

Research Article

Preparation and Characterization of Modified Polyethyleneimine Magnetic Nanoparticles for Cancer Drug Delivery

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Magnetic nanoparticles with polymeric coating have great significance in drug delivery purpose. We intended to prepare a modified amphiphilic polymer with targeting susceptibility to reduce side effects to normal cells. In this study polyethyleneimine (PEI) as a polycationic polymer reacted with sebacoyl chloride to make a new amphiphilic polymer and folic acid as a targeting agent to reduce cytotoxicity of polymer and increase specific entrance of nanoparticles to cancerous cells. The obtained polymer (PEI-Sb-FA) was then coated on magnetic nanoparticles (MNPs) to stabilize them. The core-shell nanoparticles were characterized by different methods such as scanning electron microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), and vibrating sample magnetometer (VSM). Curcumin was finally loaded on PEI-Sb-FA-MNPs and the release behavior was studied in different pH. Curcumin loading of 28.2% was obtained and released drug in acidic pH = 4.5 was more than pH = 7.4, showing drug release sensitivity toward pH of media.

1. Introduction

Efficiency of many existing drugs is restricted by their poor solubility in water and toxicity against normal cells [1]. Traditional chemotherapy is not commonly successful in cancer therapy due to the severe side effects of anticancer drugs and induced toxicity. However nanotechnology can reduce these side effects including dermatologic, cardiac, gastrointestinal, nervous system-related, and hematologic effects. In drug delivery, it is important to deliver a medicine to the target site of therapeutic action with a specific dose [2]. For this purpose different carriers have been designed such as liposomes, hydrogels, biodegradable polymers, and magnetic nanoparticles. Polymeric nanoparticles have been widely used in drug delivery because they have small sizes that enable them to be transferred into the body from different routes [3].

The concept of drug delivery by magnetic nanoparticles was proposed by Widder et al. in 1978 [4]. They coated magnetic nanoparticles with polymeric shell as suitable carriers for drug delivery due to their high stability and super paramagnetism behavior and more biocompatibility. Polymers have emerged as an efficient biocompatible coating agent with the ability of tuning the surface functionality. Yallapu et al. [5] introduced magnetic drug carriers with a pluronic polymer (F127) shell for controlled delivery of curcumin. Iron oxide is one of the nanoparticles that is used for magnetic resonance imaging and cancer treating. The polymeric nanoparticles can be prepared via two different strategies including (a) Fe₃O₄ nanoparticles coating with a layer of polymer and (b) Fe₃O₄ nanoparticles precipitated in the presence of a porous polymer [6]. Polyethyleneimine (PEI) is a synthetic polymer that is frequently used in gene delivery systems. Amine

groups can be functionalized through different methods to control its toxicity [7].

For decades there has been rapid increase in using natural products for cancer therapy. One of these natural products is curcumin. Curcumin is extracted from *Curcuma longa*, possessing a number of beneficial biological characteristics [8].

In this work, Fe₃O₄ magnetic nanoparticles coated with a pH sensitive polymer and folic acid were used to increase targeting. The solubility of curcumin was increased by inclusion in the iron nanoparticles coated with modified polymer.

2. Materials and Methods

2.1. Materials. PEI (MW = 60000), sebacoyl chloride, acetone, folic acid, dimethyl sulfoxide (DMSO), ethanol, sorbitan monooleate (Span 80), triethylamine, ninhydrin, cyclohexane, N-hydroxysuccinimide, methanol, iron (III) chloride hexahydrate (FeCl₃·6H₂O), iron (II) chloride tetrahydrate (FeCl₂·4H₂O), disodium hydrogen phosphate, curcumin, (Na₂HPO₄·12H₂O), potassium dihydrogen phosphate (KH₂PO₄), potassium iodate (KIO₃), and acetonitrile were used. All of the reagents were analytical grade, purchased from Sigma Aldrich and used as soon as received without further purification. Deionized water was used to prepare all aqueous solutions.

2.2. Synthesis of Fe₃O₄ Nanoparticles. The coprecipitation method was used for the preparing of Fe₃O₄ nanoparticles. This method applies ferric and ferrous ions in a 1:2 molar ratio [9]. According to the procedure, 3.17 g of FeCl₂·4H₂O (0.016 mol) and 7.68 g of FeCl₃·6H₂O (0.008) were dissolved in 50 mL of deionized water. The mixture was stirred under N₂ at 85°C for 1 hr. Then 40 mL of NH₄OH solution was added and stirred under N₂ for 1 hr and then cooled to room temperature. The precipitated particles were washed three times with deionized water and were separated by a magnet and then dried under vacuum at 65°C.

2.3. Synthesis of PEI-Sb. Modified branched PEI network was prepared via interfacial polycondensation reaction between PEI (MW = 60,000) and sebacoyl chloride. A 2% v/v solution of Span 80 in cyclohexane (100 mL) was mixed with 30 mL of 10% wt aqueous solution of PEI (pH = 8.0). The mixture was stirred and 1.92 g (8 mmol) of sebacoyl chloride (sb) in 4 mL of cyclohexane was added. The reaction was allowed to proceed for 15 min under argon in ice bath and then it was diluted with 100 mL of cyclohexane. The resultant product in the form of beads settled within 1 day and was then separated from the liquid and rinsed several times with 100 mL of cyclohexane. The beads were transferred into the aqueous phase by dispersing the product in 100 mL of polysorbate 20 (Tween 20) (30% v/v) and 500 mL of deionized water was gradually added. The beads were then filtered and rinsed with water to eliminate traces of impurities [10].

2.4. Ninhydrin Test. The content of primary amino groups in PEI-Sb was determined using ninhydrin colorimetry test [11]. Polyethyleneimine was used as standard to prepare the

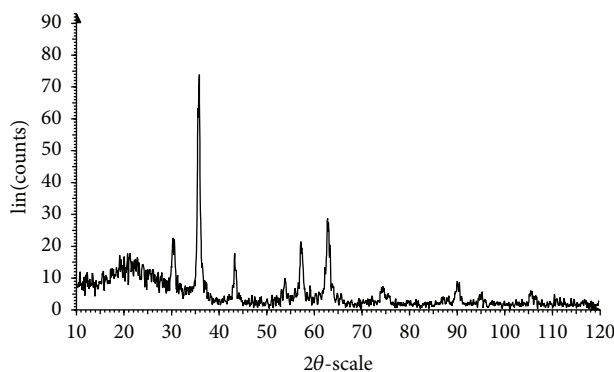
calibration curve (40, 55, 120, and 150 ppm). Absorbance measurements were determined at 570 nm wavelength. 2 mL of PEI in mentioned concentrations and PEI-seb solution (150 mg/mL) were prepared and then 1 mL of ninhydrin (0.005 g/L) reagent was added to every solution. The samples were subsequently heated in a boiling water bath for exactly 15 minutes and cooled to 20°C for 20 minutes. Afterwards 5 mL of diluting solution was added and mixed and the absorbance was measured at 570 nm by a UV-Vis spectrophotometer.

2.5. Preparing of Folate-Conjugated Polymer (Folate-PEI-Sb). Folic acid was initially activated by NHS [12]. In summary, folic acid (1 g) was dissolved in anhydrous DMSO (30 mL) and then NHS (0.9 g) and DCC (0.5 g) were added to the solution and the reaction was completed overnight by stirring at room temperature and under argon. 1,3-Dicyclohexylurea (DCU) was removed by filtration. To 5 mL of polymer solution containing PEI-Sb (0.4 g), triethylamine (0.05 mL) and then the filtered activated folate solution (3 mL) was added. The reaction proceeded at room temperature for 10 h under argon. Subsequently, the obtained solution was centrifuged and then filtered. After purification and rinsing, the obtained polymer was freeze-dried. The conversion of NH₂-PEI-Sb into folate-PEI-Sb was evaluated by dissolving the polymer in DMSO. The absorbance was measured at 363 nm by Cecil UV-Vis spectrophotometer to confirm the presence of folate in the sample [13].

2.6. Preparation of Core-Shell Magnetic Nanoparticles. To prepare core-shell FA-PEI-Sb-MNP, 5 mL MNP (10 mg/mL) was dispersed in water helping ultrasonication. Then, FA-PEI-Sb solution in DMSO (5.5 mg/mL) was mixed with the MNP dispersion. The suspension was stirred for 12 h at room temperature [14]. MNPs covered by polymer were collected by a permanent magnet and washed three times by deionized water and dried under vacuum desiccator.

2.7. Characterization of Magnetic Nanoparticles. Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and thermal gravimetric analysis (TGA) were taken to characterize the magnetic nanoparticles. The magnetic properties measurements were carried out by vibrating sample magnetometry (VSM) at room temperature. Fourier transform infrared spectra (FTIR) were recorded in Nicolet 870 by a KBr pellet. XRD spectra were taken by a Bruker axs D8 advance diffractometer utilizing the X-ray beam from Cu K α radiation. TGA results were obtained using L70/2003 thermal analyzer. The temperature range was raised up to 800°, at a ramp of 10°C/min with argon as inert gas. The VSM was applied to measure the magnetic properties of nanoparticles.

2.8. Drug Loading. 10 mg curcumin was dissolved in 1 mL acetonitrile and added dropwise to 10 mL of an aqueous dispersion of PEI-Sb-FA-MNPs (10 mg). Afterwards, the suspension was stirred overnight to allow penetration of curcumin into polymeric layer in core-shell nanoparticles at 12000 rpm. The obtained nanoparticles were washed three times in water to remove unloaded curcumin. The loaded

FIGURE 1: XRD of Fe_3O_4 -Polymer.

curcumin was estimated at 450 nm wavelength by a UV-Vis spectrophotometer (Cecil 9000) [15].

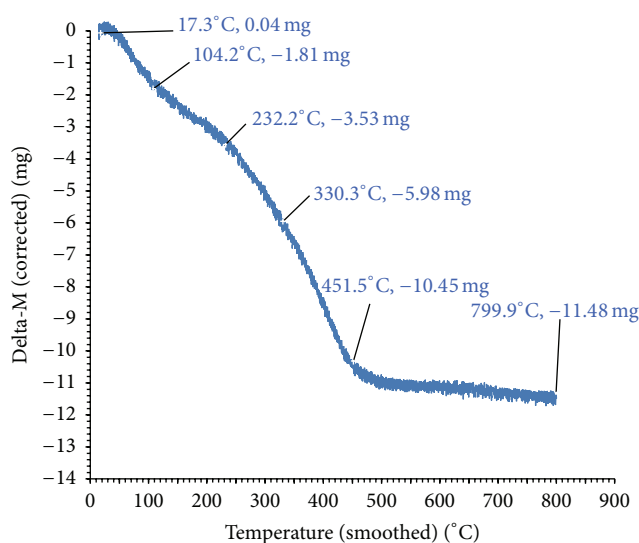
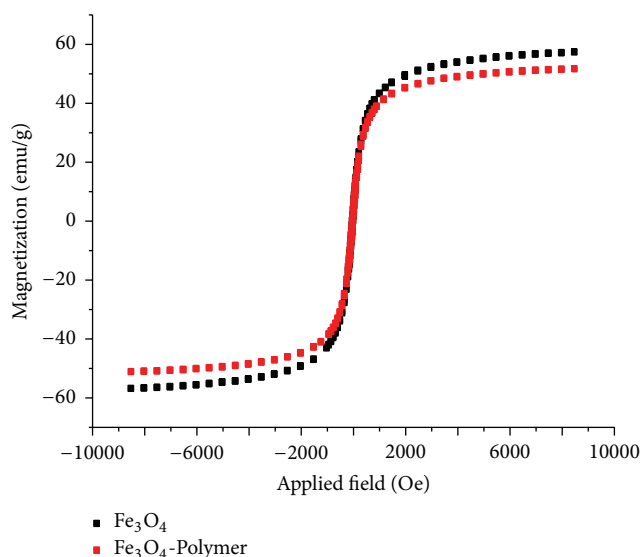
2.9. In Vitro Release Experiments. Curcumin release from core-shell polymeric magnetic nanoparticles was accomplished in PBS at different pH levels (pH 4.5 and pH 7.4). Briefly, 10 μg of MNPs containing curcumin was dispersed in 50 mL PBS solution in a tube. The sample was divided into centrifuged tubes and then placed in a shaking water bath with 120 rpm at 37°C. Because free curcumin is not soluble in water, the released curcumin in buffer at predetermined time intervals was centrifuged at 5000 rpm for 15 minutes to remove the released curcumin and redissolved in 1 mL of DMSO and the absorbance of solutions was measured at 450 nm using a UV-Vis spectrophotometer. Concentration of the solutions was determined using a calibration curve obtained from curcumin solutions (0–10 $\mu\text{g}/\text{mL}$) [13, 16].

3. Results and Discussion

The ninhydrin assay confirms that 54.63% of amine groups have been functionalized by carboxylic groups.

The XRD pattern shows magnetite peaks for iron oxide nanoparticles. The seven characteristic diffraction peaks (20.1, 30.1, 35.6, 43.1, 53.8, 57.2, and 62.8) are indicating that the crystalline iron oxide was formed completely. The crystalline size was approximately 28 nm calculated by Scherrer's equation. Finally, the results indicate preservation of crystalline structure of Fe_3O_4 for the functionalized Fe_3O_4 NPs (Figure 1).

Thermal gravimetric analysis was performed to evaluate the thermal stability of the nanoparticles. This analysis was recorded from ambient temperature to 800°C. Figure 2 shows the TGA curves for magnetic nanoparticles in nitrogen atmosphere at 20°C/min ascending temperature. The weight-loss areas were observed at 104.2°C, 232.2°C, 330.3°C, and 451.5°C, respectively. The first weight loss at the range of room temperature to 232°C could be attributed to the absorbed solvent evaporation. The second weight loss until around 451°C could be due to the polymer degradation on the surface of magnetic nanoparticles. Based on weight loss of about 10 mg at the range of 232–451°C, the coating amount of modified PEI on Fe_3O_4 was measured to be about 10%.

FIGURE 2: TGA curve of Fe_3O_4 -Polymer.FIGURE 3: Magnetic hysteresis curve of Fe_3O_4 and Fe_3O_4 -Polymer.

From Figures 1 and 2, we can realize that the nanoparticles have core-shell structure.

Magnetic hysteresis curves of Fe_3O_4 and modified PEI Fe_3O_4 NPs at room temperature are shown in Figure 3.

The nanoparticles display saturation magnetization equal to 57.09 and 51.4 for Fe_3O_4 and modified PEI- Fe_3O_4 emu/g. Saturation magnetization of FA-PEI-Sb-MNPs was lower than that of Fe_3O_4 that could be attributed to the modified polymer on the surface of nanoparticles. Paramagnetic behavior can use for carrier targeting and make them suitable for use in the pharmaceutical field.

The SEM micrograph of Fe_3O_4 -PEI nanoparticles is shown in Figure 4. The SEM images exhibit that the mean particle size is 17 nm. These images show that magnetic nanoparticles have spherical structure and enough monodispersity.

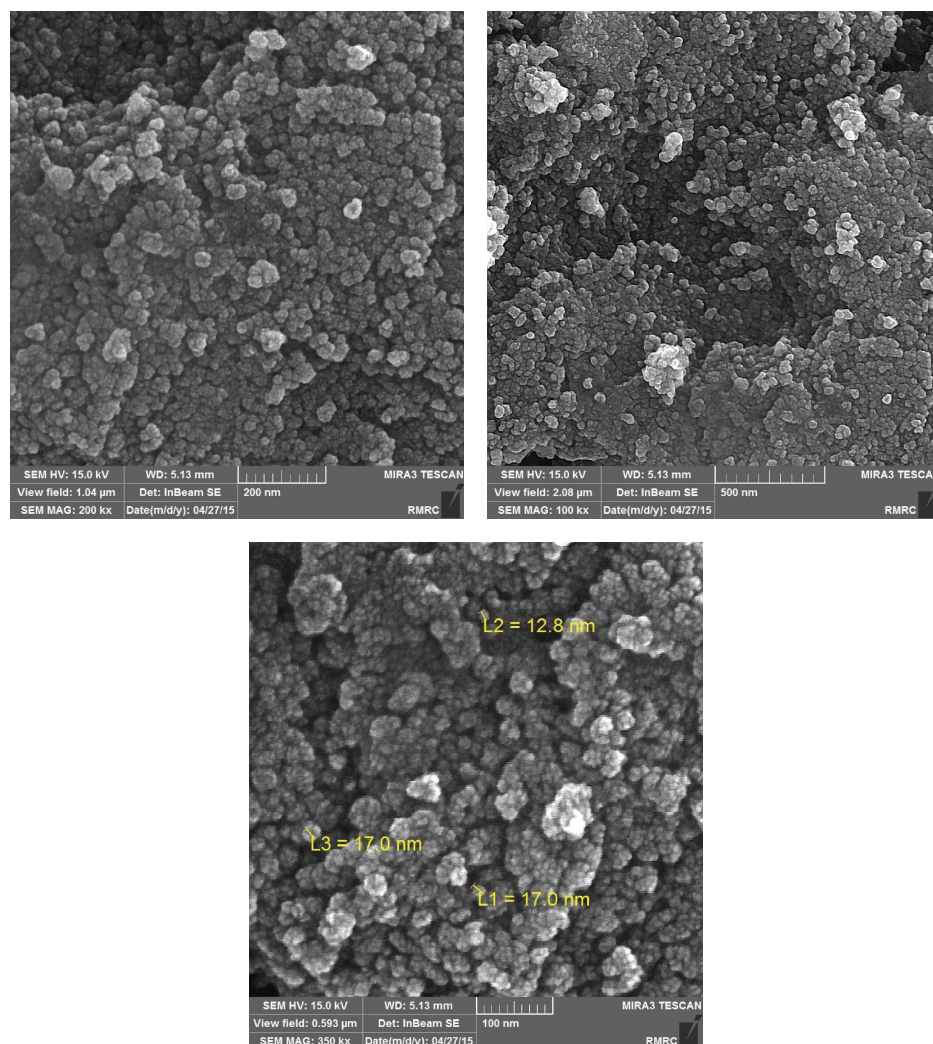


FIGURE 4: The SEM micrographs of FA-PEI-Sb-MNP nanoparticles and hydrodynamic size of them.

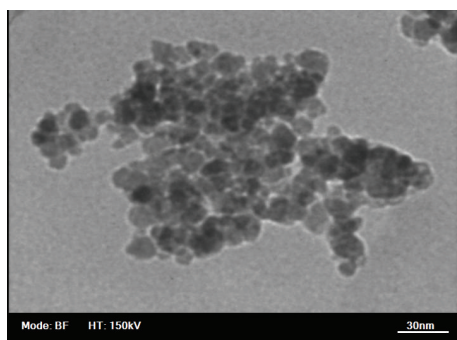


FIGURE 5: Transmission electron microscopy images of FA-PEI-Sb-MNP.

TEM (transmission electron microscopy) images of nanoparticles are shown in Figure 5.

The FTIR spectra of PEI, PEI-Sb, and FA-PEI-Sb are shown in Figure 6(a) and the FTIR spectrum of polymer coated- Fe_3O_4 is shown in Figure 6(b).

The band at 1609.01 can be ascribed to amide bond and the bands at 2916.83 and 2849.10 could be related to the stretching vibration of C-H bonds of the alkyl chain. The peak at 1705.49 shows the presence of carboxyl group (Figure 6(a), FTIR spectrum of PEI-Sb-FA). The major peaks for FA-Sb-PEI- Fe_3O_4 NPs can be determined as follows: 3353.65 (N-H stretching vibration), 2923.04, and 2837.53 (C-H stretching), 1026.56 (C-N vibration), and 1661.91 (C=O amide) (Figure 6(a), FTIR spectrum of PEI-Sb).

The calibration curve was used to calculate the drug loading in nanoparticles (Figure 7). The drug loading content of obtained magnetic nanoparticles showed a value equal 28.2%. The presence of Sb moiety conjugated with PEI- NH_2 produce amphiphilic character for the polymer which can control drug loading and drug release due to the pH sensitivity of the polymer as well as high affinity to cell penetration due to the positive charge of the carrier surface interaction with.

The release study of curcumin showed that release of curcumin in acidic medium was more sensitive than neutral

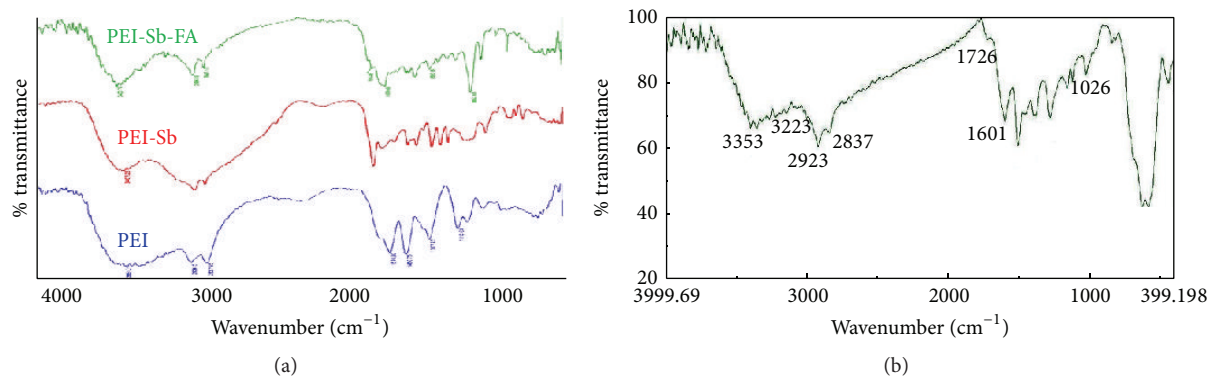


FIGURE 6: FTIR spectra of PEI with different ligands (a) and core-shell Fe₃O₄-modified PEI nanoparticles (b).

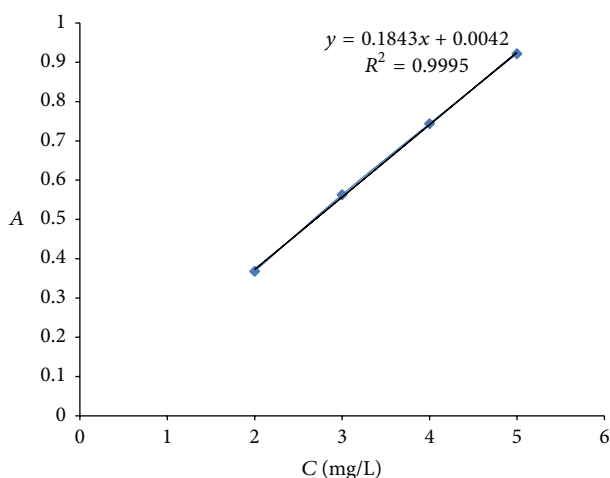


FIGURE 7: Curcumin loading calibration curve.

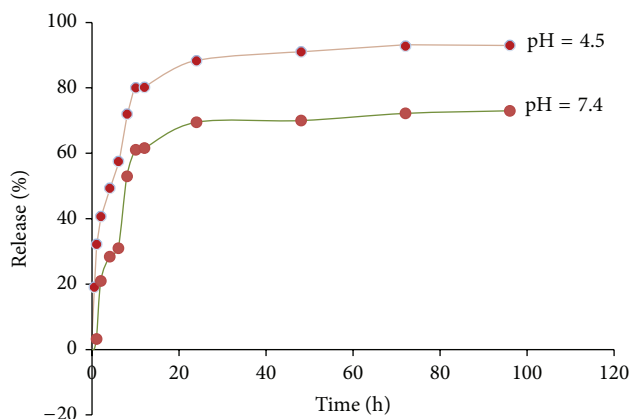


FIGURE 8: Evaluation of curcumin release from FA-PEI-Sb-MNPs.

medium and that is in accordance with cancer cell conditions (Figure 8).

4. Conclusion

Briefly, we synthesized a modified PEI coated magnetic in which PEI was modified by sebacyl chloride and targeted by folic acid. Particles diameter can be controlled by the relative amount of polymer. Nanoparticles were characterized by FTIR, XRD, VSM, SEM, and TGA. The results adapted each other. Curcumin was loaded into nanoparticles and release behavior was investigated.

Competing Interests

The authors declare that they have no competing interests.

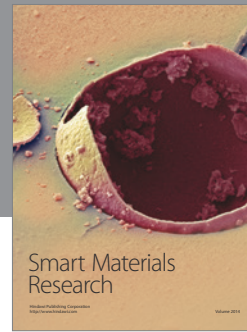
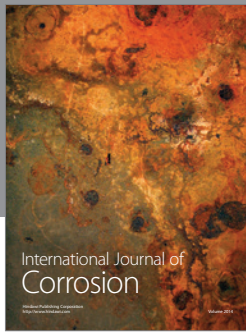
Acknowledgments

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