Significance of free radicals in amine plasma polymers deposited under mild ion bombardment

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Abstract: We investigated the presence and dynamics of the free radicals in the amine plasma polymer films using electron paramagnetic resonance (EPR). The films were deposited in radio-frequency (RF) capacitively coupled plasma under mild conditions of relatively high pressure, i. e. decreasing the energy of incoming ions by collisions in the plasma sheath. We compare the density and decay of the free radicals for different substrate potentials, RF power and pulsed or continuous mode of the discharge.

Keywords: cyclopropylamine, plasma polymers, electron paramagnetic resonance.

1. Introduction

Amino-group containing plasma polymers have been of particular interest for various biomaterials applications because they allow amide covalent coupling and, under certain pH conditions, offer a positively-charged surface for electrostatic binding [1]. Therefore, they were successfully applied to the immobilization of DNA [2] and various proteins [3]. However, it is difficult to assess what plasma parameters and the film structural properties are important for successful and stable immobilization.

Plasma polymers (PPs) do not have a well-defined structure of conventional polymers that can be described as a repetition of a particular unit and the molecular weight of the polymer chain. Since they are deposited from a complex environment of many gaseous reactants created by the dissociation of original gas feed, their chemical structure cannot be derived only from the starting reactants. It depends on the electron concentration and energy given by the discharge configuration and power. The deposition is also influenced by the collision frequencies related to the pressure and the energy flux transmitted by ions accelerated within the plasma sheath adjacent to the substrate.

Considering the most used discharge, low-pressure radio-frequency (RF) capacitively coupled plasma (CCP), the questions remain about the role of ions, their energy, density, and chemistry in the particular experiment. The comparison among the experimental set-ups is hindered by the varied substrate potential and the pressure (collisional/collisionless regimes) or not well-defined transitions between the discharge modes (CCP/ICP or α/γ of CCP).

We studied the plasma polymerization of cyclopropylamine (CPA) mixed with Ar in three CCP reactor setups and different plasma conditions. The response of immunosensors constructed with the plasma polymerized CPA (PP-CPA) films revealed the surprisingly long reactivity of the film surfaces. The response of immunosensors constructed with the plasma polymerized cyclopropylamine (PP-CPA) films revealed the surprisingly long reactivity of the film surfaces. It opened the question of whether such phenomena could be explained by the free radicals (species with unpaired spins) trapped in the PP films. Therefore, we aimed here to investigate the presence and dynamics of the free radicals in the PP-CPA films deposited under mild varied conditions using electron paramagneti c resonance (EPR).

2. Experimental

The amine PPs films were deposited in radio frequency (RF) discharge with capacitive coupling from cyclopropylamine (CPA) vapours mixed with Ar (PP-CPA films). The three different CCP setups used for the film deposition covered were described in detail in our previous publications [4,5,6]. The film deposition conditions differed in the RF power invested into the plasma-chemical reaction. The RF (13.56 MHz) discharge was operated in the continuous mode or pulsed using the duty cycle 33 % and the repetition frequency of 500 Hz. The on-time RF power varied between 30 and 150 W. To simplify the space of the deposition parameters, the films are identified by the average RF power (P_{av}) calculated as the on-time RF power multiplied by the duty cycle.

The films investigated by EPR were deposited on 1 x 10 cm polyethylene terephthalate (PET) foil with the thickness of 0.023mm (GoodFellow product No. ES301230). The EPR experiments were conducted using a Magnettech X-band EPR spectrometer. The foil with the film was rolled and inserted into quartz tubes, 5 mm in diameter (Merck product No. Z567396-5EA). The tubes were sealed with parafilm. To accurately quantify the number of spins in the polymeric samples, each sample was paired with a sealed glass capillary of 1 mm diameter and 3 mm length containing MgO:Cr(III) with a known number of spins, which served as a reference standard. The spectra were recorded using a microwave power of 3.9 mW and a modulation amplitude of 2.7 G at 100 kHz. The number of spins in the samples was determined by

comparing the areas of the EPR signals of the reference standard to those of the samples and fitting the peaks using Lorentzian lineshapes.

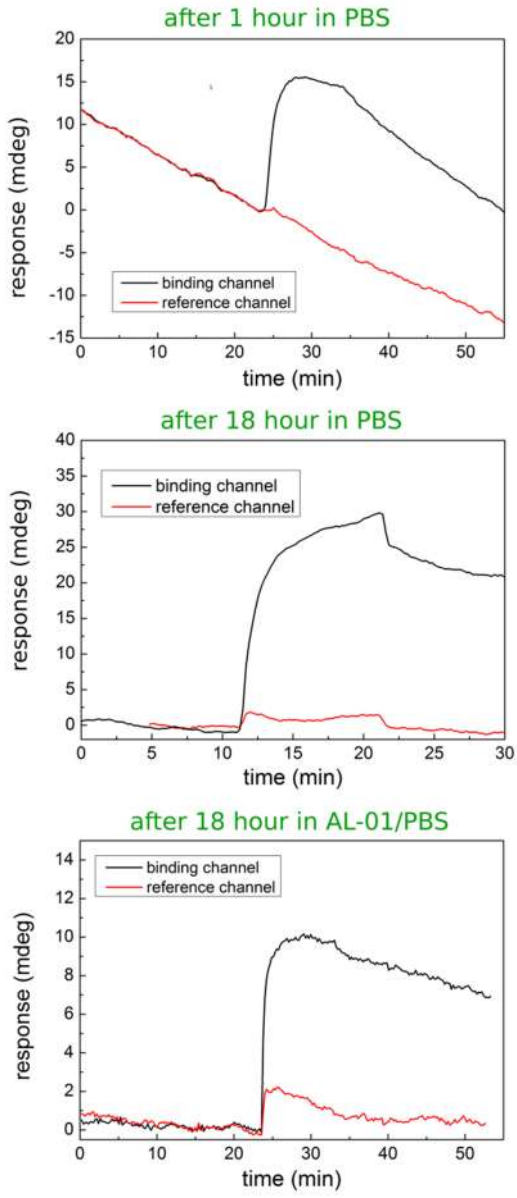

Fig. 1 SPR immunosensor response to 10 µg/ml solution of HAS for the PP-CPA film after various stabilizations.

3. Results and Discussion

The immunosensors are extremely sensitive to the specific surface reactivity and film stability in PBS. Therefore, their construction using plasma polymer thin films reveals exciting details about the complex chemical structure of PPs. We compared performance of the quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) immunosensors constructed with the amine PPs prepared under various conditions.

The PP-CPA films deposited at the floating potential and the RF electrode had similar atomic composition (the C:N:O ratios were 77:20:3 and 78:18:4) and similar concentrations of NH_2 groups, but their performance in the QCM sensing was significantly different. Much better signal was obtained with the film deposited at the floating potential [3]. It raised the question to which parameters the film chemical structure should be optimized and what fine detail of the film chemical structure is responsible for the significantly different sensor behaviour.

The QCM with the PP-CPA film was immersed in 5% glutaraldehyde/phosphate buffer saline (PBS) solution for 1 h at room temperature and then left 18 hours at 4 °C in 100 µg/ml AL-01 antibody/PBS solution. This procedure ensured that the detection baseline of human serum albumin (HSA) was stable for both the films mentioned above. The experiments with SPR immunosensors confirmed the importance of the film reactivity stabilization in PBS [7]. The film deposited at the floating potential was immersed for 1 and 18 hours in PBS, before the procedure with glutaraldehyde coupling started. The detection of HSA exhibited a drifting baseline for only 1 hour immersion (Fig. 1 top). The crucial point was the stabilization of the film in pure PBS because the immersion in antibody/PBS solution for 18 hours stabilized the baseline but the sensor signal to HAS was low (Fig. 1 bottom).

When comparing the immunosensing for the optimized PP-CPA and the C_2H_4/NH_3 films optimized for water stability and high functionality (double layer structure with highly cross-linked bottom film) [8]. The baseline of the SPR signal could not be stabilized for the C_2H_4/NH_3 film even after immersion in the PBS for a long time. Since the highly cross-linked film was deposited under higher ion assistance, it opened the question of whether such phenomena could be explained by the free radicals trapped in the PP films.

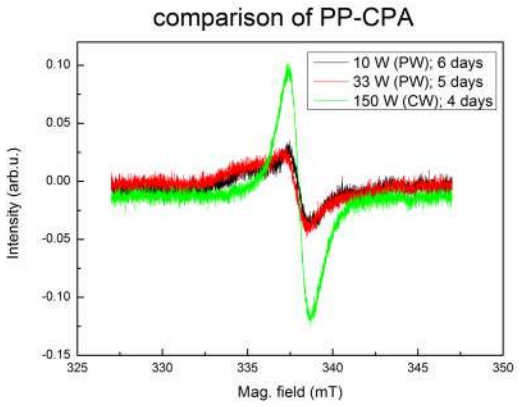

Fig. 2 EPR measurements of free radicals in the PP-CPA films deposited at three different average powers P_{av} in the pulsed (PW) or continuous wave (CW) RF discharge.

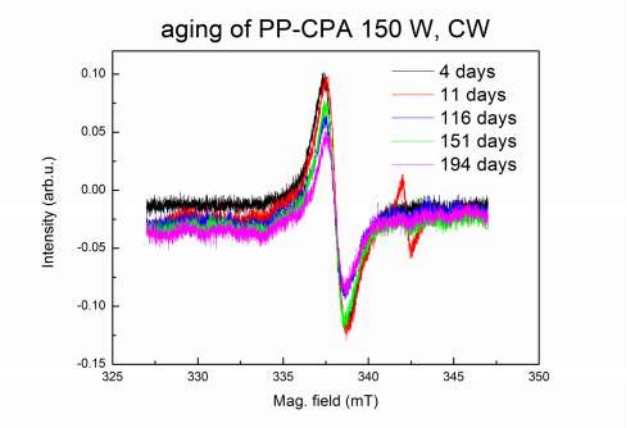

Fig. 3 Decay of free radicals in the PP-CPA films deposited at 150 W (continuous wave mode of the RF discharge). The EPR measurement was carried 4-194 days after the deposition.

The EPR measurements of the PP-CPA films confirmed the presence of free radicals (Fig. 2). These films were deposited at 50 Pa. At 50 Pa, the dc bias-voltage was maximum -40 V (for the maximum power used, 150 W) and the ions underwent many collisions in the plasma sheath, keeping their energy low. The spin density in the 150 W (CW) film, calculated using an EPR standard, was about $5x10^{20}$ cm^{-3}. It is somewhat surprising that the spin density is so high in the films deposited at quite mild conditions. The spin amount decreased with the decrease of the RF power. It can be related to both, decreased ion density due to lower RF power and decrease of the maximum ion energy because the dc bias was lower at lower RF power. The radicals exhibited long life time as seen by the EPR measurements after several months (Fig. 3).

4. Conclusion

The response of immunosensors constructed with the plasma polymerized cyclopropylamine (PP-CPA) films revealed the surprisingly long reactivity of the film surfaces. It opened the question of whether such phenomena could be explained by the free radicals (species with unpaired spins) trapped in the PP films. The EPR measurements confirmed that

5. References

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