DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 20(16), 2527-2534 (1994)

DETERMINATION OF RETINOIC ACID - LIPOSOMAL ASSOCIATION LEVEL IN A TOPICAL FORMULATION

F. Bonté[1], J. M .Chevalier, A . Meybeck

LVMH Recherche, 50 rue de Seine, 92703 Colombes, France

ABSTRACT

A new liposomal formulation containing a lower dose of drug and possessing increased skin bioavailability has recently been proposed. This paper describes a method to quantitatively determine the levels of retinoic acid associated with liposomes in a marketable topical gel.

INTRODUCTION

Retinoic acid has long been used in topical formulations for the treatment of acne (1). More recently, a new application for this molecule has opened up in the treament of aged skin (2). Unfortunately, many side effects such as skin irritation have limited its use. A topical liposomal formulation containing low dose of drug has been shown to retain its pharmacological properties while improving its tolerance (3,4). This phenomenon might be explained by increased skin bioavailability with the liposomal preparation (5,6).

Within the scope of development of topical liposomal formulations, we focused on a simple method to evaluate the entrapment efficiency of

1. Author to whom correspondence should be addressed

retinoic acid in a liposomal suspension and to quantitavely determine its levels following its formulation in a dermatological product for topical use.

MATERIALS AND METHODS

Chemicals

Trans-retinoic acid was purchased from Hoffmann La Roche, Basel , Switzerland and complies with the French Pharmacopoeia X ed. monograph.
Soy bean lecithin and cholesterol were purchased respectively from American Lecithin Company, Woodside, NY, USA, and Prochimex , Paris, France.
Carbomer 940 was from Polyplastic, Rueil Malmaison France.
All other chemicals used were of analytical grade unless otherwise specified.

Preparation of liposomal formulations

The retinoic-acid liposomal suspension composed of soybean lecithin - cholesterol (9:1 , w / w) was prepared by spray drying followed by size homogenisation as previously described (7,8).
For preparation of a dermatological product, the liposomal suspension was mixed with a carbomer 940 0.8 % gel (pH 5.5) containing antioxidants and preservatives.
Liposomal size distribution was checked on the suspension and on the gelified product by photon autocorrelation with a Malvern Autosizer 2 (Coultronics , les Ulis, France).
Liposomal structural integrity was controlled by freeze fracture electron micrographs as classically described (9)

Measurement of gel viscosity

Viscosity was determined with a Brookfield viscometer LVT (Stoughton, MA, USA) using a mobile LV4 and speed 6.

Separation of retinoic acid liposomes from free retinoic acid

The separation of associated or entrapped retinoic acid from free retinoic acid was performed using a size exclusion Sepharose 4 B column (Pharmacia, Uppsala, Sweden), 25 mm in diameter and 400mm in length.

Prior the sample injection, the column was saturated with empty (drug free) liposomes.The liposomal suspension or the gel after treatment was eluted with 2mM HEPES buffer pH 7.55 , 0.02% sodium azide , and the collected fractions were analysed .
Flow rate: 3 ml per hour
100 fractions of 1 ml were collected.

In order to process the dermatological gel on the column , it was necessary to dilute it in HEPES buffer in the presence of NaCL.
0.5 g of gel was diluted with 5 ml of 2mM HEPES pH 7.55 containing 0.77% NaCl and the sample was injected on the column.

Assay of phospholipids:

Turbidimetry:

The eluted fractions were first assayed by measurement of the optical density at 600 nm versus HEPES buffer which was correlated to a standard curve of 150 nm soybean lecithin cholesterol (9-1) liposomal suspension.

Assay of Inorganic phosphorus :

This assay was performed according to the method of Chen et al. as previously described (10).

Assay of retinoic acid :

The collected fractions were treated with methanol - triton X100 (95:5,v:v),and filtered on a Millex SR 0.45µm filter. Retinoic acid was

quantitatively determined by UV spectrophotometric measurement at 351 nm and HPLC on a C18 column as described by Bonhommme et al. (11).

RESULTS AND DISCUSSION

This paper describes the determination of the entrapment efficiency of retinoic acid by gel permeation in a liposomal suspension before and after formulation in a topical form.

Methods to determine the entrapment efficiency of drug in liposomes suspensions have already been referred (12,13), but no applications to final marketed topical products have been described .

The entrapment efficiency can be defined as the number of moles of drug encapsulated or intimately associated with the lipids constituting the liposomes.

Since the viscosity of the gel does not permit its direct injection on the column, we proposed that it be diluted in sodium chloride buffer to within the range of the limits of the detection's methods .

As shown in Fig 1, the viscosity of the gel decreased rapidly with increasing salt concentration and an increase in the size of the liposomes , a known phenomenon was observed .

Nevertheless we found that the addition of 0.77% of sodium chloride to the gel decreased its viscosity at a level permitting injection without destabilizing the liposomal structure, as controlled by freeze fracture microscopy (data not shown).

At this concentration of sodium chloride in the HEPES buffer the liposomal size is 172 nm.

Fig 2 shows the elution profile of a gel, the liposomal peak being evaluated by turbidimetry and the retinoic acid by UV absorbance at 351

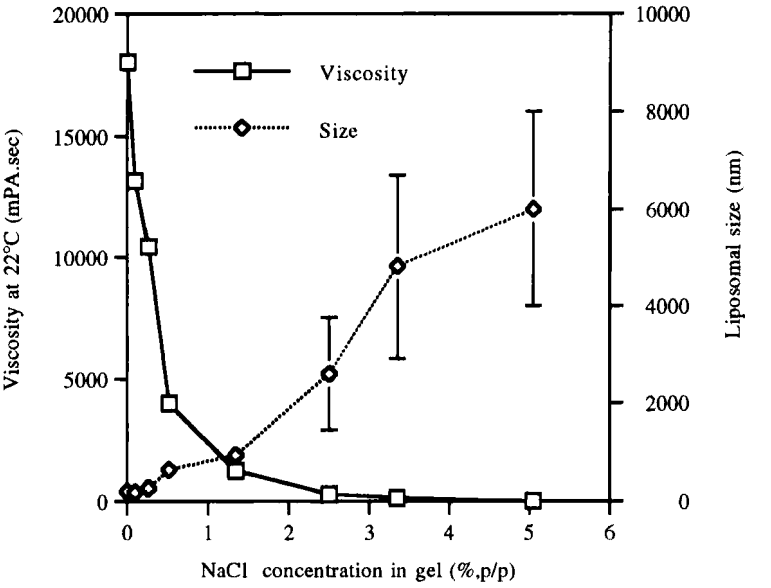

FIGURE 1:

Viscosity and size variations as a function of the sodium chloride concentration in the gel

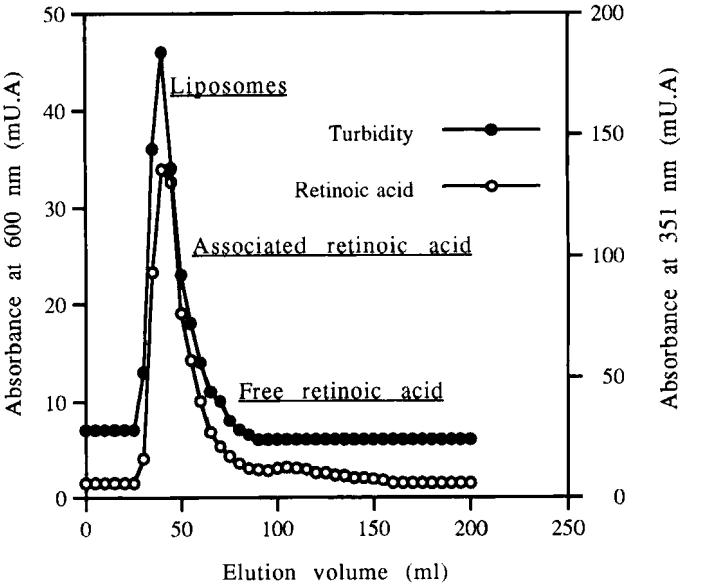

FIGURE 2 :

Elution profile of a liposomal gel

TABLE 1:

Phosphorus content, associated and free retinoic acid contents. Entrapment efficiency is expressed as the number of mmol of retinoic acid per mole of phosphorus

	phosphorus content mM x 10^{-2}	associated retinoic acid mM x 10^{-4}	free retinoic acid mM x 10^{-4}	entrapment efficiency
liposomal suspension	2.18 ± 0.11	1.32 ± 0.1	0.18 ± 0.1	6.10 ± 0.6
gel	2.08 ± 0.11	1.30 ± 0.10	0.15 ± 0.10	6.25 ± 0.6
gel +0.2 10^{-4} mM retinoic acid	2.15 ± 0.11	1.35 ± 0.1	0.35 ± 0.10	6.30 ± 0.10

nm. A similar profile was obtained with the original liposomal suspension . This figure clearly shows that retinoic acid is primarily eluted with the liposomes .

Following collection of the fractions, inorganic phosphorus was assayed to check that the amounts determined by turbidimetry were related. Furthermore 99% of the lipids and 100% of the retinoic acid were recovered from rhe original formulation after elution. Note that the inorganic phosphorus assay was first standardised with the raw material of the liposomes soybean lecithin .

Retinoic acid content was also checked by HPLC following extraction of the fractions .

The same experiments were performed with the original liposomal suspension with or without NaCl in HEPES buffer. In this case 0.25 g of

suspension were applied on the column .The results obtained were similar (n= 3).

From table 1 it is possible to calculate that 6. 3 m moles of retinoic acid were associated or entrapped in 1 mole of lipids (expressed in phosphorus) in the liposomal gel formulation , the theoretical value being 7. Similar results obtained with the original liposomal suspension prove that such a final formulation in gel form does not modify the entrapment of retinoic acid.

The methodology was validated by checking that the liposomal suspension diluted in the same conditions with a saline HEPES buffer saturated with retinoic acid and immediately injected, presented a proportionally increased free retinoic acid peak (Table 1). This means that retinoic acid measured by the present method is inside or very intimately associated with the liposomes .

The method proposed here to determine the entrapment efficiency of retinoic acid in the final liposomal formulation is suitable for industrial development.

REFERENCES

1 J.J. LEYDEN, J.AM.ACAD.DERMATOL., 19 ,164 -168,(1988)

2 J.S. WEISS, C.ELLIS, J.H. HEADINGTON, J.J. VORHEES, J. AM.ACAD. DERMATOL 19, 169 - 175, (1988)

3 A.MEYBECK, F. BONTE, G. REDZINIAK, INTERNATIONAL CONFERENCE ON PHARMACEUTICAL TECHNOLOGY, PARIS, FRANCE, (1989)

4 A. MEYBECK, P.MICHELON, C. MONTASTIER, G. REDZINIAK, US PAT 5,034 228 (1991), PRIORITY FR 85 18362

5 V. MASINI, F. BONTE, A. MEYBECK J.WEPIERRE, J.PHARM.SCI.82,17-21,(1993)

6 M. MEZEI, in LIPOSOMES IN DRUG DELIVERY , G. GREGORIADIS ED, HARWOOD ACADEMY PUBLISHERS, CHUR, SWITZERLAND, (1993)

7 G. REDZINIAK, A. MEYBECK , US PAT 4,508703,(1985) PRIORITY FR 8202620

8 G. REDZINIAK, A. MEYBECK, US PAT 4,621023, (1986), PRIORITY FR 8217311

9 B . STERNBERG, in LIPOSOME TECHNOLOGY 2ND EDITION , G GREGORIADIS, CRC PRESS, BOCA RATON, USA (1993)

10 P.S. CHEN, T.Y. TORIBARA, H.WARNER, ANAL. CHEM., 28,1756-1758,(1956)

11 L. BONHOMME , B. DULEBA,T.BEUGRE, G.FREDJ, INT.J PHARM, 65,R9-R10, (1990)

12 E. FATTAL, P. COUVREUR, F. PUISIEUX, in LIPOSOMES, INSERM TEC DOC LAVOISIER EDITION, PARIS , FRANCE, (1993)

13 C.NASTRUZZI, P. WALDE, E. MENEGATTI, R. GAMBARI, FEBS LETTER, 259,293-296,(1990)