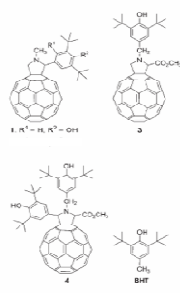


INTRODUCTION

- It has been described that fullerenes (C₆₀) present interesting properties with potential application in clinical conditions related to oxidative stress.¹
- One of the most prominent features of fullerenes is the ability to quench free radicals.
- However, because of its poor solubility, this has been studied mostly in organic solutions, while the antioxidant activity and cytotoxicity of fullerenes and their derivatives in aqueous medium is not well characterized.¹
- The antioxidant capacity of synthesised C₆₀-conjugates has been investigated and it was higher comparing to C₆₀ isolated.
- The aim of this study was to assess the viability of C₆₀-conjugates by determining its antioxidant activity and cytotoxicity in bio-relevant media**

MATERIAL AND METHODS

- Fullerene (C₆₀) and C₆₀-Butylated hydroxytoluene conjugates (C₆₀-BHT1, C₆₀-BHT2 and C₆₀-BHT3)² were solubilized either through:
 - Surfactants addition (Polyvinylpyrrolidone (PVP) or Tween20);³
 - Encapsulation in liposomes, type multilamellar vesicles (MLV's), suitable for pharmaceutical formulation;⁴
 - Suspension in water.³



- Antioxidant activity was assessed by oxygen radical absorbance capacity (ORAC)⁵ and by the assay of thiobarbituric acid reactive substances (TBARS)⁶.
- Biologic tests consisted in cytotoxicity assay⁷ and intracellular antioxidant activity⁸, using Caco-2 cell lines.

Fig. 1- Fullerene compounds used in this study: 1 - C₆₀-BHT2; 3 - C₆₀-BHT1; 4 - C₆₀-BHT3 (C₆₀-BHT conjugates were synthesized by Enes R. et al.²). BHT (Butylhydroxytoluene, a phenolic antioxidant) was used as a control.

RESULTS

SOLUBILIZATION AND ENCAPSULATION OF C₆₀ AND C₆₀-BHT CONJUGATES

- Fullerenes were successfully solubilized. The presence of C₆₀ and C₆₀-BHT conjugates in micellar solutions was confirmed in a concentration of 27,5 μM.

EVALUATION OF ANTIOXIDANT CAPACITY (ORAC ASSAY)

- C₆₀BHT2-MLV and C₆₀BHT1-PVP were the most efficient in the inhibition of free radical damage (6,3% in relative ORAC level).

Table 1. Relative ORAC value (Eq. 1) for solubilized C₆₀, C₆₀BHT1, C₆₀BHT2 and C₆₀BHT3, at the concentration of 2.75 μM. CV = coefficient of variation, n=3.

	C ₆₀	C ₆₀ BHT1	C ₆₀ BHT2	C ₆₀ BHT3	BHT
MLV	5.2 (7.9%)	4.9 (3.9%)	6.3 (6.4%)	2.5 (15.0%)	2.9 (26.6%)
PVP	5.07 (0.30 %)	6.3 (2.8%)	5.1 (9.6%)	5.0 (4.5%)	6.3 (4.3%)

$$\text{relative ORAC value} = \frac{[AUC_{\text{sample}} - AUC_{\text{blank}}] / (AUC_{\text{Trolox}} - AUC_{\text{blank}})}{\text{molarity}_{\text{Trolox}} / \text{molarity}_{\text{sample}}} \quad (\text{Eq. 1})$$

EVALUATION OF LIPID PEROXIDATION (TBARS ASSAY)

- C₆₀-MLV showed better antioxidant activity than C₆₀-aq suspensions.
- C₆₀BHT2-MLV was the most efficient against lipid peroxidation (less 40% in TBARS).

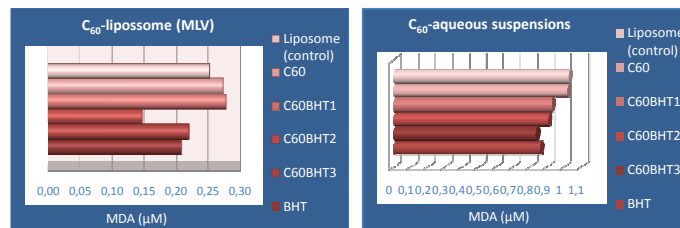


Fig. 2- TBARS value (malondialdehyde (MDA) concentration) after MDA-TBA reaction in the presence of fullerenes.

EVALUATION OF CYTOTOXICITY TO CELLS (MTS ASSAY)

- None of the tested compounds revealed cytotoxicity for up to 2.75 μM.

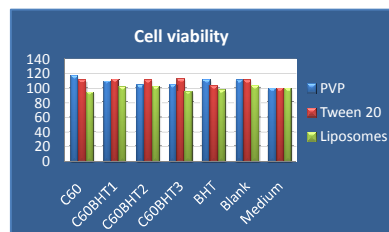


Fig. 3 - Cell viability after 4 hours contact with tested compounds, assessed by tetrazolium compound (MTS) bioreduction. Cytotoxicity would be considered if the percentage of viable cells decreased below 80%, relative to controls, in the presence of the tested compound.

EVALUATION OF INTRACELLULAR ANTIOXIDANT CAPACITY (CAA ASSAY)

- Both C₆₀BHT3-MLV and C₆₀-PVP have high intracellular antioxidant capacity, better than BHT.

Table 2 - Antioxidant activity of 2.75 μM C₆₀ and C₆₀-BHT conjugates in liposomes (MLV) and in PVP. CV = coefficient of variation, n=3.

Compounds	MLV		PVP	
	%ROS	CV (%)	%ROS Surfactant - %ROS	CV (%)
C ₆₀	95.6	3.7	10.0	61.2
C ₆₀ BHT1	89.7	1.4	15.8	66.4
C ₆₀ BHT2	88.0	8.7	17.5	62.9
C ₆₀ BHT3	78.6	4.0	26.9	63.1
BHT	88.9	5.2	16.6	63.8
MLV/PVP	105.5	7.1	0.0	73.2

$$F_{\text{rel}} = (F_{60\text{min}} - F_{0\text{min}}) / F_{0\text{min}} \quad (\text{Eq. 2})$$

$$\% \text{ ROS} = (F_{\text{rel sample}} / F_{\text{rel control}}) \times 100 \quad (\text{Eq. 3})$$

FINAL REMARKS

- The problem of fullerenes poor solubility was solved. After homogeneous dissolution in biorelevant media, we were able to assess their antioxidant activity and cytotoxicity. The obtained results were strongly dependent on the solubilization system.
- Encapsulated fullerenes (C₆₀BHT2-MLV) showed the highest antioxidant activity, even better than BHT.
- The biological tests revealed that fullerenes and their conjugates are not toxic in a concentration of 2.75 μM.
- Some compounds were able to reduce intracellular activity of ROS to a lower level than BHT.

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