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Carbon synthetized by RF PACVD method enhances the activity of antioxidants

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Abstract: *Carbon synthetized by RF PACVD method enhances the activity of antioxidants.* The aim of the presented study was to evaluate the reductive activity of antioxidants in the presence of carbon manufactured by Radio Frequency Plasma Activated Chemical Vapor Deposition method (RF PACVD). 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was employed to estimate antioxidants reductive potential. Based on the experimental results, the antioxidant activity of carbon colloid was not confirmed. Nevertheless the reductive activity of antioxidants measured in time manner, in the presence of carbon, was significantly elevated comparing to the antioxidants alone. The presented results suggest that the surface of carbon synthetized by RF PACVD method suspended in water, creates more friendly reductive environment for antioxidants with hydroxyl groups (ascorbic acid – AA and butylated hydroxyanisole – BHA) then with sulfhydryl groups (glutathione – GSH).

Key words: carbon powder particles, RF PACVD method, antioxidant, ascorbic acid, BHA, glutathione, DPPH

INTRODUCTION

The external environmental factors like UV, optical radiation, temperature, organic and inorganic oxidants as well as internal cell oxidant factors are the potential reasons of the oxidation stress, which interfere the cell redox homeostasis. Such interferences may cause the serious antioxidant deficiencies and they may initiate tumor processes or cell apoptosis. The limited amount of reducers in the organic matter leads to the unstable redox homeostasis. Growing oxidative strength of the environmental factors causes the rapid decrease of the antioxidant potential. The strength of the reducers in living organisms is limited by the outside factors and endogenous biosynthesis. That is why, the organism diet rich with the natural antioxidants (AA and GSH) is necessary for the regular organism functioning. The organic matter should be protected by synthesized antioxidants (BHA) to extend their time of stability. The environment may also be protected by slowing the oxidation reactions. In the living organism this may be achieved by the establishment of proper ratio between the lipid antioxidants and unsaturated acids. In the organic matter it maybe achieved by proper storage conditions like the controlled temperature or the light protection. There is a large demand for the research on the factors which possess both the characteristics of the antioxidant and the ability of sustaining the proper environment for reduction reactions.

The recent study presents the additional biological benefits of carbon particles which are commonly considered as the potential carrier of the molecules. There are also a few studies evaluating the specific characteristics of RF PACVD carbon particles which could be used in biomedicine or pharmaceutical industry. Czerniak-Reczulska et al. (2010) noted the influence of RF PACVD carbon on the process of the proliferation of endothelial cells. Bakowicz-Mitura et al. (2007) observed that RF PACVD carbon may act as an antioxidant or anti-inflammatory factor. Catalitic properties of this type of carbon in organic environment, have not been precisely studied yet. Perhaps, as in the case of diamond obtained by detonation method used for proteolytic enzyme immobilization, the immobilization reducers may modulate redox reaction in simple *in vitro* systems. We suspect that carbon synthetized by RF PACVD method may actively transfer electrons in oxidoreductive reactions in the organic matter. Therefore we presume that RF PACVD carbon can protect antioxidant activity from environmental stress. The hypothesis was tested by determining the effect of RF PACVD carbon colloid on ascorbic acid, glutathione and tert-butyl-4-hydroxyanisole activity by measurements of reducing power of antioxidants on 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the iron ions.

MATERIAL AND METHODS

Manufacturing parameters of RF PACVD carbon powder were described by Czerniak-Reczulska et al. (2010). The samples were divided into three groups. Carbon particles was suspended in water. Investigated antioxidants (AOX): AA, GSH were dissolved in water. BHA was dissolved in ethanol. In the solution of 0.5 $\mathrm{m\cdot mol^{-1}}$ AA, 0.7 $\mathrm{m\cdot mol^{-1}}$ BHA and 100 $\mathrm{m\cdot mol^{-1}}$ GSH, carbon particles (RF PACVD) were suspended in the concentration of 50 $\mathrm{mg\cdot l^{-1}}$. The experiment was also performed with the incubation of all the solutions in 100°C for 10 min.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The free radical scavenging activity of the mix sample was measured by the decrease in absorbance of methanolic DPPH solution at 517 nm (Krings and Berger 2001) in time manner (after 0.25; 0.5; 1; 2; 3; 4; 5 min). The antioxidant activity was expressed as: % disappearance $= [(A_{\mathrm{control}} - A_{\mathrm{sample}})/A_{\mathrm{control}}] \times 100\%$ (A – absorbance).

The results were subjected to statistical analysis by two-way ANOVA and Duncan’s range test.

RESULTS AND DISCUSSION

According to Mitura (1987) and Bakowicz (2003), RF PACVD particles exhibits the reducing activity. In our study, the analytical methods did not identified reducing strength of the RF PACVD carbon. AA possesses two active hydroxyl groups that participate in the redox reactions (Niemiec et al. 2005). RF PACVD carbon catalyzes reaction with AA in the DPPH test. Carbon elevates AA activity after 15 s incubation and significantly increases the vitamin activity after 3 min incubation. High temperature decreased the activity of AA from 50 to 10%. However in the presence of carbon powder, the activity of ascorbic acid decreased in about 30% (Table 1).

Carbon particles did not affect the change in activity of glutathione in any of the experiments. BHA synthetic antioxidant strength reduction depends on the active $^{-}$OH groups. Carbon significantly elevates antioxidant activity of BHA from 30 s to 2 min incubation. High temperature decreased activity of BHA as it was expected. However within first minute, reduction reaction was elevated comparing to the group without carbon particles (Table 2).

GSH is a multifunctional thiol group containing tripeptide that is a powerful antioxidant (Table 3), found in most aerobic organisms (Banhegyi et al. 1997).

The living organisms are exposed to the external environmental factors which is the source of oxidative stress. This is the one of the harmful principal issues in the healthcare, where excess of ROS (chemical species with unpaired electrons on the molecular orbitals) generated in various pathogenic processes are recognized as an indicator in cytotoxicity and the cellular disorder. At the intracellular level, ROS are balancing between the biochemical antioxidants such as ascorbic acid or glutathione. In the organic matter (food) redox homeostasis is remains unstable due to the limited of amount of reducers. Growing oxidative strength of the environmental factors leads to rapid decrease in the antioxidant potential. The strength of the reducers in living organisms is limited by delivered factors from the organism diet and possibility of endogenous biosynthesis. Simultaneously, the known strategies for the preservation of the organic matter (food products) are

TABLE 1. Reducing activity of AA on DPPH radical in time manner

TIME (min)	Reducting activity (%) of						ANOVA				
							influence				
	RF PACVD CARBON		ascorbic acid (AA)		RF PACVD CARBON + AA		RF PACVD CARBON		temperature		inter--action
	–	100°C	–	100°C	–	100°C	SEM	p	SEM	p	p
0.25	0.13^{a}	0.40^{a}	52.04^{b}	10.47^{b}	59.11^{c}	29.56^{c}	2.033	0.0191	0.798	0.0000	0.0000
0.5	0.14^{a}	0.27^{a}	1.90^{b}	0.18^{b}	4.72^{c}	2.83^{c}	0.426	0.0004	0.195	0.0000	0.0000
1	0.16^{a}	0.09^{a}	0.38^{b}	0.15^{b}	6.02^{c}	1.86^{c}	0.203	0.0000	0.329	0.0000	0.0000
2	0.16^{a}	0.05^{a}	0.38^{b}	0.16^{b}	5.08^{c}	0.76^{c}	0.359	0.0033	0.316	0.0017	0.0079
3	0.14^{a}	0.13^{a}	0.12^{a}	0.08^{b}	0.32^{b}	0.28^{b}	0.0232	0.0063	0.500	0.0011	0.0235
4	0.13^{a}	0.12^{a}	0.11^{a}	0.10^{b}	0.17^{b}	0.34^{b}	0.0241	0.0021	0.060	0.1380	0.3045
5	0.17	0.11^{a}	0.05	0.15^{b}	0.10	0.29^{b}	0.0229	0.6231	0.047	0.1217	0.3246

a,b,c – RF PACVD CARBON + AA > RF PACVD CARBON and AA ($p < 0.05$).

TABLE 2. Reducing activity of BHA on DPPH radical in time manner

TIME (min)	Reducting activity (%) of						ANOVA				
							influence				
	RF PACVD CARBON		BHA		RF PACVD CARBON + BHA		RF PACVD CARBON		temperature		inter-action
	–	100°C	–	100°C	–	100°C	SEM	p	SEM	p	p
0.25	0.10^a	0.197^a	4.94^b	3.751^b	5.60^b	8.941^c	0.306	0.0000	0.306	0.0000	0.0000
0.5	0.00^a	0.097^a	5.26^b	5.092^b	6.74^c	6.843^c	0.178	0.0000	0.178	0.0000	0.0000
1	0.01^a	0.060^a	7.02^b	7.134^b	8.66^c	8.246^c	0.154	0.0000	0.154	0.0000	0.0000
2	0.16^a	0.222^a	12.12^b	9.509^b	13.82^c	9.523^b	0.189	0.0000	0.189	0.0000	0.0000
3	0.03^a	0.272^a	12.32^b	7.461^b	12.81^b	6.970^b	0.148	0.3110	0.148	0.3110	0.2235

a,b,c – RF PACVD CARBON + BHA > RF PACVD CARBON and BHA ($p < 0.05$).

TABLE 3. Reducing activity of GSH on DPPH radical in time manner

TIME (min)	Reducting avtivity (%) of			ANOVA	
	RF PACVD CARBON	glutathione (GSH)	RF PACVD CARBON + GSH	SEM	p
0.25	0.013^a	45.33^b	43.07^b	1.832	0.0211
0.5	0^a	12.87^b	13.65^b	1.945	0.0000
1	0^a	10.46^b	12.57^b	1.734	0.0000
2	0^a	10.80^b	13.21^c	0.956	0.0014
3	0^a	4.05^b	3.44^b	0.531	0.0088

a, b, c – means with different superscripts are significantly different ($p < 0.05$).

the supplemented synthetic antioxidants such BHA. The effectiveness of antioxidants depends on the accompanied carriers employed to reach the target. Number of studies presents the improvement of the antioxidant efficiency, in the way of binding them to the nano-carriers consisting of different carbon allotropes which exhibit reducing properties. According to Kato et al. (2009), water-soluble derivative of fullerene (C60) exhibit antioxidant activity. Flavonoids bind to hydroxyl nanotubes revealed significant increase in the antioxidant properties *in vitro* (Nichit and Stamatin 2013).

We observed the significant increase in the reducing force of the ascorbic acid and BHA in the RF PACVD colloidal environment. Probably the high reactivity of the obtained complexes is the result of molecular self-assembly into supramolecular structures. Self-assembly begins at the level of atoms, where it relies on the chemical complementarity. On the molecular level, it allows the materials to precipitate and form highly organized structures. We presume that non--covalent interactions (such as hydrogen bonding and ionic interactions, hydrophobic interactions) between carbon and antioxidants, enhance the thermal stability of the new formed new complexes. This happens in the process of stabilizing the engaged electrons on the carboxylic acid groups by electronic properties of RF PACVD carbon structure, especially as the active interaction occurs between the experimental factors. Many allotropes of carbon (graphite, diamond, fullerenes, nanotubes or graphen) are presented as the interesting materials for electrochemical applications, like the energy storage. Moreover, the amphoteric character of carbon allows for applying its electrochemical properties from the donor to the acceptor state (Frąckowiak and Beguin 2001).

CONCLUSIONS

According to the present results, carbon colloid increases the activity of antioxidants, especially in the environment that affect their activity (high temperature). Moreover, the effect of RF PACVD carbon powder depends on the biochemical structure of the antioxidant. The presented results suggest that the surface of carbon particles suspended in water, creates friendly reductive environment for antioxidants with hydroxyl group (AA and BHA), but not GSH.

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Streszczenie: *Węgiel wytworzony metodą RF PACVD zwiększa aktywność antyoksydantów.* Celem doświadczenia było zbadanie aktywności redukującej wybranych antyoksydantów (kwasu askorbinowego, butylowanego hydroksyanizolu i glutationu) w obecności cząstek węgla wytworzonego metodą RF PACVD testem DPPH. Test ten polega na pomiarze kinetyki reakcji między przeciwutleniaczem a wolnymi rodnikami. Im skuteczniejszy przeciwutleniacz, tym stała szybkości powstawania zredukowanej formy difenylopikrylohydrazyny (DPPH-H) jest większa. Jakkolwiek nie stwierdzono aktywności redukującej hydrokoloidu węgla RF PACVD to jednak rozpuszczone w nim antyoksydanty istotnie zwiększyły swój potencjał redukujący w porównaniu do próbek zawierających czysty antyoksydant. Uzyskane wyniki sugerują, że cząstki węgla RF PACVD zawieszone w wodzie stwarzają korzystne środowisko dla aktywności związków redukujących z aktywną grupą hydroksylową (AA i BHA), ale nie dla związków z grupą sulfhydrylową (GSH).

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