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Selective Enrichment of Phosphopeptides and Phospholipids from Biological Matrixes on TiO_2 Nanowire Arrays for Direct Molecular Characterization by Internal Extractive Electrospray Ionization Mass Spectrometry

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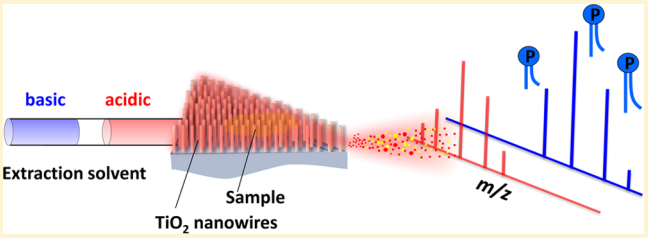
Supporting Information

ABSTRACT: Rapid analysis of phosphopeptides and phospholipids in biological matrixes is of significant interest in multiple disciplines of life sciences. Herein, trace phospholipids in human plasma, whole blood, and undiluted human urine as well as phosphopeptides in protein digest were selectively captured on a homemade array of TiO_2 nanowires for sensitive characterization by internal extractive electrospray ionization mass spectrometry (TiO_2-iEESI-MS). Sequential release of captured chemicals from TiO_2 array was achieved by tuning pH of the extraction solvent. A single sample analysis, including sample loading, chemical extraction and MS detection, was accomplished within 3 min. As far as the quantification of phospholipids, acceptable linearity ($R^2 \geq 0.9985$) and relative standard deviations (RSDs $\leq$ 8.9%) were obtained within the range of 0.1−500 μg L^{-1} for LysoPC(14:0) and LysoPC(16:0) in raw urine samples. Limit of detection (LOD) $\leq$ 0.025 μg L^{-1} and recovery rates of 94.8−101.6% were obtained for these phospholipids. As far as the quantification of phosphopeptides, $R^2 \geq 0.9994$ and RSDs $\leq$ 9.2% within the range of 0.3−200 μg L^{-1} were obtained for two phosphopeptides in nonphosphopeptides mixtures. LODs $\leq$ 0.09 μg L^{-1} and recovery rates of 83.4−107.0% were obtained for these phosphopeptides. On the basis of the orthogonal partial least-squares discriminant analysis, TiO_2-iEESI-MS patterns from the blood of 46 patients with ovarian cancer were confidently discriminated from the MS patterns of 46 healthy volunteers. Our results indicate the strong potential of TiO_2-iEESI-MS approach for the selective detection of trace phosphopeptides and phospholipids in various biological matrixes with high sensitivity, high specificity, low sample consumption, and high throughput.



The study of phosphopeptides and phospholipids in biological matrixes is of significant interest to reveal the essence of life activities.[1−5] Protein phosphorylation plays an important role in cellular growth, division, and intercellular signal transduction. Phospholipids are essential molecules responsible for membrane structure, energy fueling, and cellular signaling.[2,3,6] The deregulations of both protein phosphorylation and phospholipids metabolism are associated with a variety of pathophysiological processes (e.g., diabetes and cancer).[7,8] Due to the substoichiometric abundance of phosphorylated peptides compared with nonphosphorylated peptides and the complexity of biological matrixes, rapid detection of trace phosphopeptides or phospholipids is commonly challenging.[9,10]

Mass spectrometry (MS) is increasingly employed for the analysis of biological matrixes due to the high selectivity and sensitivity of chemical detection.[11−14] Conventionally, MS analysis of biological matrixes involves multiple sample pretreatment steps (e.g., centrifugation, filtering, chemical extraction, desalting, dilution, fractionation, etc.) as well as chromatographic separation (e.g., gas or liquid chromatography), which takes several hours for the analysis of one sample.[10,15−17] The analysis of biological samples has become considerably simpler and faster with the development of ambient mass spectrometry (AMS).[18,19] Currently, various ambient ionization technologies have been introduced for the analysis of complex gaseous, liquid, and solid samples.[18−20] These methods have found broad applications in food safety analysis,[21−23] forensics,[19,24,25] biological science,[13,26,27] etc.

The detection sensitivity and chemical selectivity of direct MS analysis has been significantly improved with the adoption

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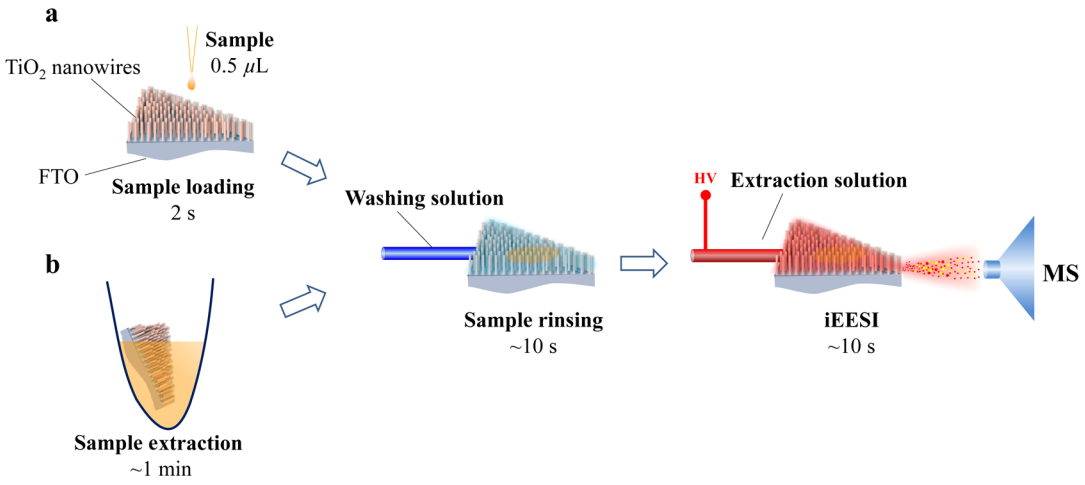

Figure 1. Schematic illustration of TiO_2-iEESI-MS analysis. (a) Direct sample loading and (b) extractive sampling.

of functional materials.[28,29] Various functional materials have been used for the selective chemical extraction of biosamples prior to MS detection.[30−34] Also, use of molecularly imprinted membranes,[35] surface modified wooden tips,[36] and octadecyl silica coated blades[37,38] allowed MS detection of target compounds at sub-ppb level in complex samples.[28,29,37] Recently, titanium dioxide (TiO_2) particles were demonstrated for the enrichment of analytes with phosphoryl group based on the Lewis acid−base interactions between the phosphoryl group and Ti ions.[39−41] Thus, the development of ambient ionization method combined with the TiO_2 material should be a potential solution for the rapid determination of phosphopeptides or phospholipids in biological matrixes.

In this study, a novel method was developed for the rapid and sensitive determination of phosphopeptides and phospholipids in biological matrixes based on the selective preconcentration of phosphopeptides and phospholipids by the arrays of TiO_2 nanowires in combined with internal extractive electrospray ionization mass spectrometry (TiO_2-iEESI-MS). The analytical performance and potential applications of the method were characterized for human plasma, whole blood, undiluted human urine, and protein digest.

EXPERIMENTAL SECTION

Materials and Chemicals. This study involved experiments on biofluids, including human whole blood, plasma, urine, and casein digest. Note that the experiments on biofluids of human whole blood, plasma, and urine samples adhered to the tenets of Helsinki Declaration[42] and were approved by the Ethics Committee of the Jilin University and the First Hospital of Jilin University. For details about the materials and chemicals, refer to the Supporting Information.

Preparation of TiO_2 Nanowire Arrays. In accordance with earlier literature reports,[43,44] TiO_2 nanowire arrays were synthesized on FTO coated glass with the shape of an isosceles triangle with length of 10 mm and base width of 6 mm. For details about the preparation and characterization of TiO_2 nanowire array, refer to the Supporting Information.

Sampling Method. For small-volume samples (e.g., less than 1 μL) such as human plasma and whole blood, a direct loading method was used for sampling (shown in Figure 1 workflow a). Briefly, a 0.5 μL aliquot of plasma sample containing 1% TFA was added on the TiO_2 nanowire arrays, and the deposited plasma sample was washed using 0.5 mL of 50% acetonitrile/water (v/v) solution containing 1% TFA (v/v) for matrix cleanup. Ammonia methanol (1.5%, w/w) biased with a high voltage (+4.0 kV) was used as the extraction solution for iEESI-MS analysis. For the analysis of whole blood sample, first, a 0.5 μL aliquot of whole blood sample was added on the TiO_2 nanowire arrays. Second, extraction solution of 1% TFA methanol (v/v) was applied for iEESI over ca. 5 min. Third, the extraction solution was changed to 1.5% ammonia methanol (w/w). The extraction solution was pumped through the TiO_2 nanowire arrays at a flow rate of 8 μL min^{-1} using a syringe pump (Harvard Apparatus, Holliston, MA, United States).

Alternatively, an extraction sampling method was used for the analysis of large volume samples (e.g., 2 mL) such as human urine and protein digest (shown in Figure 1 workflow b). For the quantification of urinary phospholipids, the TiO_2 nanowire arrays were dipped into a vial containing 2 mL urine sample and vortexed for 1 min. Then, the TiO_2 nanowire arrays were washed using 0.5 mL of 50% acetonitrile/water (v/v) solution containing 1% TFA (v/v) to remove matrix components that were physically attached to the nanowire surface. After this quick washing step (~5 s), TiO_2-iEESI-MS was performed using extraction solution of 1.5% ammonia methanol (w/w). For the analysis of β-casein digest, the experimental procedure was the same as the analysis of the urine samples.

Mass Spectrometry. The experiments were carried out using an Orbitrap Fusion Tribrid mass spectrometer (Thermo Scientific, San Jose, CA, United States). Details about instrumental parameters refer to the Supporting Information. For the comparison purpose, paper spray and nano-ESI experiments were performed according to literature.[45,46]

Chemical Identification and Data Analysis. Chemical assignment of the peaks on the mass spectra was done based on high resolution mass data, collision induced dissociation (CID) experiments, authentic compounds, and earlier literature reports[2,8,26,47] as well as by consulting databases of Human Metabolome Database (HMDB, www.hmdb.ca) and LIPID MAPS (www.lipidmaps.org). Briefly, high resolution MS1 spectra were collected for all the samples, and specific ions of interest were isolated for CID experiments. Then, the m/z value and its corresponding characteristic fragment ions were obtained. These data were employed to search on the HMDB and LIPID MAPS for chemical assignments. The

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comparison of earlier literature reports[22,40−42] was used for reference to further validate the identifications. Similar to previous studies,[48,49] the mass spectral data were exported for OPLS-DA analysis. Briefly, the mass spectral data were exported into Microsoft Excel and arranged using the m/z values as independent variables, in which the m/z value and its signal intensity exported from each sample case were matched, respectively. The exported data in the Excel were aligned based on the m/z value of each sample case using Matlab (version 7.8.0, Mathworks, Inc., Natick, MA) prior to OPLS-DA analysis by SIMCA (version 13.0, Umetrics, Sweden). Furthermore, Q^2 parameter and 200 permutation tests were performed to verify the robustness, predictive power, and validity of the OPLS-DA model.

RESULTS AND DISCUSSION

TiO_2-iEESI. Schematic illustration of TiO_2-iEESI-MS is shown in Figure 1. The synthesized TiO_2 nanowire arrays were characterized using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD). The top and cross-sectional SEM views clearly show a highly uniform and densely packed array of nanowires with the length of ca. 3 μm (Figure S1a and S1b). Both EDX and XRD data indicate that the synthesized TiO_2 nanowires belong to the class of tetragonal rutile (JCPDS file 75-1748) (Figures S1c and S1d).

In TiO_2-iEESI, phosphopeptides and phospholipids are adsorbed on a TiO_2 nanowire array under acidic conditions (e.g., containing 1% TFA, pH ≈ 1−2) and readily released under alkaline conditions (e.g., 1.5% ammonia/methanol, w/w, pH ≈ 9) during the online extraction/ionization of iEESI. Retention of phosphopeptides and phospholipids on the TiO_2 nanowire arrays is based on the Lewis acid−base interactions: the phosphate moiety of the phospholipids, being a strong Lewis base, interacts with the empty d-orbitals of the transition metal, which acts as a Lewis acid under acidic conditions.[39−41] Accordingly, binding of the phosphopeptides or phospholipids with TiO_2 nanowire should occur at low pH, and disruption of these interactions should be achieved by a pH shift toward basic conditions under which the transition metal oxides no longer exhibit Lewis acid characteristics but behave as Lewis bases.

To test-proof the performance of TiO_2-iEESI-MS, equal amounts of the same plasma sample (0.5 μL) were independently analyzed by paper spray-MS (PS-MS) and TiO_2-iEESI-MS approaches (Figure 2) under identical MS settings. The PS-MS mass spectrum displayed strong background signal at m/z 163.1 from diethylene glycol monobutyl ether (DGBE), which is a common MS contaminant,[50] and relatively weaker signals of phospholipids (Figure 2a). In the case of TiO_2-iEESI-MS, the signal intensity of phospholipids was remarkably increased (Figure 2b). For example, the signal intensities of the dominant ions, including PC(34:2) (m/z 758.6), PC(36:2) (m/z 786.6), and PC(36:4) (m/z 782.6), were increased by 12−15 fold relative to PS-MS. These results suggest that the TiO_2-iEESI-MS is a potentially powerful approach for the sensitive analysis of phospholipids in plasma samples.

Analysis of Phosphopeptides in Tryptic Digests. The detection of trace phosphopeptides in β-casein digest using TiO_2-iEESI-MS was also demonstrated. The mass spectra for direct analysis of tryptic digest of β-casein (4×10^{-7} M) by nanoESI-MS and TiO_2-iEESI-MS are shown in Figure 3.

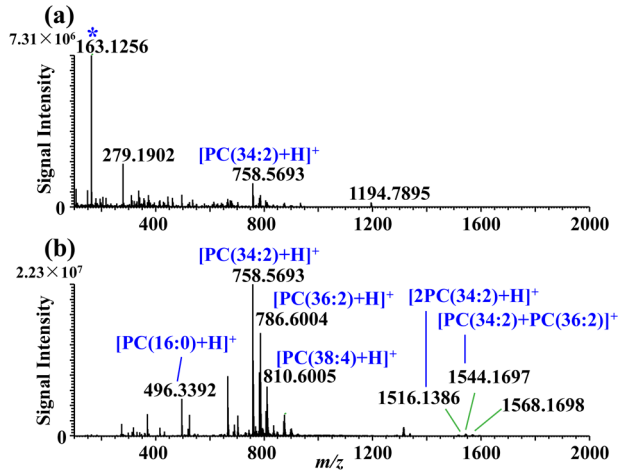

Figure 2. Mass spectra of human blood plasma sample: (a) PS-MS and (b) TiO_2-iEESI-MS. An asterisk (*) indicates noise.

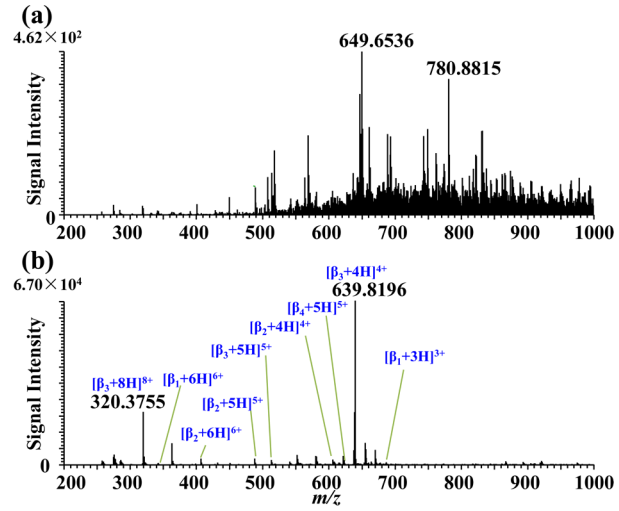

Figure 3. Mass spectra of tryptic digest of β-casein (4×10^{-7} M): (a) nanoESI-MS and (b) TiO_2-iEESI-MS.

Without the TiO_2 nanowire arrays extraction, the non-phosphorylated peptides and sample matrix signals were dominant in the mass spectrum obtained by nanoESI-MS (Figure 3a). In contrast, in TiO_2-iEESI-MS, four phosphopeptides (detailed information in Table S1) with the signal-to-noise ratio (S/N) enhanced more than 2 orders of magnitude were observed (Figure 3b). The same list of phosphopeptides was reported in earlier studies.[46,51] As a result, TiO_2-iEESI-MS is a potentially useful approach for the rapid determination of phosphopeptides in tryptic digests.

Analysis of Plasma from Ovarian Cancer Patients. Here, TiO_2-iEESI-MS was applied for the rapid scanning of phospholipids in the plasma samples donated by ovarian cancer patients and healthy volunteers. Full scan mass fingerprints were recorded from a set of samples from 32 patients with ovarian cancer and 32 healthy individuals. Typical mass spectral fingerprints are shown in Figure 4.

The OPLS-DA score plot showed that two group specimens were completely separated from each other (Figure 4c). To further validate this model, random permutation tests with the PLS-DA model were performed. Validation with 200 permutation tests generated intercepts R^2 = 0.59 and Q^2 =

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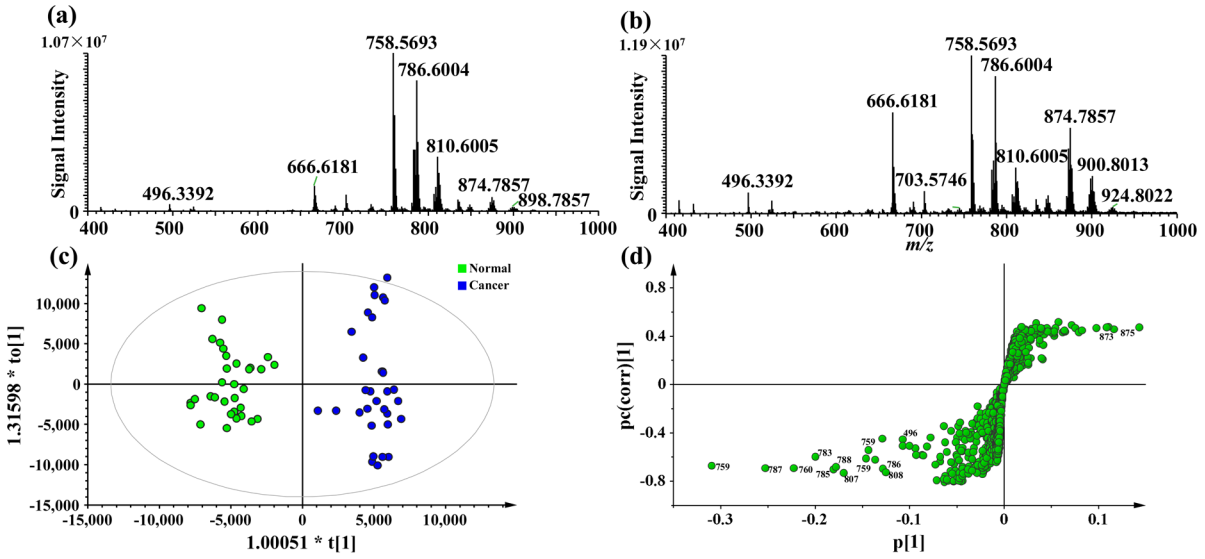

Figure 4. Differentiation of ovarian cancer in human by TiO_2-iEESI-MS analysis of plasma. (a) Mass spectra obtained from healthy volunteers, (b) mass spectra obtained from patients with ovarian cancer, (c) OPLS-DA score plot of MS data collected from ovarian cancer plasma samples (blue) and normal plasma samples (green), and (d) the S-plot loading plot of the MS data.

−0.29, which suggested that the model was not overfitted (Figure S2). Furthermore, S-plot revealed that the peaks at *m/z* 782.6 (PC(36:4)), 784.6 (PC(34:3)), 806.6 (PC(38:6)), 760.6 (PC(34:1)), 810.6 (PC(38:4)), 808.6 (PC(38:5)), 788.6 (PC(36:1)), 496.3 (LysoPC(16:0)), etc. notably contributed to the differentiation of two kinds of plasma samples (Figure 4d), which suggests that the corresponding phospholipids with higher VIP value might act as potential diagnostic biomarkers of ovarian cancer. Of course, the study on a larger group of samples is needed to validate the method for the clinical diagnosis of ovarian cancer. This work is currently underway in our laboratory.

Analysis of Human Whole Blood. The mass spectra obtained from a 0.5 μL aliquot of whole blood sample are shown in Figure 5. With the acidic solution of 1% TFA methanol (v/v) initially employed for the extraction of the whole blood sample, small molecules such as alkaloids (choline (*m/z* 104, $[M]^+$)), amino acids (e.g., arginine (*m/z* 175.1, $[M + H]^+$), lysine (*m/z* 147.1, $[M + H]^+$), valine (*m/z* 118.1, $[M + H]^+$), and leucine (*m/z* 132.1, $[M + H]^+$)), and sugars (glucose (*m/z* 203.1, $[M + Na]^+$) and sucrose (*m/z* 381.1, $[M + K]^+$)) were detected (Figure 5a). Additionally, blood lipids including diglycerides (DGs) (e.g., DG(34:0) (*m/z* 635.5, $[M + K]^+$) and DG(36:0) (*m/z* 647.6, $[M + Na]^+$)) as well as abundant signals of triglycerides (TGs) such as TG(52:3) (*m/z* 879.7, $[M + Na]^+$), TG(52:5) (*m/z* 891.7, $[M + K]^+$), TG(52:4) (*m/z* 893.7, $[M + K]^+$), TG(52:3) (*m/z* 895.7, $[M + K]^+$), TG(52:2) (*m/z* 897.7, $[M + K]^+$), TG(54:5) (*m/z* 903.7, $[M + Na]^+$), TG(54:4) (*m/z* 905.7, $[M + Na]^+$), TG(54:3) (*m/z* 907.7, $[M + Na]^+$), TG(54:6) (*m/z* 917.7, $[M + K]^+$), TG(54:5) (*m/z* 919.7, $[M + K]^+$), TG(54:4) (*m/z* 921.7, $[M + K]^+$), TG(56:8) (*m/z* 941.7, $[M + K]^+$), TG(56:7) (*m/z* 943.7, $[M + K]^+$), etc., were detected (detailed in Supplementary Table S2). Also, α and β subunits of hemoglobin were observed in multiply charged ion states, along with the abundant signal of heme (*m/z* 616.2) in the low mass region. The observation of abundant signals for triglycerides and hemoglobin from the blood sample indicates that the proposed method could be a potential tool for the diagnosis of diseases such as hyperlipidemia and hemoglobinopathy.[52,53]

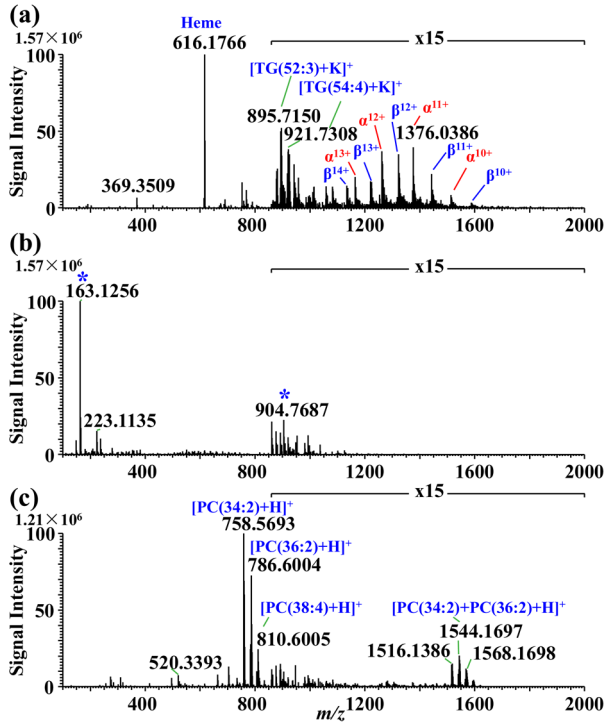

Figure 5. Analysis of human whole blood using TiO_2-iEESI-MS. (a) Mass spectra obtained initially using the extraction solution of TFA/methanol solution (1% v/v), (b) mass spectra obtained after 5 min of online extraction with TFA/methanol solution (1% v/v), (c) mass spectra obtained upon follow-up extraction with ammonia/methanol solution (1.5% w/w). An asterisk (*) indicates noise.

Upon continuous TiO_2-iEESI-MS analysis over 5 min, the intensities of signals such as heme and triglycerides decreased by ca. 90% relative to the original level, suggesting that those compounds were almost completely extracted within 5 min of extraction with 1% TFA/methanol solution (Figure 5b). Interestingly, abundant signals were detected in the mass

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Table 1. Quantitation of Phospholipids and Phosphopeptides in Biological Matrixes by TiO_2-iEESI-MS/MS

compound	precursor ion (m/z)	product ions for quantification (m/z)	slope	intercept	R^2	linear ranges ($\mu g\ L^{-1}$)	LODs ($\mu g\ L^{-1}$) (S/N = 3, n = 6)	RSDs (%, n = 6)[a]
LysoPC (14:0)	468.3	184.1	736.27	125.19	0.9989	0.1−500	0.025	5.4−8.9
LysoPC (16:0)	496.3	184.1	667.33	115.64	0.9985	0.1−500	0.021	4.8−7.8
phosphopeptide I[b]	600.2	502.2	36.09	2.73	0.9994	0.3−200	0.09	4.6−9.1
phosphopeptide II[c]	1195.6	931.5	55.88	14.57	0.9997	0.3−200	0.07	6.0−9.2

[a]The RSDs are derived from the analysis of samples with different known concentrations of analytes used for the establishment of calibration curve. [b]Phosphopeptide I (sequence: NPE[pY]). [c]Phosphopeptide II (sequence: KEAPPAPPQ[pS]P-NH_2).

range of m/z 600−900 when the extraction solution was changed to 1.5% ammonia/methanol (w/w) (Figure 5c). The detected signals belong to protonated phospholipids ($[M + H]^+$) such as LysoPC(16:0) (m/z 496.3), PC(34:2) (m/z 758.6), PC(34:1) (m/z 760.6), PC(36:4) (m/z 782.6), PC(34:3) (m/z 784.6), PC(36:2) (m/z 786.6), PC(36:1) (m/z 788.6), PC(38:4) (m/z 810.6), etc., while other compounds such as sugars, triglycerides, or proteins were not detected under this conditions. Signal intensity variation of typical triglycerides (TG(52:3) (m/z 895.7) and TG(54:5) (m/z 919.7)) and phospholipids (PC(34:2) (m/z 758.6) and PC(36:2) (m/z 786.6)) clearly shows chromatographic separation of triglycerides and phospholipids during the TiO_2-iEESI-MS process upon changing the extraction solution (Figure S3), indicating that phospholipids were retained on the TiO_2 nanowire arrays while triglycerides and sugars were extracted with an acidic solvent (i.e., 1% TFA methanol, pH ≈ 1). The retained phospholipids were successfully extracted and ionized using basic extraction solution (i.e., 1.5% ammonia methanol, pH ≈ 9). The capture of phospholipids by TiO_2 nanowire arrays could be explained by the Lewis acid−base interaction between phosphate groups and Ti ions.[9] In earlier studies, metal oxides like TiO_2, ZrO_2, and Fe_2O_3 have been applied to separate phosphorylated proteins and peptides from the complex samples.[54] TiO_2-based metal oxide affinity chromatography (TiO_2-MOAC) is probably the most widely used method for phosphopeptides separation.[9,55] However, only few studies reported TiO_2-based metal oxide for the analysis of phospholipids.[56,57] Our results indicate that TiO_2-iEESI-MS could be a simple, rapid, and low sample consumption method for the detection of various types of compounds in blood sample.

Analytical performance of TiO_2-iEESI-MS for the analysis of whole blood sample was compared with PS-MS under identical experimental conditions (Figure S4). The signal intensities and signal-to-noise ratios of hemoglobin, triglycerides, phospholipids, and the subunits of hemoglobin were weaker in PS-MS than in TiO_2-iEESI-MS (Figures S4a and S4b). After ca. 5 min of online extraction, similar mass spectra were detected (Figures S4c and S4d). Upon the follow-up extraction by 1.5% ammonia/methanol solution, high abundance of phospholipids signals emerged in TiO_2-iEESI-MS (Figure S4e), whereas no phospholipids signals were detected in PS-MS under identical conditions (Figure S4f). The signal intensities of typical phospholipids of PC(34:2) (m/z 758.6) and PC(36:2) (m/z 786.6)) increased more than 1 order of magnitude compared with PS-MS over a change of the extraction solution, and the signal-to-noise ratios (S/N) of phospholipids were greatly enhanced (Figure S5). We explain the poor signal intensities of phospholipids in PS-MS by the fact that paper substrate has very low binding affinity to phospholipids from the blood sample. The results indicate that the proposed method is of high sensitivity and selectivity for the detection of phospholipids in whole blood sample.

Furthermore, phospholipids in the whole blood samples donated by ovarian cancer patients and healthy volunteers were also tested using TiO_2-iEESI-MS. Full scan mass fingerprints were recorded from a set of samples from 14 ovarian cancer patients and 14 healthy individuals (Figure S6). The OPLS-DA score plot of MS data displayed clear differentiation between the ovarian cancer samples and the healthy samples (Figure s7a). Also, the permutations tests result indicated that the separation among the two groups was statistically significant (Figure S7b). These results suggest that, besides the blood plasma samples, even highly complex whole blood samples can be tolerated and directly analyzed by TiO_2-iEESI-MS.

Quantification of Phospholipids and Phosphopeptides. Urinary phospholipids are an important group of phospholipids associated with various clinical and physiological conditions.[2] While the majority of previous studies have focused on phospholipids in cellular membranes, tissues, and blood samples, undoubtedly, urine samples are convenient for noninvasive diagnosis.[2,7] Here, we used TiO_2-iEESI-MS for the direct quantification of target phospholipids in undiluted human urine. Spiked LysoPC(14:0) and LysoPC(16:0) (5 $\mu g\ L^{-1}$) in 2 mL of undiluted urine samples were successfully detected as protonated ions at m/z 468.3 and m/z 496.3, respectively. In CID experiments, the precursor ion at m/z 468.3 yielded characteristic fragment ions at m/z 184.1 and m/z 450.3, while the precursor ion at m/z 496.3 yielded fragment ions at m/z 184.1 and m/z 478.3 (Figure S8). The fragment ion at m/z 184.1 belongs to protonated PC headgroup, which is consistent with literature.[8] Thus, the signal intensity of m/z 184.1 was selected as target signal to establish the quantitative method for LysoPC(14:0) and LysoPC(16:0), respectively. Analytical parameters including the extraction/ionization solution and extraction time were optimized. Methanol containing 1.5% proportion of ammonia (w/w) was found superior to other concentration ratios (see Supplementary Figure S9a). Extraction time of 1 min was needed for the adsorption of target phospholipids from undiluted urine sample (see Supplementary Figure S9b).

For the TiO_2-iEESI-MS/MS detection of LysoPC(14:0) and LysoPC(16:0) in urine, standard solutions of LysoPC(14:0) and LysoPC(16:0) were spiked into blank undiluted urine samples (2 mL) to make a series of working solutions in the range of 0.01−500.0 $\mu g\ L^{-1}$. As can be seen in Table 1, satisfactory linearity was attained for both LysoPC(14:0) and LysoPC(16:0) over the range of 0.1−500.0 $\mu g\ L^{-1}$, with R^2 values of 0.9989 for LysoPC(14:0) and 0.9985 for LysoPC(16:0), respectively (detailed in Figure S11). The LODs defined by S/N ≈ 3 were estimated to be 0.025 $\mu g\ L^{-1}$ for LysoPC(14:0) and 0.021 $\mu g\ L^{-1}$ for LysoPC(16:0),

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respectively. The RSDs of 6 replicates were ≤8.9% for LysoPC(14:0) and ≤7.8% for LysoPC(16:0), respectively (detailed in Tables S3 and S4). The measurement of one urine sample took less than 3 min. Recovery rates at three spiked concentrations were from 97.1 to 101.0%, with RSDs ≤ 9.6% (n = 6) for LysoPC(14:0), and from 94.8 to 101.6%, with RSDs ≤ 8.9% (n = 6) for LysoPC(16:0) (detailed in Table S7).

Also, quantitative TiO_2-iEESI-MS/MS detection was demonstrated for two synthetic phosphopeptides (phosphopeptide I (M_w: 601.2, sequence: NPE[pY]) and phosphopeptide II (M_w: 1196.6, sequence: KEAPPAPPQ[pS]P-NH_2)) from the nonphosphopeptides mixture (Gly-Tyr, Val-Tyr-Val, Tyr-Gly-Gly-Phe-Met, Tyr-Gly-Gly-Phe-Leu, and Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, each peptide at concentration 1 mg L^{-1}). As illustrated in Figure S10, tandem mass spectrometry experiments were employed to generate the characteristic fragment ions at m/z 502.2 and m/z 931.5 for the quantitative detection of phosphopeptide I and phosphopeptide II, respectively. The calibration curves are illustrated in Figure S11, and the results are summarized in Table 1. The linearity ranges were 0.3−200.0 μg L^{-1} for the both phosphopeptides, with R^2 values of 0.9994 for phosphopeptide I and 0.9997 for phosphopeptide II, respectively (Figure S11). The LODs defined by S/N ≈ 3 were estimated to be 0.09 μg L^{-1} for phosphopeptide I and 0.07 μg L^{-1} for phosphopeptide II, respectively. The RSDs of 6 replicates for different concentrations samples were ≤9.2% for these 2 phosphopeptides (detailed in Tables S5 and S6). Recovery rates at three spiked concentrations were from 91.0 to 107.0%, with RSDs ≤ 7.9% (n = 6) for phosphopeptide I, and from 83.4 to 90.9%, with RSDs ≤ 9.0% (n = 6) for phosphopeptide II (detailed in Table S7).

Table S8 presents a comprehensive comparison of analytical performance for the proposed method with regard to the performance of previously reported MS methods[39,40,58−66] based on titanium enrichment for the study of phosphopeptides and phospholipids. The data shows that the method established in this work is of higher speed, more sensitive for phospholipids, and with similar sensitivity for phosphopeptides compared with previously reported methods. Typically, the TiO_2 particles or SPE cartridge filled with TiO_2 sorbent are used for the off-line enrichment of phosphopeptides and phospholipids prior to HPLC-MS analysis,[40,58,59,63] requiring tedious sample preparations such as sample desalting, centrifugation, eluent drying, and chemical reconstitution, which greatly limits the speed of analysis for complex matrixes. Alternatively, online enrichment methods based on TiO_2 nanoparticle-deposited capillary column have also been developed,[61,65,66] but the sample flow rate in those methods should be maintained at a very low flow rate (e.g., 100 nL min^{-1}) for the efficient trapping of phosphorylated peptides, thus resulting in a long time of analysis.

CONCLUSIONS

To conclude, rapid analysis of biological samples such as human whole blood, plasma, urine, and protein digest using TiO_2-iEESI-MS was demonstrated in this study. Chromatographic separation of chemicals and solvent-specific release of analytes were observed during the sample extraction/ionization process, demonstrating good selectivity and sensitivity for the analysis of phospholipids and phosphopeptides in complex biological matrixes. The possibility of targeted phospholipid quantification and biofluid sample differentiation was also demonstrated. The presented method is featured by the high speed of analysis, high sensitivity, low sample consumption, and minimal sample preparation, indicating potential for application in biofluids analysis involved in clinic diagnosis, plant science, proteomics, etc. Furthermore, owing to phosphoryl-containing compounds strong adsorption to TiO_2,[41,67] TiO_2-iEESI-MS should be generally suitable for the analysis of other phosphate compounds in biofluid samples. A related study is currently underway in our laboratory.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b03022.

Additional information on materials and chemicals, biofluid samples, preparation of TiO_2 nanowire arrays, and mass spectrometry methods (PDF)

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Notes

The authors declare no competing financial interest.

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