Synthesis and characterization of nanometric zinc oxide for a stationary phase in liquid chromatography

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Abstract. The increasing demand for equipment to remove organic compounds in industry and research activity has led to evaluate nanometric zinc oxide (ZnO). In this work, we present the ZnO nanoparticles synthesis for reusing of discarded columns, as a low-cost alternative. The compound was obtained by sol-gel technique using zinc chloride and sodium hydroxide as precursors and a drying temperature of 169°C. An X-ray diffractometer was used to estimate the average particle size at 20.3±0.2nm; the adsorption capacity was 0.0144L/g and the chemical resistance was tested with HCl and NaOH. The ZnO nanopowder was packed with 100psi pressure in an empty C-18 column cavity. The column packing resolution was evaluated using a high performance liquid chromatographer (HPLC-Thermo Scientific Dionex UltiMate 3000); using a caffeine standard, the following parameters were established: solvent flow: 1.2mL/min, average column temperature: 40°C, running time: 10 minutes, mobile phase acetonitrile-water composition (9:1). These results validate the potential of ZnO nanopowder as a column packing material in HPLC technique.

1. Introduction
The stationary phases of silica are the most common packing material of columns used in high performance liquid chromatography (HPLC). However, its usage is limited to pH ranges from 2.5 to 7.5 due to the structural chemical instability of this material. For this reason, some ceramic materials have been studied for replacing the silica [1]. The development of new instruments and stationary phases for the HPLC technique is a subject of interest, but they are being produced with complex manufacturing and are expensive [2].

Several inorganic oxides have a high potential in the development of new columns as alternative to the silica such as titanium dioxide (TiO₂), zirconium oxide (ZrO₂) and zinc oxide (ZnO) [3]. ZnO has similar properties to both TiO₂ and silica, but unlike TiO₂, it is cheaper and more resistant to pH changes than silica [4,5]. In this work, ZnO nanopowder for application as a stationary phase in HPLC was synthesized by sol-gel technique, which is a chemical route widely used because of its simplicity, reproducibility, commercial viability, high yields, product homogeneity, industrial adaptation, and nanometric size control [6,7].

2. Experimental section
2.1. Zinc oxide preparation
The reagents were zinc oxide, sodium hydroxide, zinc chloride, hydrochloric acid, analytical-grade nitric and sulfuric acid distributed by Merck KGaA. Acetonitrile, methanol, caffeine and water, with HPLC quality, were acquired from Merck KGaA.
Sol-gel process was used as described by Gel [6]. However, in this work some modifications were taken: firstly, the reactor was placed during one hour under ultrasonic agitation; the reaction temperature was 90°C; methanolic solution concentration was 90% methanol/10% water; 0.1M NaOH was heated at 90°C and 0.25M ZnCl₂ (dipped during 30 minutes) was added into the methanolic solution. Finally, the obtained white precipitate was washed with water nine times, under ultrasonic agitation and dried at 200°C.

2.2. Characterization
An X-Ray diffractometer (Bruker D8 Advanced) with Cu Ka radiation (\(\lambda=1.5418\)Å) was used to determine the crystallographic phase and crystal size of the synthetized ZnO powder and a scanning electron microscope (SEM-Model JSM-5610 LV, Electron Optics Corporation, Japan) was utilized to observe crystal shapes and size distribution. The initial solid crystallization temperature of the samples was measured using a differential scanning calorimeter (NETZSCH DSC 214 POLYMA) and the decomposition temperature was determined with a thermogravimetric analyzer (NETZSCH TG 209 F1). Finally, an HPLC (Thermo Scientific Dionex UltiMate 3000) was used to identify caffeine, using the nanocrystalline ZnO as column packing material.

2.3. Column packing
The packing of the stationary phase of ZnO nanopowder into a stainless steel cylinder (HPLC column, 110mm high with a radius of 2mm), was done using the ZnO nanoparticles in a water/ethanol mixture 30-70%, which was propelled by an air compressor at constant pressure of 100psi.

2.4. Resistance to alkaline and acidic eluents test
The sample of zinc oxide (100mg) was deposited in test tubes; 10mL of hydrochloric acid with 0.1M and 1M concentration and sodium hydroxide solution with 50mM and 0.5 M concentration were added, respectively. Then, the test tubes were agitated and heated for 48 hours at 40°C; later the supernatant was separated by centrifugation. The residue was washed with distilled water and then centrifuged. Finally, the oxide was dried during 2 hours at 105°C [1].

2.5. Adsorption isotherm measuring
The determination of the adsorption isotherms was performed as follows: 20mg of the ZnO sample (adsorbent) were placed into individual test tubes and heated for 2 hours at 105°C. Then, into each tube, 2mL of methylparaben solution were added; this solution was prepared in different concentrations using hexane-2-propanol (9:1) as diluent. Finally, the tubes were placed into an ultrasonic bath during 15 minutes and after 1 hour in a water bath at 40°C [1].

3. Results and analysis

3.1. Characterization and morphology
Figure 1 shows the thermogravimetric curve of the sample; it indicates a 24.23% of mass loss at 78.16°C, which corresponds to losing methanol in the synthesis process; the other inflection point in this curve, around 168°C, indicates a mass loss of 16% corresponding to water evaporation. According to DSC curve in Figure 2, the evaporation of solvents that were used in the synthesis is confirmed; the ZnO sample is initially crystalline and it does not need a synthesis process because there is not any exothermic peak in the curve.

In Figure 3, the X-Ray diffractogram (XRD) of the ZnO nanopowder is shown; the representative peaks of ZnO suggest high purity and crystallization degree. The average particle radius of 20.3±0.2nm was calculated indirectly using the Scherrer Equation (1) [8,9]:

\[ r = \frac{0.9\lambda}{2\beta\cos(\theta)} \]  \hspace{1cm} (1)
Where 0.9 is a shape factor, $\lambda$ is the wavelength of X-rays, $\theta$ is the Bragg diffraction angle and $\beta$ is the full width at half-maximum (FWHM) of the diffraction peak corresponding to the plane (1 0 1).

The spherical morphology of the nanoparticles was observed from the SEM micrograph in Figure 4. From this picture, it is possible to see that the nanoparticle size is around 25nm, which is similar to the order of estimated value using the Equation (1).

**Figure 1.** Thermogravimetric curve: mass loss versus temperature for synthesized ZnO powder.

**Figure 2.** DSC curve: heat flow versus temperature for synthesized ZnO powder.

**Figure 3.** X-ray diffractogram of ZnO nanopowder.

**Figure 4.** Micrograph of ZnO nanoparticles, magnification X 20000.

3.2. Test of resistance to alkaline and acidic eluents

The endurance of zinc oxide powder to moderately basic and acid pH was registered in Table 1. It was proved that ZnO did not dissolve neither in 0.5 nor in 0.05M NaOH, the ZnO mass recovered completely, whereas it dissolved in 1M HCl solution. The comparison with the percentage recovery values of zinc oxide and silica that was reported by Kawara et al. [1] shows that the ZnO nanoparticles have better stability to contact with higher pH solutions than silica gel.
Table 1. Percentage recovery of the zinc oxide nanopowder after contact with a medium (HCl and NaOH) at different concentration and pH during 48 h at 40 °C.

| Material     | HCl concentration | pH | Recovery (%) | NaOH concentration | pH | Recovery (%) |
|--------------|-------------------|----|--------------|-------------------|----|--------------|
| ZnO          | 1                 | 0  | 0            | 0.5               | 14 | 100          |
|              | 0.1               | 1  | 96.3         | 0.05              | 13 | 100          |
| Silica gel*  | 1                 | 0  | 95.5         | 0.5               | 14 | 0            |
|              | 0.1               | 1  | 98.5         | 0.05              | 13 | 73.0         |

*Values reported by Kawara et al. for silica gel [1].

3.3. Adsorption isotherm measurement

The adsorption isotherm of zinc oxide nanoparticles was measured by shake-flask method; the adsorbed methylparaben per unit of adsorbent weight (Cs, nmol/mg) versus methylparaben concentration in the supernatant (CM, M) was plotted as can be seen in Figure 5. These experimental points were fixed to a straight line using Henry Equation (2) [1,10]:

\[
C_s = K_d \cdot C_M
\]  

(2)

Where \( K_d \) is the distribution constant. From the fixed straight-line slope, 0.0144Lg\(^{-1}\) was estimated for this parameter, which is similar to 0.0188Lg\(^{-1}\) reported for titania [1], that have much larger adsorption capacity than silica gel.

Figure 5. Adsorption isotherm of the zinc oxide nanopowder. The solid line corresponds to the best fit to equation (2).

Figure 6. Caffeine chromatogram measured with the column filled with the zinc oxide nanopowder.

3.4. HPLC analysis

The chromatogram obtained from the caffeine in Figure 6 shows a retention time of 2.23 minutes. The analysis conditions for separation profiles of caffeine were column temperature: 40°C, flow-rate: 1.2mL min\(^{-1}\), mobile phase composition (acetonitrile-water): 9:1 and absorption wavelength: 253nm.
4. Conclusion
Zinc oxide spherical nanocrystals with an average radius of 20.3±0.2 nm were synthesized using sol-gel technique without sintering process. The found data suggests that this nanopowder has better properties than silica gel for application in HPLC separation; for instance, it has high resistance to basic pH eluents (12.69-13.69) and 23% more of adsorption capacity than silica gel. Chromatographic analysis with this nanopowder as a stationary phase was acceptable for caffeine separation.

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