VI ENCONTRO DAS CIÊNCIAS E TECNOLOGIAS DA SAÚDE
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and its conjugates with butylated hydroxytoluene

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Escola Superior de Tecnologia da Saúde de Lisboa — Instituto Politécnico de Lisboa — A Desafiar o Futuro!

Introduction

- It has been described that fullerenes (C_{60}) 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 derivates in aqueous medium is not well characterized [1].
- The antioxidant capacity of synthesised C_{60}-conjugates has been investigated and its was higher comparing to C_{60} isolated.

- **The aim of this study was to assess the viability of C_{60}-conjugates by determining its antioxidant activity and cytotoxicity in bio-relevant media**

Material and Methods

I. Fullerene (C_{60}) and C_{60}-Butylated hydroxytoluene conjugates (C_{60} -BHT1, C_{60}-BHT2 and C_{60}-BHT3) [2] were solubilized either through:

A. Surfactants addition (Polyvinylpyrrolidone (PVP) or Tween20); [3]

B. Encapsulation in liposomes, type multilamellar vesicles layers (MLV's), suitable for pharmaceutical formulation; [4]

C. Suspension in water. [3]

II. Antioxidant activity was assessed by oxygen radical absorbance capacity (ORAC) [5] and by the assay of thiobarbituric acid reactive substances (TBARS) [6].

III. Biologic tests consisted in cytotoxicity assay [7] and intracellular antioxidant activity [8] , using Caco-2 cell lines.

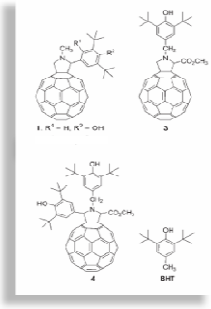

Fig. 1– Fullerene compounds used in this study:
1 - C_{60}-BHT2; 3 - C_{60}-BHT1; 4 - C_{60}-BHT3
(C_{60}-BHT conjugates were synthesized by Enes R. *et al* [2].
BHT (Butylhydroxytoluene, a phenolic antioxidant) was used as a control.

Results

Solubilization and encapsulation of C_{60} and C_{60}-BHTconjugates

- **Fullerenes were successfully solubilized.** The presence of C_{60} and C_{60}-BHT conjugates in micellar solutions was confirmed in a concentration of 27,5 µM.

Evaluation of antioxidant capacity (ORAC assay)

- **C_{60}BHT2-MLV and C_{60}BHT1-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 C_{60}, C_{60}BHT1, C_{60}BHT2 and C_{60}BHT3, 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%)

relative ORAC value = $[(AUC_{Sample} - AUC_{Blank}) / (AUC_{Trolox} - AUC_{Blank})] \times (molarity_{Trolox} / molarity_{sample})$ **(Eq. 1)**

Evaluation of lipid peroxidation (TBARS assay)

- **C_{60}-MLV showed better antioxidant activity than C_{60}-aq suspensions.**
- **C_{60}BHT2-MLV was the most efficient against lipid peroxidation** (less 40% in TBARS).

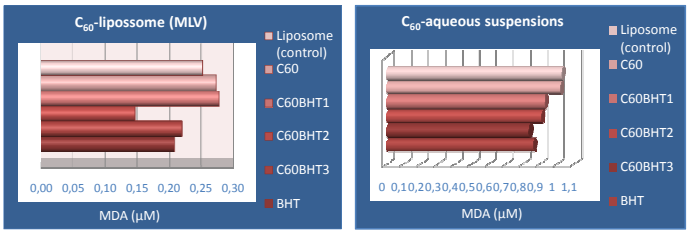

Fig. 2– TBARS value (malondialdehyde (MDA) concentration) after MDA-TBA reaction in the presence of fullerenes.

Evaluation of cytotoxicity to cells (MTS assay)

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

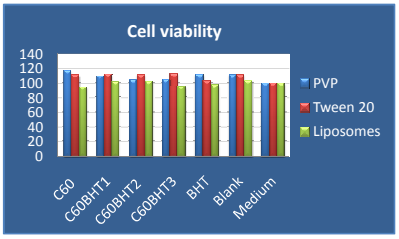

Fig. 3 – Cell viability after 4 hours contact with tested compounds, assessed by tetrazolium compound (MTS) bioreduction. Citotoxicity would be considered if the percentage of viable cells decreased below 80%, relative to controls, in the presence of the tested compound.

Evaluation of intracellular antioxidant capacity (CAA assay)

- **Both C_{60}BHT3–MLV and C_{60}-PVP have high intracellular antioxidant capacity, better then BHT.**

Table 2 -Antioxidant activity of 2.75 µM C_{60} and C_{60} -BHT conjugates in liposomes (MLV) and in PVP. CV = coefficient of variation, n=3.

	MLV			PVP		
Compounds	%ROS	CV (%)	%ROS Surfactant - %ROS	%ROS	CV (%)	%ROS Surfactant - %ROS
C_{60}	95.6	3.7	10.0	61.2	**3.4**	**12.0**
C_{60}BHT1	89.7	1.4	15.8	66.4	3.2	6.8
C_{60}BHT2	88.0	8.7	17.5	62.9	7.4	10.3
C_{60}BHT3	78.6	**4.0**	**26.9**	63.1	9.0	10.1
BHT	88.9	5.2	16.6	63.8	1.3	9.4
MLV/PVP	105.5	7.1	0.0	73.2	4.9	0.0

$F_{rel} = (F_{60min} - F_{0min}) / F_{0min}$ (Eq. 2); **% ROS** = $(F_{rel}$ sample$/F_{rel}$ control$) \times 100$ (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 (C_{60}BHT2-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.**

Acknowledgements

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