ISSN 1392–1320 MATERIALS SCIENCE (MEDŽIAGOTYRA). Vol. 18, No. 2. 2012

Formation of Conversion Coatings on Magnesium Alloy AZ31 in Solutions Containing Nicotinic Acid

Gedvidas BIKULČIUS [1]*, Alma RUČINSKIENĖ [1], Virginija BUKELSKIENĖ [2], Algirdas SELSKIS [1], Asta GRIGUCEVIČIENĖ [1]

[1] *Department of Materials and Corrosion Research, State research institute Center for Physical Sciences and Technology, A. Goštauto 9, LT-01018 Vilnius, Lithuania*
[2] *Department of Developmental Biology, Institute of Biochemistry, Mokslininku 12, LT-08662 Vilnius, Lithuania*

crossref http://dx.doi.org/10.5755/j01.ms.18.2.1912

Received 23 December 2010; accepted 18 September 2011

The coating of nicotinic acid (NA), sodium fluoride (SF) and sodium fluoric - nicotinic acid (SF-NA) deposited from a sodium fluoric solution with subsequent treatment in nicotinic acid on magnesium alloy AZ31 has been investigated. The corrosion resistance of coated AZ31 in a simulated body fluid (SBF) solution was studied using potentiodynamic polarization tests. A comparison polarization tests results of conversion coatings have shown a sharp reduction in corrosion current density from SF to NA, respectively 70.1×10^{-6} A/cm^2 to 20.4×10^{-6} A/cm^2. The morphology of the coatings was studied by a scanning electron microscope (SEM). The results revealed that there are cracks in the NA coatings. Despite the presence of cracks, the NA coatings better protect the AZ31 magnesium alloy against corrosion than as SF coatings. The cytotoxicity tests showed that AZ31 magnesium alloy with NA conversion coatings have no any toxic effect on the normal adult stem cells and this means that the magnesium AZ31 alloy with NA is distinguished for its good biocompatibility in a cell culture *in vitro*.
Keywords: magnesium alloys; conversion coatings; corrosion; degradable implant material, cytotoxicity.

INTRODUCTION

Bone plates are commonly used in bone fracture healing for internal fixation. They primarily provide mechanical support at the location of bone fracture before bone reunion has completed. Bone plates can be either non-degradable or degradable. Non-degradable or permanent bone plates are made of metallic materials such as Ti alloys and stainless steel. These materials have a high mechanical strength and are used in high load-bearing parts. In addition, they possess an excellent corrosion resistance and will remain inside the human body after bone reunion has completed. After the healing stage, the presence of a permanent bone plate results in a number of adverse effects, the most serious one being the occurrence of osteoporosis in the neighboring bone tissues due to mismatch in elastic modulus and hence stress shielding [1, 2]. For young patients, permanent bone plates also restrict bone growth [3]. In view of these adverse effects, a second surgery to remove the bone plate is usually recommended.

However, second surgery is accompanied by risk, economic burden, and psychological stress. Whereas, the degradable bone plates would gradually degrade and disappear inside the body. In this case, no second surgery is required, and the retention-removal dilemma in using permanent bone plates is solved. However, the degradable bone plates currently in use are made of polymeric materials, which are inherently of lower mechanical strength and are suitable for low load-bearing parts only. Development of degradable bone plates for high load-bearing parts only. Development of degradable bone plates for high load-bearing applications is thus a challenging problem in biomedical materials science.

Magnesium is potentially a wonderful implant material for its non-toxicity to the human body. Mg^{2+} is an essential element which is present in large amounts in the human body [4].

However, pure magnesium can corrode too quickly in the physiological (pH 7.4 – 7.6) and high chloride environment of the physiological system, losing mechanical integrity before the tissue has sufficiently healed and producing hydrogen gas in the corrosion process at a rate that is too fast to be dealt with by the host tissue [5].

Several possibilities exist to tailor the corrosion rate of magnesium by using alloying elements and protective coatings, processes that of course must lead to a non-toxic, biologically compatible material. It is clear that combination of magnesium alloy with preservative coating well helps at achieving corrosion resistance of implant.

A well-known method for the improvement of bone growth on metallic implant surfaces is one with fluoride conversion coating [6]. But long preparation time (24 h) in needed and working with HF acid is hazardous.

It is known [7] that nicotinic acid (C_5H_4NCOOH) as vitamin is a good inhibitor for mild steel [8], aluminium [9] and zinc [10]. Nicotinic acid, also known as pyridine-3-carboxylic acid, forms different types of metal complexes [11]. Besides, NA is very cheap, easily available, and what is the most important, nontoxic.

The present study is an attempt to modify the surface of magnesium alloy AZ31 implants by conversion treatment in sodium fluoride (SF), in nicotinic acid (NA) and in sodium fluoride with subsequent treatment in nicotinic acid (SF and NA).

*Corresponding author. Tel.: +370-5-2729389; fax.: +370-5-2648890.
E-mail address: *gbikulcius@chi.lt* (G. Bikulčius)

EXPERIMENTAL DETAILS

Samples of dimensions 10 mm × 40 mm × 2 mm were cut from commercial AZ31 magnesium alloy. The AZ31 samples were mechanically polished to a 2000 grit finish, degreased with acetone and were rinsed in distilled water.

The coating of sodium fluoride was deposited from 0.3 M sodium fluoride for 2 h (pH 8.5) on magnesium alloy AZ31 [12]. The coating of nicotinic acid was deposited at a concentration of 0.05 M for 2 h (pH 2.1). And the coating of sodium fluoride and nicotinic acid was deposited from a sodium fluoride solution (1 h) with subsequent treatment in nicotinic acid (1 h) on magnesium alloy AZ31. Samples of untreated AZ31 and sodium fluoride and/or nicotinic acid-coated AZ31 were immersed in a simulated body fluid (SBF) solution [4]: 8.0 g/l NaCl, 0.4 g/l KCl, 0.14 g/l $CaCl_2$, 0.35 g/l $NaHCO_3$, 1.0 g/l $C_6H_6O_6$ (glucose), 0.2 g/l $MgSO_4$ $7H_2O$, 0.1 g/l KH_2PO_4 H_2O, 0.06 g/l Na_2HPO_4 $7H_2O$ at 37 °C ±1 °C (pH 7.0).

The potentiodynamic polarization studies were carried out in naturally aerated SBF at 37 °C using a PGSTAT302 AUTOLAB(The Netherlands). A saturated $Ag\,|\,AgCl\,|\,KCl_{sat}$ electrode was used as reference. A platinum foil served as a counter electrode. Potentiodynamic polarization experiments were performed at a low scan rate of 2 mV/s. The corrosion current density (i_{corr}) was estimated by linear fit and Tafel extrapolation to the cathodic and anodic parts of the polarization curves.

Immersion tests of AZ31 samples coated with NA, SF-NA and SF were carried out in SBF solution at pH 7.0, kept at 37 °C in open air. The volume of solution was calculated based on a volume-to-sample area ratio of 50 mL/cm^2, which well exceeded the minimum ratio required by ASTM G31 [13]. After the immersion test for 4 h, the samples were then cleaned for removing the corrosion products formed on the samples using a standard chromium trioxide (CrO_3) solution recommended in ASTM G1-90 [14]. The corroded surface of the samples was studied using SEM.

SEM images were obtained using a digital scanning electron microscope Model EVO 50 XVP (Oxford Instruments, Analytical Ltd, UK). EDS X-ray maps were obtained at 20 kV on a scanning electron microscope EVO-50 EP (Carl Zeiss SMT) with Inca X-Sight spectrometer (Oxford Instruments). X-ray lines of Mg K_α, Al K_α, Zn K_α, C K_α ,N K_α, F K_α and O K_α were used for the characterization of elements distribution on the sample surface.

A screening test on *in vitro* cell culture was performed to obtain a preliminary evaluation of the acute cytotoxicity of the samples. Cytotoxicity tests were carried out by indirect contact [15]. Primarily, the NA samples were sterilized in a laminar camera with UV radiation, both sides for 15 min. The extracts of test samples were prepared using a cell growth medium as the extraction medium with the surface area of extraction medium ratio of 1.25 ml/cm^2 and incubated for 48 h [16].

After each procedure the sample-extract was withdrawn and diluted 1 : 9 in the growth medium. Such examples of diluted extracts were used for indirect cytotoxicity tests. The control cell groups involved the use of regular growth medium as positive control and 0.64 % phenol in growth medium as negative control.

In the study of cytotoxicity, primary adult myogenic stem cell line was applied. It was prepared from an adult rabbit muscle [17]. The cells were grown in Iscove's modified Dulbeco's medium (IMDM, Gibco) supplemented with 10 % of fetal calf serum (Gibco), penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were maintained at 37 °C in humidified atmosphere with 5 % CO_2 and passaged twice a week detaching cells from the plate by a 0.25 % (w/v) trypsin/EDTA solution (Gibco).

The cells were inoculated into 96-well tissue culture test plates (Orange Scientific) at a density of 3×10^3 cells per 100 μl in the growth medium in each well and incubated for 24 h. Then, the medium was replaced with 100 μl of prepared extracts and incubated for 24 h or 72 h. After that, the culture medium was removed and 10 μl of MTT was added to each well. MTT (3-(4.5-dimethyl-2-thiazolyl)-2.5-diphenayl-2H-tetrazolium bromide, Sigma) test is a colorimetric method for the quantitative evaluation of cell proliferation and viability *in vitro*. The cell samples were incubated with MTT for 1 h at 37 °C, the solution was removed and insoluble formazan was dissolved in the ethanol. The absorbance of colored solution was quantified by measuring at a 570 nm wavelength by a microplate reader (Tecan Infinite 200).

A qualitative analysis of cell viability was assessed in neighboring samples using an inverted optical and fluorescence microscope (Nicon). Cell visualization was accomplished by using a dye-mix solution of 100 μg/ml acridine orange (AO, Molecular probes) and 100 μg/ml ethidium bromide (EB, Sigma). A volume of 3 μl of this dye mixture was added to 50 μl of growth medium in the well. AO is taken up by viable cells. It intercalates into double-stranded DNA and makes it appear green. EB is only taken up by nonviable cells; it intercalates into DNA, making it appear orange [18].

RESULTS AND DISCUSSION

The polarization curves of the coatings obtained from different solutions are demonstrated in Fig. 1 and the corresponding corrosion potential and corrosive current are tabulated in Table 1.

The corrosion potential (E_{corr}) of NA coating have shown a negative shift to 70 mV as compared with that of magnesium alloy substrate with SF. The corrosion current density i_{corr} decreased sharply from 70.1×10^{-6} A/cm^2 of the substrate with SF coating to 20.4×10^{-6} A/cm^2 for NA coating.

Fig. 2 reveals the morphology of the conversion coatings on magnesium alloy after various treatments. Fig. 2, a, b, displays the morphology of AZ31 after 2 h treatment using the NA (0.05 M). A protective layer with "dry-mud" morphology is seen. Fig. 2, c, d, shows coatings on the magnesium alloy treated for 1 h in the SF solution with subsequent one-hour treatment in the NA solution. Network-like cracks as those in Fig. 2, a, b, are uniformly distributed all over the surface of the conversion-coated layer. It can be seen that some irregular cracks are formed on the surface. The conversion coatings were no longer continuous with some patches on the magnesium alloy surface. These cracks may possibly be due to hydrogen evolution during the conversion treatment and/or the

dehydration of the surface layer after treatment. Where as Fig. 2, e, f, presents the morphology of magnesium alloy after treatment in SF (0.3 M) without any cracks.

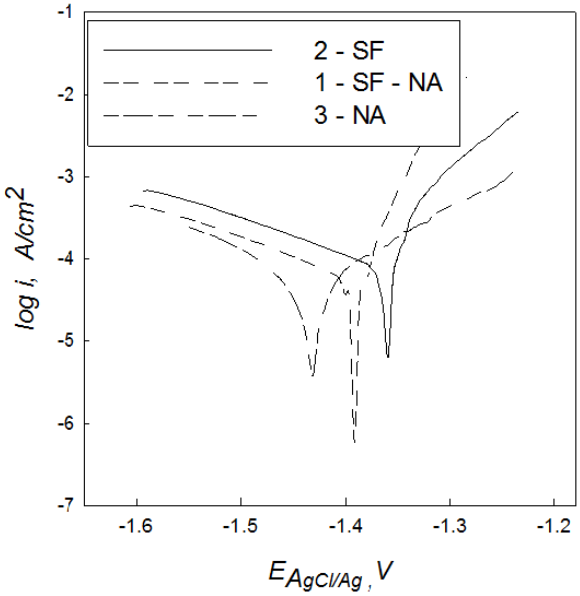

Fig. 1. Polarization curves of AZ31 in SBF: 1 – SF-NA; 2 – SF; 3 – NA

Table 1. Corrosion potentials and corrosion current densities obtained from the electrochemical potentiodynamic polarization curves

Sample	$-E_{corr}$ ($V_{Ag/AgCl}$)	i_{corr} (A/cm^2)
AZ31 treatment by immersing in nicotinic acid solution	1.43	20.4×10^{-6}
AZ31 treatment by immersing in NaF solution and in nicotinic acid solution	1.39	48.6×10^{-6}
AZ31 treatment by immersing in NaF solution	1.36	70.1×10^{-6}

Element distribution of the coating was analyzed using electronic probe microanalysis. Fig. 3 shows the EDS X-ray maps of magnesium, aluminum, zinc, fluorine, carbon, nitrogen and oxygen at the same place of the coating. The distribution images of the elements resembled the micrograph obtained by SEM which also presented a net-like structure. The distribution of the net coincided with the cracks in the conversion coatings. The quantities of magnesium, fluorine and zinc were increased while aluminum was sensed at the same sites. The carbon and oxygen elements mainly concentrated on the islands surrounded by the cracks.

So, the results of morphological studies seemingly contradict the ones of polarization studies. In our opinion the reason is that the protective coating of Mg alloy is formed of two layers. The former being unsoluble MgF_2 (in the cracks), and the latter being formed by NA and compounds of magnesium alloy elements (in islets). Thus, the two-layer coating is distinguished for its barrier protection of magnesium alloy.

The morphology of the sample surface is shown in the SEM micrographs in Fig. 4. It could be observed that all samples are similar in their corrosion behavior. To characterize the corrosion resistance of immersion samples of AZ31 we exploited the method of intersections lines based on the Saltykov method [19]. This method consists in calculating of the length of intersections across uncorroded areas in relation to the unit of intersection length. A linearly size (L) was calculated according to the equation:

$$L = \frac{l_1 + l_2 + ... + l_n}{l}, \quad (1)$$

where l_n is the length of intersections on uncorroded areas, mm; l is the total length of all intersections lines, mm.

According to (1), L decreases in the following order: NA > SF-NA > SF or 0.237>0.227>0.129, respectively. Thus, the immersion test has shown that the corrosion resistance of coatings NA is higher than that of SF coatings.

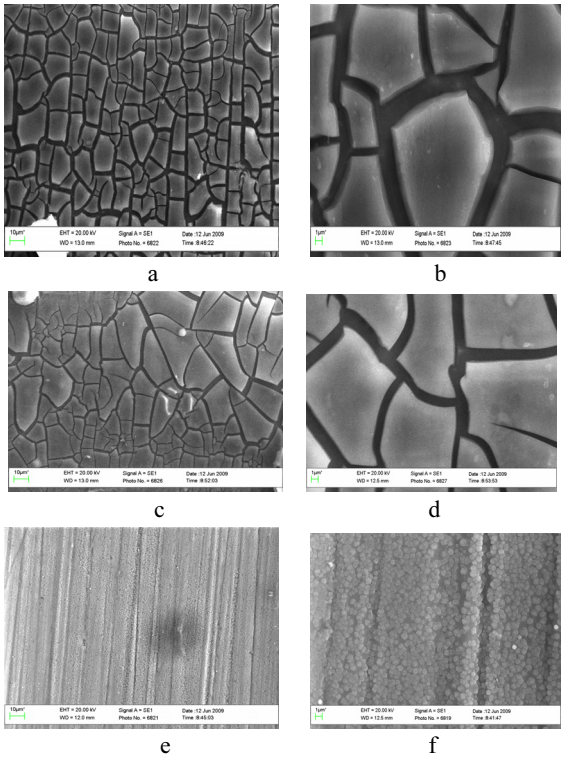

Fig. 2. SEM micrograph of AZ31 alloy after immersion in solution at 25 °C: (a, b) NA (2 h), (c, d) 0.3 M SF (1 h) and NA (1 h), (e, f) 0.3 M SF (2 h). Magnitude: a, c, e – ×400; b, d, f – ×2000

The experimental results suggest that 10 % of tested extracts did not indicate cytotoxic activity in normal adult stem cell culture. Fig. 5 shows the results of the indirect cytotoxicity test for magnesium alloy AZ31 samples. Qualitative analysis of the cell monolayer had no signs of lesion (Fig. 5, a – control culture, c – monolayer after the treatment). The study of cell viability certified the same cell viability level: after AO&EB staining the cells appeared green (viable) both in control and in tested cell cultures (Fig. 5, b and d). MTT quantitative analysis of the cells after 24 h and 72 h treatments (Fig. 5, e and f) demonstrated the same proliferative activity in the tested and control (negative) cultures. So, it can be concluded that these materials did not liberate any toxic components which could inhibit cell growth or provoke cell death.

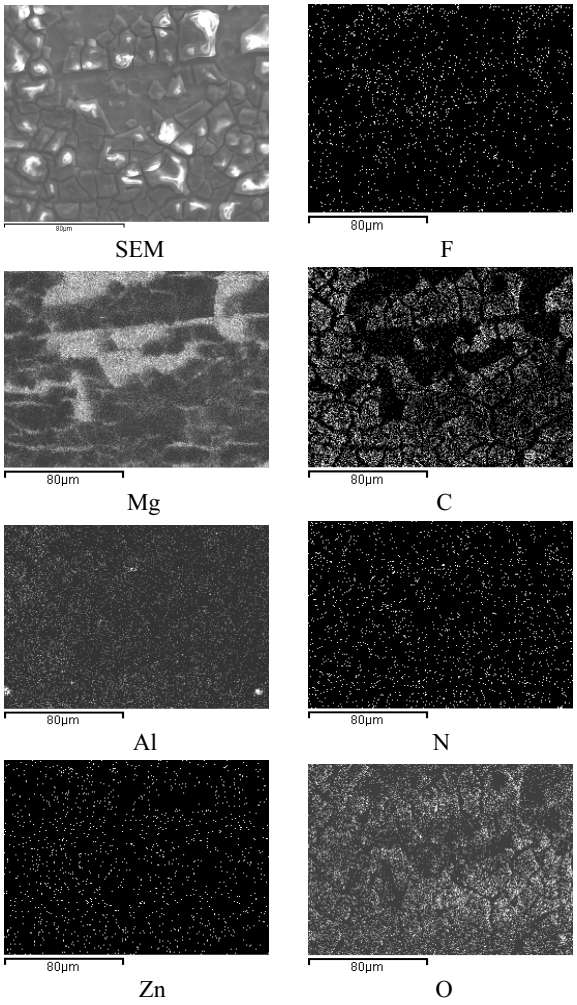

Fig. 3. Mapping pictures Mg, Al, Zn, F, C, N, O elements distribution of NaF-NA coating obtained from solutions 0.3 M SF (1 h) and 0.05 M NA (1 h). Magnitude: a – h – ×400

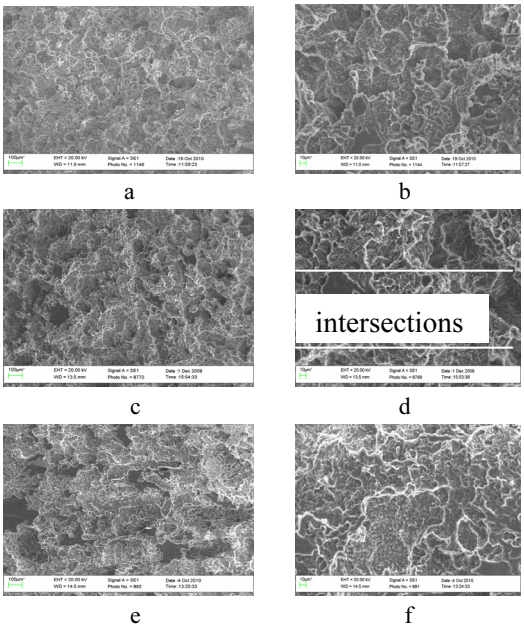

Fig. 4. SEM micrographs surface morphology after immersion in SBF at 37 °C, in open air, for 4 h, with corrosion products removed according to ASTM G1-90: (a, b) NA –coated, (c, d) (SF – NA) and (e, f) SF. Magnitude: a, c, e – ×400; b, d, f – ×2000

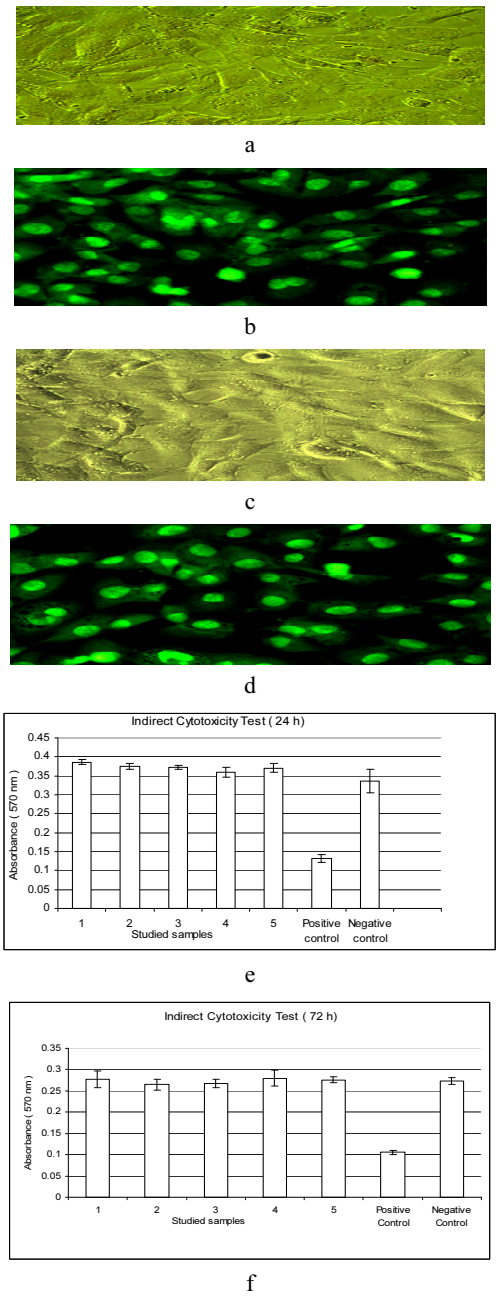

Fig. 5. Indirect cytotoxicity test for magnesium alloy AZ31 with coating obtained from 0.05 M NA solutions (2 h): qualitative analysis of cell culture assessed using an inverted optical (a and c) and fluorescence microscope (b and d; viable cells appear green); a and b – control adult myogenic stem cell culture; c and d – the cells after magnesium alloy AZ31 extract treatment; e and f – quantitative analysis of cytotoxic effect (MTT test, e – 24 h, f – 72 h extract treatment). Magnitude: a – d – ×200

CONCLUSIONS

A comparison polarization tests results for conversion coatings have shown a sharp reduction in corrosion current density from SF to NA, 70.1×10^{-6} A/cm^2 to 20.4×10^{-6} A/cm^2 respectively.

The morphology results revealed that the NA coatings possess cracks. Despite the presence of cracks, the NA coatings protect the AZ31 magnesium alloy against corrosion better than SF coatings.

Immersion tests recorded a significant increase in corrosion resistance due to the nicotinic acid conversion coating.

The cytotoxicity tests have shown that AZ31 magnesium alloy with NaF-NA conversion coatings in 10 % of the tested extracts did not indicate cytotoxic activity in a normal adult stem cell culture. This means that the magnesium AZ31 alloy with NaF-NA conversion coatings is distinguished for its good biocompatibility in cell culture *in vitro*.

Nicotinic acid is an efficient and environment-friendly inhibitor for magnesium alloy AZ31 in a simulated body fluid (SBF).

REFERENCES

1. **Nagels, J., Stokdijk, M., Rozing, P. M.** Stress Shielding and Bone Resorption in Shoulder Arthroplasty *Journal of Shoulder and Elbow Surgery* 12 2003: pp. 35 – 39.
2. **Lindsey, R. W., Gugala, Z. G., Torga-Spak, R.** Long-term Retention Versus Removal of Orthopedic Trauma Fixation Implants. In: M. R. Baugaertner and T. Tornetta, Editors, Orthopaedic Knowledge Update: Trauma 3 (3rd edition), American Academy of Orthopaedic Surgeons, 2005.
3. **Van der Elst, M., Klein, C. P. A. T., Patka, P., Haarman, H. J. T. M.** Biodegradable Fracture Fixation Devices. In: D. L. Wise, Editor, Biomaterials and Bioengineering Handbook, Marcel Dekker, 2000.
4. **Song, G.** Control of Biodegradation of Biocompatable Magnesium Alloys *Corrosion Science* 49 2007: pp. 1696 – 1701.
5. **Witte, F., Kaese, V., Haferkamp, H., Switzer, E., Meyer-Lindenberg, A., Wirth, C.J., Windhagen, H.** In Vivo Corrosion of Four Magnesium Alloys and the Associated Bone Response *Biomaterials* 26 2005: pp. 3557 – 3563.
6. **Chiu, K. Y., Wong, M. H., Cheng, F. T., Man, H. C.** Characterization and Corrosion Studies of Fluoride Conversion Coating on Degradable Mg Implants *Surface & Coating Technology* 202 2007: pp. 590 – 598. http://dx.doi.org/10.1016/j.surfcoat.2007.06.035
7. **Ju, H., Li, Y.** Nicotinic Acid as a Nontoxic Corrosion Inhibitor for Hot Dipped Zn and Zn-Al Alloy Coatings on Steels in Diluted Hydrochloric Acid *Corrosion Science* 49 2007: pp. 4185 – 4201.
8. **Subrahmanyam, D. V., Rama Char, T. L.** Nicotinic Acid as an Inhibitor for Corrosion of Mild Steel in Hydrochloric Acid Solutions *Anti-Corrosion Methods and Materials* 14 1993: pp.19 – 20.
9. **Desai, M. N., Desai, S. M., Gandhi, M. H., Shah, C. B.** Corrosion Inhibitors for Aluminium and Aluminium Based Alloys: Part 2 *Anti-Corrosion Methods and Materials* 18 1993: pp.4 – 10.
10. **Ju, H., Li, Y.** Nicotinic Acid as a Nontoxic Corrosion Inhibitor for Hot Dipped Zn and Zn-Al Alloy Coatings on Steels in Diluted Hydrochloric Acid *Corrosion Science* 49 2007: pp. 4185 – 4201.
11. **Abu-Youssef, M. A. M**. Two New 3D Network Structures: $[Cd_3(nic)_4(N_3)_2(H_2O)]_n$ and $\{Zn(nic)(N_3)]_n$ (nic=nicotinate anion) *Polyhedron* 24 2005: pp. 1829 – 1836. http://dx.doi.org/10.1016/j.poly.2005.05.026
12. **El-Taib Heakal, F., Fekry, A. M., Fatayerji, M. Z.** Influence of Halides on the Dissolution and Passivation Behavior of AZ91D Magnesium Alloy in Aqueous Solutions *Electrochemica Acta* 54 2009: pp. 1545 – 1557.
13. ASTM G31-72(2004), Standard Practice for Laboratory Immersion Corrosion Testing of Metals, ASTM Standards, Philadelphia, PA, USA, 2004.
14. ASTM G1-03, Standard Practice for Preparing, Cleaning, and Evaluation Corrosion Test Specimens, ASTM Standards, Philadelphia, PA, USA, 2003.
15. **Gu, X., Zheng, Y, Cheng, Y., Zhong, S., Xi, T.** In Vitro Corrosion and Biocompatibility of Binary Magnesium Alloys *Biomaterials* 30 2009: pp. 484 – 494.
16. EN ISO 10993-12:2004, Biological Evaluation of Medical Devices — Part 12: Sample Preparation and Reference Materials (ISO 10993-12:2002).
17. **Širmenis, R., Bukelskienė, V., Domkus, V., Sirvydis, V.** Cellular Cardiomyoplasty: Isolation and Cultivation of Skeletal Muscule Satellite Cells *Acta Medica Lituanica* 6 1999: pp. 178 – 181 (in Lithuanian).
18. **Mercille, S., Massie, B.** Induction of Apoptosis in Nutrient-Deprived Cultures of Hybridoma and Myeloma Cells *Biotechnology and Bioengineering* 44 1994: pp. 1140 – 1154.
19. **Saltykov**, **S. A.** Stereometrische Metallographie**.** VEB: Deutscher Verlag für Grund- stoffindustrie, Leipzig**,** Germany, 1974 (in German).