Seryum Oksit Nanoparçacıklarının Sentezlenmesinin CaCo2 Hücrelerine Sitotoksit Etkisi (in Vitro)

Effect of Ceria Nanoparticles Synthesis on their Cytotoxicity against CaCo2 Cells in Vitro

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Özetçe

Son yıllarda seryum oksit katı yakıt pilleri, katalitik malzemler, güneş pilleri ve biyomedikal uygulamalar gibi bir çok mühendislik ve biyolojik uygulamaalrda kullanılmaktadır. Bu çalışmanın amacı nanoseryumun çeşitli sentezlenme yöntemleri ile üretiminin CaCo2 hücre hattına sitotoksik etkisini incelemektir. Seryum nanoparçaçıklarının X-Ray Kırılımı, Fourier Dönüşümlü İnfraret Spektrometre, boyut analizi, zeta potansiyel ölçümü ve yüzey alanı ölçümleri ile morfolojik ve yapısal karakterizasyonları yapılmıştır. Sitotoksisite testleri CaCo2 kolon kanseri hücreleri kullanarak yapılmıştır. Düşük konsantrasyonlarda nanoser-yum sitotoksik etki göstermemiş ve sentezleme yöntemlerine göre sitotoksisitenin etkisi araştırılmaktadır.

Abstract

In recent year's cerium oxide has been used productively in various engineering and biological applications, such as solid oxide fuel cells, catalytic materials, solar cells and biomedical applications as biological antioxidants [1-4]. Aim of this study is to investigate effects of synthesis conditionsof nanoceria on cytotoxicity against CaCo2 (human colon adenocar-cinoma) Cells in vitro. The morphological and structural characterization of ceria nanopowders were performed by X-Ray Diffraction (XRD), Fourier Transform Infrared Spectra (FTIR), size distribution, electrokinetic analysis (zeta potential measurements), surface area. Cytotoxicity test using colon cancer cells showed that nanoceria have no cytotoxic effect against Caco-2 cells at low concentration and cytotoxicity change with respect to synthesis conditions.

1. Introduction

In recent year's cerium oxide has been used productively in various engineering and biological applications, and biomedical applications because of unique optical properties and oxidizing capacity. The motivation of this study is to investigate effects of synthesis conditions on cytotoxicity against CaCo2 (human colon adenocar-cinoma) cells in vitro. The morphological and structural characterization of ceria nanopowders were performed by X-Ray Diffraction (XRD), Fourier Transform Infrared Spectra (FTIR). Also further measurements were obtained in terms of size distribution, zeta potential measurements.

2. Experimental

Cerium oxide nanoparticles that starting material Cerium nitrate hexahydrate were synthesized using two different precipation routes. First technique was forward precipitation, briefly, the base is added to the acid under strirring (Ce1). Cerium nitrate hexahydrate was dissolved in ultrapure water (18.2 MΩ) to obtain 1M, then amonium hydroxide was added to solution. The sol was stirr continously at room temperature 4 hours and then was centrifuged and dried at 80°C overnight. On drying, the precipitate was lemon yellow in color. This sample was then suspended in water and peptized using citric acid solution. These sol was dried at 80°C. Second route was reverse precipitation techniques, briefly, the acid is added to base(Ce2). Cerium nitrate hexahydrate was dissolved in ultrapure water to obtain 1M solution, then citric acid was added to solution. The resulting solution was pored under stirring into amınonia solution and allowed to mixed 4 hours. On drying, the precipitate was light brown-yellow in color, these sol was dried at 80 °C overnight. Cerium oxide nanoparticles were synthesized by starting material Cerric amonium nitrate (Ce3). Citric acid was dissolved in ultrapure water to obtain 1M solution, then Cerric amonium nitrate was added to solution allowed to mix 4 hours. Then sol was dried at 80 °C overnight. On drying, the precipitate was light brown-yellow in color. All ceria powders were calcined 100, 200 °C, 2 hours.

CaCo2 cell line was maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) containing 15% FBS (BIO-IND), 1µg/mL streptomycin/100 IU/mL penicillin incubated at 37°C in the dark with 5% CO_2 humidified incubator. To investigate the cytotoxic activity of the compounds, 95 µl of cell suspension was inoculated into 96-well microculture plates at $1x10^4$ cells density per well in culture media containing FBS, penicillin/streptomycin. Dilutions of compounds were freshly prepared before each experiment. After 24 h cultivation for cell attachment, extracts were added at final concentrations 2, 1, 0.1, 0.01, 0.001, and 0.0001 µg/ml for triplicate assay. Cells were treated with the ceria for 24 and 48 h and cytotoxic effects were determined by tetrazolium(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma, USA) based colorimetric assay. The absorbance was determined at 540 nm. Results were represented as percentage cell viability.

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3. Results and Discoussions

Figure 1 shows the powder XRD pattern of the synthesized powder. The diffraction peaks of the sample assigned to cubic fluorite CeO_2 are consistent with the JCPDS file of CeO_2 (JCPDS No. 34-0394). The patterns in Figure 1 the ceria nanoparticles were dried at 80 ^oC the XRD signals are weak. High background and the rather weak intensity of the reflections suggest considerable XRD-amorphous content to the samples. And also for Ce1 and Ce2 show that same precursor; different route was affected in the crystallinity. Reverse precipitation technique can provide small crystal size. Precursor difference (Ce3) causes the different XRD- pattern.

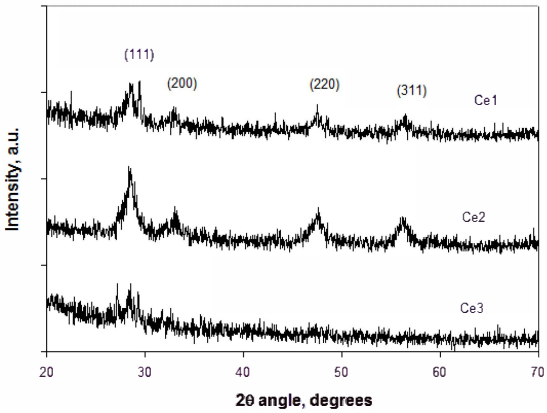

Figure 1: XRD pattern of nanoceria at heat treated 80^oC.

Figure 2. gives the zeta potential of three nanoceria samples (Ce1, Ce2, Ce3) as a function of pH in ultra-pure water. The zeta potential values of three nanoceria were in the same range. This is due to the chemicals involved in the synthesis process. Patil et al. also showed that the chemicals involved in the synthesis process were affected the difference in the zeta potentials [18]. Three nanoceria (Ce1,Ce2,Ce3) were treated with citric acid solution, this was influenced the zeta potential results of the powders. The isoelectric point (IEP) of nanoceria powders were approximately 2.5.

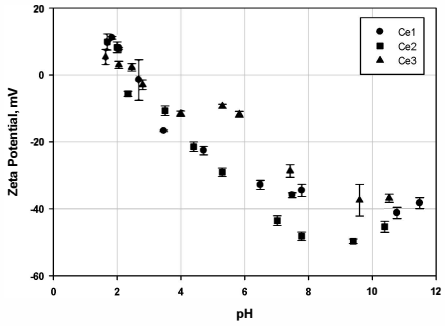

Figure 2: Zeta potential of the nanoceria samples in ultra pure water.

The effect of ceria naoparticles on cell viability of human colon adenocarcinoma cells (Caco-2) were investigated. Cytotoxicity measurements were performed and results were given in Figure 3.Cytotoxicity test using colon cancer cells showed that nanoceria have no cytotoxic effect against Caco-2 cells at low concentration and cytotoxicity changes with respect to synthesis conditions.

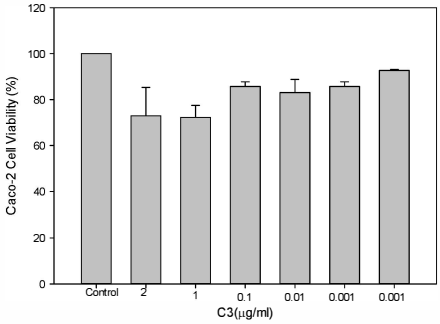

Figure 3: Cell viability (%) for Caco-2 cells incubated with ceria (C3) treated culture media (incubation times: 24h; concentrations: 2,1,0.1,0.01,0.001,0.0001 mg/ml)

4. Conclusion

Ultrafine ceria nanoparticles have been successfully synthesized by cerium nitrate hexahydrate and ammonium cerium(IV) nitrate as precursors. Effect of precursor and routes and calcination temperature was investigated, which showed that precursor and routes influence the size and morphology of the synthesized nanoparticles. Nanoceria powder which were produced by forward precipitation techniques led to larger crystallite sizes (8.6 nm) than reverse precipitation (7 nm). On the other hand nanoceria powders which were produced by ammonium cerium(IV) nitrate was shown amorphous content, low crystallinity and biggest size (12 nm. The advantages of the method, rapid synthesis, in a normal atmosphere, with low cost, give a potential avenue for further practical scale-up of the production process and applications. Cytotoxicity test using colon cancer cells showed that nanoceria have no cytotoxic effect against Caco-2 cells at low concentration and cytotoxicity changes with respect to synthesis conditions.

Kaynaklar

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