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Solid-Phase Extraction of Biogenic Amines using Sorbent Material Immobilized with Salicylaldehyde 2,4-Dinitrophenylhydrazone

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Abstract

Sorbent material based on a novel hydrazone compound, $C_{13}H_{10}N_4O_5$, was prepared by immobilizing the ligand into gel matrix (SG). Characterization of SG by FTIR, and solid UV-vis proved the presence of the hydrazone in the sol-gel silica. The competitive sorption characteristics of eight biogenic amines (BAs) using batch method was studied using HPLC. The extraction was optimized for key parameters such as pH, contact time, BAs concentration, ligand concentration and selectivity among the studied species. The results show that the sorbent material has special selectivity towards SPD > PUT > CAD.

Keywords: *Sol-gel, hydrazones, biogenic amines, SPE*

1. Introduction

Biogenic amines represent a group of low molecular weight organic bases occurring in all living organisms. Putrescine (PUT), spermidine (SPD), spermine (SP) and cadaverine (CAD) are natural polyamines that occur in animals and plants, while putrescine and spermidine also found in most bacteria [1]. They are synthesized and degraded as a result of normal metabolic activities in the cells of microorganisms, plants and animals [2]. Therefore, they are ubiquitous in animals, plants and microorganisms [3]. Polyamines such as PUT, SPD, SP and CAD are important in the regulation of nucleic acid function, membrane stabilization, protein synthesis, brain development, nerve and tissue growth and regeneration [2,4]. They also play a major role in the body's response to brain injury and stress, and in the regulation of neuronal ion channels and brain neurotransmitter receptors. Some studies also shown that the high concentration of polyamines found in human milk

play a role in the apparent protective effect of human milk against allergies [5]. In plants, they also have been associated with pH and thermic or osmotic stress responses, cell division, flowering, and may function as allelochemical compounds and as components of the chemical and physical defenses against herbivores and pathogens [6].

There is a continuous need to find out a suitable technique which can selectively extract, separate and recover biogenic amines from food samples. Organic-inorganic hybrid materials (sol-gel) had drawn attention of researchers now a days as a new class of material. It is involved hydrolysis and condensation reactions that closely controlled by the initial synthesis condition and parameters such as temperature, acid or base catalyst, alkoxide concentration and presence of active groups. Solid phase extraction techniques are considered to be superior to the liquid-liquid extraction due to their simplicity, rapidity, and the ability to provide a high enrichment factor. This paper describes the extraction of BAs from pure aqueous solutions containing biogenic amine mixture with sol-gel silica immobilized with salicylaldehyde 2,4-dinitrophenylhydrazone.

2. Experimental

Analytical grade chemicals, reagents and solvents were used as received without additional purification. 18 mΩ ultrapure water was used throughout. A Perkin Elmer 200-series HPLC unit and an Ultraviolet-visible lambda 35 UV -vis spectrometer were used for separation and detection, respectively. Carbon, hydrogen, and nitrogen analysis for the ligand was performed using CHN Analyzer. Stuart Scientific flask shaker SFI (500 osc. min^{-1}) was used for the extraction. The chromatographic separation in the isocratic mode was carried out using a C_{18} ODS Hypersil column (250 × 4.5 mm, 5 µm) at ambient temperature. The mobile phase was 60:25:15 (v/v/v) acetonitrile:water:methanol at a flow rate of 1.0 mL min^{-1}; wavelength, 254 nm; injection volume, 50 µL. The studied ligand, the sorbent material and stock solution (1000 mg L^{-1}) of a mixture of seven BAs containing salts (TRY, PEA, PUT, CAD, HIS, TYR and SPD in water) were carried out as previously reported [7,8].

2.1 Characterization of the ligand and sorbent

From the CHN analysis of the ligand, the carbon, hydrogen and nitrogen percentage was found to be 50.21, 3.05 and 17.90%, respectively, comparing to the theoretical values (51.66, 3.33 and 18.54%). The ^{1}H NMR spectra of the ligand was measured in DMSO. The ^{1}H NMR spectrum of the compound (Fig.1), along with the signals of aromatic protons of phenol and benzene ring signals of protons of NH and N=CH groups are observed. The values of chemical shifts for OH, NH, N=CH were; 11.71, 10.23 and 8.94ppm, respectively. The chemical shift for the aromatic

group was 6.80-8.85. The ^{1}H NMR spectrum of the ligand showed a broad singlet centered at 11.72 ppm which assigned to the resonance of the proton of the OH group attached to benzene ring. The singlet peak at 10.25 ppm is due to the ⟩C–NH proton signal.

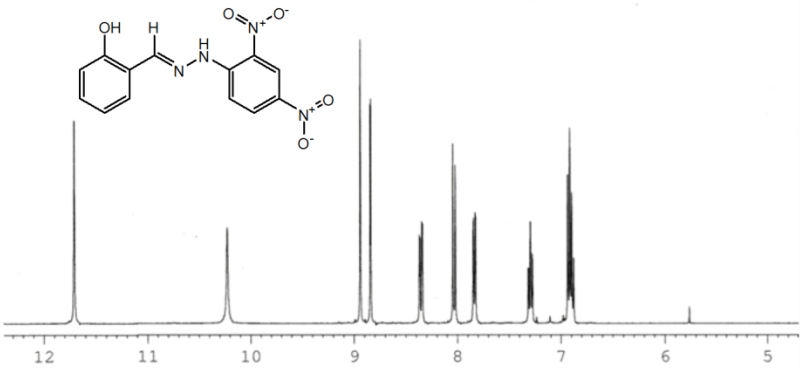

Figure 1. 400 MHz ^{1}HNMR spectrum of the studied ligand in DMSO-d_6 at 25°C.

The incorporation of the ligand into the sol-gel network was confirmed by UV-Vis technique. The free ligand showed red shift with maximum absorption (λ_{max}) around 420 nm. However, incorporating the ligand into the silica-gel causes a shift in the wavelength (λ_{max}) of the free ligand ($\Delta\lambda_{max}$= -29).

2.2 Extraction method

Batch method of extraction was conducted. The sorbent (25 mg) was placed in a glass vial along with 1 mL BAs standard mixture and 4 mL of 0.1 M Tris buffer (pH 9). The mixture was shaken mechanically at room temperature for 15 min. After the equilibrium time, the mixture was filtered and the filtrate (1 mL) was derivatized as previously reported [9]. The amount of the un-extracted BAs left in the solution after the extraction was determined by reversed phase HPLC. The extracted amount was calculated by the difference.

3. Discussion

3.1 Optimized parameters of extraction

The extraction was optimized by using 20 mg L^{-1} BAs standard solution. Important parameters that affects the extraction efficiency were investigated, i.e., sample pH, ligand

concentration within the sorbent material, extraction time, and sorbent capacity.The extraction was also carried out using blank sorbent (i.e. does not contain the studied ligand) as control.

3.2 Effect of pH

The effect of sample pH (3 – 11) on the extraction efficiency was studied. At pH 3-6 no significant extraction was observed (< 50%). The pH range for the quantitative extraction of BAs was found to be from 8 – 11 (Fig. 2). The sorbent exhibited an extraction close to 100% mostly for SPD followed by PUT and CAD. Within this pH range (pH 8 – 11), deprotonation occurs at the (N–H); amino group [10] which maximizes the delocalization resonance and provides extra charge/charge interaction between the protic BA and the N–H group. This result proved that, the ligand polarity plays an important role in the interaction of the studied sorbent material with amines at this range of pH [11]. However, the low extraction results at the acidic medium, could be due to the highly ionization abilities of BAs at lower pH.

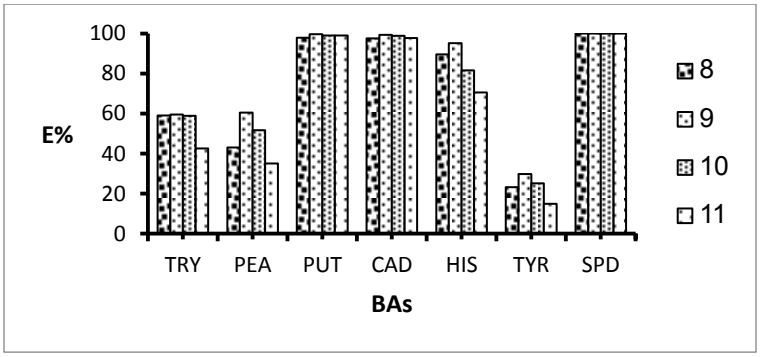

Figure 2. Effect of pH

3.3 Effect of contact time

The effect of contact time on the extraction efficiency was studied by shaking the BAs mixture with each sorbent ranging from 5 to 60 min at the optimum pH. Good extraction was observed after 5 min of contact time (%E >79 %) for all the studied BAs (results not shown). However, maximum extraction was achieved after 15 min. Increasing the contact time further has no significant improvement of the extraction. Therefore, 15 min contact time was chosen for subsequent studies.

3.4 Effect of ligand concentration in the sol-gel network

Sorbent materials containing different concentrations of ligand (0.85% - 6.8%) were prepared and their extraction efficiency were examined at the optimum pH 9 and extraction time of 15 min. It was observed that the highest extraction (%E >80) for all BAs was obtained when 0.85% ligand in the sorbent was used (Fig.3). Further increase of the ligand concentration does not seem to affect the extraction efficiency.

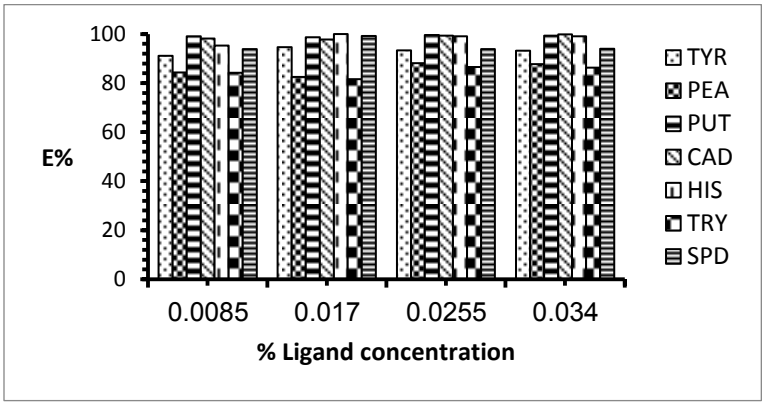

Figure 3: Effect of ligand concentration

3.5 Effect of BAs concentration

Five different concentrations (5 – 500 mg L^{-1}) of BAs were used to study the extraction efficiency and the capacity of the sorbent (Fig. 4). It was found that the aliphatic BAs (PUT, CAD, and SPD) were quantitatively extracted (%E 100 %) up to 200 mg L^{-1}. At concentrations higher than 200 mg L^{-1} a decrease in the extraction of PUT and CAD was observed, while no decrease was found for SPD. The presence of the aromatic ring resulted in a decrease in the extraction efficiency due to steric effects. HIS was extracted more than the other aromatic BAs due to its smaller size.

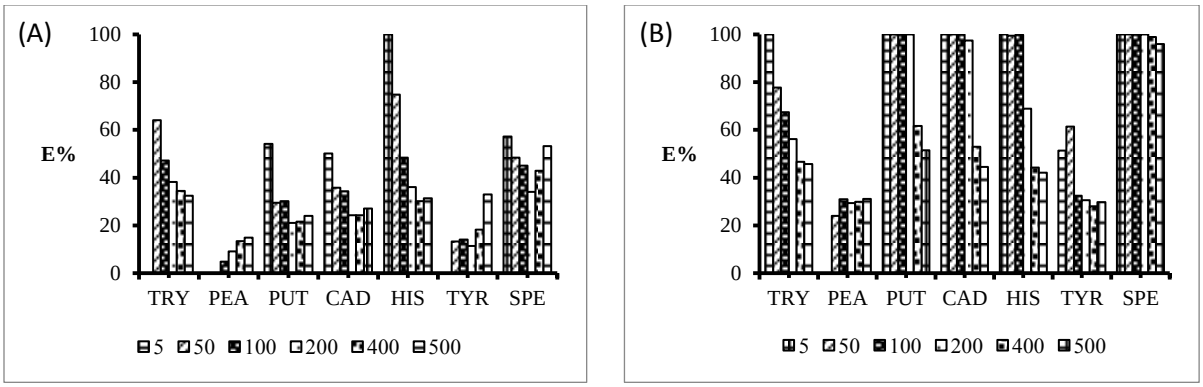

Figure 4. Effect of BAs concentration. (A) blank and (B) immobilize sol-gel

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