Specification

The present invention relates generally to hydrated lipidic lamellar phases and their process of fabrication, as well as to compositions notably for pharmaceutical, cosmetic or like use comprising said lipidic lamellar phases.

The present invention has more specially for an object a process of preparation of pulverulent mixtures of amphiphilic lipidic constituents and of hydrophobic constituents, the said mixture being utilized particularly for the production of hydrated lipidic lamellar phases.

The hydrated lipidic lamellar phases have been abundantly described in scientific publications, notably by Perron R. (Revue Française du Corps Gras, 1980, 27 (4) 173-183). Their formation results from a property characteristic of certain amphiphiliic lipids in the presence of an aqueous medium. The molecules of the amphiphilic lipids are constituted of a polar or non-polar hydrophilic part and of a hydrophobic part. According to M.M. Rieger (Cosmetics and Toiletries, 1981, 96 35-38), one of the conditions so that the amphiphilic lipid be likely to form lamellar phases and not only micellar aggregates is that the dimensions of its hydrophobic part must be at least as important as that of its hydrophilic part. These lipids are capable of forming bimolecular lipidic layers (lamellae or bilayers), separated from one another by a more or less important quantity of water or of aqueous solution which may be reduced to a simple interstitial film. The molecules of said lipids forming the lipidic layers are placed next to one another and

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oriented in a way that their hydrophobic portion be situated inside the said layer. One calls the whole of these lipidic layers and of the interstitial aqueous medium a hydrated lipidic lamellar phase.

The liposome is a particular configuration of a lamellar phase appearing in the form of a lipidic spherule constituted of one or several concentric bilayers alternating with aqueous compartments. The liposomes are dispersed in an aqueous medium and constitute a very hydrated lamellar phase. The conditions under which non-polar amphiphilic lipids can form liposomes or "niosomes" have been exposed by G. Vanlerberghe et al. (Colloques Nationaux du CNRS, N° 938, Bordeaux 1978, Ed. CNRS 1979, p 303-311).

It is also known that the liposomes can be obtained by dispersion in an aqueous medium of a lamellar phase following various processes, notably by means of ultrasonic waves.

The liposomes have been the object of a great number of publications, such as for example the articles already mentioned and the following articles : Sessa G. et al., J. Lipid Res. 1968, 9, 310-318; Bangham A.D. et al., Meth. Membr. Biol. 1974, 1-68; D.A. Tyrrell et al., Biochim. Biophys. Acta 1976, 457, 259-302; Strianse S.J., Cosmetics & Toiletries 1978, 93, 37-41; Puisieux F., Labo-Pharma 1978, 281, 899-904; Nicolau C. et al., La Recherche 1981, 123, 748-749.

The industrial interest of liposomes or of hydrated lamellar phases, resides in the fact that substances possessing interesting properties may be incorporated, either in the lipidic bilayers for the substances partially or totally hydrophobic, or in solution in the aqueous medium located between two bilayers or encapsulated in liposomes for the water-soluble substances.

The applications of these products are very numerous particularly in foodstuffs, cosmetic or pharmacy.

Very varied substances may be incorporated in the lamellar phases or in the liposomes, notably for the purpose of protection of fragile substances against exterior agents or of improvement of the penetration of certain substances in the biological tissues. It is

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also possible to modify the physico-chemical properties of the lamellar phases or of the liposomes themselves by incorporation to the lipidic bilayers of certain compounds such as sterols, for exemple cholesterol (Venderberghe G. et al. cited hereabove; Gregoriadis G., Biochem. J, 19, 196, 591) notably to control the permeability and the stability of the lipidic bilayers.

The addition of hydrophobic compounds such as sterols increases the hydrophobic group/hydrophilic group ratio in the lamellar phase which complies with the condition of stability of the lamellar phases exposed by M.M. Rieger (cited hereabove). Thus, the addition of such hydrophobic compounds makes possible the formation of lamellar phases with amphiphilic lipids which could not form them if they were employed alone. It is also possible to incorporate to the lamellar phases amphiphilic compounds of which the hydrophilic part will be charged positively or negatively (French patent applications N° 78-13632, FR-A-2 390 159 and 78-22984, FR-A-2 399 241),for example, the dicetyl phosphate imparts to the liposomes a negative charge, whereas stearylamine provides a positive charge.

A certain number of processes of production of such lamellar phases, and of liposomes are already known. One of the processes most currently in use consists in a first stage in dissolving the amphiphilic lipid or lipids and possibly the hydrophobic substance or substances in an appropriate volatile solvent. The solution obtained is placed in the flask of a rotary evaporator. After evaporation of the solvent, one obtains a film adhering to the wall of the flask. One then introduces in this latter water or possibly the aqueous solution to be encapsulated and one subsjects the whole to a vigorous agitation. One thus obtains a suspension of liposomes which can be possibly homogenized by ultrasonic waves. One of the inconveniences of this process resides in the obtention of the film which can be obtained only with great difficulty on an industrial scale.

According to another process described notably in the French patent application N° 78-22984 (FR-A-2 399 241) the aqueous solution

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to encapsulate is dispersed in a solvent insoluble or slightly soluble in water in the presence of a lipid or of an amphiphilic surfactant, there are thus formed microdrops of the said aqueous solution, which one thereafter emulsifies in an aqueous medium in the presence of an excess of a lipid or surfactant which may be identical or different to the one used previously. The insoluble solvent is eliminated before or after the emulsification, for example by the blowing of air at the surface of the liquid. This process necessitates a total elimination of the solvent used which presents numerous difficulties, especially on an industrial scale. Further, it does not permit the production of slightly hydrated lamellar phases.

It is also possible to prepare liposomes according to the Swiss patent N° 623 236 by putting in contact, on the one hand, at least one liquid amphiphilic lipid dispersible in water, of which the hydrophilic part is ionic, and on the other hand, the aqueous phase to be encapsulated. The mixture is then subjected to a vigorous agitation to obtain a lamellar phase which thereafter may be dispersed in water or an aqueous solution. In this process, to incorporate hydrophobic compounds, it is necessary to use surface-active compounds with emulsifying property that much stronger as the hydrophobic character of the compound to be incorporated is more marked. These surface-actives are very often incompatible with numerous applications of the liposomes, for example pharmaceutical or cosmetic.

According to another process described in the French application N° 78-13632 (FR-A-2 390 159), one prepares a solution containing at least one amphiphilic lipid, at least one biologically active compound or possibly, at least one adjuvant. One lyophilizes this solution to eliminate the solvent and the mixture of the constituents is obtained under the form of a powder which may be stored in sealed containers until it is used to form in particular liposomes. This last stage consists in dispersing the said powder in an appropriate aqueous medium. This process presents the

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inconveniences involved particularly in the classical techniques of lyophilization which are relatively complex and expensive and further which have a production rate relatively limited.

The different known processes described hereabove present therefore numerous inconveniences not permitting notably an easy or inexpensive industrial production of lamellar phases. These inconveniences are principally due to the difficulties in obtaining an intimate mixture of the basic constituents forming the lamellar phases, in other words the amphiphilic lipids and possibly the hydrophobic substances. To facilitate the dispersion of the basic constituents of the lamellar phases in an aqueous medium,, it is often necessary to utilize additional products with surface-active property. As these products have often undesirable effects, for example with regard to the organism, it is necessary to eliminate them completely, which is not always possible even by dialysis. One understands by the expression basic constituents, the compounds utilized to form the lipidic layers of the lipidic lamellar phase, namely at least one amphiphilic lipid or mixture of one or several amphiphilic lipids and possibly, of one or several compounds partially or totally hydrophobic.

The present invention has for aim to remedy all these inconveniences by proposing a new process of preparation of hydrated lipidic lamellar phases, and more particularly a new process of preparation of a pulverulent mixture of amphiphilic lipidic constituents with possibly constituents hydrophobic or partially hydrophobic. This process is of a great simplicity and permits to produce continuously large quantities of mixtures and therefore of hydrated lipidic lamellar phases or of liposomes, without however necessitating notably the utilization of additional products with surface-active property.

To this effect, the present invention has for object a process of preparation of a pulverulent mixture of at least one amphiphilic lipidic constituent and possibly of at least one constituent of hydrophobic character characterized in that it comprises the putting

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in solution of the said amphiphilic lipidic constituent or constituents and possibly of the said constituent or constituents of hydrophobic character, in a non-aqueous solvent and the atomization of the solution thus obtained in a current of a gaseous fluid to produce the said pulverulent mixture.

Thus, it is possible to obtain an extremely fine powder which can be very easily dispersed in an aqueous medium by known methods of dispersion to give birth to a lamellar phase more or less hydrated following the ratio of quantity of dispersed product/quantity of aqueous medium utilized.

Further, this process permits to obtain a powder product which can easily be stored with a view to its later use and therefore thus to produce the hydrated lipidic lamellar phase by dispersion of the powder in a suitable aqueous medium, containing possibly an active compound to be encapsulated immediately before its use for example as medicine, or treating product in dermatology, cosmetics or the like, more particularly when the compound with interesting active property is relatively unstable and can be prepared only immediately before its utilization.

The process of the invention consists therefore in dissolving in a solvent or a mixture of appropriate solvents the basic constituents, in other words at least one amphiphilic lipid and possibly one constituent hydrophobic or partially hydrophobic which one wishes to incorporate to the lipidic bilayers.

The solution obtained is then atomized. This operation consists particularly in introducing under the form of very fine droplets for example by means of a spray nozzle, the solution into an enclosed space traversed by a gaseous fluid carried to a temperature higher than the boiling of the solvent used. Under the action of the heat, the solvent is vaporized and entrained by the gaseous current. In the region provided for of the apparatus where the temperature of the current of gaseous fluid becomes inferior to the temperature of transition of the mixture of said basic constituents an extremely fine powder forms, this powder being recovered by a device for example, a cyclone and collected at the base of this device. The

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mixture thus collected of basic constituents dissolved in the starting solution is homogeneous and constituted of extremely fine particles.

Thus, the process of the invention permits to treat important quantities of solution and therefore to produce industrially a pulverulent mixture which can be used in particular for the production of hydrated lipidic lamellar phases such as liposomes or the like. Indeed, as the basic constituents are dissolved in a solvent, there exists no problem of agitation of an important volume of solution, and it is possible to atomize a relatively high flow of solution without using a complex and expensive apparatus. Further, the energetic power necessary to the atomization of a solution and the evaporation of the solvent is markedly inferior to the energetic power consumed by the prior technique of lyophilization.

Advantageously, the temperature of atomization is comprised between 60°C and 100°C.

It is of course possible to atomize according to the process of the invention a solution containing only one constituent, that is to say an amphiphilic lipid, if one desires to produce lipidic bilayers constituted only of this single constituent. However, the lipidic bilayers more generally used are constituted by a mixture of several constituents, for example at least one amphiphilic lipidic constituent, and possibly at least one constituent hydrophobic or partially hydrophobic.

Any amphiphilic lipid capable of forming lamellar phases can be used in the process of the invention. However, it is necessary that these amphiphilic lipids either alone or in the presence of substances partially or totally hydrophobic be suitable for atomization, that is to say that the temperature of transition of the amphiphilic lipid or of the mixture be suitable to obtain an atomized product under the form of powder.

In the case of the production of a mixture containing at least one substance hydrophobic or partially hydrophobic, to permit the formation of lamellar lipidic phases, the weight proportion of the

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amphiphilic lipidic constituent or constituents in the whole of the basic constituents dissolved in the starting solution, that is to say the whole of the amphiphilic lipidic constituents and hydrophobic constituents, must be greater than 50% at least and preferably greater than about 70%.

As suitable amphiphilic lipid, one can use for example compounds belonging to the family of phospholipids; glycolipids or phospho-aminolipids such as for example an egg or soja lecithin, a phosphatidylserine, a cerebroside or a sphyngomyelin.

The substances hydrophobic or partially hydrophobic which are suitable to be mixed with at least one amphiphilic lipidic substance in the solution before atomization are extremely varied and are chosen according to the properties which one seeks to impart, on the one hand to the pulverulent mixture, and on the other hand, to the lamellar phases or to the liposomes produced from the pulverulent mixture.

By way of example, the substances hydrophobic or partially hydrophobic which can be used are sterols or their esters, such as cholesterol, ß-sitosterol, oestradiol, lanosterol, stigmasterol, γ-oryzanol; aliphatic fatty acids or their esters such as for example stearic acid, ximeninic acid, isopropyl myristate or the like; triterpenic fatty acids or their esters such as for example glycyrrhetinic acid or the like; primary or secondary fatty alcohols or their esters such as for example dicetyl phosphate; aliphatic fatty amines such as for example stearylamine; aminated acids acylated by a fatty acid such as for example stearoyl-L-glutamic acid; polypeptides such as for example elastin hydrolysate polypeptides; vitamins, for example a tocopherol, advantageously α-tocopherol; extracts of animal or vegetable origin such as for example embryonic extracts of extracts of aloe; or phenols which may comprise one or several constituents chosen among the radical groups alcoxy, alkyl comprising from 1 to 4 carbon atoms, carboxy, formyl, such as for example vanilline.

However, this list of hydrophobic constituents which can be used to form a pulverulent mixture according to the process of the

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invention is not limitative.

Further, it may be advantageous but not indispensable to choose as amphiphilic lipidic constituent and/or as hydrophobic constituent, a compound biologically active and/or possessing interesting organoleptic and/or physicochemical properties.

Furthermore, the solvent destined to prepare the solution to be atomized is chosen on the one hand according to its capacity of dissolving the basic constituents described hereabove and, on the other hand, depending on its boiling point which must not be too high in order to avoid the utilization of a temperature of atomization which would tend to deteriorate the said basic constituents. It is also possible, according to the invention, to utilize a mixture of solvents miscible between them, in the proper proportions to meet the conditions set forth above. The dissolution of the constituents is performed before or after the mixing of the solvents, this dissolution possibly requiring a slight heating, for example at a temperature of about 40°C.

The solvents currently used are, particularly, chloroform, methanol, ethanol, dichloromethane, or a mixture of chloroform with methanol, chloroform with ethanol, at 33% methanol or ethanol, respectively.

According to another characteristic of the invention, the gaseous fluid used in the atomization must be substantially and chemically inert with regard to the basic constituents dissolved in the starting solution. However, since the atomization step is relatively short, the time of contact between the basic constituents and the carrier gas is very short, it is generally possible to use air without risk of oxidation of the said constituents. Of course, it may be necessary to select more inert gases than air, for example nitrogen or the like, especially in case the temperature of atomization is relatively high or where one of the basic constiuents is very sensitive to oxidation.

Advantageously, the gas flow is adjusted so as to avoid a part of the powder formed being lost by being carried away in the flow of

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gaseous fluid.

As already described previously, the gaseous fluid at the inlet into the enclosed space of the atomizing apparatus, must be at a temperature referred to as the atomizing temperature, higher than the boiling temperature of the solvent used. However, the temperature of this gaseous fluid must not prevent the formation of the powder in the region provided therefor in the apparatus, that is to say the gaseous fluid in that region must be at a lower temperature than the temperature of transition of the mixture of constituents dissolved in the starting solution.

According to a variation of the process of the invention, there are added to the lipidic solution, prior to its atomization, fine solid preferably microalveolate particles, for example kieselguhr, in an amount at least equal in weight to that of the whole of the lipids contained in the solution and preferably at least three times greater than the said amount of lipid.

Of course, a furtther amount of solvent will be added, if need be, so that the final suspension may be vaporizable in the atomizing apparatus. Furthermore, the dimensions of the said solid particles must not be too important in order to avoid obstructing the spraying nozzle of the apparatus.

After atomization, the lipidic complex is intimately mixed with the said solid particles in the form of powder.

One advantage of the present variation of the process is that it allows to obtain a powder through atomization even using amphiphilic lipids whose melting temperature is lower than 50°C.

Of course, the processes for producing a pulverulent mixture according to the invention may be carried out in a continuous or discontinuous way, the powder being collected directly at the outlet of the cyclone either to be stored as a product, or to be used immediately, in particular for the formation of hydrated lamellar phases such as liposomes.

The invention also has for object a pulverulent mixture of lipidic constituents with possibly at least one hydrophobic or

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partially hydrophobic constituent obtained by the above-described process. This mixture presents in particular the characteristic of being very easily dispersed in an aqueous solution.

The invention has further for object a process of preparation of hydrated lipidic lamellar phases such as liposomes or the like, characterized in that it consists in dispersing the pulverulent mixture described above in an appropriate aqueous medium.

The pulverulent mixture obtained by atomization is dispersed in the aqueous medium by a conventional method such as for example stirring, homogenization or sonication.

However, the pulverulent mixture obtained following the variation of the atomizing process of the invention, dispersed by means of mere agitation, for example with the aid of a magnetic bar, allows obtaining a very hydrated phospho-lipidic lamellar phase constituted of particles in the form of liposomes of particularly reduced dimensions, of the order of 100 to 300 nanometers, without it being necessary to use the usual homogenization techniques, such as ultrasonication for example.

The solid microalveolate particles contained in the said dispersion may be easily eliminated by mere filtration, for example on a 10 µm porous filter, which may be followed, if necessary, by a sterilizing filtration.

Advantageously, the aforesaid pulverulent mixture may be collected directly in the container where the dispersion operation takes place in the aforesaid aqueous medium, after the means of recovery of the powder, for example after the cyclone, in its lower part. Thus, one performs a continuous production of either slightly hydrated lamellar phases or of liposomes.

The different aqueous media used for the preparation of hydrated lamellar phases may be either water, or solutions of various substances the choice of which depnds upon the properties which one desires to impart to the hydrated lamellar phases. For example, the aqueous solution intended to be encapsulated in the lamellar phases or liposomes may contain substances having

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interesting biological, organoleptic, physicochemical or physiological properties. By way of non-limitative example, the solution to be encapsulated may contain enzymes, antibiotics, polypeptides such as elastin polypeptides. The dispersion medium may also be an isotonic solution of sodium chloride.

According to a first form of embodiment of the invention, it is possible to disperse the pulverulent mixture obtained through atomization directly in a relatively important quantity of aqueous medium to obtain directly the desired weight concentration in lamellar phases.

For example, the atomized pulverulent mixture is dispersed in an aqueous medium in which is dissolved, possibly, a substance to be encapsulated to obtain a weight concentration in the pulverulent mixture in the suspension lower than about 50%. There is thus obtained a suspension of highly hydrated lamellar phases in which the dissolved substance is encapsulated. One prefers to carry out suspensions of a weight concentration in pulverulent mixture comprised between 10% and 25%.

Thus, the yield of encapsulation of the substance dissolved in the aqueous medium is comprised between about 20% an about 50%. This encapsulation yield is is high, for it is possible to obtain hydrated lamellar phases, to disperse the atomized pulverulent mixture in a relatively small quantity of aqueous medium.

Following a variation of the process of the invention, the atomized pulverulent mixture is dispersed in a relatively small quantity of an aqueous medium advantageously containing the substances which one wishes to encapsulate, the said dispersion being homogenized to ensure a very good mixture, for example with the aid of a roller grinder. The weight concentration in pulverulent mixture in the mixture obtained is for example higher than 50% approximately. There is thus obtained a slightly hydrated lamellar phase.

This lamellar phase may be either used directly as industrial products, and in that case, the weight concentration in pulverulent

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mixture is comprised advantageously between 60% and 75%, or this slightly hydrated lamellar phase may be diluted in a fresh quantity of the aqueous medium already used, or in a fresh aqueous dispersion medium, to form a highly hydrated lamellar phase, that it to say a suspension of liposomes, advantageously to obtain a weight concentration in pulverulent mixture comprised between 0.1% and 10%. Thus, in this variation, one obtains a very high encapsulation yield, which is higher than about 50%.

According to a preferred form of embodiment of the invention, the aforesaid aqueous dispersion medium is iso-osmotic with regard to the aqueous solution to be encapsulated. Furthermore, this process allows to obtain lamellar phases or liposomes of very small size, for example smaller than or equal to three μm which it has been possible to verify by an electronic microscope study.

It is also possible, if desired, to obtain a more thorough homogenization of the suspension of liposomes, for example by means of a prolonged sonication.

The present invention has further for object hydrated lipidic lamellar phases such as liposomes or the like prepared according to the process described above. Advantageously, the lamellar phases contain encapsulated substances possessing useful properties, notably in the pharmaceutical, cosmetic, foodstuffs or other fields.

Lastly, the invention also has for object pharmaceutical or cosmetic compositions containing lipidic lamellar phases prepared according to the above process notably used as a vehicle for active substances.

According to the processes of the invention, it is possible to carry out an intimate mixture of the basic constituents of the lipidic lamellar phases, namely a mixture of one or several amphiphilic lipidic compounds and of one or several hydrophobic compounds, without necessitating the use of surface-active compounds. Furthermore, it is possible to produce hydrated lipidic lamellar phases from the atomized pulverulent mixture of the invention, by dispersion of the latter in a relatively small

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quantity of aqueous medium and, consequently, to obtain a high yield of encapsulation of the compounds dissolved in the aqueous medium.

Furthermore, these processes allow producing hydrated lipidic lamellar phases according to a continuous or discontinuous method of manufacture. They may also be used very easily on an industrial scale with a manufacturing cost inferior to that of the known processes.

Furthermore, the pulverulent mixture obtained by atomization may be easily dispersed in an aqueous medium, which allows to obtain slightly hydrated lipidic lamellar phases or highly hydrated lipidic lamellar phases.

The invention will be better illustrated and other characteristics, details and advantages thereof will appear more clearly from the following non-limitative examples, and given solely by way of illustration of the present invention.

Example 1

In a 100ml beaker are introduced 9g of phosphatidylserine and 1g of ß-sitosterol which are dissolved, with the aid of magnetic agitation, in 50ml of chloroform. To the solution obtained (solution A) is added, maintaining the agitation, 0.1g of sodium stearoylglutamate in solution in 25ml of methanol (solution B). The mixture obtained is vaporized in the enclosed space of an atomizer supplied with air whose temperature will have been adjusted to 75°C. There is collected in a receptacle placed at the base of the apparatus a very fine white powder. This powder thus obtained is introduced into a 500ml beaker containing 100ml of a solution containing 2% of hyaluronidase and 0.9% of sodium chloride (solution C). The homogenization of the mixture is ensured at standard temperature by means of a magnetic agitation maintained during one hour. There is obtained a dispersion having the aspect of a gel. The latter poured into a 1.5 l container provided with a propeller agitation is diluted in 900ml of a physiological solution (0.9% aqueous solution of sodium chloride). One thus obtains an opalescent suspension.

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Observation by electronic microscope of this suspension reveals the presence of liposomes of a size smaller than 3 µm.

Example 2

In a 100ml beaker containing a mixture composed of 50ml of chloroform and of 25ml of methanol are introduced 9.9g of sphingomyelin and 0.1g of dicetyl phosphate and are agitated.The solution obtained is atomized as in the preceding example at 75°C. The powder collected at the outlet of the apparatus is intimately mixed with 100ml of an aqueous solution at 1% of alpha-chymotrypsin and 0.9% of sodium chloride, with the aid of a magnetic agitation during one hour followed by a sonication during 20 minutes. One dilutes as in the preceding example, in 900ml of a physiological solution. There is obtained a blueish solution. The size of the liposomes, determined by evaluation of the Brouwnien movement of the particles (apparatus of the Nano-Sizer type), is slightly smaller than 0.1 µm. One determines the yield of encapsulation of the ∝-chymostrypsin after filtration on gel (sepharose CL-4B) of the blueish solution. The yield of encapsulation of the ∝ -chymotrypsin with respect to the ∝-chymotrypsin dissolved in the aqueous dispersion solution is 40%.

Example 3 to 9

One carries out liposome preparations according to the method described in example 1, with the constituents indicated in the appended Table 1.

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TABLE 1

Ex.	SOLUTION A in 50ml of chloroform				SOLUTION B in 25ml of methanol		C° Atomization	SOLUTION C Quantity used: 100 ml
N°	Nature of product	weight (g.)	nature of product	weight (g.)	nature of product	weight (g.)	°C	nature of products and concentrations
3	Cerebroside	8	Cholesterol	2	None	-	75	0.1%Streptomycin 0.9% Na Cl
4	Soya lecithin	9	β-oestradiol	1	None	-	75	0.9% Na Cl
5	Soya lecithin	9.9	None	-	Vanillin	0.1	60	0.9% Na Cl
6	Soya lecithin	9	γ-oryzanol	1	None	-	80	0.9% Na Cl
7	Soya lecithin	8	Cholesterol	2	None	-	75	10% aloe extract 0.9% Na Cl
8	Soya lecithin	9.5	Cholesterol	0.5	None	-	75	1% elastin hydrolysate 0.9% Na Cl
9	Soya lecithin	8.9	Cholesterol α-tocopherol	1 0.1	None	-	75	0.9% Na Cl

Example 10

Into a 100ml beaker containing 30ml of dichloromethane are introduced 2g of soya lecithin and 0.2g of sitosterol. They are stirred by means of a magnetic bar until complete dissolution. 7g of Kieselguhr are thereafter added and the stirring is maintained for 5 minutes in order to obtain a homogeneous suspension.

This suspension is vaporized within the enclosed space of an atomizer supplied with a current of air whose temperature is adjusted to 60°C.

A very light powder is collected.

The powder thus obtained is introduced into a 500ml beaker containing an aqueous solution of 1% milk glycoproteins and 0.9% sodium chloride. The mixture is subjected to magnetic stirring for 15 minutes. It is thereafter filtered on a 10 micrometers porous filter. There is thus obtained an opalescent liquid the observation of which by electronic microscope reveals the existence of a suspension of liposomes whose size, measured by means of an apparatus of the Nano-Sizer® type, is in the order of 220 nanometers.

This suspension of liposomes may be sterilized through sterilizing filtration on a 0.45 micrometer Millipore® filter.

Example 11

The preparation of an atomized powder is carried out according to the method described in Example 1 from 15g of soya lecithin and 3g of lanosterol in 50ml of chloroform. Atomization is conducted at 75°C. One mixes with a spatula the pale yellow powder obtained, with 6ml of an aqueous solution containing 5% sodium carboxylate Pyrrolidone, 5% sodium lactate and 0.9% sodium chloride, after which the mixture is homogenized by means of a roller grinder.

There is obtained a lamellar phase having the consistency of a viscous gel whose characteristics are checked through X-ray diffraction. This lamellar phase can be used in this form, notably for preparing cosmetic products.

It is also possible to disperse this slightly hydrated lipidic lamellar phase in 200ml of an aqueous solution containing 0.9%

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sodium chloride to obtain a suspension of highly hydrated lamellar phases or liposomes. The measured yield of encapsulation of sodium carboxylate Pyrrolidone is 60%.

Example 12

Into a 500ml beaker, is introduced 0.17g of elastin hydrolysate dissolved in 100ml of 96% ethanol. One thereafter adds while stirring 200ml of chloroform and 0.34g of soya lecithin. The mixture is atomized at 80°C. The white powder obtained is placed in suspension in 50ml of a physiological solution by means of magnetic stirring for one hour. Observation by electronic microscope reveals the existence of liposomes smaller in size than one μm. Sonication of this suspension allows obtaining a blueish opalescent solution containing liposomes whose size does not exceed 0.2 μm.

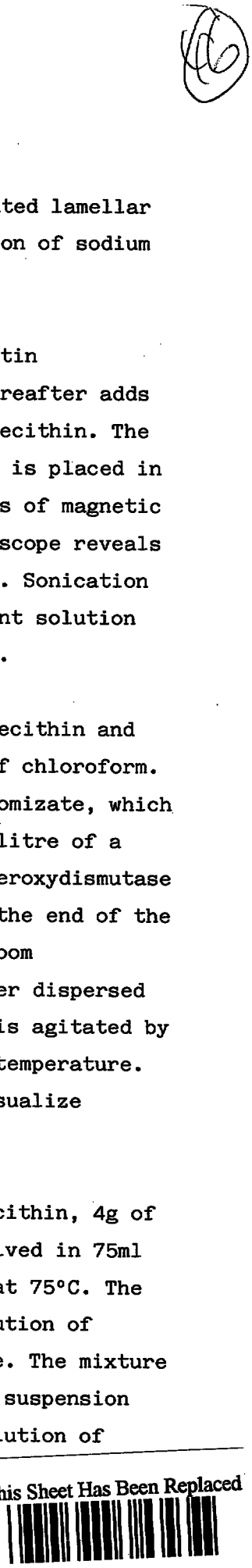
Example 13

Into a 500ml beaker are introduced 90g of soya lecithin and 10g choloesterol. This mixture is dispersed in 400ml of chloroform. The solution thus obtained is atomized at 75°C. The atomizate, which appears as a white powder, is directly collected in 1 litre of a 0.9% solution of sodium chloride, containing 1g of superoxydismutase (S.O.D.), subjected to vigorous magnetic stirring. At the end of the atomization, the stirring is continued for 1 hour at room temperature. The milky suspension obtained is thereafter dispersed in 9 litres of a 0.9% solution of sodium chloride. It is agitated by a rotor and paddle homogenizer for 15 minutes at room temperature. Observation with an electronic microscope allows to visualize liposomes of medium size of the order of a micrometer.

Example 14

Into a 100ml beaker are introduced 5g of soya lecithin, 4g of cholesterol and 1g of γ-oryzanol. The mixture is dissolved in 75ml of chloroform. The solution thus obtained is atomized at 75°C. The atomizate is collected directly in 100ml of a 0.9% solution of sodium chloride containing 1g of a collagen hydrolysate. The mixture thus obtained is subjected to homogenization, then the suspension obtained is thereafter dispersed in 900ml of a 0.9% solution of

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sodium chloride.

Example 15

Into a 100ml beaker are introduced 4g of soya lecithin, 4g of sphyngomyelin and 2g of cholesterol dissolved in 75ml of chloroform. The solution thus obtained is atomized at 75°C. The atomizate is collected directly in 1 litre of a 0.5% solution of d-glucose. This solution is subjected to vigorous agitation to obtain a homogenization.

Example 16

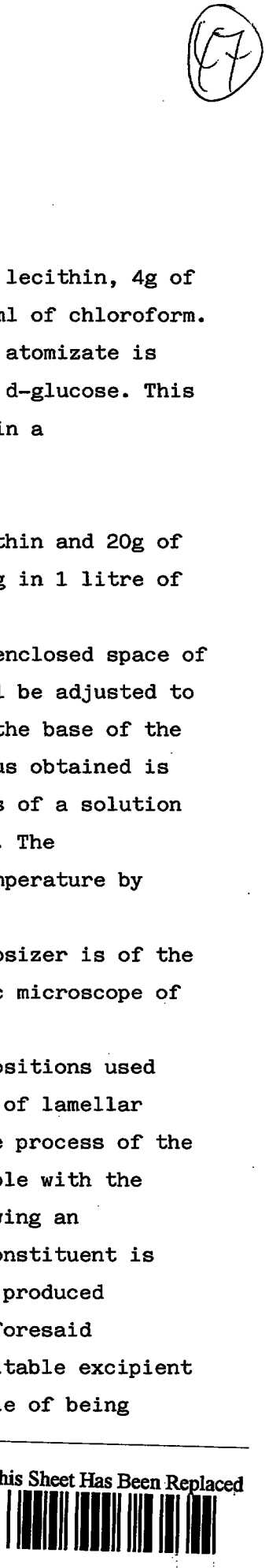
Into a 2l beaker are placed 180g of soya lecithin and 20g of stigmasterol dissolved by means of magnetic stirring in 1 litre of dichloromethane (CH_2Cl_2).

The mixture obtained is vaporized within the enclosed space of an atomizer supplied with air whose temperature will be adjusted to 60°C. There is collected in a receptacle placed at the base of the apparatus a very fine, white powder. This powder thus obtained is introduced into £ 50l container containing 20 litres of a solution of 0.9% sodium chloride and 1% elastin polypeptides. The homogenization of the mixture is ensured at room temperature by means of a turbine rotor for 1 hour.

The size of the particles measured with a nanosizer is of the order of 450nm. The visualization with an electronic microscope of these particles shows the existence of liposomes.

The following Examples 17 to 22 indicate compositions used notably in cosmetics, and obtained from suspensions of lamellar phases or lamellar phases themselves obtained by the process of the invention which are mixed with an excipient compatible with the human organism, and notably with the skin, and allowing an application of this on the skin. Thus, the active constituent is incorporated or encapsulated in the lamellar phases produced industrially by the process of the invention. The aforesaid suspension or the lamellar phase is mixed with a suitable excipient to produce for example creams, milks or balms capable of being applied particularly to the skin.

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According to a similar process, there can also be manufactured pharmaceutical compositions, the encapsulated or incorporated constituent having interesting properties from a biological or physiological point of view. These compositions may be in a solid, pasty or liquid form according to the excipient used.

Example 17

Preparation of moisturizing cream for skin care

Composition: Elastin suspension
(according to example 12) 10g
Emulsified excipient oil
in water 90g
Use: daily applications to the skin

Example 18

Cream for skin care with stimulating properties

Composition: Oryzanol suspension 10g (according to example 6)
Emulsified excipient oil in
water 90g
Use : daily applications to the skin

Example 19

Cream for skin care with protecting properties

Composition: Tocopherol suspension 10g
(according to example 9)
Emulsified excipient oil in
water 90g
Use: evening applications for preparing the skin to exposure to weather conditions the next day.

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Example 20

Moisturizing milk for the body

Composition: Aloe-extract suspension 10g
(according to example 7)
Emulsified excipient oil in
water 90g
Use: application after the bath or after the sun.

Example 21

Lip protecting balm

Composition: Lamellar phase with Lanosterol 10g
(according to Example 11)
Fatty excipient 90g
Use : application to the lips for protection against weather conditions.

Example 22

Stimulating antiwrinkle treatment

Composition: Enbryonic extract suspension
(according to Example 8) 30g
Gelled excipient 70g
Use : application to the face once a week

According to the invention, the solvent used to produce the pulverulent mixture through atomizing in a non-aqueous solvent.

This is why the examples only mention in this regard non-aqueous solvents such as chloroform for example.

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Claims

1. Process of preparation of a pulverulent mixture of at least one amphiphilic lipidic constituent and, possibly, of at least one constituent of hydrophobic character, characterized in that it comprises the putting into solution of the said amphiphilic lipidic constituent(s), and possibly of the said constituent(s) of hydrophobic character, in a non-aqueous solvent and the atomization of the solution thus obtained in a current of a gaseous fluid to produce the said pulverulent mixture.

2. Process according to claim 1, characterized in that the aforesaid gaseous fluid is brought to a temperature called atomization temperature higher than the boiling temperature of the aforesaid solvent, and in that in the region of formation of the powder, the temperature of the current of the aforesaid gaseous fluid is lower than the transition temperature of the aforesaid mixture of amphiphilic lipidic constituent and possibly of hydrophobic constituent.

3. Process according to claim 2, characterized in that the temperature of atomization is comprised between 60°C and 100°C.

4. Process according to one of the preceding claims, characterized in that the weight proportion of the aforesaid amphiphilic lipidic constituent(s) in the whole of the constituents dissolved in the solvent before atomization is greater than 50%.

5. Process according to claim 4, characterized in that the aforesaid weight proportion of amphiphilic lipidic constituent is greater than 70%.

6. Process according to one of the preceding claims, characterized in that at least one of the amphiphilic lipidic constituents or at least one of the aforesaid hydrophobic constituents is a biologically active compound and/or possesses organoleptic and/or physico-chemical properties.

7. Process according to one of the preceding claims, characterized in that the aforesaid amphiphilic lipidic constituents

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are selected among the compounds belonging to the family of glycolipids, phospholipids or phospho-amino-lipids.

8. Process according to claim 7, characterized in that the aforesaid amphiphilic lipidic constituents are selected among the group comprising: soya or egg lecithin, a phosphatidylserin, a cerebroside or a shpyngomyelin.

9. Process according to one of the preceding claims, characterized in that the aforesaid constituents of hydrophobic or partially hydrophobic character are selected among the group comprising sterols or their esters, aliphatic fatty acids or their esters, triterpenic fatty acids or their esters, primary or secondary fatty alcohols or their esters, aliphatic fatty amines, aminoacids acylated by a fatty acid, polypeptides, vitamins, extracts of animal or vegetable origin, or phenols which may comprise one or several substituents selected among the alcoxy, alkyl comprising 1 to 4 carbon atoms, carboxy or formyl groups.

10. Process according to claim 9, characterized in that the aforesaid hydrophobic constituents are selected by the group comprising cholesterol, ß-sitosterol, oestradiol, lanosterol, stigmasterol, γ-oryzanol, stearic acid, xymeninic acid, isopropyl myristate, glycyrrhetinic acid, dicetyl phosphate, stearylamine, stearoyl-L-glutamic acid, elastin hydrolysate polypeptides, a tocopherol, embryonic extracts, aloe extracts or vanilline.

11. Process according to one of the preceding claims, characterized in that the aforesaid solvent consists of a solvent or of a mixture of solvent of which at least one is a solvent of the aforesaid amphiphilic lipidic constituents and/or the hydrophobic constituents, the said solvent or mixture of solvent having a boiling point lower than the temperature of deterioration of the said dissolved amphiphilic or hydrophobic constituents.

12. Process according to claim 11, characterized in that the solvents are selected notably among the group comprising chloroform, methanol, ethanol, dichloromethane, or mixtures of chloroform-methanol, chloroform-ethanol, preferably comprising 33%

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of methanol or of ethanol.

13. Process according to one of the preceding claims, characterized in that the gaseous fluid used to atomize the aforesaid solution is a gas substantially chemically inert with regard to the constituents dissolved in the said solution, such as air or nitrogen.

14. Process according to one of the preceding claims, characterized in that, there is added before atomization to the aforesaid solution of said amphiphilic lipidic constituents and possibly of said hydrophobic constituent(s), an amount of solid preferably microalveolate particles, such as kieselguhr, at least equal in weight to that of the whole of the lipidic constituents contained in the said solution, and preferably in an amount at least three times as great as the said amount of lipidic constituents.

15. Pulverulent mixture of at least one amphiphilic lipidic constituent and possibly of at least one constituent of hydrophobic character , characterized in that it is obtained by the process according to one of the preceding claims.

16. Process of preparation of hydrated lipidic lamellar phases, such as liposomes for example, characterized in that it consists in dispersing the pulverulent mixture according to claim 11 in an appropriate aqueous medium.

17. Process according to claim 16, characterized in that the pulverulent mixture is collected directly in the aforesaid aqueous medium subjected to agitation to disperse the said pulverulent mixture.

18. Process according to claims 16 or 17, characterized in that the pulverulent mixture is dispersed in a determined quantity of the aforesaid aquaous medium to obatin a desired weight concentration of pulverulent mixture.

19. Process according to one of claims 16 to 18, characterized in that the aforesaid aqueous medium contains at least one substance biologically active and/or having organoleptic and/or

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physoco-chemical properties.

20. Process according to claim 18 or 19, characterized in tat the aforesaid weight concentration of pulverulent mixture in the suspension of hydrated lipidic lamellar phases is equal at most to about 50%, preferably is comprised between about 10% and about 25%.

21. Process according to claim 20, characterized in that the yield of encapsulation in the aforesaid hydrated lipidic lamellar phases of the aforesaid substance dissolved in the aforesaid aqueous medium is comprised between about 20% and about 50%.

22. Process according to one of claims 16, 17, 18, 19, characterized in that the aforesaid pulverulent mixture is dispersed in a small amount of aqueous medium to obtain slightly hydrated lamellar phases.

23. Process according to claim 22, characterized in that the weight concentration of pulverulent mixture in the aforesaid aqueous medium is comprised between about 60 and 75%.

24. Process according to claim 22 or 23, characterized in that the said slightly hydrated lamellar phases are obtained by dispersion of the aforesaid pulverulent mixture in the aforesaid aqueous medium by crushing, for example by means of a roller mill.

25. Process according to one of claims 22 to 24, characterized in that the aforesaid slightly hydrated lamellar phase is dispersed in the aforesaid aqueous medium or another suitable aqueous medium, to obtain a weight concentration of pulverulent mixture comprised between 0.1 and 10% approximately.

26. Process according to claim 25, characterized in that the yield of encapsulation in the aforesaid hydrated lipidic lamellar phases of the aforesaid substance dissolved in the aforesaid aqueous medium of formation of slightly hydrated lamellar phases, is higher than 50% approximately.

27. Hydrated lipidic lamellar phases, characterized in that they are obtained by the process according to one of claims 16 to 26.

28. Pharmaceutical composition containing a therapeutically

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efficient dose of an active constituent, characterized in that the said active constituent is incorporated or encapsulated in hydrated lipidic lamellar phases according to claim 27, the said lamellar phases being mixed with at least one excipient acceptable by the organism.

29. Composition for cosmetic use comprising an efficient quantity of an active substance, characterized in that the said substance is incorporated or encapsulated in hydrated lipidic lamellar phases according to claim 27, the said lipidic lamellar phases being mixed with at least one excipient suitable to obtain a milk, an unguent, a cream, or a balm.

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