Supplementary Materials of

***In vitro* Antioxidant Potential Evaluation of   
Non-Functionalized Fullerenes and Endofullerenes**

by

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# Quenching fullerene ability in aqueous media

## Quenching of ABAP-Luminol system in the presence of fullerene

Interactions between the components of free-radical systems (ABAP, luminol) and aqueous fullerene dispersions (AFD) C60, C70, and Gd@C82 have been studied by fluorescent spectroscopy. Stern-Volmer approach (KSV) for quenching fluorescence analysis has been applied. Quenching occurs in static (with constant KS) or dynamic (with constant KD) interactions mechanism. To more speciation, fluorescence lifetime measurement should be applied. The magnitude of KS demonstrates that dynamic quenching cannot account for the decrease in intensity [1].

Normalization of fluorescence data at excitation 350 nm in Stern-Volmer coordinates

where

is the fluorescence intensity without a quencher;

*I* is the fluorescence intensity with quencher addition;

*Q* is quencher concentration (M).

All known data are presented for 293K. Quenching of 1-pyrenencarboxilic acid by tris(malonate)-substituted C60 were KSV~4.4÷3.4)×104 M–1 [2]; for pristine C60 and fullerenol C60(OH)n with RNase A were 8.2×104 M–1 and1.7×104 M–1 accordingly [3]; bovine (BSA) and human (HSA) serum albumins interaction between Gd@C82(OH)22 2.3 and 2.5×104 M–1 accordingly by a dynamic mechanism [4].

In this work, we conducted an estimation of quenching fullerene ability for AFD C60, C70, and Gd@C82 in the ABAP, luminol system. The magnitude of constant value was C60 ~ C70 > Gd@C82 (3.7 ± 0.1, 3.8 ± 0.1, and 2.9 ± 0.1)×104 M–1 which have good accordance with [2-4]. The fluorescence spectra are presented below (Figure S. 1). The different values of the Stern-Volmer constants for Gd@C82 are due to the different nature of the interactions [5]. It can be electrostatic interaction prevails and hydrogen bonds and van der Waals interactions [6] compared to non-endohedral fullerenes.

Figure S. 1. The fluorescent spectra of aqueous fullerene dispersions C60, C70, and Gd@C82 act as a quencher at 350 nm excitation wavelength, emission spectra registered 400–700 nm with maxima signal at 494 nm. Main areas:

(1) Raman scattering peak of water at 497 nm;

(2) transparent blue area is luminol signal (c=2.0 mM);

(3) transparent green area is ABAP signal (c=2.5 mM).

Registration conditions were: excitation and emission slits 5 nm, scanning pitch 1 nm, integration time 0.1s, detector voltage 950 V, pH 7.4 (phosphate buffer), at 293K.

## Quenching of Luminol system in the presence of fullerene

In addition, we have estimated of quenching fullerene ability for AFD C60, C70, and Gd@C82 in only the luminol added system. The linearity of the Stern-Volmer plot indicates that only one quenching mechanism is possible; it could be either dynamic or static for more speciation lifetime measurements is needed. Luminol quenching (Figure S. 2) the magnitude of constant value was C70 > C60 > Gd@C82 (7.4 ± 0.1, 5.7 ± 0.3, and 2.9 ± 0.1)×104 M–1 which have good accordance with ABAP behavior, fullerene systems only for Gd@C82.

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|  | Figure S. 2.  The fluorescent spectra of aqueous fullerene dispersions C60, C70, and Gd@C82 act as a quencher at 350 nm excitation wavelength, emission spectra registered 400–700 nm with maxima signal at 428 nm.  Registration conditions were: excitation and emission slits 5 nm, scanning pitch 1 nm, integration time 0.1s, detector voltage 950 V, pH 7.4 (phosphate buffer), luminol c=2.0 mM; at 293K. |
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