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(54) **REPULSIF POUR LES INSECTES**

(54) **CUTANEOUS DELIVERY OF ORGANIC MATERIALS**

(57) L'invention concerne un distributeur à libération temporelle d'un agent de répulsion d'insectes ancré sur la couche cornée, l'agent répulsif étant distribué sur une période de temps et résistant à l'élimination par la transpiration, l'abrasion ou le lavage avec un détergent. Un exemple est une membrane bipolaire ayant une configuration de liposome qui enveloppe et cloisonne la molécule organique ayant normalement une solubilité très limitée dans un milieu aqueux. La molécule organique peut être, par exemple, LN-diéthyle-m-toluamide de répulsion des moustiques et connue par l'acronyme "DEET". Un autre exemple est un insecticide ou agent répulsif d'animaux. Le système d'apport liposomique de l'art antérieur dépend de la formation des liposomes dans un milieu aqueux, capturant ainsi une partie du milieu dans un volume d'un noyau. Dans le mode préféré de cette invention, une "charge" ou substance chimique à action biologique consistant en une substance sensiblement insoluble dans l'eau est séquestrée dans une vessie lipide bipolaire, lequel lipide et laquelle charge peuvent être utilisés en tant que tels ou ciblés sur un site désigné. De préférence, une molécule cible telle que la pyridinethione est utilisée pour ancrer la structure liposomique sur la couche cornée.

(57) A time release dispenser of insect repellant and anchored to the stratum corneum, whereby the repellant cargo is dispensed over a period of time, and resists removal by perspiration, abrasion, or detergent wash. An example is a bipolar lipid membrane formed into liposome configuration, and caused to envelope and partition an organic molecule that normally has a very limited solubility in aqueous media. The organic molecule may be, for example, a mosquito repellant N, N-Diethyl-m-toluamide known by the acronym "DEET". Another example is an insecticide or repellant for animals. The prior art liposomal delivery system depends upon forming liposomes in an aqueous media, thereby capturing some of the media in the core volume. In the preferred embodiment this invention, a burden of a substantially water insoluble substance is sequestered in a bipolar lipid bladder, which lipid and burden may be used as such or targeted to a designated site. Preferably, a target molecule, such as pyridinethione, is used to anchor the liposomal structure to the stratum corneum.

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(57) Abstract

A time release dispenser of insect repellant and anchored to the stratum corneum, whereby the repellant cargo is dispensed over a period of time, and resists removal by perspiration, abrasion, or detergent wash. An example is a bipolar lipid membrane formed into liposome configuration, and caused to envelope and partition an organic molecule that normally has a very limited solubility in aqueous media. The organic molecule may be, for example, a mosquito repellant N, N-Diethyl-m-toluamide known by the acronym "DEET". Another example is an insecticide or repellant for animals. The prior art liposomal delivery system depends upon forming liposomes in an aqueous media, thereby capturing some of the media in the core volume. In the preferred embodiment this invention, a burden of a substantially water insoluble substance is sequestered in a bipolar lipid bladder, which lipid and burden may be used as such or targeted to a designated site. Preferably, a target molecule, such as pyridinethione, is used to anchor the liposomal structure to the stratum corneum.

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INSECT REPELLANT

BACKGROUND OF THE INVENTION

Field of the Invention

Emulsions are formed from molecules that are not very soluble in aqueous media, but are soluble in organic solvents.

A species of the field is the establishment of a delivery system using a liposomal emulsion delivery system for targeting and anchoring aromatic hydrocarbons, such as mosquito repellants and insecticides, to the stratum corneum.

Liposome and vesicle will be used interchangeably herein, because the art is not sufficiently exact with respect to the parameters of nomenclature.

Description of Prior Art

Liposomes are known to encapsulate and thereby sequester water soluble pharmacological agents. Thus, these agents are separated from the surrounding media. The liposomes have potential for cite specific delivery, as presented by the prior art, for their use as carriers to enhance therapeutic and protective indices.

Achieving controlled release of volatile substances such as insect repellants, has been the object of intense effort for several decades. However, only a few reports of actual success to limited systems are known. Literature studies indicate that most success in this area has been through the use of polymers, resins and impregnated gel beads and silica. These prior art systems provide only minimal accuracy and afford less than satisfactory control over release of the product.

This invention distinguishes over all known prior art, including intensive literature studies, by the discovery that formation of bipolar lipids into vesicles in the presence of organic molecules that are minimally soluble in aqueous media, exhibit a phenomena of physical entrapment of the organic solvent in the manner illustrated in the

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drawings. The organic solvent would not ordinarily be expected to fill the core of a vesicle/liposome during formation, because the outermost portion of a lipid membrane, both on the exterior surface and on the core volume wall, is a hydrophilic sphere and therefore it is generally assumed that an organic solvent would not be encapsulated.

It is an object of this invention to provide a slow release delivery system for chemicals which are soluble in an organic solvent, by sequestering the chemical in a bipolar vesicle.

Another object of the invention is to provide a target molecule for such sequestered agent, the target molecule being selected to have affinity for the desired host surface under consideration.

SUMMARY OF THE INVENTION

The term "burden" as used herein shall refer in general to a biological effective chemical for application to a living host skin surface, to elicit a desired response.

The burden is sequestered in a bipolar lipid vesicle, or entrapped in a fragment thereof.

Application of an environment enhancement chemical to the skin of a warm blooded host is accomplished by performing liposomal emulsification of the enhancement chemical, and anchoring the liposome to the stratum corneum by means of a targeting molecule having a lipophilic moiety engaged into the liposome, and having a moiety with affinity for the stratum corneum.

A specific example is the emulsification of molecules that are not very soluble in aqueous media but show solubility for lipid phase and organic solvents. For example, one embodiment is 6,12 (2 Ethyl-1, 3,-hexanediol) and N,N-Diethyl-m-toluamide (DEET).

Flea and tick repellants and/or insecticides are further examples.

The drawings illustrating this invention show the discovered unique sequestering of the described class of water insoluble burden. Liposomes cannot be formed in an organic solvent, and water insoluble chemicals cannot be trapped in a core volume of a lipid vesicle. This invention discloses the phenomena of physically sequestering organic soluble material.

Then, by attaching a targeting molecule an article of manufacture is produced which will attach to a targeted skin cell. For example, by targeting the human stratum corneum with pyridinethione, sequestered DEET will gradually escape its sequestering lipid, and repel or kill insects for an extended period of time.

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BRIEF DESCRIPTION OF THE DRAWINGS

The single figure is a five step sequence taken from electron micrographs.

DEFINITION

Because the invention is in the unique means for dispensing a biologically active substance such as an insect repellant or insecticide, and not in the substance per se, reference hereinafter for disclosure will be limited to "insect repellant" for economy of expression, and shall include any substance embraced in the generic term "biologically active substance."

DETAILED DESCRIPTION

First Example

One preferred embodiment of the invention is to provide an article of manufacture for repelling or killing insects, comprising a substantially water insoluble organic compound DEET, or a pesticide, loaded to a bipolar lipid body. A targeting molecule, for anchoring the lipid body, has a lipid soluble moiety united to the lipid body, and a target moiety having affinity for stratum corneum.

As stated, the prior art method of making vesicles or liposomes is to subject a mixture of bipolar lipid, such as egg lecithin, and an aqueous solution of the intended burden, to high energy input such as sonication or microfluidization. The bipolar lipid breaks into smaller units and forms bladders known as a vesicle or liposome. In the process some of the aqueous media is captured in the core volume of the liposomes. This basic known system is satisfactory for aqueous media but is inoperative for organic molecules that are minimally soluble in aqueous media.

In this example, a lecithin is first polytroned and then microfluidized or sonicated to create very large multilamellar liposomes. Although a separate step may be employed, it has been found practical to carry out a simultaneous reaction by including in the mixture of lecithin and aqueous media, a burden such as DEET. The DEET molecule possesses hydrophobic (lipid seeking) groups such as the ethyl moiety and the paramethylbenzene rings. The lipid seeking nature of the DEET molecule allows it to seek and penetrate a large multilamellar liposome so that the lipid domain of the liposome actually is disrupted and penetrated by the DEET molecule. The capture of the DEET molecule is illustrated in the five-step illustration in the drawing, which are reproductions of actual images from electron miscroscopy. The small black spot represents a particle of DEET and the multilamellar rings represent a larger liposome.

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The liposome, no longer being intact, is simply a large multilamellar array of lipid which seeks to repair itself thermodynamically and in so doing emulsifies the DEET particle or molecule by surrounding the DEET with the large multilamellar array. The DEET particle thus has been sequestered and protected by lipid. This is not a liposome or a vesicle in the classical sense because the core volume is not aqueous but rather is organic. The organic particle of DEET seeks to exclude core volume water.

This concept may be considered as a semi-classical emulsion. It has all the characteristics of an emulsion. It breaks upon freezing and warming causing the DEET to coalesce and partition into a larger separate phase. The liposomal lamellae are acting as an emulsifying agent. The major portion of the lamellae evidence a distorted non-spherical pattern, as illustrated in the drawing, as they attempt to emulsify the DEET particles progresses.

The manner in which the emulsification effect differs from classical emulsions is that the emulsifying agent has lamellae or onion skin kinds of structures and these structures have been preformed in a single step. A multiple protective layer pattern has been developed that will emulsify the DEET.

The described liposomal emulsion system has captured or sequestered DEET in a new way based on emulsion chemistry. It is apparent that once the DEET has been emulsified by the onion skin lamellae, its escape to the atmosphere is restricted and slowed. This concept forms the basis for timed release of water insoluble substances such as DEET and prolongs their duration of action.

The above-described article of manufacture may be employed in the described state and can cling to skin and hair for a period of time. However, a vastly improved result is obtained by employing a variety of skin substantive molecules such as pyridinethione and the derivatives described herein to anchor the lipid and its burden to skin of the host for the purpose of maintaining a slow time-

release of the active product onto the skin surface and surrounding area.

The target pyridinethione is a molecule having a lipid soluble moiety and a hydrophilic moiety and therefore the lipid soluble moiety will penetrate the wall of the emulsified array and extend the hydrophilic moiety away from the surface to act as a targeting agent for attaching the completed article of manufacture to the corneum stratum.

Second Example

Close examination of the mixture which has been polytroned and subjected to either sonication or microfluidization reveals that not all of the lipid material has been formed into perfect liposomes as illustrated in the drawing. There remains fragments of the liposomes which have a slightly curved configuration, as well as sheet-like fragments of bipolar lipid. If the intensity of the sonication or microfluidization is off of ideal, the resultant product can be essentially all fragments or bits of sheet bipolar lipid with few well formed liposomes. It is this type of product which heretofore has been considered to be essentially useless because of the small amount of well formed liposomes. It has been discovered, in working with this material, however, that contrary to expectations, a very effective delivery of DEET to the stratum corneum has actually been achieved.

The explanation for this unexpected phenomena is known as partitioning within multilamellar bilayers. It is a known fact that highly immiscible fluids, such as oil and water, do have some degree of solubility one in the other. The amount of solubility will vary according to the particular ingredients, which solubility is obtainable from standard chemical tables.

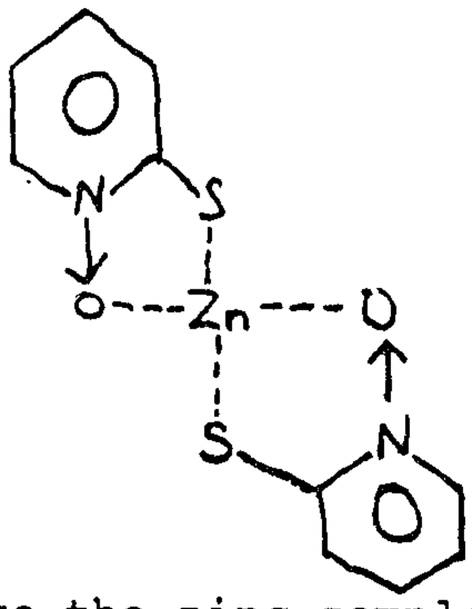
Therefore, it has been discovered that those fragments and sheets which are not sufficiently large to envelope the DEET as illustrated, for example by the completely formed vesicles, will nevertheless carry some of the DEET and also will accept the targeting molecule. Therefore, the

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degree of perfection toward forming of perfect vesicles is greatly reduced and the efficiency of the manufacturing process greatly increased.

Targeting Procedure

Applicants recognize the ability of chelated zinc pyridinethione (ZPT) to adhere to stratum corneum. ZPT has the following structural formula:



Since the zinc complex of ZPT shown above is much less soluble in aqueous media because of the ligand formation by the ionizable and hydrophilic functional groups, such as the sulfhydrals and oxygens, this molecule is an appropriate candidate as a liposome targeting agent. It can be incorporated in a one-step procedure into the liposome membrane during the time of sonication and microfluidization.

The procedure used for making the product as tested is set forth below:

1. 120 mg of the sodium salt of pyridinethione is dissolved in 525 ml of deionized water and allowed to stir at ambient temperature for 15 minutes.

2. 120 g of hydroxylated lecithin is weighed into an 800 ml beaker while 525 ml of solubilized sodium pyridinethione is added in 100 ml aliquots. During pyridinethione addition, the sample is slowly polytroned with an ultra-Turrax TP-18/1051 polytron from Janke and Kunkel IKA Laboratory, at setting #4 for 15 minutes.

3. After the lecithin had been mixed, 180 ml of 100% diethyl-m-toluamide (DEET) was added to the 600 ml of sodium pyridinethione lecithin solution in three aliquots of 60 ml each, five minutes apart. After the last aliquot, the total

volume of 825 ml was allowed to mix while polytroning on setting #4 for 10 minutes. Then, 495 ml of deionized water was added and allowed to mix while polytroning on setting #4 for 30 minutes. The total volume of the solution at this point was 1320 ml.

4. The 1,320 ml of the pyridinethione-lecithin DEET suspension was processed through the microfluidizer at a head pressure of 20 psig and a shear pressure of 2,500 psig. The total recoverable volume of 1,320 ml was recycled three times through the microfluidizer under the above conditions. The initial starting temperature of the suspension was 20 degrees C, and the final temperature following microfluidization was 63 degrees C.

EMBODIMENTS OF THE INVENTION IN WHICH EXCLUSIVE PROPERTY AND PRIVILEGE IS CLAIMED ARE AS FOLLOWS:

1. An article of manufacture, comprising:

an insect repellent which is substantially insoluble in water and soluble in organic solvents;

a first phase which is a liposome lamellae enveloping a second phase which is a bio-organic insect repellent soluble in organic solvents and substantially insoluble in water; and

an anchoring molecule of sodium pyridinethione having a moiety embedded in said liposome.

2. A protective structure for stratum corneum, comprising a liposome and a burden of substance selected from the class consisting of insect repellents, insecticides, burn medication, and sunscreen, and an anchor molecule having a first moiety anchored to the vesicle, and a second moiety that has the potential to anchor to the stratum corneum when applied thereto.

3. A lamellae sequestered burden of claim 2, with anchor molecules having a lipophilic moiety engaged with the membrane structure and a moiety attracted to stratum corneum.

4. An article of manufacture comprising:

an insect repellent which is substantially insoluble in water and soluble in organic solvents;

a first phase which is a liposome lamellae enveloping a second phase which is 6,12(2-ethyl-1-3) hexanediol insect repellent soluble in organic solvents and substantially insoluble in water; and

and anchor molecule of sodium pyridinethione having a moiety embedded in said liposome.

5. An article of manufacture comprising:

an insect repellent which is substantially insoluble in water and soluble in organic solvents;

a first phase which is a liposome lamellae enveloping a second phase which is N,N,diethyl-m,toluamide insect repellent soluble in organic solvents and substantially insoluble in water; and

an anchor molecule of sodium pyridinethione having a moiety embedded in said liposome.

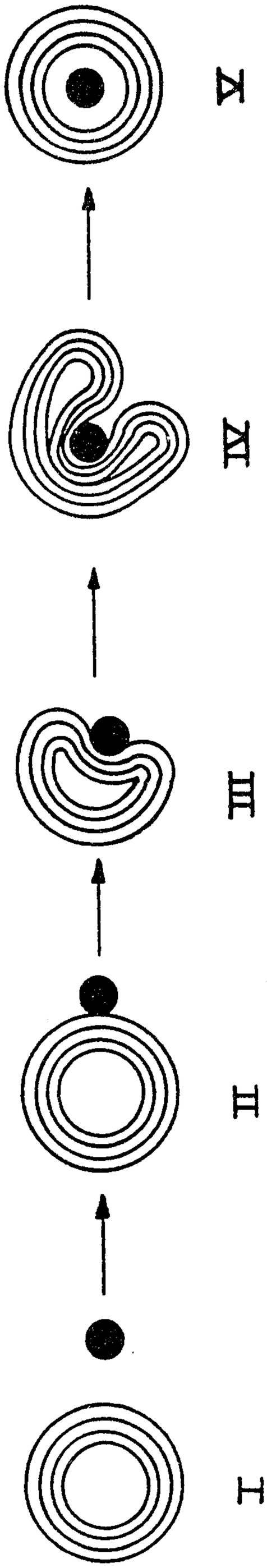


FIG. 1