ENCAPSULATION OF LEAD IONS IN NANOTECHNOLOGY STRUCTURES

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ABSTRACT

Lead is a heavy metal which shows a multi-system tissue toxicity. In this work, we focused on the potential use of electrochemical method for determination of free and liposome-encapsulated lead and on determination of the encapsulation efficiency preventing the lead toxicity.

INTRODUCTION

- **Lead**
  - heavy metal
  - toxic at extremely low dose
  - acute and chronic effects on human health multi-system tissue toxicity (neurological, cardiovascular, renal, gastrointestinal, hematologic, reproductive, genotoxic and carcinogenic effects) [1]
- **Liposomes**
  - spherical nanoshells composed of lipid bilayers that enclose an aqueous phase
  - stable in solution for a long period of time [2]
  - small and controllable size (from tens for thousands of nm)
  - presence of internal cavities
  - the most investigated organic nanoparticles [3]

RESULTS AND DISCUSSION

- Measured values of lead trapped in liposome were distinctly lower in comparison with the originally embedded lead concentrations (Fig. 2 A). Application of lower originally embedded lead concentrations (2.4 or 4.8 mM) resulted in greater capture of lead ions in the liposome.
- The signal of lead encapsulated in the liposome was reduced in comparison to the signal of lead standard (Fig. 2 B, C).
- With increasing scan rate the lead signal increased (Fig. 3 A, B). The change of the lead peak potential is not expressly influenced by scan rate (Fig. 3 C). Signals of lead in liposomes were lower than signals in samples containing only lead standard (Fig. 3 D).
- The calculated IC 50 values for evaluating the lead cytotoxicity show significant differences between the lead enclosed in liposome (29.1 μM) and free ions lead (198.3 μM). From the cytotoxic studies to the bacterial strain of *S. aureus* was observed that the free lead ions are less toxic in comparison with lead encapsulated in liposomes. The bar graph of growth speed shows a very gradual increase in the start of measurement with all lead ions concentrations applied in both enclosed and free form (Fig. 4).

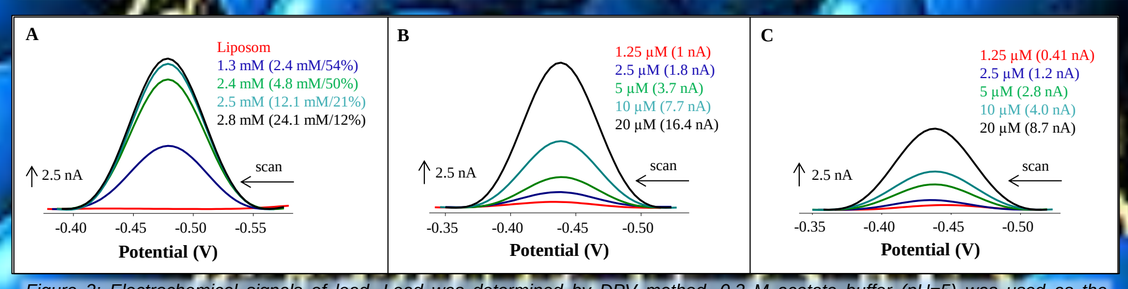

*Figure 2: Electrochemical signals of lead. Lead was determined by DPV method. 0.2 M acetate buffer (pH=5) was used as the supporting electrolyte. Characteristic peak for lead was at potential of -0.4 V. (**A**) Voltammograms of lead encapsulated in the liposome with concentration range 0 – 2.8 mM (applied concentration/percentage of capture), (**B**) voltammograms of lead standards with concentration range 1.25 – 20 μM, (**C**) voltammograms of lead encapsulated in the liposome with concentration range 1.25 – 20 μM.*

CONCLUSION

Using electrochemical methods, we confirmed the lead encapsulation into the liposome structure during the sample preparation and the influence of the matrix to the lead detection. Lead encapsulated in the liposome is due to the matrix more easily accessible to the cells and therefore its toxicity in comparison with the free lead ions is higher. The electrochemical methods could be very suitable tools for determination of electro-active compounds enclosed in liposomes.

REFERENCES

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[2] Vamvakaki, V., D. Fournier, and N.A. Chaniotakis, *Fluorescence detection of enzymatic activity within a liposome based nano-biosensor.* Biosensors & Bioelectronics, 2005. **21**(2): p. 384-388.
[3] Livney, Y.D., *Milk proteins as vehicles for bioactives.* Current Opinion in Colloid & Interface Science, 2010. **15**(1-2): p. 73-83.

MATERIAL AND METHODS

Preparation of liposome filled with lead (Fig. 1)
Electrochemical determination
- *Differential pulse voltammetry (DPV)*
- *Cyclic voltammetry (CV)*

Atomic absorption spectrometry (AAS) - comparative method for lead determination.
Toxicity determination of free lead ions and lead encapsulated in the liposome
Staphylococcus aureus (NCTC 8511, Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic). Bacterial culture was diluted by cultivation medium to OD_{600} = 0.1 and used in the following experiments: Evaluation of the antimicrobial effect of tested compounds - Multiskan EX (Thermo Fisher Scientific, Germany) and subsequent analysis in the form of growth curves. The concentrations of lead: 0 - 250 μM, measurements: every 30 minutes for 24 hours at 37 °C at the wavelength of 620 nm.

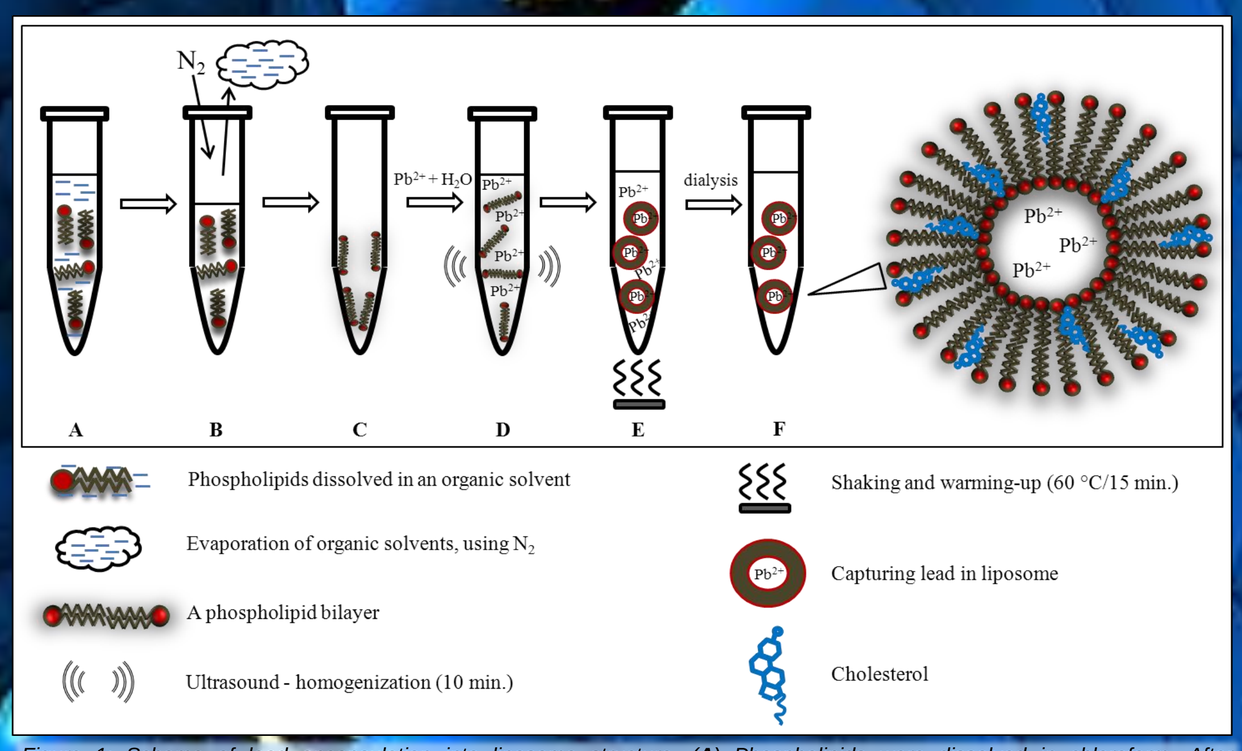

*Figure 1: Scheme of lead encapsulation into liposome structure. (**A**) Phospholipids were dissolved in chloroform. After evaporation of the solvent (**B**) lipid film was obtained (**C**). (**D**) Lead solution were added to the phospholipids bilayer. Samples were homogenized for 10 minutes using ultrasound. (**E**) The homogenized mixtures were heated and shaken for 15 min at 60 °C at Thermomixer Comfort (Eppendorf). (**F**) Non-captured lead ions were removed from the solution by dialysis.*

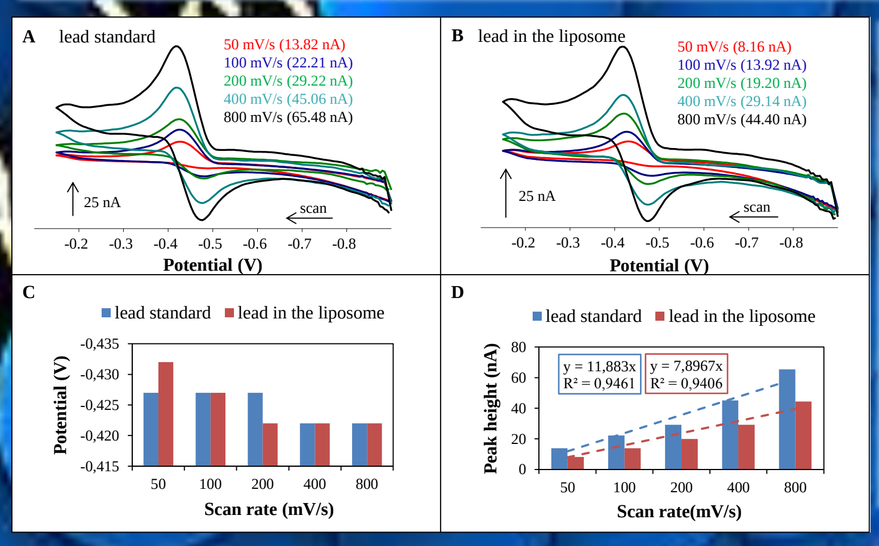

*Figure 3: Electrochemical signals of lead. Lead was determined by CV method using different scan rate (50 – 800 mV/s). 0.2 M acetate buffer (pH=5) was used as the supporting electrolyte. Characteristic peak for lead was at potential of -0.4 V. (**A**) Voltammograms of lead standards with concentration of 20 μM, (**B**) voltammograms of lead in the liposome with concentration of 20 μM, (**C**) Changing the position of the lead standard peak and peak of lead encapsulated in the liposome depending on the applied scan rate. (**D**) Linear dependence for the peak heights of the lead standard signals and lead encapsulated in liposome signals on the applied scan rate.*

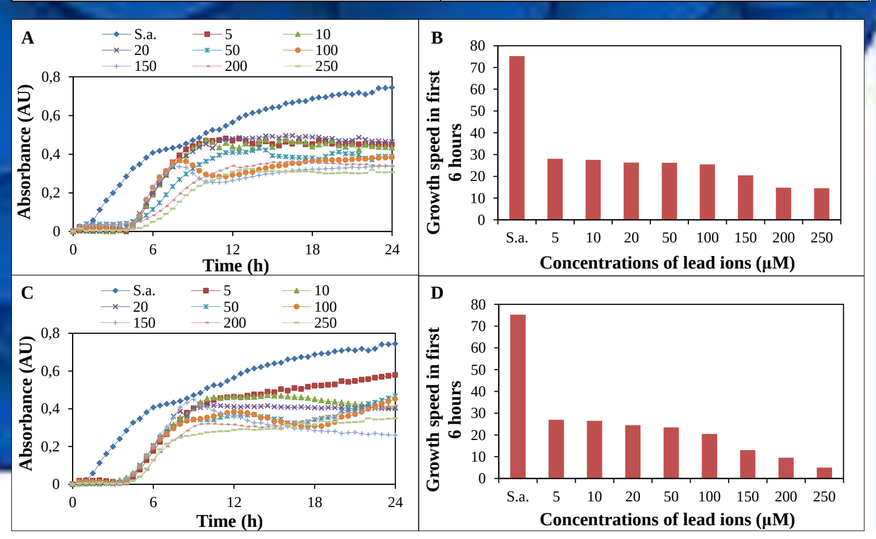

***Figure 4** Spectrophotometric analysis of growth of the Staphylococcus aureus with different concentrations of lead. **(A)** growth curves of S.aureus with different concentrations of lead without liposome, **(B)** Spectrophotometric analysis of the growth of Staphylococcus aureus bacterial culture with lead ions concentrations 5, 10, 20, 50, 100, 150, 200 and 250 μM in 6 hours,**(C)** growth curves of S.aureus with different concentrations of lead closed in liposome, **(D)** Spectrophotometric analysis of the growth of Staphylococcus aureus bacterial culture with lead ions concentrations 5, 10, 20, 50, 100, 150, 200 and 250 μM in 6 hours closed in liposomes*

ACKNOWLEDGMENT

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