comparison of the two rows of images showed that the crystals on the VCP were more compact and the diameters were much smaller; this would have resulted in concentration of the analyte and, thus, played an especially important role in overcoming the loss of analyte during the washing process. Although the process was also coupled with concentration of the contaminants, as shown in Fig. 6A (image h) and Fig. 6B (image h), this problem was solved by enhancement in both the signal intensity and tolerance to salts that have been well investigated in our work. Photographs of the dried spots on VCP in Fig. 6B (images f–j) show that the coat produced more homogeneous spots, suggesting that the analyte was best incorporated into those matrix crystals that are marked with arrows to indicate the existence of the Vaseline. When using the two protocols of on-plate desalting, Vaseline also acts as an adhesive to prevent the analyte loss by means of sticking the crystallization to the plate tightly. The same order of microscope images for MCP and GCP is followed in Fig. 7 (images a–e) and Fig. 7 (images f–j), respectively, and the sizes of particles are seen to be larger, more regular, and more concentrated. In Fig. 7, MCP and GCP also produced more highly homogeneous spots, reducing the time consumed looking for the sweet spots. Mineral oil, glycerol, and Vaseline made the particles more "sticky" and also ensured that the matrix could withstand stronger

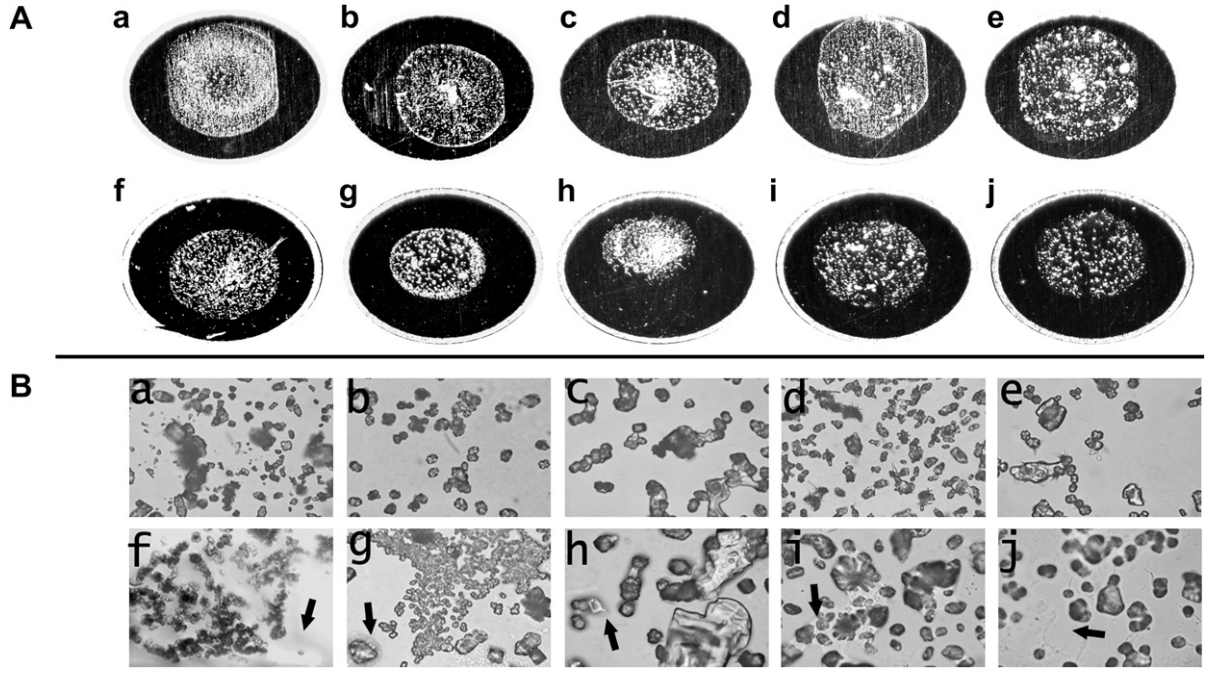

Fig. 6. Stereomicroscope and microscope images of the droplets deposited onto a stainless steel plate (A, images a–e, and B, images a–e) and on VCP (A, images f–j, and B, images f–j). The analyte order of each row is as follows: CHCA, CHCA and analyte mixture, mixture with 1 mol/L of NaCl, and mixture with DBC and DAC washing protocols to remove NaCl. The arrows in images f to j mark the presence of Vaseline.

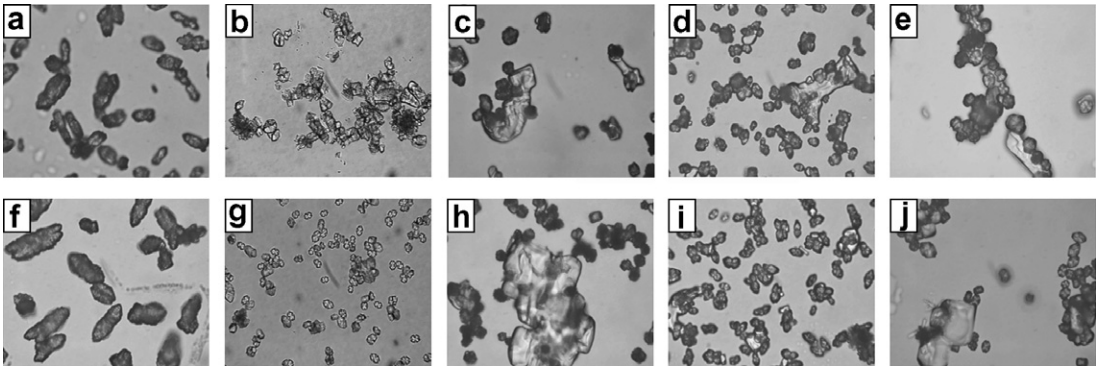

Fig. 7. Microscope images of the droplets deposited onto MCP (a–e) and GCP (f–j). The analyte order of both rows is as follows: CHCA, CHCA and analyte mixture, mixture with 1 mol/L of NaCl, and mixture with the DBC and DAC washing protocols to remove NaCl.

laser intensity and that the ion signals could be obtained for extended periods of time. Adhesives have been used to immobilize inorganic materials such as graphite particles and porous silicon [26,27]. Han and Sunner introduced glycerol to make the carbon particles more sticky [27]. Ren and coworkers introduced NIPPOLAN-DC-205 to immobilize carbon nanotubes on the target [11]. As a consequence, these coats may play a role in eliminating the potential influence of the matrix, which may fly off under stronger laser intensity. Furthermore, the three adhesives formed a thin layer between the steel plate and the peptide/CHCA cocrystallization, acting as a coat on the plate. In such a situation, there was less opportunity for the matrix and analytes to be absorbed onto the plate.

Conclusion

A simple method has been introduced to prepare MALDI–TOF MS plates for the analysis of peptides using three adhesives—mineral oil, glycerol, and Vaseline—that are efficient in sticking the CHCA matrix to the target. The distinct advantages of the procedure are as follows. First, it takes only a few minutes for three adhesive coats to be preprepared on a single plate, they can be stored at room temperature for use, and they can be easily washed. Second, it improves the signal intensity, the detection limit of peptides, and the tolerance to contaminants (with a reduced spot size of the peptide samples), and it binds the matrix and affects the formation of crystals. Third, it reduces the loss of peptides during the procedure of on-plate desalting, which is especially beneficial in the case of larger molecular mass peptides. Fourth, it is robust, simple to use, and suitable for any MALDI plates. In conclusion, the method offers an excellent sample preparation prior to MALDI–TOF MS.

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