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Low-temperature microwave microplasma for bio-decontamination

***Abstract**. This paper presents results of the investigations of an atmospheric pressure Ar and Ar/O_2 microwave (2.45 GHz) microplasmas which can be used in the biomedical applications. The microplasma in the form of a column was generated using a simple, coaxial microwave microplasma source (MMS). The gas temperature at the microplasma tip was as low as about 300 K. This makes the microwave microplasma suitable for many applications, including bio-medical. Preliminary test with Escherichia coli K-25 indicated antibacterial effect of Ar and Ar/O_2 microplasmas.*

Streszczenie.** Prezentowana mikrofalowa (2,45 GHz) mikroplazma Ar oraz Ar/O_2 może znaleźć zastosowanie w medycynie, np. przy dezynfekcji. Mikroplazmę w kształcie kolumny wytwarza prostej konstrukcji, współosiowy mikrofalowy generator mikroplazmy. Temperatura na szczycie kolumny mikroplazmy jest niska, rzędu 300 K. To czyni mikroplazmę użyteczną do zastosowań w medycynie. Wstępne testy z użyciem bakterii Escherichia coli K-25 wskazują na antybakteryjne działanie mikroplazmy Ar i Ar/O_2. **(Niskotemperaturowa mikrofalowa mikroplazma do biodekontaminacji).

Keywords: plasma sources, microwave discharges, tuning characteristics, gas processing.
Słowa kluczowe: generatory plazmy, wyładowania mikrofalowe, charakterystyki strojenia, obróbka gazów.

Introduction

The interest in the atmospheric pressure low-temperature microplasmas is growing because of many merits of such a microplasma: small size (from µm to several mm), portability of the source, easy to use, low investment and operation costs. The microplasmas can be used for microwelding and surface modification, as a light sources, and in atomic spectroscopy systems. Also there is interest in the microplasmas used in the biomedical applications such as sterilization of medical instruments, high-precision surgery, cells treatment and deactivation of bacteria and viruses [1-7].

Sterilization is a physical or chemical process that impairs or eliminates, especially microorganisms like bacteria. Many sterilization methods such as autoclaving, γ–irradiation, ethylene oxide (EtO) and UV sterilization, as well as plasma sterilization are currently being used. However, most of methods for sterilization need lengthy time and can be operated only in closed space while other methods are toxic to the human body [8, 9]. A significant advantage of plasma sterilization is the possibility to achieve a high level of sterilization at low temperatures with no residual toxicity. The sterilization is achieved due to the reactive species in the plasma (electrons, ions, radicals, reactive molecules, UV light, etc.) [10]. Many plasma sterilization systems were developed: low [11] or atmospheric pressure [12], DBD [13], RF [14] or microwaves [15]. One of challenges is the application of non-thermal plasmas directly on the surface of human body or on internal organs [16]. Whereas for surface modification and biological decontamination both low-pressure and atmospheric pressure plasmas can be used. For direct therapeutic applications only small size atmospheric pressure microplasma sources are applicable.

In this paper we report results of the experimental investigation of a simple coaxial microwave microplasma source (MMS). The main advantages of the presented MMS are simplicity and low cost. The paper is focused on low-temperature Ar and Ar/O_2 microwave microplasma.

Microwave microplasma source MMS

The presented MMS (Fig. 1) is a more advanced version of previous MMSs developed by us and described in [17-20]. The structure of the MMS shown in Fig. 1 is based on a coaxial line, formed by the inner (a brass rod ended with a thinner rod top) and outer (a brass cylinder) conductors. The top of inner conductor was made of tungsten. The inner conductor is fixed inside the outer conductor tightly with a PTFE centring disc. The operating gas was supplied through a void duct between the inner and outer conductors. The MMS was connected to the coaxial cable using N-type connector. The microwave power was supplied through a 50 Ω coaxial cable from a 2.45 GHz microwave magnetron generator.

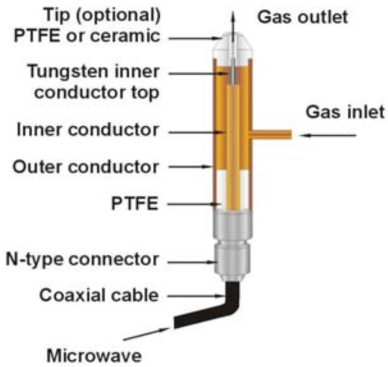

Fig.1. The sketch of coaxial microwave microplasma source

The MMS could work with various gases like Ar or N_2. Also working with their mixtures with O_2 (up to 5%) was possible. The microplasma was generated by the MMS in the form of a tiny candle-like flame (in Ar, Ar/O_2 at low absorbed powers) or a plasma jet (in Ar/O_2 at high absorbed powers, N_2, N_2/O_2) above the inner conductor top (see Fig. 2). The length and diameter ranged from 5 - 25 mm and 0.5 - 2 mm, respectively for Ar microplasma and from 2 - 30 mm and 2 - 16 mm, respectively for Ar/O_2, N_2, N_2/O_2 microplasma, depending on the operating parameters. The N_2 and N_2/O_2 microplasmas had high gas temperatures, when Ar microplasma was relatively low-temperature. Additive of O_2 to Ar microplasma caused mild increase of temperature for comparable absorbed microwave power levels.

Optionally, when worked with the Ar, the MMS could be operated with a PTFE or MACOR® ceramic tip. This tip played three functions: it formed a kind of nozzle that increased the velocity of argon in plasma forming zone, it prevented breakdowns between the inner and outer conductors, specially in case operating in the Ar, and it covered the hotter part of the microplasma column, thus exposing only the lowest temperature microplasma (i.e. its tip). The MACOR® ceramic tip allowed also for work in

Ar/O_2 mixture, although the tested tip was to high and exposing only a little part of microplasma at high absorbed power levels (see Fig. 2.). That was the reason that operating in Ar/O_2 mixture was tested without a tip.

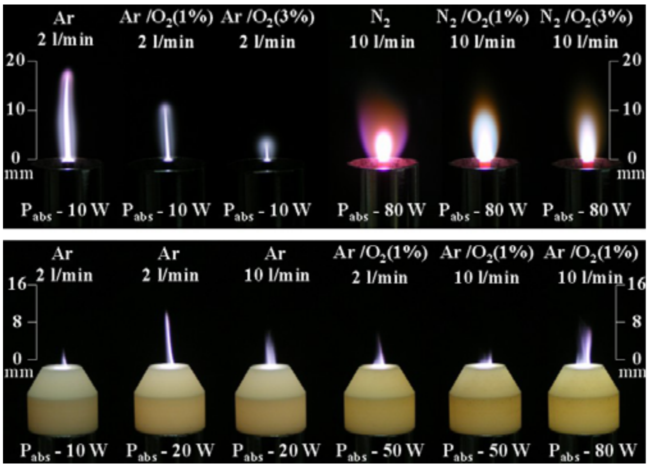

Fig.2. The photos of the microwave microplasma for various operating gases. MMS without a tip and with the MACOR® ceramic tip.

Experiments

The main parts of the experimental setup (see Fig. 3) used in these measurements were the magnetron generator (2.45 GHz), microwave power measuring system, the MMS, gas supplying and flow control system, and spectrometer (CVI DK-480 with 1200 gr/mm and 3600 gr/mm grating), equipped with a CCD camera and a PC computer, for emission spectra analysis. The microwave power P_{abs} absorbed by the microplasma was determined from the difference (P_I - P_R), where P_I and P_R are the incident and reflected microwave powers, respectively. The incident P_I and reflected P_R microwave powers were directly measured using directional coupler and dual-channel power meter.

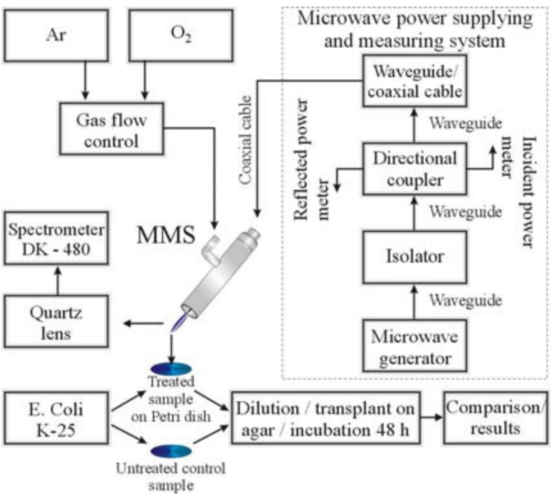

Fig.3. Diagram of the experimental setup for diagnostics of low-temperature microplasma for bio-sterilization.

To assess the usefulness of the argon microwave plasma for the biomedical applications, e.g. for sterilization, first we performed spectroscopic measurements (Optical Emission Spectroscopy) of the electron density, microplasma temperatures and active species identification.

The electron density in the argon microplasma was determined from the Stark broadening of H_β spectral line of the hydrogen Balmer series [20] using either GKS theory of Griem, Kolb and Shen [21] or Gig-Card theory of Gigosos and Cardenoso [22]. The H_β spectral line was observed in the emission spectrum due to the presence of water vapour in the microplasma (from the ambient air). The rotational spectra of OH radicals ($A^2\Sigma^+ \rightarrow X^2\Pi$) and N_2 molecules second positive system ($C^3\Pi \rightarrow B^3\Pi$) were employed for the determination of rotational temperatures of OH and N_2 species which were present in the microplasma due to the absorption of gases, including water vapour, from the ambient air. The measured spectra were compared with those simulated in SPECAIR program [23] in order to determine rotational temperatures of OH radicals and N_2 molecules.

Preliminary tests of biocidal effect with *Escherichia coli* K-25 bacteria were performed. *E. coli* used as the bio-indicator was obtained from Chemical Faculty, Gdansk University of Technology. The 0.1 ml of 10^9 CFU/ml (Colony Forming Unit/ml) dilution were placed on four sterile Petri dishes. One of them was treated with Ar, second with Ar/O_2(0.5%) and third with Ar/O_2(1%) microplasma. After treatment samples were transport to laboratory in Chemical Faculty, Gdansk University of Technology. There, each Petri dish was rinsed with 1 ml of physiological saline. The saline obtained after rinsing was diluted with fresh saline of dilution ratio 1:10. The 1 ml of this dilution were transplanted to Tryptic Soy Agar and then incubated for 48 hours. The last sample was processed parallel the same way, excluding microplasma treatment. At the end all samples were compared. The test was simple and not aimed to quantity results but only to indicate biocidal effect.

Results

The MMS was not equipped with any tuning element. Nevertheless the device was sufficiently matched for various gases in various configuration. As seen in Fig. 4, the reflection coefficient P_R / P_I (P_R - reflected microwave power, P_I – incident microwave power) for Ar and N_2 as a working gas was less than 5 %.

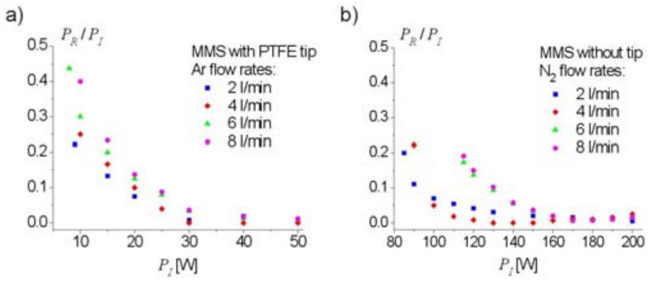

Fig.4. The reflection coefficient P_R / P_I (P_R - reflected microwave power) as a function of the incident microwave power P_I for the different argon (a) and nitrogen (b) flow rate.

The operating in Ar and Ar/O_2 (up to 5%) was tested without MMS tip for flow rates from 1 l/min up to 10 l/min. In Fig. 5 required absorbed powers for different O_2 additive are presented. The required absorbed powers were defined by: the minimum power necessary for stable discharge and maximum power, when breakdowns between the inner and outer conductors occurred. The MMS worked stable with Ar and Ar/O_2 for gas flow higher than 2 l/min. Below this value breakdowns between the inner and outer conductors occurred for relatively low absorbed microwave powers in pure Ar microplasma and Ar/O_2 microplasma become very hot due to the fact that the air surrounding it entered into it. As seen in Fig. 5, increasing O_2 additive caused increase of required minimal absorbed power. On the other hand increasing O_2 prevented breakdowns between the inner and outer conductors for higher absorbed powers. Figure 6 shows the reflection coefficient P_R / P_I for Ar/O_2 as a working gas. This coefficient was higher than that in Fig. 4, but it was still less than about 15%.

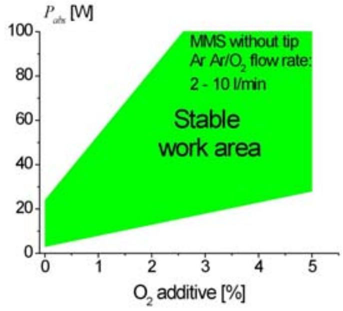

Fig.5. Required absorbed microwave powers for stable microplasma generation for different O_2 additive. MMS without tip. Ar, Ar/O_2 flow rate from 2 l/min up to 10 l/min.

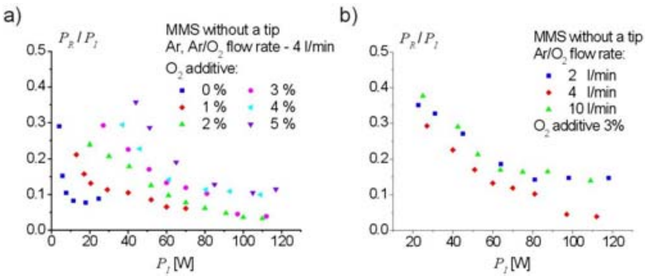

Fig.6. The reflection coefficient P_R / P_I (P_R - reflected microwave power) as a function of the incident microwave power P_I for the different O_2 additive at Ar, Ar/O_2 flow rate of 4 l/min (a) and for different flow rates of Ar, Ar/O_2 at 3% of O_2 additive (b).

The measured electron density for Ar microplasma varied from 6*10^{14} to 1.4*10^{15} cm^{-3}, depending on operating parameters and location within the microplasma column. Figure 7 shows the electron number density n_e as a function of distance above the inner conductor top (distance AICT) in Ar microplasma column (P_{abs} – 10W, Ar flow rate – 2 l/min). Generally, the electron number density increased with increasing absorbed power and no influence of Ar flow rate for it was observed.

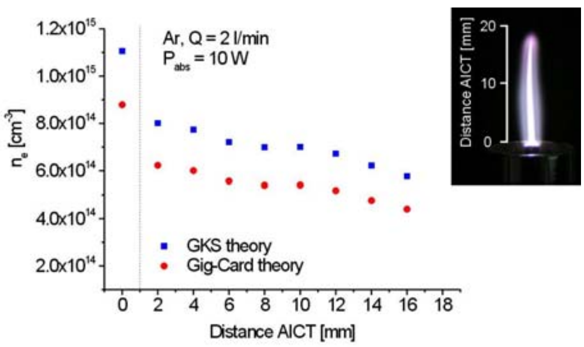

Fig.7. Electron number density n_e as a function of distance above the inner conductor top (AICT) in Ar microplasma column. P_{abs} - 10 W, Ar flow rate - 2 l/min. MMS without tip.

Figure 8 presents the influence of O_2 additive on emission spectra at 602 – 618 nm. The identification of lines were performed with NIST database [24]. The presents of O atoms (O I lines at 615.7 nm) in microplasma was observed. The lines at 615.59, 615.67 and 615.82 nm are very close to each other and was observed as a one due to DK-480 spectrometer (1200 g/mm grating) spectral resolution. This lines could be observed even without additive of O_2 to working gas. The O_2 in this case was coming from air surrounding microplasma. As seen in figure the intensity of this lines (proportional to the excited O I atoms population in microplasma) increased with increasing O_2 additive.

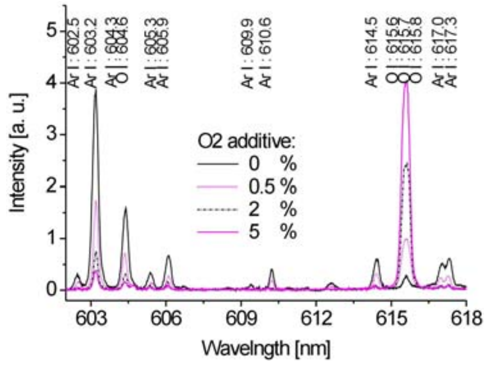

Fig.8. Influence of O_2 additive on emission spectra at 602 nm – 618 nm. P_{abs} – 20 W, Ar flow rate – 4 l/min. MMS without tip.

In Fig. 9. comparison of the measured and simulated emission spectra of N_2 second positive system (b) and OH (A-X) band (c) are presented. The rotational temperatures were determined to be about 500 K for OH radicals and 800 K for N_2 molecules at the microplasma core of visible part of column, when MMS was operated with MACOR® ceramic tip. These values were measured at an absorbed microwave power of 10 W and Ar flow rate of 10 l/min. The obtained allows us to estimate the microplasma gas temperature. In our previous works [17] using a thermocouple we found that the microplasma gas temperature at the microplasma tip could be as low as 303 K. Fig. 9a presents the possibility of skin treating using Ar microplasma.

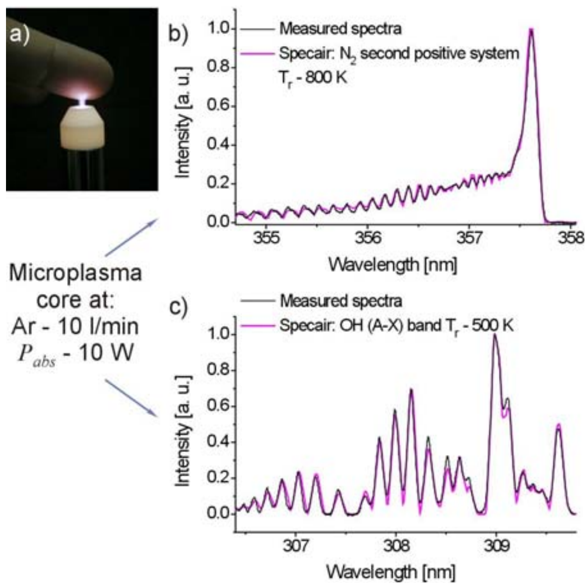

Fig.9. Photo of Ar microplasma (a), comparison of the measured and simulated emission spectra of OH (A-X) band (b) and N_2 second positive system (c). P_{abs} −10 W, Ar flow rate – 2 l/min. MMS with PTFE tip.

The biocidal effect test was performed for Ar, Ar/O_2(0.5%) and Ar/O_2(1%) microplasma. The sample treatment time was 300 sec, the absorbed microwave power was 10 W and the flow rate was 4 l/min. The result indicated reduction of bacteria population for all treated samples. The reduction seemed to be grater for Ar/O_2(0.5%) and Ar/O_2(1%) than for pure Ar microplasma. Fig. 10 presents comparison of samples after transplant to agar and incubation. In Fig. 10a the untreated sample, in Fig. 10b the sample treated with Ar microplasma and in Fig. 10c the sample treated with Ar/O_2(0.5%) microplasma are shown. Bacteria colony distribution in untreated sample result was uniform, while in treated with microplasma sample results distribution of bacteria colony became spotted due to the lower number of CFU in treated samples.

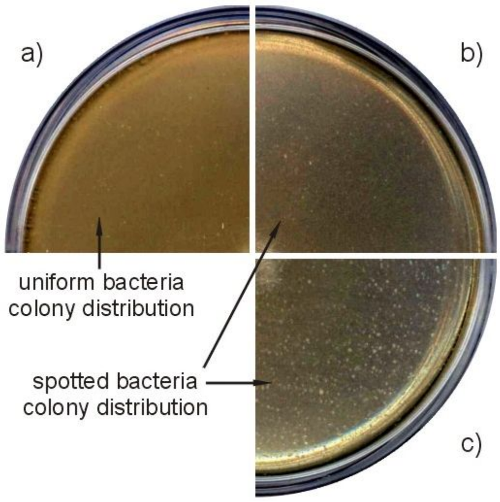

Fig.10. Comparison of incubated samples (a) in the untreated sample (a) and in Ar (b) and Ar/O_2(0.5%) (c) microplasma treated samples.

Conclusions

The high density Ar and Ar/O_2 microplasma generated by coaxial microwave microplasma source is presented in this paper. The simplicity of the source, stability of the microplasma and wide range of its parameters allow the conclusion that the MMS can find practical applications in various fields. The relatively low gas temperature (from 303 K) in Ar microwave microplasma allows using it in medicine for treatment of alive tissues without burning them. The presents of active radicals like OH or O indicate that it could be useful in sterilization. Preliminary test with *Escherichia coli* K-25 indicated antibacterial effect of Ar and Ar/O_2 microplasma. The sterilization quantity investigation are under going.

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REFERENCES

[1] Becker K.H., Kogelschatz U., Schoenbach K.H., Barker R.J., Non-Equilibrium Air Plasmas at Atmospheric Pressure, IOP Publishing, Bristol, 2005
[2] Fridman A., Plasma Chemistry, Cambridge University Press, New York, 2008
[3] Kawai Y., Ikegami H., Sato N., Matsuda A., Uchino K., Kuzuya M., Mizuno A., Industrial Plasma Technology, WILEY-VCH Verlag GmbH & Co. KgaA, Weinheim, 2010
[4] Bogaerts A., Neyts E., Gijbels R., van der Mullen J., Gas discharge plasmas and their applications, *Spectrochim. Acta Part B*, 57 (2002), No. 4, 609-658
[5] Tendero C., Tixier C., Tristant P., Desmaison J., Leprince P., Atmospheric pressure plasmas: A review, *Spectrochim. Acta Part B*, 61 (2006), No. 1, 2-50
[6] Ehlbeck J., Schnabel U., Polak M., Winter J., von Woedtke Th., Brandenburg R., von dem Hagen T., Weltmann K.-D., Low temperature atmospheric pressure plasma sources for microbial decontamination, *J. Phys. D: Appl. Phys.*, 44 (2011), No. 1, 013002-013002-18
[7] Lee H.W., Park G.Y., Seo Y.S., Im Y.H., Shim S.B., Lee H.J., Modelling of atmospheric pressure plasmas for biomedical applications, *J. Phys. D: Appl. Phys.*, 44 (2011), No. 5, 053001-053001-27
[8] Choi J.H., Han I., Baik H.K., Lee M.H., Han D.-W., Park J.-C., Lee I.-S., Song K.M., Lim Y.S., Analysis of sterilization effect by pulsed dielectric barrier discharge, *J. Electrostat.*, 64 (2006), No. 1, 17-22
[9] Xu L., Terashita F., Nonaka H., Ogino A., Nagata T., Koide Y., Nanko S., Kurawaki I., Nagatsu M., Discharge conditions for CW and pulse-modulated surface-wave plasmas in low-temperature sterilization, *J. Phys. D: Appl. Phys.*, 39 (2006), No. 1, 148–152
[10] Moisan M., Barbeau J., Moreau S., Pelletier J., Tabrizian M., Yahia L'.H., Low-temperature sterilization using gas plasmas: a review of the experiments and an analysis of the inactivation mechanisms, *Int. J. Pharm.*, 226 (2001), No. 1-2, 1-21
[11] von Keudell A., Awakowicz P., Benedikt J., Raballand V., Yanguas-Gil A., Opretzka J., Flotgen C., Reuter R., Byelykh L., Halfmann H., Stapelmann K., Denis B., Wunderlich J., Muranyi P., Rossi F., Kylian O., Hasiwa N., Ruiz A., Rauscher H., Sirghi L., Comoy E., Dehen C., Challier L., Deslys J.P., Inactivation of Bacteria and Biomolecules Low-Pressure Plasma Discharges, *Plasma Process Polym.*, 7 (2011), No. 3-4, 327–352
[12] Lee K.-Y., Park B.J., Lee D.H., Lee I.-S., Hyun S.O., Chung K.-H., Park J.-C., Sterilization of Escherichia coli and MRSA using microwave-induced argon plasma at atmospheric pressure, *Surf. & Coat. Technol.*, 193 (2005), No. 1-3, 35-38
[13] Laroussi M., Leipold F., Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure, *Int. J. Mass Spectrom.,* 233 (2004) , No. 1-3, 81-86
[14] Yang L., Chen J., Gao J., Guo Y., (2009) Plasma sterilization using the RF glow discharge, *Appl. Surf. Sci.*, 255 (2009), No. 22, 8960–8964
[15] Sato T., Fujioka K., Ramasamy R., Urayama T., Fujii S., Sterilization Efficacy of a Coaxial Microwave Plasma Flow at Atmospheric Pressure, *IEEE Trans. on Industry Appl.*, 42 (2006), No. 2, 399-404
[16] Nastuta A.V., Topala I., Grigoras C., Pohoata V., Popa G., Stimulation of wound healing by helium atmospheric pressure plasma treatment, *J. Phys. D: Appl. Phys.*, 44 (2011), No. 10, 105204-105204-9
[17] Goch M, Jasiński M, Mizeraczyk J, Zakrzewski Z., Microwave Microdischarge Generator Based on Coaxial Line, *Przegląd Elektrotechniczny*, 84 (2008), nr 3, 80-82
[18] Jasiński M, Kroplewski L, Zakrzewski Z, Mizeraczyk J (2008) Atmospheric Pressure Microwave Microplasma Sources. *Chemicke Listy*, 102 (2008), S1322-S1326
[19] Jasiński M., Zakrzewski Z., Mizeraczyk J., New Atmospheric Pressure Microwave Microplasma Source, *Acta Technica CSAV*, 53 (2008), 347-354
[20] Hrycak B., Jasiński M., Mizeraczyk J., Spectroscopic investigations of microwave microplasmas in various gases at atmospheric pressure, *Eur. Phys. J. D*, 60 (2010), No. 3, 609-619
[21] Griem H.R., Spectral Line Broadening by Plasmas, Academic Press, New York, 1974
[22] Gigosos M.A., Cardenoso V., New plasma diagnosis tables of hydrogen Stark broadening including ion dynamics n.20, *J. Phys. B: At. Mol. Opt. Phys.*, 29 (1996), No. 20, 4795
[23] http://www.specair-radiation.net Accessed 7 September 2011
[24] http://www.nist.gov/pml/data/asd.cfm Accessed 7 September 2011

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