Eur. Phys. J. Appl. Phys. (2016) 75: 24711
DOI: 10.1051/epjap/2016150590

THE EUROPEAN PHYSICAL JOURNAL APPLIED PHYSICS

Regular Article

Inactivation of Escherichia coli on PTFE surfaces by diffuse coplanar surface barrier discharge*

Zlata Tučeková[1,a], Zuzana Koval'ová[2], Anna Zahoranová[1], Zdenko Machala[2], and Mirko Černák[1,3]

[1] Department of Experimental Physics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská Dolina, 842 48 Bratislava, Slovakia
[2] Division of Environmental Physics, Faculty of Mathematics, Physics and Informatics, Comenius University, Mlynská Dolina, 842 48 Bratislava, Slovakia
[3] R&D Center for Low-Cost Plasma and Nanotechnology Surface Modifications, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Received: 30 November 2015 / Received in final form: 19 April 2016 / Accepted: 9 May 2016

Abstract. The non-equilibrium plasma of diffuse coplanar surface barrier discharge (DCSBD) was tested for decontamination of bacteria *Escherichia coli* on polymer surfaces. We investigated the optical parameters of DCSBD plasma generated in synthetic air with different relative humidity. Our study was provided to estimate the main plasma components active during the DCSBD plasma degradation of *E. coli* contamination prepared on polytetrafluoroethylene (PTFE, Teflon) surface, in ambient air at atmospheric pressure. The DCSBD plasma was characterized by means of electrical measurements and optical emission spectroscopy. The inactivation of *E. coli* bacteria was evaluated by standard microbiological cultivation (CFU plate counting). The experimental results of the germicidal efficiency obtained for short plasma exposure times proved the effectiveness of DCSBD plasma for the polymer surface decontamination.

1 Introduction

The non-thermal plasma sources expanded nowadays to new biological areas of application, such as microbial decontamination and disinfection, ready-to-eat food and packaging materials treatment, cancer cells treatment, dentistry and wound therapy [1]. Thus the application of different types of dielectric barrier discharges and plasma jets [2–11] on microorganism inactivation on heat-sensitive surfaces, even on tissue [12,13], have recently attracted a lot of attention in the field of plasma medicine.

Diffuse coplanar surface barrier discharge (DCSBD) belongs to the non-thermal plasma sources widely tested for industrial, environmental and bio-medical applications. The eminent volume density of electric power (100 W cm^{-3}) of the non-equilibrium plasma of DCSBD [14,15] generated in ambient air at atmospheric pressure allows short time treatment of different planar materials, also thermally degradable or biocompatible materials. The DCSBD electrode system arrangement eliminates the safety hazard associated with the treated material manipulation and the DCSBD plasma is cold enough to touch with hand [15].

In the DCSBD plasma generated in ambient air the non-elastic collisions of the energetic electrons and molecules induce dissociations, excitations and ionizations of the molecules present in the used gas (mainly N_2, O_2 and H_2O molecules in the air). Air humidity and chemical reactions of H_2O molecule in non-thermal plasma volume are important parameters for sample treatment [16,17]. The essential particles for inactivation of microorganisms [18], such as radicals, ions and reactive oxygen (ROS) and nitrogen (RNS) species, e.g., O, O_3, O_2^-, H, OH, HO_2, H_2O_2, N^+ and N_2^+, can be produced. The accumulation of charge generated in the discharge region on the surface of a cell membrane and the electrostatic tensile strength can also contribute to microorganism inactivation [19]. The role of different inactivation agents in the plasma, such as reactive radicals, ROS and RNS, UV radiation and high energy electrons, as well as the synergic effect of plasma components is widely discussed [20–24].

It is known from the literature that an important role can also be played by natural ambient air humidity [20], which affects the formation of active species in the plasma. In order to verify this effect, the OES of DCSBD plasma generated in dry synthetic air with different percentages of water vapor admixtures and the inactivation of

[a] e-mail: zlata.tucekova@fmph.uniba.sk

* Contribution to the topical issue "6th Central European Symposium on Plasma Chemistry (CESPC-6)", edited by Nicolas Gherardi, Ester Marotta and Cristina Paradisi

bacterial contamination on PTFE surface by DCSBD plasma generated in ambient air (with relative humidity ≈50%) at atmospheric pressure were investigated.

2 Experimental

2.1 Generation of DCSBD plasma

The DCSBD plasma electrode system with the active plasma area of 200 mm × 80 mm was formed by silver electrode strips 1.5 mm wide with 1 mm electrode gap. The electrode system was placed on the planar ceramics (Al_2O_3, 96% purity). To supply DCSBD plasma electrode system, the AC high voltage power supply (20 kV – peak to peak, ∼14 kHz) *Lifetech Generator VF 700 W* (from Lifetech, Brno, Czech Republic) was used. The voltage $U(t)$ was estimated as the subtraction of both voltage signals measured by *Tektronix P6015A* (1:1000) high voltage probes and the current $I(t)$ was measured by Rogowski's coil *Pearson electronic 4100* during the inactivation of bacteria in ambient air at 400 W of input power. These signals were displayed using *Tektronix 2024 B* oscilloscope. The sine simulation of measured waveforms was used for calculation of the period T, frequency f, radial frequency ω of power supply, phase shift $\Delta\varphi$ of actual current and voltage waveforms, evaluation of voltage/current maxima U_A/I_A and discharge current peak I_{peak} value.

2.2 Optical emission spectroscopy of DCSBD plasma

The emission spectra of DCSBD plasma with the dominant 2nd positive system N_2 (SPS) were measured using spectrometer *Avantes 2048 TEC* with spectral range 300–400 nm. The intensities of N_2 SPS ($\Delta v = -2$, head 0–2 at 380.5 nm) were compared. The values of vibrational temperature were estimated from N_2 SPS ($\Delta v = -2$, heads 0–2, 1–3, 2–4, starting at 380.5 nm) by *Spectrum Analyzer* [25] and the rotational temperatures were evaluated by simulation of N_2 SPS ($\Delta v = -1$, head 0–1 at 357.42 nm) using *Specair* [26]. To observe the emission spectra in the range 230–950 nm, *Ocean Optics SD2000* two-channel spectrometer was used.

The measurement of optical emission spectroscopy of DCSBD plasma generated in dry synthetic air (80% nitrogen and 20% oxygen) with different relative humidities (RH 1%, 50%, 85%) at atmospheric pressure (Fig. 1) was carried out using a reactor chamber (volume of 1.5 L) with the quartz glass window. The gas flow of synthetic air was kept at 1.5 L min^{-1} and the RH was adjusted using the bubbler with distilled water. The relative humidity was measured by industrial temperature and humidity transmitter *T3319* (COMET SYSTEM, Czech Republic).

2.3 Preparation of bacterial cells

The bacterial suspension of *Escherichia coli* BW 25113 was cultured overnight in Lysogeny Broth (LB) at 37 °C

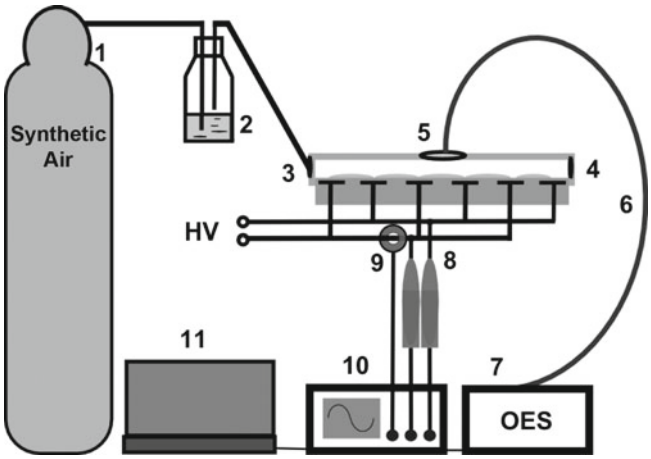


Fig. 1. The scheme of the measurement of DCSBD parameters: 1 – synthetic air, 2 – bubbler with distilled water, 3 – gas input, 4 – gas output, 5 – quartz glass window, 6 – optical fiber, 7 – spectrometer, 8 – high voltage probes, 9 – Rogowski's coil, 10 – oscilloscope, 11 – computer.

on a shaker platform (400 rpm). The PTFE plates (15 × 50 mm) were contaminated by 5 droplets of the suspension (5 × 10 μL, the initial concentration of bacteria was $4.99 \pm 1.15 \times 10^8$ CFU/mL) and dried at 35 °C for 20 min. The control samples were prepared by the same procedure. After plasma treatment each plate was put inside a test tube with 20 mL of physiological saline solution (0.85% NaCl). Two conditions were distinguished: the samples were kept on air after the plasma treatment for a few minutes before putting into saline solution and further processing – "Air + NaCl solution" or immediately put into saline solution after the plasma treatment – "NaCl solution", then, the solution was thoroughly mixed by the vortex, serially diluted and plated on agar medium in petri dishes. After incubation of bacteria at 37 °C for 16–20 h, the number of colonies (CFU) was counted. Five independent repetitions were performed for each exposure time (except 10 s in air – three repetitions). The bactericidal effect was calculated as $\log_{10}$ reduction of bacterial population, it is equal to $\log_{10}$ ($CFU_{control}/CFU_{sample}$), where $CFU_{control}$ and CFU_{sample} are number of bacterial colonies grown from control and plasma treated samples, respectively.

2.4 Inactivation of bacterial cells

The experimental apparatus used for the inactivation of *E. coli* bacteria on the contaminated PTFE surface is shown in Figure 2. The PTFE plates with dimensions 15 mm × 50 mm were treated using the plasma of DCSBD generated in ambient air at input power 400 W. The sample was fixed on the movable holder and the contaminated PTFE surface was moved 0.7 mm above the ceramics of DCSBD plasma system for 10–60 s.

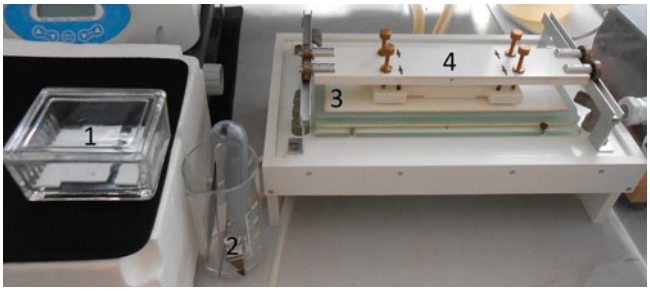


Fig. 2. DCBSD reactor for inactivaton of *E. coli* on the contaminated PTFE surface: 1 – contaminated PTFE samples, 2 – sterille forceps, 3 – DCSBD plasma system, 4 – movable sample holder.

3 Results and discussion

3.1 Parameters of the DCSBD plasma during E. coli inactivation

The voltage and current time dependence (Fig. 3) measured at the power 400 W allowed us to estimate the power supplied to the DCSBD system during the inactivation in ambient air. The power supplied to the DCSBD electrode system was estimated to 90% of the used input power. The surface and volume power density of DCSBD plasma was determined from the dimensions of plasma layer and calculated to be 2.25 W cm^{-2} and 75 W cm^{-3}, respectively. Using sine function simulation of the measured waveforms, the parameters of the discharge and parameters of the used power supply were calculated and are listed next to the Figure 3. Labelled current peaks originate from DCSBD microdischarges. It should be noted that the shown waveforms are an accumulation of the signal from several periods of discharge ignition.

3.2 Emission spectra of the DCSBD plasma and temperature evaluation

The typical emission spectra of the DCSBD plasma generated in synthetic air with different RH are shown in Figure 4. These spectra are dominated by the 2nd positive system (SPS) of molecular nitrogen N_2 ($C^3\Pi_u$-$B^3\Pi_g$). We can see NOγ ($A^2\Sigma^+$-$X^2\Pi_r$) system emission in detail in the spectra and OH ($A^2\Sigma^+$-$X^3\Pi_{3/2}$) emission partially overlapped by SPS of N_2 ($\Delta v = 1$). These emissions occurred due to the presence of H_2O in the synthetic air and as a result of recombination of dissociated N_2 and O_2. The intensity of N_2 SPS ($\Delta v = -2$, head 0–2) was, as expected, increasing with the rise of the input power and the maximum of intensity was attained in the synthetic air with RH 50%.

The dominant N_2 SPS accompanied by 1st positive system (FPS) of N_2 ($B^3\Pi_g$-$A^3\Sigma_u{}^+$) are characteristic for many atmospheric non-equilibrium discharges generated in air. The spectrum in Figure 5 dominated by the presence of N_2 FPS indicates further formation of N_2 $A^3\Sigma_u{}^+$ metastables.

The above mentioned molecules and metastables participate in a number of chemical reactions in air plasma, and the bactericidal effect of these systems is an important attribute for biochemical decontamination and sterilization [27]. Previous conclusions in references [20,28] indicate, that the presence of water is required to achieve fast inactivation. Subsequently as the humidity of air increases, the dominant bactericidal factors change from O_3 and UV radiation to generated OH radicals.

The values of vibrational temperature T_V of N_2 SPS measured in the DCSBD plasma generated in synthetic air with different RH (1%, 50% and 85%) at various input powers (300 W, 350 W and 400 W) were the same. The value of T_V was estimated to 2700 K (±300 K). Such T_V determined using three vibrational bands can be used as the indicator of the non-equilibrium plasma character.

The values of rotational temperature T_R of N_2 SPS ($\sim$ gas temperature) have increased slightly with the input power and with the rise of relative humidity (Tab. 1). The value of T_R estimated for synthetic air with high RH indicates the possibility of thermal damage of *E. coli* membranes and that the gas temperature in the upper layer of DCSBD plasma may be responsible for the bactericidal effect. Taking the T_R *error* value into account, the gas temperature in DCSBD plasma layer generated in humid air is applicable for thermally degradable materials treatment. It should be noted that these measured T_R apply for the plasma volume and the upper layer of DCSBD plasma (0.3 mm). The global gas temperature near the sample (0.7 mm) can reach considerably lower values.

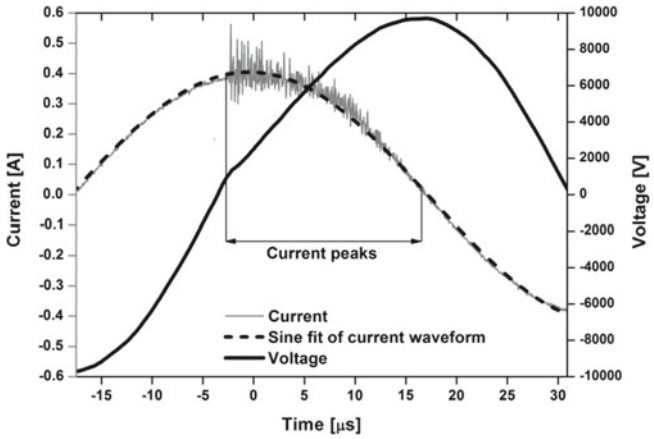
3.3 Germicidal efficiency of DCSBD plasma

To test decontamination efficiency of DCSBD discharge, PTFE plates were contaminated with 50 μL of *E. coli* suspension. Half of the samples was put inside the test tube with saline solution right after the plasma treatment and the other half was kept outside the solution for a few minutes. Figure 6 shows the comparison of $\log_{10}$ reduction efficiencies between the two groups of samples.

The decontamination efficiency was increasing with the increasing exposure time from 1.6 and 1.4 $\log_{10}$ for 10 s to 5.1 and 3.0 $\log_{10}$ reduction for 60 s, for "Air + NaCl solution" and "NaCl solution" groups, respectively. The decontamination effect of DCSBD plasma was more pronounced for the samples "Air + NaCl solution". This effect was caused by air incubation of sample after the treatment and the after-effect of plasma products that may have led to an additional inactivation of *E. coli*.

The 99% decontamination efficiency was reached within 20 s for the group "Air + NaCl solution" and 30 s for the group "NaCl solution". In comparison with previously published results [2,9], the germicidal efficiency of DCSBD plasma for *E. coli* inactivation on PTFE surface was achieved in shorter exposure time.

The statistical analysis of the decontamination results (Mann-Whitney test on the level of significance 0.05) showed no significant difference between the two groups



U_A [kV]	10
I_A [mA]	400
I_{peak} [mA]	130
T [μs]	70
f [kHz]	14.3
ω [rad.10^3s^{-1}]	90
Δφ [rad]	1.34

Fig. 3. Voltage/current waveforms and discharge current peaks of DCSBD plasma generated in ambient air at input power 400 W and characteristic parameters obtained using sine simulation of voltage/current waveforms.

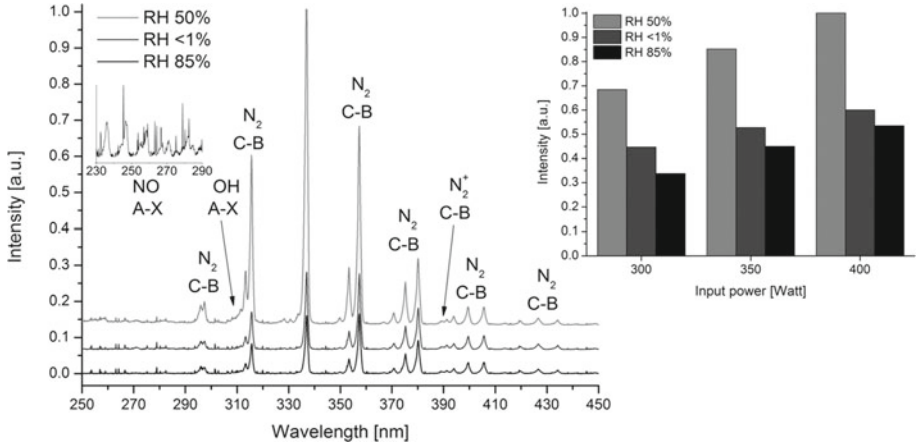


Fig. 4. Emission spectra of DCBSD plasma generated in synthetic air with different RH (input power 400 W) (left) and N_2 SPS (380.5 nm) intensity at various input powers (right).

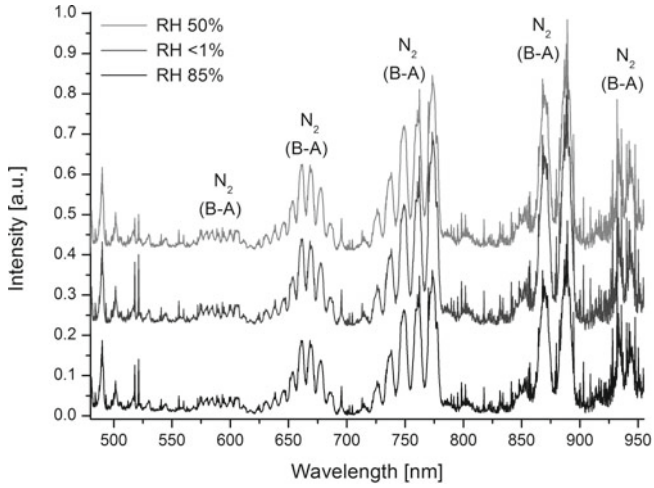


Fig. 5. Emission spectra of DCBSD plasma generated in synthetic air with different RH (vis-NIR region).

with one exception: 50 s exposure time. Within the first group (samples exposed to air and processed), the significant difference (Kruskal-Wallis test for analysis of variance) was found between exposure times 10 vs. 50 s ($p < 0.05$), and 10 vs. 60 s ($p < 0.01$). For the second group (sample put directly into saline solution), a significant difference was found only between 10 vs. 60 s ($p < 0.05$).

3.4 Discussion

The DCSBD plasma in humid air produced ROS and RNS detected by optical emission spectroscopy (OH, NO-γ, N_2^+) and in references [29,30] reported O_3, which are known for their bactericidal effect, when diluted in water-based medium [7,24,31]. Additionally, de-excitation of excited hydroxyl radical (OH^*) emits UV-B (280–315 nm) radiation, which contributes to the bactericidal effect [32]. On the other hand, UV-B radiation may have reached and affected only upper layers of bacterial cells.

The inactivation of lower layers of the bacterial sample was driven by the diffusion of the reactive species into the cells [33]. The reactive species in samples kept on air had longer time to diffuse and to react within the bacterial sample causing the permanent damage of bacterial cells [34,35]. The insignificant decrease of the decontamination efficiency of samples placed inside the NaCl solution right after the plasma treatment can be caused

Table 1. T_R of DCSBD plasma generated in synthetic air with different relative humidity at various input power.

Input power [W]	Synthetic air relative humidity 1%		Synthetic air relative humidity 50%		Synthetic air relative humidity 85%	
	T_R [K]	Error [K]	T_R [K]	Error [K]	T_R [K]	Error [K]
300	375	75	400	75	450	75
350	375	75	400	75	450	75
400	425	75	425	75	450	75

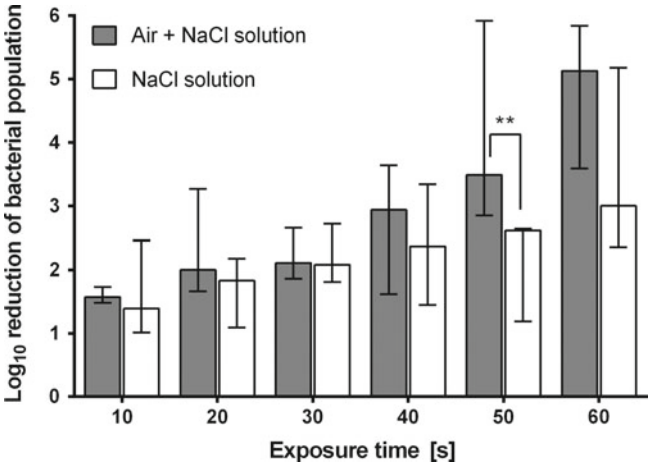


Fig. 6. The comparison of $\log_{10}$ reduction efficiencies of *E. coli* between two groups: the samples kept on air after treatment ("Air + NaCl solution") and the samples put directly into the NaCl solution after treatment ("NaCl solution") for various exposure times (graphed median ± IQR).

by the dilution of ROS and RNS concentrations directly affecting the cells in the NaCl solution and by shorter reaction time of reactive species while in the gas phase.

The input power used for biological experiments (400 W) in ambient air (RH ≈ 50%) corresponds to the gas temperature in the plasma (0.3 mm distance from the dielectric surface) equal to 425 ± 75 K. Although this temperature of plasma is quite high, the gas temperature near the samples and especially the PTFE surface temperature can be lower, but it was not measured. Therefore, we cannot completely dismiss the effect of the temperature on bacterial samples.

The effect of drying on the bacterial loss was accounted in the control sample, which was prepared on Teflon plate and dried the same way as the plasma treated samples. The concentration of bacteria on the control sample was used as a reference for the calculation of the $\log_{10}$ reduction efficiency. The 13% bacterial loss due to the effect of drying was estimated from the difference between control sample and initial bacterial load and the presented bactericidal efficiency is cleared from this effect.

4 Conclusion

The bactericidal effect of the DCBSD plasma generated in ambient air with high relative humidity (≈50%) on *E. coli* bacteria has shown high germicidal efficiency (>99%) and high $\log_{10}$ reduction of bacterial population for short exposure times (<1 min). Using DCSBD plasma generated in humid air the agents such as ROS (O_3, OH), RNS (NO-γ, N_2^+) and UV radiation are produced which contribute to microorganism inactivation [24]. As was estimated from the optical emission spectroscopy, the values of gas temperatures increased with the rise of relative humidity, which can also result in the thermal damage of *E. coli* cell membranes.

This work was supported by Slovak Research and Development Agency Grant SK-CZ-2013-0147, APVV-0134-12 Grant and VEGA Project No. 1/0904/14.

References

1. V. Scholtz, Biotechnol. Adv. **33**, 1108 (2015)
2. Y. Song, IEEE Trans. Plasma Sci. **43**, 821 (2015)
3. A. Zahoranová, Plasma Chem. Plasma Process. **36**, 397 (2015)
4. E. Dolezalova, Bioelectrochemistry **103**, 7 (2014)
5. Z. Koval'ová, Eur. Phys. J. Appl. Phys. **61**, 24306 (2013)
6. W. Ni, Surf. Coat. Technol. **234**, 120 (2013)
7. D. Ziuzina, J. Appl. Microbiol. **114**, 778 (2013)
8. J. Pawłat, Trans. Mater. Res. Soc. Japan **38**, 625 (2013)
9. H. Miao, G. Yun, Appl. Surf. Sci. **257**, 7065 (2011)
10. Y. Akishev, Pure Appl. Chem. **80**, 1953 (2008)
11. J.H. Choi, J. Electrostat. **64**, 17 (2006)
12. G. Fridman, Plasma Chem. Plasma Process. **26**, 425 (2006)
13. G. Daeschlein, J. Hosp. Infect. **81**, 177 (2012)
14. M. Černák, Plasma Phys. Control. Fusion **53**, 124031 (2011)
15. M. Černák, Eur. Phys. J. Appl. Phys. **47**, 22806 (2009)
16. V. Prysiazhnyi, Appl. Surf. Sci. **258**, 5467 (2012)
17. J. Čech, Plasma Phys. Technol. **2**, 304 (2015)
18. S. Perni, Appl. Phys. Lett. **90**, 073902 (2007)
19. D.A. Mendis, IEEE Trans. Plasma Sci. **28**, 1304 (2000)
20. D. Dobrynin, New J. Phys. **11**, 115020 (2009)
21. M. Laroussi, Int. J. Mass Spectrom. **233**, 81 (2004)
22. M. Laroussi, Plasma Process. Polym. **2**, 391 (2005)
23. M. Laroussi, IEEE Trans. Plasma Sci. **37**, 714 (2009)
24. Z. Machala, Plasma Process. Polym. **10**, 649 (2013)
25. Z. Navrátil, Czech. J. Phys. **56**, 944 (2006)
26. C. O. Laux, Von Karman Institute Lecture Series 2002–07, Rhode-Saint-Genèse, Belgi, 2002

27. Z. Machala, J. Mol. Spectrosc. **243**, 194 (2007)
28. H. Eto, Appl. Phys. Lett. **93**, 13 (2008)
29. M. Šimek, in *Proc. 28th ICPIG, Prague, Czech Republic, 2007*, p. 934
30. S. Lazović, Publ. Astron. Obs. Belgrade **84**, 401 (2008)
31. K. Oehmigen, Plasma Process. Polym. **7**, 250 (2010)
32. Z. Kovalova, J. Phys. D: Appl. Phys. **48**, 464003 (2015)
33. P. Pacher, Physiol. Rev. **87**, 315 (2007)
34. M. Yusupov, New J. Phys. **14**, 093043 (2012)
35. M. Yusupov, J. Phys. D: Appl. Phys. **47**, 025205 (2014)