Technical Information

Version 1.1
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Doxosome™ Kit-Doxorubicin Liposomes (Non-PEGylated)

DESCRIPTION

Non-PEGylated doxorubicin liposome comes in a three-vial kit and has a lipid composition and liposome size similar to commercial Myocet®. Doxorubicin drug cannot be pre-loaded to the liposomes in the same way that is pre-loaded into the PEGylated liposomes. The matrix liposome in PEGylated liposome is hydrogenated soy PC which is a saturated lipid and creates a tight packed liposome suitable for pre-loading and holding the drug inside the liposomes. The matrix lipid in this formulation is L-α-phosphatidylcholine which is an unsaturated lipid. It makes a loose packed membrane and therefore, it cannot hold the drug for a long time. The doxorubicin drug should be loaded into the liposomes moments before use.

The kit comes in three vials. The first vial contains a very concentrated solution of liposomes. The liposomes are made in citrate buffer at pH 4. The second vial is composed of sodium carbonate at pH 11.4. Adding vial 2 to vial 1 makes a pH gradient around the lipid membrane. Due to the difference in the pH inside and outside of the liposomes, there will be a constant flux of proton from the inside to the outside of the liposomes. Due to this flux, doxorubicin molecules move in the opposite direction from the outside to the inside of the liposomes. During the remote loading process, due to pH gradient, the doxorubicin drug is able to freely pass into the liposomes where it is bound by hydrogen and nanocrystals of doxorubicin citrate salt to form larger doxorubicin compounds. As the new doxorubicin complexes are formed, they come together to form one of the three different shapes inside the liposomes. A long singular band that stretches the membrane, a closed circular band or an opened U-shaped band. The circular and U-shaped complexes are the most common ones. Doxorubicin liposomes that are formed by remote loading using pH gradient should be used immediately.

FORMULATION INFORMATION

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3-Vial Kit for Doxosome™ (Non-PEGylated)	Specification
Vial 1	Preformed liposomes composed of HSPC and cholesterol (55:45 molar ratio)
Vial 2	Sodium carbonate for making a pH gradient
Vial 3	Doxorubicin HCl in 0.9% saline
Instructions: Add 0.4 ml of the liposome (vial 1) to 0.6 ml of the sodium carbonate solution (vial 2). Then add 10 mg of the doxorubicin in 4 ml of the 0.9% saline solution (vial 3) to the mix, heat at 55-60 °C for 7 min, and shake it for 10 s. The final concentration of doxorubicin in the liposomes is 2 mg/ml and the total volume of the reagent is 5 ml. Over 95% of the drug is loaded to the liposomes. The drug to lipid w/w ratio is 0.27:1.	

Lipid Composition for Vial 1	Concentration (mg/ml)	Concentration (mM)	Molar Ratio Percentage
L-α-Phosphatidylcholine	71.3	92.6	55
Cholesterol	28.7	74.2	45
Total	**100 mg/ml**	**166.8 mM**	**100**

Buffer and Liposome for Vial 1	Specification
Buffer	Citrate Buffer
pH	4 (in 300 ml buffer)
Liposome Size	180 nm

Vial 2	Specification
Sodium Carbonate	166 mM
pH	11.4

Vial 3	Specification
Doxorubicin HCl	Solution in 0.9% Saline

APPEARANCE

Doxosome™ is a red translucent liquid both made of nano size unilamellar liposomes. Usually due to the small size of liposomes no settling will occur in the bottom of the vial. The liposomes are packaged in amber vials.

STORAGE AND SHELF LIFE

Storage

Doxosome™ products should always be stored at in the dark at 4 °C, except when brought to room temperature for brief periods prior to animal dosing. DO NOT FREEZE. If the suspension is frozen, the encapsulated drug can be released from the liposomes thus limiting its effectiveness. In addition, the size of the liposomes will also change upon freezing and thawing.

Shelf Life

Doxosome™ is made on daily basis. The batch that is shipped is manufactured on the same day. It is advised to use the products within 4 months of the manufacturing date.

REFERENCES AND BACKGROUND READING

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