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Original article

Sulfanilamide in solution and liposome vesicles; *in vitro* release and UV-stability studies

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ABSTRACT

The main goal of this study was to develop a liposome formulation with sulfanilamide and to investigate the liposomes impact on its release and stability to the UV-A/UV-B and UV-C irradiation. Liposome dispersions with incorporated sulfanilamide were prepared by thin-film hydration method and liposomes role to the sulfanilamide release was investigated by using a dialysis method. Comparatively, sulfanilamide in phosphate buffer solution was subject to release study as well to the UV irradiation providing for the possibilities of kinetics analysis. *In vitro* drug release study demonstrated that 20% of sulfanilamide was released from liposomes within 1 h that is approximately twice as slower as in the case of dissolved sulfanilamide in phosphate buffer solution. The kinetic release process can be described by Korsmeyer–Peppas model and according to the value of diffusion release exponent it can be concluded that drug release mechanism is based on the phenomenon of diffusion. The sulfanilamide degradation in phosphate buffer solution and liposomes is related to the formation of UV-induced degradation products that are identified by UHPLC/MS analysis as: sulfanilic acid, aniline and benzidine. The UV-induced sulfanilamide degradation in the phosphate buffer solution and liposome vesicles fits the first- order kinetic model. The degradation rate constants are dependent on the involved UV photons energy input as well as sulfanilamide microenvironment. Liposome microenvironment provides better irradiation sulfanilamide stability. The obtained results suggest that liposomes might be promising carriers for delayed sulfanilamide delivery and may serve as a basis for further research.

1. Introduction

Sulfanilamide, 4-aminobenzenesulfonamide (SA) belongs to a group of sulfonamides, the synthetic antimicrobial drug agents, commonly used in treatments against Gram-positive and Gram-negative bacteria as well as in the treatments of fungi and protozoa infections (Henry, 1943; Gray et al., 1995; Sukul and Spiteller, 2006; Varagić and Milošević, 2009). Because of heteroatoms, aromatic ring and other functional chromophore groups in the structure that are sensitive to solar radiation, sulfonamides are considered unstable. Photocatalytic degradation of sulfonamides may occur due to absorption of solar radiation (direct photocatalysis), or by reactive free radicals action such as singlet oxygen, hydroxyl radicals and other reactive species (indirect photocatalysis) (Lam and Mabury, 2005). Photodegradation efficiency in an aqueous medium depends on the intensity and frequency of applied radiation treatment, medium pH (Trovó et al., 2009; Baran et al., 2006) as well as of a photosensitizer, such as humic acid and nitrates, which can be present in the solution. A pH affects the degradation reaction rate, but does not affects the type of the degradation products in the reaction mentioned above (Boreen et al., 2004). Zessel et al. (2014) examined the sulfonamides stability to degradation under the influence of UV-A irradiation, a combination of UV-A and UV-B irradiation and sunlight. The results showed that all investigated sulfonamides are subject to degradation under the action of UV irradiation. Photo degradation was most pronounced after exposure to combined UV-A/UV-B irradiation, then after the UV-A, and finally after exposure to sunlight irradiation. The sulfonamides have a different inclination towards

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photo degradation depending on the chemical structure; thus, sulfathiazole and SA were almost completely decomposed after only 4 h of UV-A/UV-B irradiation. Of all investigated sulfonamides, sulfamethoxypyridazine, sulfachloropyridazine and SA had the greatest degree of degradation (31–65%) when exposed to UV-A irradiation. Photocatalytic degradation of various sulfonamides in the aqueous solution resulted in the formation of different degradation products, including the most commonly present sulfanilic acid, aniline, hydroquinone, carboxylic and dicarboxylic acids. The most possible degradation routes include severing S—N bonds and a loss of SO_2 molecule (Periša et al., 2013). In addition, hydroxylation of benzene ring (Trovó et al., 2009) and R residue that are bonded to the nitrogen of the sulfonamide groups are also possible degradation reactions (Lam and Mabury, 2005).

In addition to these data, there is generally a lack of information's about SA stability and possible degradation products. Because of that, there is a great need for new analytical methods capable to determine SA degradation products that are produced after *in vitro* or *in vivo* oxidation caused by free radicals acting. Also, there is a great need to determine the SA stability in pharmaceutical as well as cosmetics formulations because of wide use of this active compound. On the other hand, due to poor solubility of SA in the aqueous medium (Delgado et al., 2011), the SA use is limited. In order to improve the SA solubility and photostability, SA was incorporated in liposomes.

In this work we have investigated SA stability under influence of UV (UV-A, UV-B and UV-C) irradiation in phosphate buffer solution (PBS) and in liposomes the commonly used vesicular carriers for targeted therapies. After incorporation in liposomes, SA release from the liposomes was also investigated. In order to investigate the liposome size influence on distribution and activity of incorporated SA, three types of liposomes: small unilamellar vesicles (SUV), large unilamellar liposomes (LUV) and multi lamellar vesicles (MLV) were obtained. SA was irradiated in PBS and in liposomes but this time UHPLC techniques in combination with absorption spectroscopy were employed to analyze the formation of SA degradation products induced by continuous UV irradiation to provide the data for a kinetic analysis. The results obtained in this work may serve for further investigations of SA activity in active formulations. Also, applied optimized UHPLC-MS-MS method for identifying degradation products generated under UV irradiation can be regarded as selective, rapid and sensitive and may serve to further studies.

2. Materials and method

2.1. Materials

1.2-Diacyl-*sn*-glycero-3-phosphocholine (PC, critical temperature Tc = 15 °C) was purchased from Sigma Aldrich (Germany) and used without further purification. According to the declaration statement the PC mixture has the following fatty acid composition: 33% palmitic acid, 13% stearic acid, 31% oleic and 15% linoleic acid (other fatty acid were present in a negligible amount). The phosphate buffer solution (Na_2HPO_4-KH_2PO_4, pH 7.4), was prepared using analytical grade reagents (Centrohem, Serbia) and purified water from a Millipore Milli-Q system (conductivity $\leq$ 0.1 μS·cm^{-1}). All other materials and solvents were of analytical grade.

2.2. Sulfanilamide synthesis

Procedure of SA synthesis was carried out as previously described (Tačić et al., 2014). Chlorosulfonic acid together with acetanilide was heated for 1 h at 65 °C and then cooled. The separated crystals of 4-(acetylamino)benzenesulfonyl chloride were dissolved in water after ammonia was added and left for 24 h. The filtered crystals in the round flask were then treated with sodium hydroxide and heated under reflux. The concentrated hydrochloric acid was carefully added into the mixture, and then neutralized. The obtained SA was recrystallized from the water in order to obtain the pure substance.

2.3. Liposomes preparation

MLV, LUV and SUV liposomes with incorporated SA were prepared according to the thin-film hydration method, as previously described (Milenkovic et al., 2013; Petrović et al., 2014) with slight modifications. The PC lipid was dissolved in chloroform at the molar concentration of 5×10^{-4} M. The SA dissolved in PBS was added to the lipid at the concentration of 1×10^{-5} M, considering the final buffer volume of liposome suspension. The obtained liposome dispersions after preparation procedure were MLVs. In order to obtain LUVs and SUVs, the liposome dispersion was vortexed (HI 190M, Hanna instruments, Italy) at a speed of 500 rpm, and then extruded (LiposoFast-Basic Extruder, Avestin, Inc. Canada) through poly-carbon filters of 400 and 100 nm pore size respectively. UV–Vis absorption spectroscopy was used to rapidly estimate the dimension and lamellarity of liposomes with incorporated SA. All the operations have been performed above the critical temperature (Tc) of lipid in order to avoid defects. After preparation, all dispersions were stored at 4 °C and after 24 h their characterization was performed. The spectral data have been processed by using the software Origin 8.0.

2.4. In vitro SA release from liposomal dispersions

SA release from MLV liposomes was studied using a dialysis method. Before use, the dialysis bags (dialysis tubing cellulose membrane, Sigma Aldrich, *Mr.* cut-off 12,000 (Germany)) were soaked in PBS at a room temperature of 23 °C for 24 h to remove the preservative, followed by rinsing thoroughly in distilled water. Liposomal dispersion, 5 mL, was placed in dialysis bag of 11 cm initial length and 43 mm flat width. The bag was closed at both ends with plastic clips and tested for leakage. The final length of the bag after tying was 3 ± 0.2 cm. The dialysis bag was attached horizontally, fully stretched to the magnet on magnetic stirrer, which was then immersed in the 150 mL glass containing 50 mL of PBS pH 7.4. The bag was fully immersed under the surface. The temperature was set at 23 ± 0.2 °C and the rotation speed was set at 100 rpm to simulate *in vivo* conditions. Control bags were prepared and tested along with the liposomal dispersions. Aliquots of the release medium were withdrawn for analysis at different time intervals. Release runs were continued for 2 h. The absorbance of the collected samples was measured at SA max absorption at 258 nm. Control SA release was also run at the same conditions, from the SA-PBS pH 7.4 and identical SA concentration as in liposome dispersion. SA release kinetics in liposome and PBS was evaluated using different mathematical models (zero order, first order, Higuchi, Korsmeyer-Peppas, Baker-Lonsdale), using *DDSolver* package for *Microsoft Excel* application.

2.5. SA photostability study

The SA samples in PBS and in liposomes were exposed to the UV-A UV-B and UV-C irradiation. The samples were treated by UV-A irradiation in a period of 0–150 min, UV-B irradiation in a period of 0–3 min and UV-C irradiation in a period of 0–0.5 min in the cylindrical photochemical reactor "Rayonnet" with 10 symmetrically placed UV-A and UV-B and 8 UV-C lamps having an emission maximum at 350 nm (UV-A), 300 nm (UV-B) and 254 nm (UV-C), respectively. The quartz cells (1 × 1 × 4.5 cm)

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