United States Patent [19]

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[54] **METHOD FOR PRESERVING LIPOSOMES**

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Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 690,679, Jan. 11, 1985, abandoned.

[51] **Int. Cl.**[4] **A61K 37/24;** A61K 37/48

[52] **U.S. Cl.** **424/94.1;** 424/94.4; 514/2; 514/3; 514/76; 514/77; 514/78; 514/44; 514/579; 514/646; 435/4; 435/26; 436/829

[58] **Field of Search** 424/450, 94.1, 94.4; 514/76–78, 2, 3; 435/4; 436/829

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,993,754	11/1976	Rahman et al. .
4,016,100	4/1977	Suzuki et al. .
4,224,179	9/1980	Schneider .
4,229,360	10/1980	Schneider et al. .
4,235,871	11/1980	Papahadjopoulos et al. .
4,263,428	4/1981	Apple et al. .
4,348,384	9/1982	Horikoshi et al. .
4,370,349	1/1983	Evans et al. .
4,396,630	8/1983	Riedl et al. .
4,411,894	10/1983	Schrank et al. .
4,515,736	5/1985	Deamer .

FOREIGN PATENT DOCUMENTS

86/01103 2/1986 World Int. Prop. O. .

OTHER PUBLICATIONS

Szoka et al., *Ann. Rev. Biophysics Bioeng.* 9:467–508 (1980).

Chem. Abs. 97:11851g (1982).

Chem. Abs. 99:163956z (1983).

Crowe et al., *Archives of Biochemistry and Biophysics,* vol. 220, (1983), pp. 477–484.

Tsyganenko et al., *Antibiotiki,* 28(8), 1983, pp. 577–581 (Russ.)

Chem. Abs. 101:216308h (1984).

Archives of Biochemistry and Biophysics, vol. 232, No. 1, Jul. 1984, pp. 400–407.

Crommelin et al., *Pharmaceutical Research,* No. 3, (1984), pp. 159–163.

Crowe et al., *Biochimica et Biophysica Acta* 769, (1984), pp. 141–150, 151–159.

E. Racker, "Reconstruction of Cyrochrome Oxidase Vesicles and Conferral of Sensitivity to Energy Transfer Inhibitors", *J. Membrane Biol.,* 10, 221–235 (1972).

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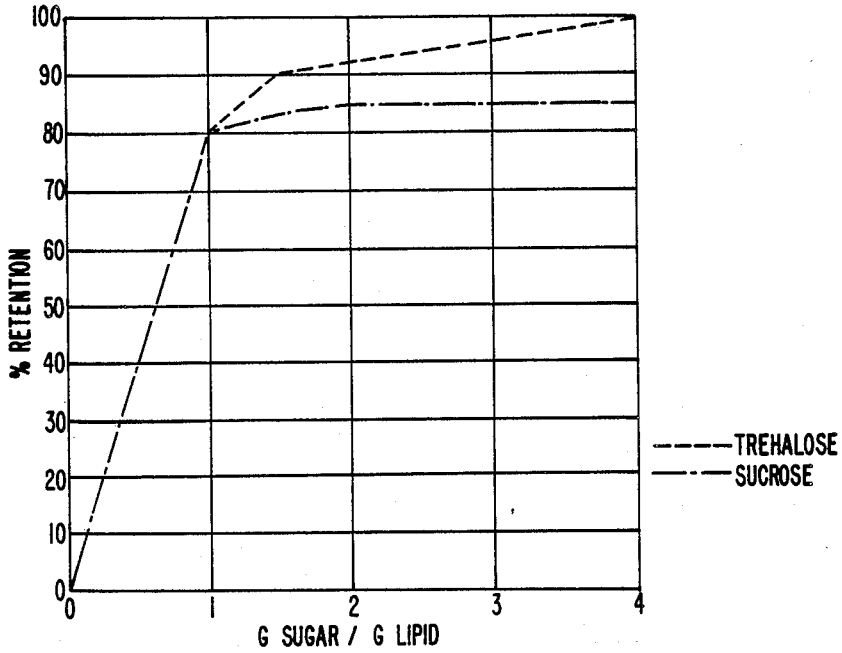
[57] **ABSTRACT**

A method for preserving liposomes includes freeze-drying liposomes most preferably having an average size of about 50 nm to 100 nm with a disaccharide preserving agent being present both internally (with the encapsulated liposomal contents) and externally. The disaccharide component is in a weight ratio with respect to the lipid component of from at least about 0.1:1 to not greater than about 4:1 and is preferably trehalose. When the lyophilizates are reconstituted by rehydration, the resultant liposomes can retain up to 100% of the originally encapsulated contents.

14 Claims, 4 Drawing Sheets