# ULTRASOUND IRRADIATION TO VISUALIZE AND TRIGGER THE RELEASE OF DOXORUBICIN FROM HYBRID MICROPARTICLES

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**Abstract**

In the presented work the simultaneous use of ultrasound in tracking and smart release was investigated. Hybrid microparticles based on an iron core coated by a polysaccharide were prepared and loaded with doxorubicin. The possibility to trigger and control the release of the payload was demonstrated by application of ultrasound cycles at different power and time length. Results clearly indicate a direct correlation between the intensity of the release with the two parameters. A preliminary test using *Sus Domestic* liver demonstrates the possibility to visualize the faith of the microparticles after injection at the target site by a linear array ultrasound transducer. The dual function of the ultrasound; to visualize the position of the microparticles and afterwards trigger the release by changing the frequency was demonstrated offering a great perspective for a future biomedical application.

**Keywords:** Ultrasound, microparticles, stimuli-responsive release, doxorubicin, iron oxide

## 1. INTRODUCTION

The diagnostic ultrasound, or sonography, represents a well-known imaging method that uses high-frequency sound waves to produce images of structures within the body. The images can provide valuable information for diagnosing and treating a variety of diseases and conditions. In the last decades a great interest has been focused on the use of ultrasound irradiation as external trigger for smart drug release by altering a number of parameters including frequency, power density, duty cycles, and application time [1]. It represents an innovative approach to addressing unmet clinical needs by providing a minimally-invasive platform for targeted delivery of pharmaceuticals [2]. It enables high concentrations of drugs to be deposited at specific locations within the body and is a preferred option for non-systemic diseases. This produces more constant and controlled drug concentration profiles with favourable pharmacokinetics [3].

Herein, the simultaneous use of ultrasound irradiation for detection and trigger the release of the anticancer drug doxorubicin (DOX) from a multifunctional system based on a hybrid microparticles was investigated. Iron oxide microparticles were modified by diazonium chemistry to improve the surface properties and favouring the polymer coat to enhance the stability in solution and the loading capacity. Prepared carrier was characterized in terms of dimension, surface charge, stability and loading capacity. The capability to trigger the release and control the intensity was evaluated by application of ultrasound cycles at different power (2 and 20 W/cm$^2$) and time length. The possibility to visualize microparticles by ultrasound technique was evaluated using *Sub domestic* (domestic pig) liver after injection of a physiological solution containing the microparticles. The positive results demonstrate the possibility of simultaneos use of ultrasound to detect the and trigger the release of the payload at the target site. The prepared system presents promising advantages to be considered as a good candidate for a future theranostic application.

## 2. MATERIALS AND METHODS

### 2.1. Materials

The following were supplied by Sigma Aldrich: low-molecular-weight chitosan (CS), ($M_w$ < $10^4$ g/mol, D.D 75-85%); *N*-HydroxySuccinimide (NHS); N-(3-Dimethylaminopropyl)-N'-Ethylcarbodiimide hydrochloride (EDC), commercial grade, powder; methane sulphonic acid (MSA), N,N-Diethylformamide 99%; *tert*-Butyl nitrite, 4-toluenesulphonic acid (p-TsOH); 4-nitroaniline, iron trichloride ($FeCl_3$); sodium borohydride; and doxorubicin hydrochloride. $C_3H_6O_3$ L-Lactic acid, 80% water solution, was purchased from Lachner Neratovice, Czech Republic. Sodium chloride, potassium dihydrogen phosphate, sodium carbonate and sodium hydroxide were acquired from Penta, Prague, Czech Republic. The $C_3H_6O$ solvent acetone, sodium hydroxide, sodium chloride, sodium phosphate and potassium phosphate were bought from IPL Lukes, Uhresky Brod, Czech Republic. Chloroform $CHCl_3$ (HPLC grade), acetic acid $CH_3CO_2H$ (HPLC grade) and hydrochloric acid were purchased from Chromservis, Prague, Czech Republic.

### 2.2. Surface modified microparticles preparation, drug loading and characterization

Microparticles were prepared following the method described elsewhere [4] with some modification. Briefly, diazonium salt prepared as reported [5] was dissolved (0.41 g) in 20 ml of distilled water and left under stirring for 15 minutes. Meanwhile, 5 ml of $FeCl_3$ (0.16 g) solution in water and 5 ml of $NaBH_4$ (0.08g) water solution were mixed under inert gas ($N_2$) for 5 minutes. Then another 5 ml of each solution was added and left under mechanical stirring for 10 minutes. Afterwards, the solution containing diazonium salt was added and left under stirring for 40 minutes. Microparticles were precipitated using a magnet and washed 3x water, 3x ethanol and 3x acetone and dried under vacuum.

The loading and coating process was performed simultaneously. A water solution of doxorubicin (1mg/ml) was prepared and mixed with a solution containing chitosan (0.5 mg/ml) in 1% v/v acetic acid. Dried microparticles were dissolved in water and mixed with the DOX and chitosan solution and left under mechanical stirring for 3h to allow the coating. Microparticles were recovered by centrifugation and the amount of DOX-loaded was evaluated indirectly by UV-Vis analysis of the supernatant at 480 nm. The presence of the organic coat was proved by FTIR-ATR and by total organic carbon analysis.

Microparticles dimension in distilled water was evaluated by laser diffraction (Mastersizer, Malvern) while the ζ–potential by dynamic light scattering (Zetasizer, Malvern).

### 2.3. Release studies

To evaluate the possibility to trigger and control the release of DOX by ultrasound application was analysed and then, compared to passive release (absence of ultrasound). Two set of microparticles solution (1 mg/ml; V: 50 ml; pH 7.4) were prepared, transferred into plastic tubes and incubated at 37°C. The effect of ultrasound on the release kinetics was evaluated by the application of 5 cycles with different power and time length. One set of microparticles undergoes cycles of 30 seconds sonication 1MHz (2W/cm$^2$ and 20 W/cm$^2$); while the other set was exposed to 5 cycles of 60 seconds each at the same powers. The time between two successive cycles was 20 minutes. After each cycle and between two consecutive cycles 3 ml of solution was withdrawn and replaced by fresh buffer solution and analysed by UV-Vis spectrophotometer at 480 nm to detect the amount of DOX.

### 2.4. Visualization

The possibility to detect the microparticles using ultrasound was investigated using *Sus Domesticus (domestic pig)* liver obtained from slaughter. A stock solution of bare microparticles 5mg/ml was prepared and 1ml injected in a designed site of the liver and subjected to ultrasound using a linear array transducer.

## 3. RESULTS AND DISCUSSION

### 3.1. Microparticles characterization and drug loading

Laser diffraction analysis showed microparticles with an average dimension of 10 μm with 0.2 PDI and good stability in physiological condition up to one week despite the ζ-potential around 12 mV.

FTIR-ATR and total organic carbon analysis demonstrated the success of the surface modification and coating. The presence of the organic layer due to the surface modification and the presence of the polymer coat were demonstrated by the representative peaks; i) for CS at 3350 $cm^{-1}$ (O-H stretch), 2870 $cm^{-1}$ (C-H stretch), 1590 $cm^{-1}$ (N-H deformation) 1060 $cm^{-1}$ (C-O-C) and ii) 1750cm-1 for C=O of the benzoic acid. Total organic carbon analysis displayed an increase in the total organic carbon of 7% and 18% following surface modification and coating, respectively.

UV-Vis evaluation at 480nm demonstrates a high encapsulation efficiency of DOX reaching overcoming 300 μg of drug per mg of microparticles.

### 3.2. Release studies and microparticles visualization

The enhanced intensity of the DOX releaseafter application of ultrasound could be ascribed to two main factors; i) the oscillatory motion of the surrounded fluid which increases the diffusivity of the unbounded drug [2] and ii) the distruption of the carrier. However, the second one plays a minor role because in case of complete wreackage of the particles a more intense burst would be detected in a narrow time interval.

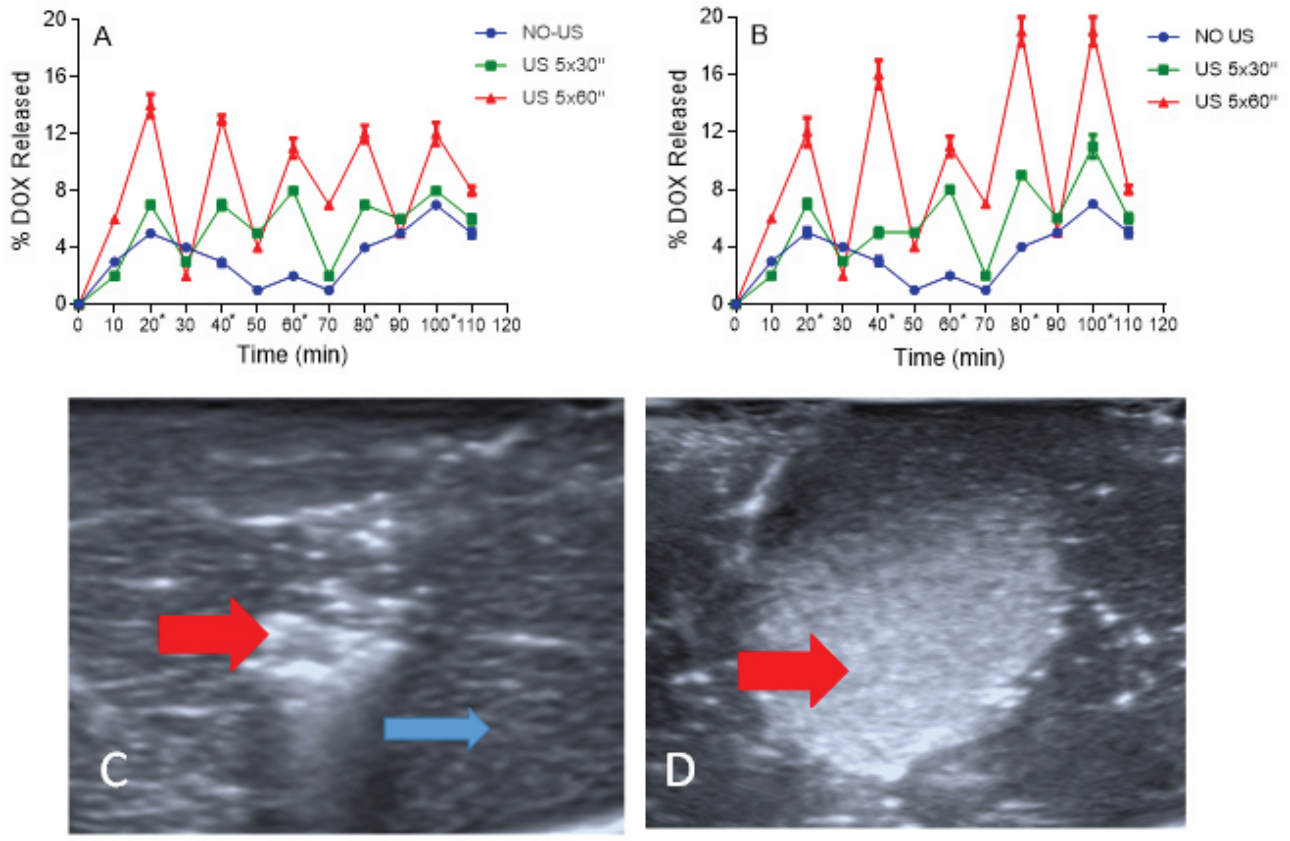


**Figure 1** Effect of different time length and power of US cycles on the release rate of DOX in physiological solution (pH 7.4).Ultrasound power A) 2W/$cm^2$ and B) 20W/$cm^2$. Echography of *Sus Domesticus* liver containing microparticles at the injection site C and D. Red arrows indicate the microparticles while blue the air bubble

The trend reported in the **Figure 1 A, B** describes the direct relation between the intensity of the release and the power and time length of the applied ultrasound. In correspondence of the ultrasound application an intensive peak is observed with a magnitude that is proportional to the ultrasound power. The ecographs reported in the **Figure 1 C, D** performed on the *Sus Domesticus* liver show the possibility to visualize the microparticles after injection. Microparticles after injection precipitate and tend to accumulate in a confined area close to the injection site.

## 4. CONCLUSION

The use of ultrasound in diagnostic medicine is well-known and a great interest in the smart delivery application has been grown. The presented study is focused on the evaluation of possible simultaneous use of ultrasound for detection and for trigger the release of the model drug doxorubicin from a hybrid system. Microparticles based on an iron core coated by polysaccharides with high loading capacity and stability in physiological conditions were developed. The results reveal the ability to visualize the location of the microparticles after injection in domestic pig liver and at the same time trigger the release and modulate the intensity by changing the power and the irradiation time. The developed microparticles represent innovative systems with great potential for future application in drug delivery, in particular for site-specific release of bioactive compounds.

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