Solid Phase Extraction and its Application in Preconcentration of Trace Environmental Pollutant from Wastewater

Today, solid-phase extraction (SPE) has become a widely acclaimed sample preparation tool for analytical chemists. This technique was developed as an alternative to liquid-liquid extraction (LLE). Historical evidences and some scientists claimed that the first literature reference to SPE was found in Bible (Riemon and Walton, 1970). But this technique was experimentally applied in the late 1940s (Liška, et al., 2000), the developments leading to its widespread use and adaptation into current analytical methods were started in the 1970s.

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Initially, SPE was used to concentrate trace amounts of organic pollutants present in wastewater samples, but its use has now extended to a wide variety of matrices including oil, serum, milk, blood, urine, plant and animal tissues, and pharmaceutical preparations (Mitra, 2003). Complex sample matrix simplification along with compound purification, reduced ion suppression or enhancement in mass spectrometric applications, capability to fractionate sample matrix to analyse compounds by class, enrichment of trace concentration level compounds are the major merits of SPE.

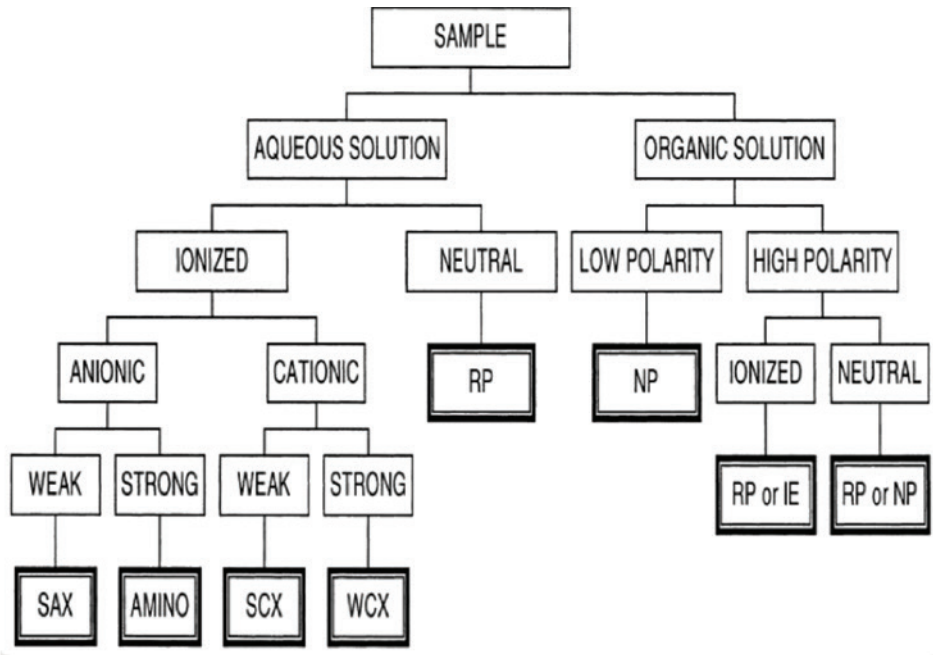

Figure 1. Conditions to isolate analyte from solution by SPE. (SAX – Strong Anion Exchanger; SCX – Strong Cation Exchanger; WCX – Weak Cation Exchanger; RP- Reversed Phase Sampling Conditions; NP – Normal Phase Sampling Conditions; IE – Ion-Exchange Sampling Conditions; AMINO – 3-aminopropylsiloxane-bonded silica) (Poole et al, 2000)

At present, commercially available cartridge, syringe barrel, and disc devices are widely used as SPE probes. Typical SPE-sorbents are based on either functionalised silica or polymeric materials. Considering economic and ecological aspects, the use of these commercial probes is not feasible. Therefore, it is essential to develop economical and ecofriendly probes so that in future SPE could be called as "Green-SPE" technique. Research is going on world over to explore the potential of biological (both plants and animals) waste materials for developing SPE cartridges and discs, as these materials are cost effective and environmentally safe. Above all, the use of these materials in SPE may open a new dimension towards their smart utilisation. It would be righteous to call it a "waste to worth" approach. Pistachio (*Pistacia vera* L.) is a very common food crop cultivated in mediterranean countries. Statistics revealed that 30 million metrics tons of pistachio shell waste (PSW) is generated annually by pistachio nut processing industries (Foo and Hameed, 2011). Here, in principle, mechanism methodology of SPE will be discussed. The application of SPE-cartridges prepared from PSW biomass for the enrichment of trace concentration level of methylene blue present in wastewater samples will also be discussed.

Principle and Mechanism of SPE

To accurately determine the concentration of desired analytes present in a sample, quantitative extraction of the analytes from the other components present in the sample matrix (which may interfere during analysis) is essential. During SPE, the analytes in a solution are enriched and separated (or purified) via a retention on sorbent (a solid phase), followed by elution with an appropriate eluent. To successfully conduct SPE process, mechanistic understanding of the interaction between sorbent and analyte of interest is essential. It is also essential to assess their properties based on hydrophobic, polar, and ionogenic characteristics. The most common retention mechanisms are based on van der Waals forces (non-polar interactions), hydrogen bonding, dipole-dipole forces (polar interactions), and cation-anion interactions (ionic interactions). These mechanisms include reverse phase-SPE, normal phase-SPE, ion exchange-SPE, and polymer based sorbent-SPE. In reverse phase-SPE, the mobile phase (sample matrix) is polar or moderately polar and stationary phase (sorbent) is non-polar. The analyte of interest is typically mild to non-polar. Normal

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phase-SPE involve a polar analyte, mild to non-polar matrix (mobile phase) and a polar stationary phase (sorbent). In ion-exchange-SPE the retention mechanism is based on the electrostatic attraction between charged functional groups of analyte, and stationary phase, polymer based sorbent-SPE is used for retaining hydrophobic compounds which contain some hydrophilic functionality, especially aromatics (Żwir-Ferenc and Biziuk, 2006). **Figure 1** summarises the conditions required for SPE (Poole et al., 2000).

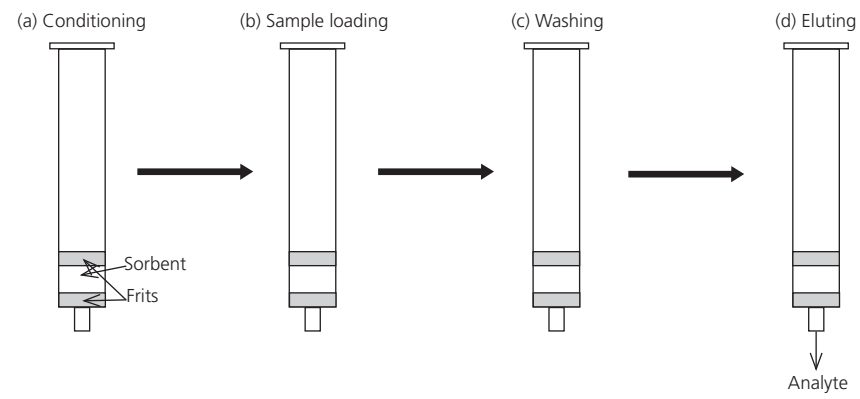

Figure 2. Scheme presenting SPE steps

Methodology

SPE column is either made or glass or polypropylene with the sorbent filled in between frits support. There are four steps which include conditioning, sample loading, washing, and finally analyte eluting (**Figure 2**). During the conditioning step, the solvent is passed through the sorbent making it wet and to solvate the functional groups present on sorbent surface. This step also removes air present in the column, filling all void spaces with solvent. Conditioning step is followed by sample loading step. 1 to 1000mL of sample may be applied to the column either by gravity feed, pumping, aspirated by vacuum, or by automated system. After sample loading step the column is rinsed in the washing step. This will remove the sample matrix from interstitial spaces of the column, while retaining the desired analyte. The desired analyte is eluted by using appropriate solvent in final step.

Applications

Previously, our research group has successfully utilised PSW biomass as a sorbent in a SPE system and tested its applicability for the enrichment of methylene blue dye traces present in different environmental wastewater samples. Quantitative determination of desired analytes was successfully carried out by ultra-performance liquid chromatography-tandem mass spectrometry (Khan et al., 2014).

SPE-Column Preparation

PSW biomass was washed several times with deionised water to remove dirt and dust, dried in an oven at 60°C for 24 hrs. The dried PSW biomass was ground and sieved to 0.2 – 0.4 mm particle size. Further, the organic content of biomass was oxidised with 30% w/w H_2O_2. The biomass was functionalised by treating it with 0.1M NaOH. The NaOH treated PSW biomass was washed with deionised water to achieve neutral pH. The treated biomass was dried overnight in an oven at 60°C. To prepare the SPE-Column, 6 mL of empty extraction column (Extrelut – 20; Merck Darmstadt, Germany) was taken. A gram of treated biomass was filled in 6 mL empty Extrelut with frit support, another frit was placed on the top of biomass. The detailed procedure is given elsewhere (Khan et al., 2014).

Sample (analyte) Preparation for UPLC-MS/MS Analysis

Wastewater samples were collected in 250mL capped glass bottles from paper, textile, laundry, and printing industries in Saudi Arabia. The samples were filtered with Whatman No. 42 filter papers and were stored at – 4°C to avoid microbial contamination. The SPE-Column was placed over SPE vacuum setup with 12 ports (Visiprep™; Supelco, Gland, Switzerland), followed by conditioning with 50mL deionised water and drying under vacuum for 10 min. 20mL of filtered industrial wastewater sample was passed through SPE-Column at 1 mL/min. flow rate to load methylene blue on PSW biomass surface. After the sample loading step the column was rinsed with 50mL deionised water and dried under vacuum for 10 min. Rinsing of the SPE-Column removed the sample matrix from interstitial spaces of the column, while retaining the desired analyte. The elution of analyte was tested by various eluents. The eluent containing analyte was evaporated to dryness under nitrogen stream and was reconstituted with 1mL of methylene violet 3RAX dye (used as internal standard for UPLC-MS/MS analysis) in methanol-water (50/50, v/v). The final extract was filtered through a 0.22 µm PTFE syringe filter prior to UPLC-MS/MS analysis.

Results

Maximum methylene blue recovery from different industrial wastewater sample was observed when1M formic acid methanolic solution was used as an eluent (**Table 1**). The analyte was analysed by ultra-performance liquid chromatography-tandem mass spectrometry having the Acquity® UPLC BEH C_{18} reversed-phase column. 65% of Milli-Q water with formic acid (0.1% v/v) and 35% acetonitrile in isocratic elution mode was selected as mobile phase. 300 µL/min. mobile phase flow rate was established to be most favorable for the analysis with retention time of < 2 min.

Conclusions

The SPE system developed using low cost waste biomass sorbent showed no matrix effects during analysis. Minimal sample pre-treatment without the loss of target analyte offered by a low-cost SPE sorbent is a significant advantage of this technique as added steps during the sample preparation would increase analysis time, solvent consumption, variability and significant losses of the target analyte and sensitivity. Overall, the results obtained during this research enable a new analytical methodology for the routine analysis of methylene blue in industrial wastewater samples. The SPE system developed during this study could be a good alternative to commercially available SPE systems.

Table 1. Methylene blue concentration levels and recovery rates in wastewater samples (Khan et al., 2014)

Sample	Methylene blue[a] (µg/mL ± SD)	Recovery (%)
Paper	0.54 ± 0.02	96
Textile	1.08 ± 0.04	99
Laundry	0.36 ± 0.01	95
Printing press	0.83 ± 0.03	98

[a]Standard deviation obtained from addition standard calibration curve

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