evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

The determination of lead ions encapsulated in liposomes

Název:

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Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

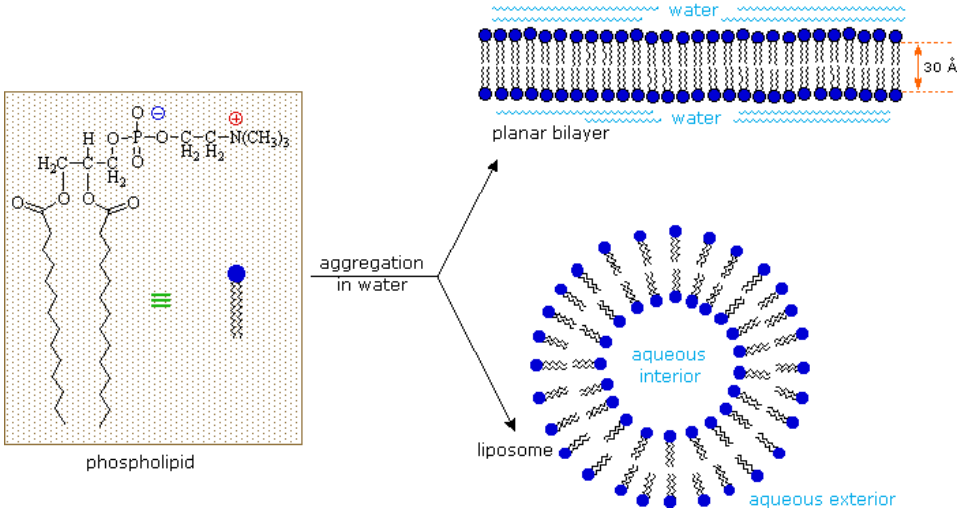
LEAD (Pb)

- 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)

Lead (II) nitrate

LIPOSOMES

- Spherical nanoparticles composed of lipid bilayers that enclose an aqueous phase
- stable in solution for a long period of time
- small and controllable size (from tens for thousands of nm)
- presence of internal cavities
- the most investigated organic nanoparticles

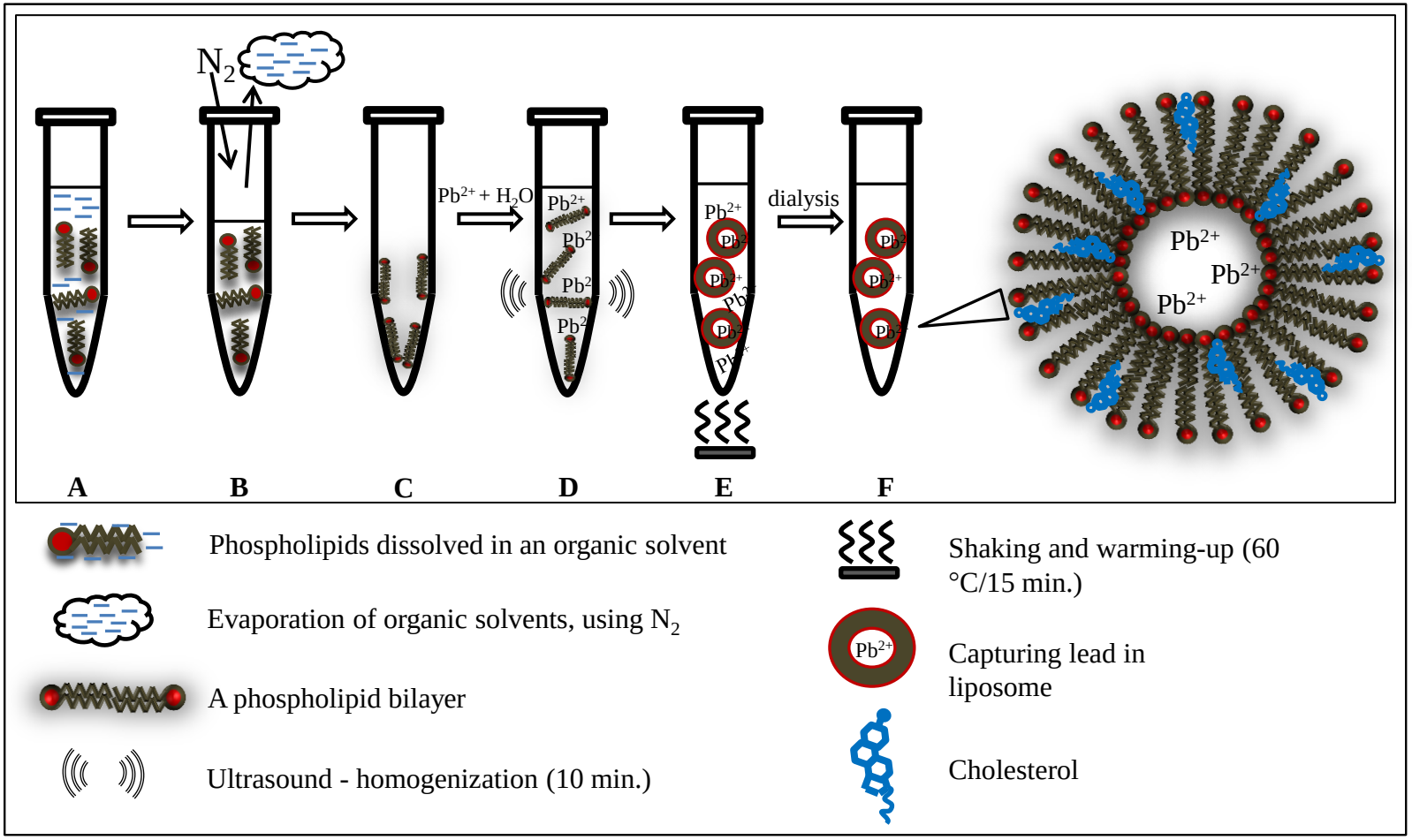


AIM OF OUR STUDY

- preparation of liposome complex with encapsulated lead ions
- potential use of electrochemical method for determination of free and liposome-encapsulated lead
- determination of the encapsulation efficiency preventing the lead toxicity

MATERIALS AND METHODS

- Preparation of liposome filled with lead

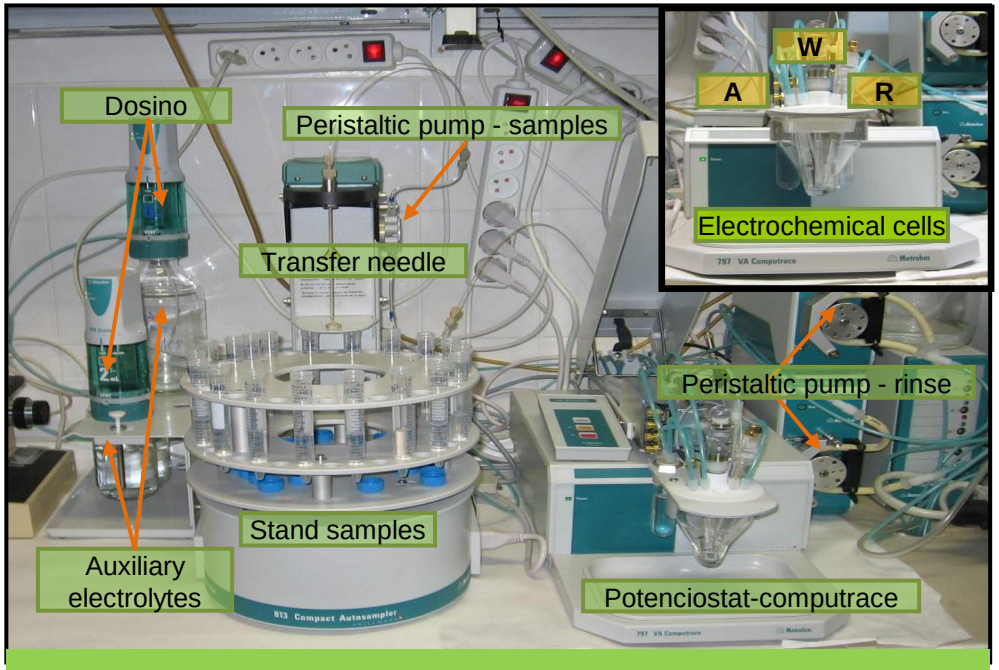


Scheme of lead encapsulation into liposome structure. (**A**) Phospholipids were dissolved in chloroform. After evaporation of the solvent (**B**) lipid film was obtained (**C**). (**D**) Lead solution were added to the phospholipids bilayer. Samples were homogenized for 10 minutes using ultrasound.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

MATERIALS AND METHODS

- Electrochemical methods (**differential pulse voltammetry** and **cyclic voltammetry**) and **atomic absorption spectrometry** were used for the detection of lead and lead encapsulated in the liposome.

DPV and CV

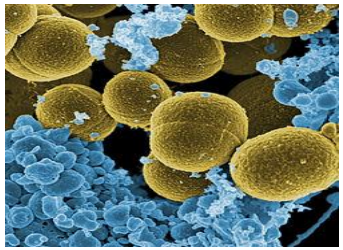


813 Compact Autosampler - Metrohm

AAS

Atomic absorption spectrometer
Agilent Technologies

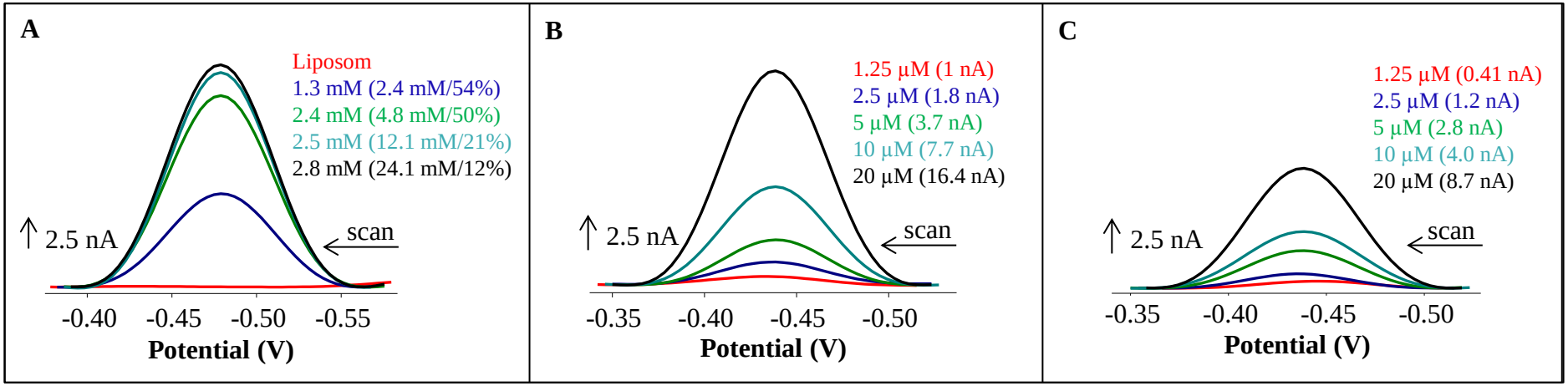
- The **toxicity** of free lead ions and lead encapsulated in the liposome was observed using bacterial strains ***S. aureus*.**



RESULTS

The creation of this nanostructure was tested using electrochemical methods and AAS.

DPV

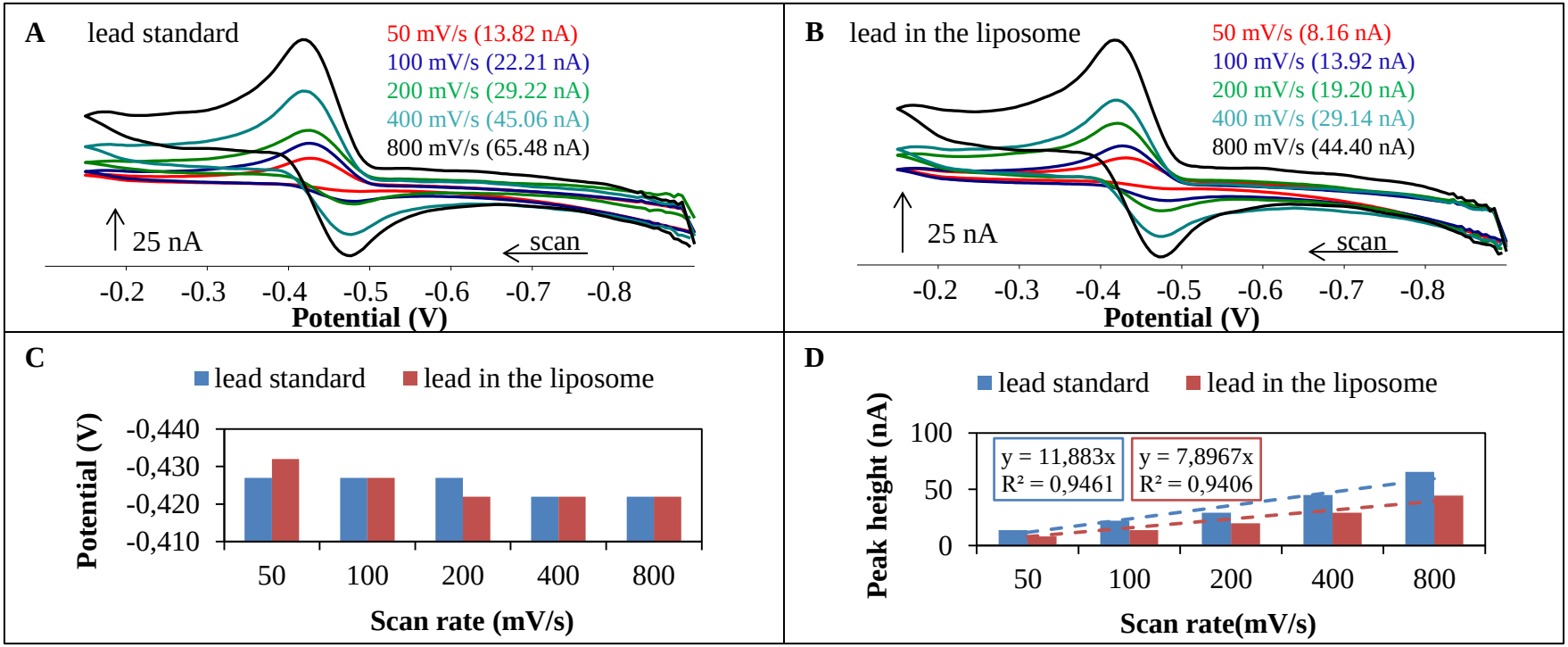


Electrochemical signals of lead. Lead was determined by DPV method. 0.2 M acetate buffer (pH=5) was used as the supporting electrolyte. Characteristic peak for lead was at potential of -0.4 V. (**A**) Voltammograms of lead encapsulated in the liposome with concentration range 0 – 2.8 mM (applied concentration/percentage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

- Measured values of lead trapped in liposome were distinctly lower in comparison with the originally embedded lead concentrations
- It was found that the application of lower original lead concentrations (**2.4** or 4.8 mM) resulted in higher efficiency of the capture of lead ions in the liposome (A)
- The signal of encapsulated lead was reduced in comparison to the signal of lead standard (B, C).

RESULTS

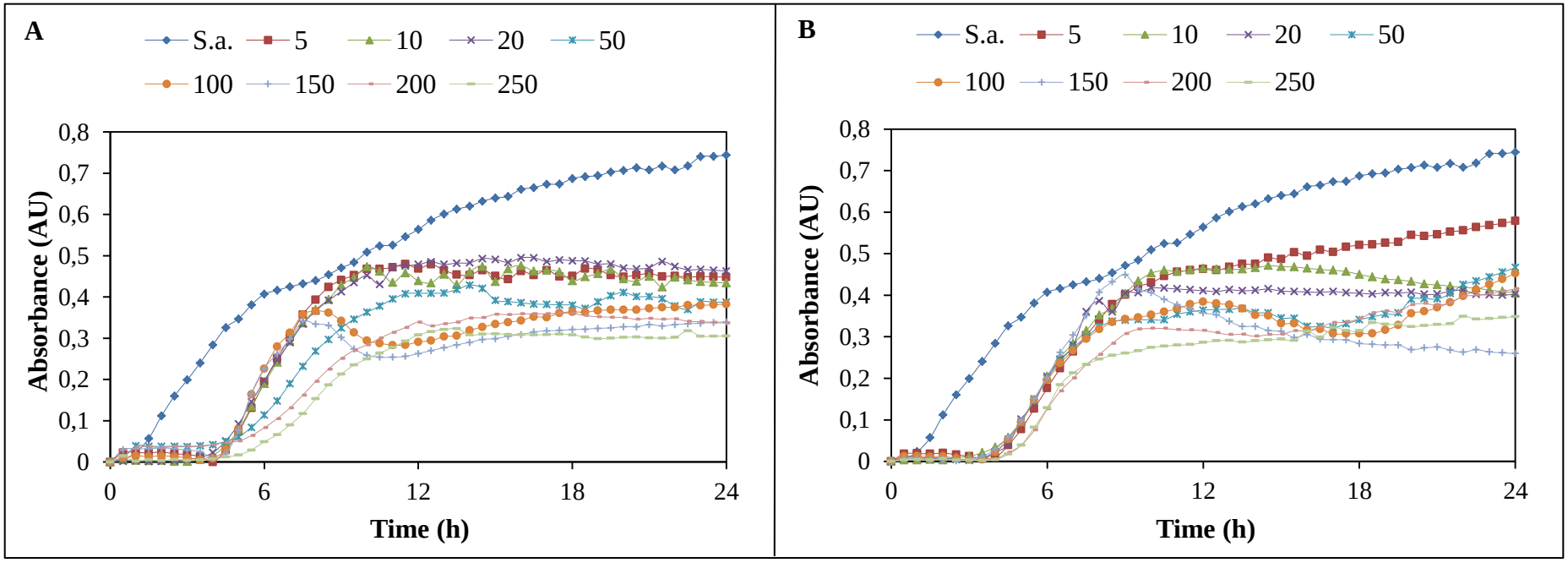
CV



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.

- With increasing scan rate the lead signal increased (A, B)
- Change of the lead peak potential is not expressly influenced by scan rate (C)
- Signals of lead in liposomes were lower than signals in samples containing only lead standard, so we can say that the matrix (liposome) reduces the detection of lead in the samples

RESULTS - toxicity



Spectrophotometric analysis of growth of the *S. aureus* with different concentrations of lead. (A) growth curves of *S. aureus* with different concentrations of lead without liposome, (B) growth curves of *S. aureus* with different concentrations of lead closed in liposome.

Pb without liposome - Abs. 6h	1.0	µM	Pb in liposome - Abs. 6h	137.5	µM
Pb without liposome - Abs. 12 h	29.1	µM	Pb in liposome - Abs. 12 h	198.7	µM
Pb without liposome - Abs. 18 h	1.0	µM	Pb in liposome - Abs. 18 h	1.0	µM
Pb without liposome - Abs. 24 h	1.0	µM	Pb in liposome - Abs. 24 h	1.0	µM

Inhibition concentration of lead ions in or without liposome in four different times.

The statistically calculated **IC 50** values for evaluating the lead cytotoxicity show significant differences between the lead enclosed in liposome (198.7 µM) and free ions lead (29.1 µM).

RESULTS - summary

- We have managed to enclose the lead to liposome and we have confirmed the encapsulation by electrochemical methods.

- We studied the reduction of lead signal affected by matrix (liposome).

- From the cytotoxic studies to the bacterial strain of *S. aureus* was observed that the free lead ions are more toxic in comparison with lead encapsulated in liposomes cytotoxic.

CONCLUSION

- Liposomes appear to be a suitable carrier of various substances through the inner cavity.
- Due to the liposome structure the lead enclosed in the liposome is easier to accept into cells structure but the toxicity of the enclosed lead is lower in comparison to free lead ions.
- Description and quantification of the enclosing process with liposome structure will continue.

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Thanks:
Pavel
Maruška
Dáša

Mendel University in Brno

Thank you for your attention!

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