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FULL PAPER

Electrophoretic removal of sodium dodecyl sulphate from peptide solution through a hydrogel-micropipette system

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SDS removal is an important process in proteomics after protein digestion. The research study evaluated an offline SDS removal, as well as, the capability of the offline electrophoretic system for SDS removal. Tyr-Tyr-Tyr, Glu-Val-Phe, and Bradykinin were three peptides to be tested at the presence of the SDS. The electrophoretic system included a 20 μL glass micropipette containing sample which was immersed into acetonitrile at one end and hydrogel at another end. The SDS removal was driven by applying voltage to the system and after removal, the sample inside micropipette can be collected for liquid chromatography, capillary electrophoresis, and mass spectrometry.

KEYWORDS

Electrophoretic sample preparation; proteomics; offline sample preparation; sodium dodecyl sulphate.

Introduction

Sample preparation is one of the most important steps in biological sample analysis. In proteomics and other biological techniques, sample preparation is so important. Due to various biological sources such as cells, blood, and tissues, extraction and solubilization are needed for most of the analysis. Triton, CHAPS, and sodium dodecyl sulphate (SDS) are considerably used for efficient solubilization of extracted proteotypes [1-4].

Surfactants, including SDS, are amphiphilic molecules containing both hydrophobic and hydrophilic groups. They have been applied for peptide solubilization in sample preparation for proteomics because they avoid peptide precipitation in aqueous media. After sample preparation, SDS removal is critical to avoid the peak suppression band broadening in liquid chromatography (LC), and mass spectrometry (MS) [2, 5-9].

There are several SDS removal methods used in sample preparation which are categorized based on their separation procedure. In the filter-aid sample preparation (FASP), a proper filter is used for performing the separation. It has some limitations especially, in using a specific filter for different analytes [3, 4, 10-16]. Precipitation techniques are so common for SDS through acidic reagents, salts, or saturated organic solvents; however, low selectivity and recovery are the limitations of the method [17-23]. Another method would be the electrokinetic removal of SDS. In this method, SDS removal can be happened by using electroosmosis flow, and DS- can be removed in high applied voltage [24-30]. The limitation will be the range of different target analytes. Transmembrane electrophoresis is

one the best methods for SDS removal which can be performed by applying the EOF through transmembrane. High efficiency and high costs are the advantages and disadvantages of this method [6, 31, 32].

In this work, offline electrophoretic removal of the SDS at the presence of peptide mixture including Bradykinin, Tyr-Tyr-Tyr, and Glu-Val-Phe was investigated to prove the capability of the system for the offline SDS removal.

Experimental

Chemicals and reagents

HPLC-grade water was provided by Millipore purification system. Trifluoroacetic acid (TFA), formic acid (FA), acetonitrile (ACN), methanol, Tyr-Tyr-Tyr peptide, bradykinin peptide, and Glu-Val-Phe peptide were purchased from Sigma-Aldrich company (Figure 1).

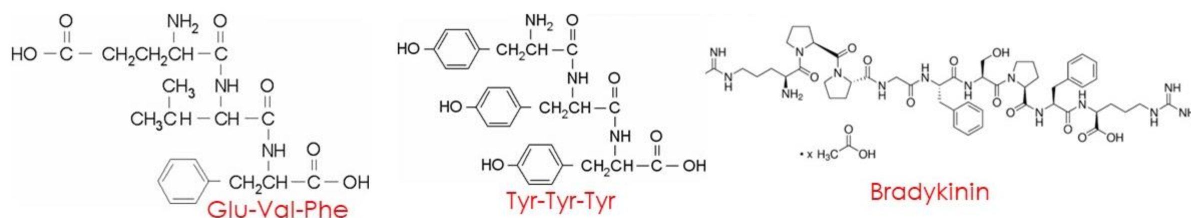

FIGURE 1 Molecular structures of the Tyr-Tyr-Tyr peptide, Bradykinin peptide, Glu-Val-Phe peptide, Paracetamol, Triamcinolone, and Trimethoprim

Preparation of hydrogel

To prepare the hydrogels directly, the mixture of acrylamide 55 wt% (monomer), N,N-dimethylacrylamide 99% (co-monomer), potassium persulfate 5 wt% (initiator), electrolyte stock solution, and purified water was used in the 2 mL capacity CE vials. The monomer, crosslinker, and initiator were used in 15:1:1.5 ratio. The other ratios are depicted in the following. The total volume was 1.2 mL so that a final concentration of which was similar to that of the separation electrolyte, and aliquots of electrolyte stock solutions (e.g., 250 mM phosphate buffer, pH 7.4) and purified water were added to make it up. The polymerization was thermally initiated at 60 ºC for 10 min. A small volume (e.g., 100 mL) of separation solution was placed into the vial to remove excess reagents after polymerization and then was removed.

Instrumentation

All capillary electrophoresis (CE) tests were conducted through Agilent Capillary Electrophoresis 3DCE model. A high voltage power supply (Matsusada, Japan) was used having the capability of providing adjustable voltages of 0–30 kV (0.1 kV increments).

Hydrogel-micropipette apparatus

20 μL micropipettes with a length of 6.4 cm and an inner diameter of 0.3 mm (Microcaps, Drummond Scientific Company, USA) and a 3 mL disposable plastic micropipette tip and 20 mL capacity scintillation vials (Sigma–Aldrich) were utilized for the tests. A hydrogel was prepared in the tip. The sample solution was stirred during electrophoretic concentration at 600 rpm. The voltage was applied through Matsusada instrument.

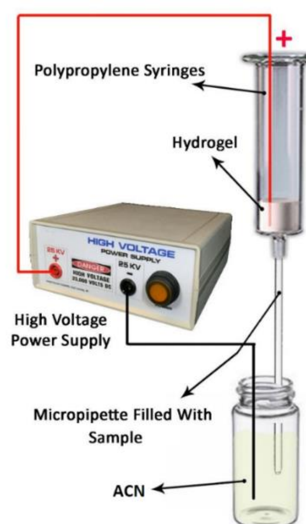

FIGURE 2 In the electrophoretic technique, the capillary containing extraction solution inserted to the vial containing sample solution.

Results and Discussion

Proof of concept

Cations of ionized silanol groups at the inner wall of fused-silica capillaries would be accumulated to provide the electroosmotic flow (EOF). The EOF has been applied for microchips and electromigration techniques in capillaries which causes cations to migrate toward the cathode under the electrical field and drives water onward from the bulk solution with a typical plug flow profile. The EOF should be controlled on the separation which has the highest effect on the electromigration technique. Applying the dynamic, semi-permanent, and permanent coatings to modify the chemical groups at the surface of the capillary wall can control the EOF. Therefore, the hydrogels can be prepared by penetration of water into the network of hydrophilic polymer networks. The polymers do not dissolve due to the chemical and physical crosslinking; however, having a high water and cations and anions content in their structure. Making a mixture of the monomers, crosslinker, and initiator with the appropriate buffer before polymerization can help hydrogels to hold electrolytes. A hydrogel coating might be used to manipulate the EOF in capillary electrophoresis (CE). Processes like separation and biocatalytic applications employed a hydrogel with EOF which meets the need to passage a solvent stream through the gel. The bulk flow inside the capillary can possibly be controlled by closing one or both ends of the capillary (Figure 2).

SDS Removal from peptide sample

With regards to some reports [33, 34] which were effectively concentrated using a hydrogel system, it can be a start point to apply the system for SDS removal using peptide solution. The researches were related to a range of anionic compounds, including inorganic ions, dye, and benzenesulfonate derivatives. First, the possible variables including the pH, voltage, timing, and quality of hydrogel which can have an effect on the separation were sorted. However, before starting, the system should be checked to be applicable or not. For this purpose, a standard peptides sample including 20 ppm of Bradykinin, Tyr-Tyr-Tyr, and Glu-Val-Phe was prepared to be examined. The sample first was tested through CE at the injection, separation, and detection at 50 mbar for 60 s, +30 kV, and 210 nm, respectively and BGE solution was 300 mM formic acid solution (Figure 3). As can be seen, the Glu-Val-Phe, Tyr-Tyr-Tyr, and Bradykinin peaks appeared at 3.5, 5.0, and 5.2 min, sequentially

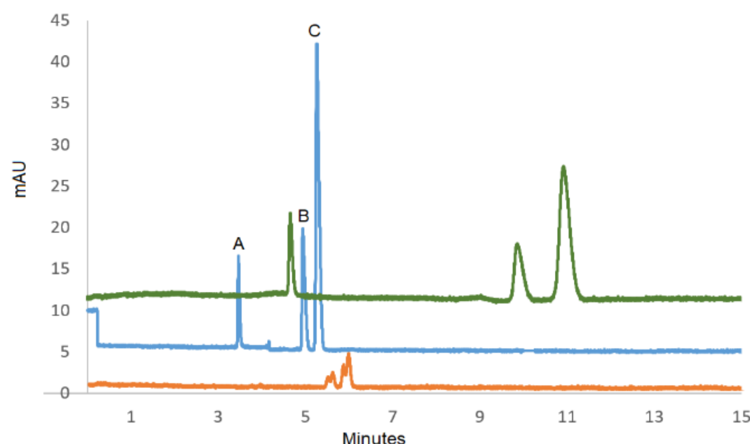

FIGURE 3 Capillary Electropherogram of standard peptides (blue), SDS-peptide (orange), and peptide after separation through the hydrogel-micropipette system (green). The samples include 20 ppm of Bradykinin, Tyr-Tyr-Tyr, and Glu-Val-Phe and 10 mM SDS at the CE injection, separation, and detection at 50 mbar for 60 s, +30 kV, and 210 nm. BGE was 300 mM formic acid. The applied voltage at the hydrogel-micropipette system was 0.8 kV for 60 seconds (green). The separation solution was ACN

Then, SDS-peptide solution was tested at the same condition. In accordance with Figure 3, electropherogram peaks of standard peptides solution (blue peak) were sharp and retention times were between 3 to 5 min. After adding SDS, the peaks were suppressed and the peptides were retained longer (orange peak). By using the hydrogel-micropipette system by applying 0.8 kV at 60 s, the extracted sample was tested through CE and the peptides were identified and analysed (green peak). But, the retention time and peak broadening were increased, showing the presence of SDS in the system. The recovery factor was found to be 95% for this separation.

Time optimization for separation

There are so many different parameters affecting the SDS removal process such as the pH, solvent, solution matrix, and voltage. However, the time of applied voltage is one of the most important parameters. After proving the capability of the system for SDS removal, the first variable was the time of applied voltage. For this purpose, the voltage was first set on 0.8 kV and applied for 60 s, 120 s, and 180 s (Figure 4). The results showed that by increasing the time of applying voltage, the peak intensity of peptides was significantly increased as the efficiency of the separation would increase significantly in higher applied voltage. Now, there has been less concentration of SDS in the sample and so, the concentration of free peptide was increased leading to decreased peak broadening and so, peptide peaks have been appeared sharper and in higher peak intensity. This effect is about different EOF in DS- and peptides. This increase continued to 120 s of applying voltage. But in higher time, peptides also were removed from the sample micropipette, which resulted in decreasing the peak intensity after 120 s.

Conclusion

It was found that, this method can remove the SDS from the peptide solution through electroosmotic flow and in comparison of other SDS removal. In addition, this method is more efficient with the recovery rate of 95%. The cost of this method is lower than other methods which makes it more attractive for biological experiments.

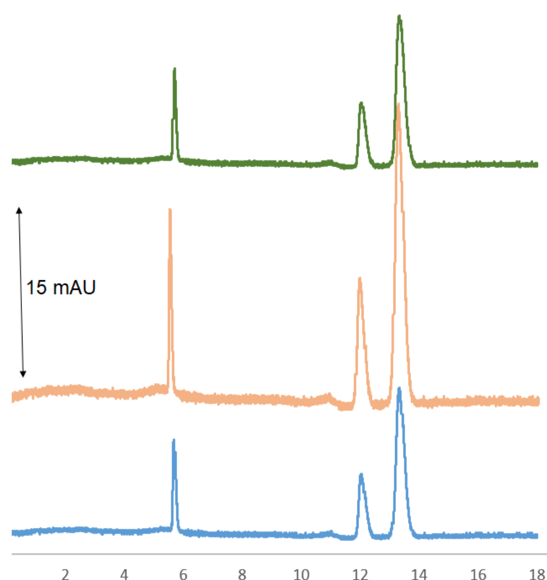

FIGURE 4 Capillary Electropherogram of SDS-peptide samples after applying 0.8 kV for 60 s (blue), 120 s (orange), and 180 s (green) through the hydrogel-micropipette system. The samples included 20 ppm of Bradykinin, Tyr-Tyr-Tyr, and Glu-Val-Phe, and 10 mM SDS at the CE injection, separation, and detection at 50 mbar for 60 s, +30 kV, and 210 nm. BGE was 300 mM formic acid

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