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Chapter

Non-polymeric Microspheres for the Therapeutic Use of Estrogens: An Innovative Technology

Salvador Espino y Sosa, Myriam Cortés Fuentes, Jacobo Alejandro Gómez Rico and Manuel Cortés Bonilla

Abstract

Non-polymeric microspheres are stable-shaped particles constituted by crystalline organic compounds. This technology allows controlled release of parental products that has its prime value on estrogen therapy. The structure is a non-polymeric crystalline microsphere that uses a low solubility fatty acid, cholesterol as a carrier. Cholesterol is a waxy lipid, a substance that is insoluble in water and has been recognized as safe as excipient by FDA for the manufacturing of drugs. Cholesterol is a lipid present in the cell membrane and subcellular organelles of tissues and serves as the building block for all steroid hormones including cortisol, aldosterone, estrogen, and testosterone; therefore, this fatty acid provides better biocompatibility than polymers. The use of cholesterol as a low solubility carrier was used to develop a first of its kind, parental HT product for the management of menopausal symptoms carrying estrogen microspheres in an aqueous suspension, which would allow an extended estrogen release maintaining plasmatic therapeutic concentrations. Estradiol doses would be up to 30 times lower than that provided by oral and transdermal routes fulfilling current recommendations regarding the use of a low dose and the nonoral route. Both intramuscular monthly administered formulations of E/P non-polymeric microspheres had favorable pharmacokinetic and safety profiles, suggesting this route as an interesting, novel, and suitable way of treating menopause-related symptoms.

Keywords: hormone therapy, estrogens, technology, non-polymeric microspheres, cholesterol

1. Introduction

1.1 Estradiol pharmacokinetics

In a classic study, the maximum concentration (Cmax) of estradiol at steady state was 161.54 and 98.82 pg/mL and reached at approximately 6 h after a single IM injection of 1.0 and 0.5 mg of estradiol, respectively. Estradiol concentrations decreased through day 4. At day 8, estradiol mean plasma concentration was between 20 and 30 pg/mL and remained stable through day 30. These concentrations are equivalent to those found naturally occurring in early follicular phase of menstrual cycles (31 pg/mL median) in premenopausal women. The estradiol

elimination kinetics, based on EC MS, is biphasic, in which the elimination phase (a) presents a mean elimination half-life of 1.34–1.57 days and the second elimination phase (β) a mean elimination half-life of 12.22–16.78 days. Estradiol concentrations remained above 10 pg/mL during the 28 days of interval administration (**Figure 1**) [1].

The safety and effectiveness in menopausal symptoms treatment have been a primary goal in the last decades [2]. Microsphere technology uses of cholesterol as a carrier substance, and the IM administration allows for doses of estradiol up to 30 times lower than those delivered by the leading oral and transdermal alternatives [3].

Due to this delivery system, which allows for an unmatched bioavailability of the active ingredients, non-polymeric microspheres provide comparable or improved efficacy outcomes than seen in the leading high dosage alternatives. At the same time, its low dose formulation results in a superior safety profile compared to other products [4].

Injectable slow release products allow for the formation of crystalline structures in the shape of microspheres, which use cholesterol as a carrier instead of the more commonly used polymers. Cholesterol NF, an inactive ingredient approved by the US Food and Drug Administration (FDA) for use in pharmaceutical specialties—including parenteral products, is generally considered safer than polymers. The highest proportion of cholesterol accepted within a parenteral pharmaceutical product is 5.2% (52 mg/mL); pharmaceutical alternatives that use this technology often contain 0.142% of cholesterol (1.42 mg/mL).

Today, HRT is coming under increasing public scrutiny as hormones in higher dosages are suspected of triggering certain forms of cancer, breast cancer; however, reduced dosages of estradiol are lowering these risks [5].

Millions of women entering menopause suffer from the well-known symptoms but are concerned that the benefits of currently available hormone replacement therapy (HRT) may not justify the suspected risks. Doctors have been looking for alternatives to HRT, such as phytopharmaceuticals, but have found them unsatisfactory in relieving menopausal symptoms [5–8].

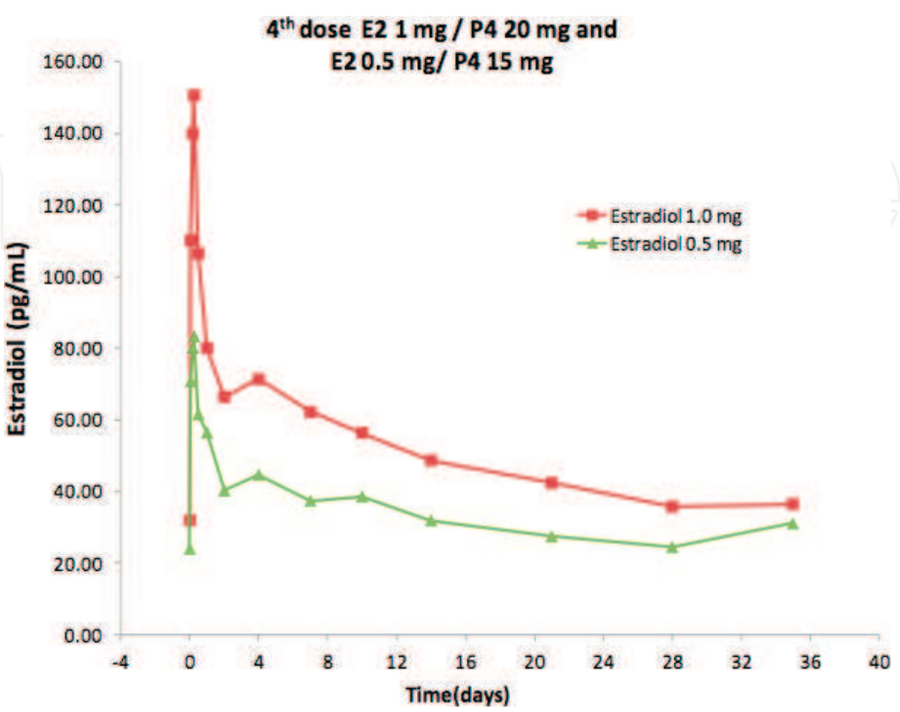

Figure 1.
Estradiol serum profile. Monthly parenteral route of administration.

A safe but effective alternative should be embraced not only by the medical community but also by the millions of women who suffer from menopausal symptoms [3, 9].

The hormonal transition process may last up to 10 years, with 3–4 years being average. Approximately, 50% of women who enter menopause suffer from menopausal symptoms (hot flushes, excessive sweating, sleep disorders, weight increase, nausea, fatigue, irritability, depression). Because these symptoms can so often be debilitating, women are inclined to seek treatment [6].

Treatments can be grouped into three classes:

1. Hormonal treatments, most commonly available as a combination of an estrogen and a progesterone, or estradiol or other estrogens alone (for women who have had a hysterectomy).

2. Nonhormonal treatments:

    a. medicines of natural origin, that is, herbal remedies such as *Cimicifuga racemosa* (leading product: Remifemin), Agnus castus; and soy and red clover isoflavones; and

    b. antidepressants.

3. Traditional remedies: herbal teas, dietary measures, etc.

While the therapeutic efficacy of hormonal therapies has been proven, their use has declined due to perceived and real, safety concerns. It should be a convenient, effective, and low dose product that also addresses the safety concerns relevant to healthy women undergoing menopause [10].

2. Stable-shaped particles of crystalline organic compounds

This technology allows the controlled release of parenteral products and consists of the formation of crystalline arrangement in spherical particles, which use a low solubility excipient as a carrier instead of polymers. Cholesterol is a waxy type of lipid, a substance that is insoluble in water and has been recognized as safe as excipient by FDA for use in the manufacture of drugs and is also a lipid present in the cell membrane and subcellular organelles of tissues and serves as the building blocks for all steroid hormones, and the use of cholesterol gives better biocompatibility than polymers.

The technology used for modification of the release of steroids has been applied in the development of products for the hormonal treatment of the climacteric symptoms (vasomotor symptoms such as night sweats, hot flushes, insomnia, memory loss/forgetfulness, mood swings), providing the clinician with an increased range of options in HRT.

2.1 About the technology

It is well known that many pharmaceutical actives are susceptible to crystallize in different manners, depending on the conditions under which they are crystallized. The polymorphs or pseudopolymorphs are crystalline structures that resulting from crystallization of a substance. When the polymorphs are melted and cooled rapidly below their melting point, that is, melt-congealed, the atoms or

molecules forming most substances need some time to arrange themselves in the order most natural for the environment in which they are placed. Therefore, they remain in unstable amorphous or semiamorphous states or organize into metastable polymorphs.

Metastable polymorphs may be enantiotropic, and they can exist in more than one crystalline form. The molecular arrangement can diverge, such that one form is stable above the transition-point temperature and the other is stable below it. As a result, the crystal habit is dynamic and reversible depending on ambient conditions [11].

A natural process that suffers the metastable polymorphs is the "aging," it is a natural crystallization process that occurs over time, transforming the metastable polymorphs into a more stable structure without human interference [12]. In the case of pharmaceutical manufacture, said process is usually costly, lengthy, unpredictable, and dangerous. This is due to the interference of various factors [13].

It is important to consider the time at which the crystallization of metastable particles occurs due to which it can be deformed or destroyed in short time, for example, hours, impacting in the bioactivity and/or bioavailability of crystallized substances. We must be careful with the different dissolution rates of distinct polymorphs of a substance, which can produce loss of uniformity and stability between the batches of the same drug.

When a compound is administered, we often require suspension in an aqueous solution suitable for injection that should not overlook the stable crystallization [14].

When a compound is administered to a patient, said compound is subject to biological fluid that contains water, even before this compound is suspended in an aqueous medium.

In the case of pellets and implants, which are placed in the body at the time of surgical procedures, the previous also applies.

Thereof to achieve full crystallization of substance prior it is administered, it is necessary to assure the physical integrity of the shaped particles and uniformity in the release of the active principle.

With the purpose of improving the stability of therapeutic compounds, some researches have induced the crystallization of them, using a dispersion drying method with temperature being controlled (Matsuda et al. [15]).

Further solubility should consider the shape and size of the therapeutics particles, due to the dissolution of a solid that is also related to the surface erosion [16].

The preservation of the shape and particle size, independently of the form in which a compound is administered either as a solid or as a suspension, is an important factor that assures the control and reproducibility of the bioavailability and biodynamics of the substance. Considering it, Kawashima et al. used two insoluble solvents for spherical crystallization of Tranilast and heat for the conversion of the resulting polymorphs of the process. Some experiments reported that the heat can accelerate the natural process of aging.

In some cases, the integrity or form of the substance could be seen compromised due to the heat requirements. Even though the reproducibility and stability is possible, the size control within the particles of the microspheres can be affected by the use of heat within the experiments.

The heat is also an important factor that can prevent reaching the desired stable polymorph of a specific substance, because in several cases, a hydrate is the most stable polymorphic form and the heat can dehydrate it. In the case of mixtures, the heating for stable crystallization is not recommended. Although the aging process is inferior to the heat method for obtaining stable polymorphs, it is also safer and has fewer limitations.

Among the studies carried out to induce crystallization of polymeric species that use solvent vapors, we highlight the putative crystallization, as well as the change

in the mechanical properties of polymeric compounds. To transform a polymer matrix, Pr4VOPc dye (vanadyl phthalocyanine having four propyl substituents), from glassy phase I to crystallized phase II, and organic solvent vapors [17] also have been used.

Tang et al. used organic solvent vapors.

2.2 Characteristics of the technology

The crystalline organic compounds might be formed by homogeneous particles of a single organic compound, or well, they might be formed by mixtures of two or more organic compounds. In aqueous suspension, it is normal that during prolonged storage, the stable particles preserve a constant shape and size. Its features are particularly essential and advantageous in pharmaceutical formulations, due to which stable particles can be fabricated to a uniform size and shape, retaining said characteristics despite of the long-term storage.

The process involves exposure to an atmosphere saturated with solvent vapors, the shaped particles above mentioned, so that one or more organic compounds are in a crystalline, amorphous, or metastable form. Of the liquids used as solvents, at least one or more organic compounds must be soluble.

Among benefits offered by process, it is found that it may be applied to hydrates, which are the most stable polymorphs. Its stability allows the integration of water molecules to crystalline web during formation, since said polymorphs will not drive off water molecules. Due to its susceptibility to high temperatures, the process is also applicable to thermolabile substances. With the exception of the compositions of mixtures-eutectic, a great variety of mixtures involved in the formation of stable structures could not be achieved by means of a process involving heat.

This leads to the utilization of a method that involves crystallization or recrystallizing of an amorphous or metastable crystalline organic compound or mixture. The method consists of the following steps:

1. exposing said compound or mixture to an atmosphere saturated with the vapors of one or more liquids, at least one of which must be a solvent for said compound or mixture, for a time sufficient for transforming the metastable compound or mixture to a stable, crystallized compound, or mixture; and

2. recovering the stable, crystallized compound or mixture for storage or use.

To carry out the process, any container can be used where they can manipulate the different variables, such as volume, pressure, temperature, and atmospheric content. The desired solvent vapors can be contained in a chamber with atmosphere saturated. Once the vapors fill the chamber without causing condensation on the surfaces of the chamber or the particles, the saturation point is reached.

The particles formed preferably acquire shapes of microsphere, pellet, or implant. Although it can be affected by melt-congealing, these are the preferred shaped particles that have uniform and reproducible surface area. The particles formed are usually configured to obtain a uniform particle size or range of sizes. Any process is susceptible to be used as long as it achieves a metastable crystalline conglomeration. The said methods should consider the crystallization of a mixture, and the mixture may be eutectic or noneutectic.

To be exposed to solvent vapors, the particles should be placed in the chamber or some other suitable container; however, said particles should not be immersed or in contact with any other liquid solvents. Once in the chamber, the particles are in a stationary or mobile state.

Depending on the consistency with the established principles, the optimal time necessary to carry out the crystallization will vary, that is, the physicochemical characteristics of the substances, such as size of the particle, the chemical makeup of the particle, the form of the solid state of the particle (i.e., amorphous, metastable crystalline), the type and concentration of solvent used, and the temperature of the treatment. These physicochemical properties will determine if the crystallization process requires seconds or even hours (normally 1–40 h are required, preferably, 1–36 h). A 24-h exposure time seems to be effective. The time ranges do not seem to be modified by the previous partial crystallization of the particles. Other important characteristics that also impact on the optimization of the exposure time of the substance and therefore on the crystallization of it are the solvent system used by the organic compound(s) to be crystallized.

As mentioned above, one of the main advantages of the process is that it can be applied to thermolabile substances, which are susceptible to high temperatures, which exactly what is sought is to avoid them. Thus, particular compound is what will define the applicable temperature range used in the process. To obtain vaporization of the solvent, it is sufficient that the temperature of the vapor in atmosphere is below the melting point of the particles.

The process requires the use of any agent classified as a solvent for the organic compound of interest. Therefore, the latter will determine the selection of the solvent. Among the conventional liquid solvents used in the laboratory are the following examples: water, alkanes, alkenes, alcohols, ketones, aldehydes, ethers, esters, various acids including mineral acids, carboxylic acids and the like, bases, and mixtures thereof. Methanol, ethanol, propanol, acetone, acetic acid, hydrochloric acid, tetrahydrofuran, ether and mixed ethers, pentane, hexane, heptane, octane, toluene, xylene, and benzene are some specific exemplary solvents. Water is an especially useful component of a solvent/liquid mixture, particularly where the most stable polymorph of a substance is a hydrate. Generally, solvents suitable for conventional liquid recrystallization of the compound of interest are suitable as a solvent in the present method.

The compound(s) of the stable particles include any organic compound capable of existing as a crystalline solid at standard temperature and pressure. The stable crystalline solid is formed by particles that are comprised by one or more organic compound(s). The said stable crystalline solid is a lattice of discrete organic molecules, i.e., nonpolymeric.

In the process, those organic compounds having some pharmacological or therapeutic activity are preferred. Even more preferred are the pharmacological compounds susceptible to the formation of polymorphs. These include particles comprised of a steroid or sterol, either, estrogen, androgen and progestogen, such as, 17P-estradiol, testosterone, progesterone, cholesterol, or mixtures thereof. Some nonsteroidal components that can be included in said mixtures are oxatomide/cholesterol, nifedipine/cholesterol, and astemizole/cholesterol.

The particles can be stored in liquid suspensions such as aqueous media, or administered directly to the patient. Due to stabilization of particles of amorphous or metastable crystalline organic compounds, the particles can be stored in liquid suspension, such as aqueous medium, or administered directly to a patient.

2.2.1 Stable-shaped particles of one or more allotropic molecular organic compounds

Allotropic organic compounds can assume two or more distinct physical forms (e.g., different crystalline forms or an amorphous versus a crystalline form). The polymorphs or polymorphic species are allotropic species.

It is important to consider the pharmaceutically acceptable excipients, stabilizers, and buffers, which conform shaped particles and contribute to the stable storage of them.

Among the advantages of stable-shaped particles is the combination of physicochemical properties. First, the particles are configured into desired shapes by means that might not result in the most stable crystalline form of the constituent organic compound. In the solid state crystallization process, the organic compound will assume the most stable crystalline structure, facilitating the retention of the size and shape of the original particle.

A configured particle that comprises of one or more molecular organic compounds, each one which has a uniform crystalline character and possessed of a high degree of storage stability, is the resultant product.

There are several characteristics that lead to the particular predictability and consistent bioavailability and associated biodynamics, such as, the combination of the uniformity of size and shape of the particle and the uniformity and stability of the crystalline structure of the constituent organic compound.

The particle size and the shape of microspheres are prefabricated to desired conditions. Thus, the particles are subject to a solid-state crystallization process that stabilizes the compounds of the particles without loses the size and shape with which they were manufactured.

The resulting particles have greater uniformity of size and shape, more uniform and predictable dissolution profiles, and greater storage stability in various forms, e.g., in liquid suspension such as aqueous media or other storage liquid, as lyophilized solid, or alone as a powder or dry solid. By storage stability, it is meant that the particles have improved shelflife without the loss of their desired uniformity in size and shape, per se. That is, if the desired particle shape is a microsphere, the particles will retain a spherical shape of constant size over periods exceeding several years.

Here, storage stability refers to retention of the original size and shape of the particle, as well as the pharmacological activity of the active agent over a period of at least one .

The technology also involves a method of crystallizing shaped particles of a metastable compound or mixture of compounds without dissolution of the particle and loss of the desired shape.

The crystallization process is affected by exposing said particles to a controlled atmosphere saturated with the vapors of a solvent or solvents. The atmosphere is optionally modified in other respects, for example, pressure, temperature, inert gases, etc. Preferably, the controlled atmosphere is saturated with a solvent vapor but not so much as to effect condensation of said solvent.

More particularly, the method affects crystallization of an amorphous or metastable organic compound in a shaped particle without alteration of the dimensions (e.g., size and shape) of said particle which comprises of: (i) exposing said shaped particle to an atmosphere saturated with the vapor of a liquid, said liquid being a solvent for said organic compound and (ii) recovering said shaped particle wherein the said organic compound is of a uniform crystalline structure.

Alternatively stated, the method involves effecting a solid-state crystallization of a molecular organic compound in a particle of definite size and shape comprising of: (i) exposing said particle to an atmosphere saturated with a solvent for said organic compound; and (ii) recovering said particle, wherein said organic compound in said recovered particle is of a uniform crystalline structure and said recovered particle has retained said size and shape. Retaining the size and shape of the particle is meant to include minor variations in the dimensions of the particle, for example, no more than about 15%, preferably, no more than about 10%.

This technology provides a means for fabricating particles of desired dimension without regard to the resulting allotropic form of the organic compound. After the particle is fabricated into the desired shape and size, the solid state crystallization can be affected to crystallize the organic compound into a storage-stable solid state of uniform crystal structure. Moreover, the solid state crystallization can be affected on particles composed of more than one allotropic organic compound.

Preferably, the shaped particle is a microsphere, and, as a result of the present process, the organic compound(s) of the microsphere are ordered into a single, homogeneous crystalline form without any deterioration in the size or shape of the microsphere.

The term "crystallization" refers to a process by which the most stable polymorph of a particular substance is achieved. Recrystallization refers to a process similar to crystallization except that the organic compound of the particle, rather than being amorphous, was initially only partially crystalline, with a mixed crystalline habit, or crystalline, but of a less stable form. Unless indicated otherwise, the term crystallization includes recrystallization.

The term "solid state crystallization" refers to a crystallization process that is affected without macroscopic dissolution of the compound being crystallized. As used herein, solid state crystallization includes a crystallization process wherein an organic compound within a shaped particle is crystallized or recrystallized by exposure to a solvent vapor without loss or alteration of the shape or size of the particle. It will be appreciated by those skilled in the art that while subtle intermolecular changes will be affected by such crystallization (e.g., creation or rearrangement of crystal lattice structure), the microscopic and/or macroscopic dimensions of the particle will not be appreciably altered. The term "saturated" when used in reference to the atmosphere wherein the crystallization is conducted means that the atmosphere within the chamber or enclosure used to hold the solvent vapors contains the maximum quantity of said solvent in the vapor phase without affecting visible condensation on surfaces within the chamber. Condensation does not include microscopic condensation on the surface of the particles that does not affect their shape.

The term "solvent" refers to a liquid at standard temperature and pressure, and one capable of solubilizing an appreciable amount of a specified solid solute. The solid solute will be a particular organic compound. Solids vary from 0 to 100% in their degree of solubility [18].

A liquid will be a considered a solvent with respect to a particular solid solute provided the solute is at least 10% soluble in said liquid.

The term "particle" refers to a discrete collection of a plurality of molecules of one or more organic compounds. As used herein, a particle may be an ordered collection (e.g., crystalline) or disordered collection (e.g., amorphous) of molecules, or any combination thereof. The term embraces, among other things, microscopic as well as macroscopic particles such as powders, microspheres, pellets, implants, and the like.

Preferably, particles are made of microspheres. The preferred microspheres range in size from 1 micron to 1 mm, more preferably 1 to 500 microns, and most preferably in the range of 1–100 microns, particularly for human use. When the particles are in pellet form, such particles are normally but not necessarily cylindrical with lengths of 1000–5000 microns and diameter of 500–1000 microns. These particles can have important applications for veterinary use and are not injected but deposited under the skin.

The size and shape of the particle will depend on the intended application and the constituent organic compound(s). For example, microsphere size is chosen for

practical reasons, that is, a size appropriate for administration using a hypodermic needle or for assuring a desired rate of dissolution.

The term "molecular organic compound" refers to an organic compound existing as stable discrete molecules (i.e., nonpolymeric) and when combined with a plurality of identical molecules, it is capable of assuming one or more ordered crystalline structures. Thus, a molecular organic compound is meant to distinguish from a polymeric species.

The term "metastable" means a pseudoequilibrium state of a solid substance where the content of free energy is higher than that contained in the equilibrium state. A "stable" substance or particle has a crystalline structure whose shape remains unchanged in a standard ambient environment, e.g., in air having varying levels of moisture, for an extended period. However, it should be understood that "stable" does not indicate infinite stability but means sufficiently stable such that the particles remain sufficiently stable for the preservation of their crystalline characteristics during storage and up to their application and use and additionally, after they have been administered to a subject, up to their total dissolution.

The technology also encompasses stable microspheres achieved using the present method. Such microspheres preferably contain a compound having pharmaceutical applications. The microspheres and pellets are useful in human, as well as in animal, therapeutic regimens.

For instance, there is currently a need for compositions that accomplish the sustained release of steroid growth promoters in food animals to promote the growth of such animals. The amount of growth hormone administered to an animal would depend on the particular animal species, hormone, length of treatment, age of animal, and amount of growth promotion desired. The particles can be particularly configured for optimal delivery by injection by varying the particle size.

As discussed above, the microspheres are stable in aqueous fluids and are thus amenable to parenteral injection. Some examples of modes of administration are (IV), intraarterial (IA), intramuscular (IM), intradermal, subcutaneous, intraarticular, cerebrospinal, epidural, intraperitoneal, etc. In addition, the compounds can be administered via an oral route, either as an aqueous suspension or a lyophilized product. Other routes of administration are also acceptable, including topical application, into the eye, or via inhalation in the form of droplets or mist.

The dosage may take the form of a microsphere powder in vials/ampoules, ready to be prepared as suspensions, or take the form of ready-prepared suspensions, packaged into injectable ampoules or directly into syringes, ready to be administered in human or veterinary medicine. The suspension medium may be water, a saline solution, an oil, containing buffers, surfactants, preservatives, commonly used by pharmacotechnicians for preparing injectable substances, or any other substance or combination, which does not threaten the physical and chemical integrity of the substances in suspension and which is suitable for the organism which will receive it. If it is desired to avoid a sudden initial increase in the level of active ingredient in the internal medium of the receiving organism, it will be preferable in the case of ready-for-use suspensions to use liquid vectors in which said active ingredients are practically insoluble. In the case of active substances, partially soluble in the lukewarm liquid vector but insoluble at cold temperature, it is preferable, from the pharmacological point of view, to avoid the formation of precipitates (called "caking effect") by preparing formulations in the form of separate microsphere powder and liquid vector, which will be mixed only at the time of injection.

For most applications in human medicine (duration of action of the active ingredient between a circadian cycle and a menstrual cycle), it is preferable to use

microspheres whose diameter is between 5 and 100 microns, depending on the combinations of active substances/carrier substances.

A separation of microspheres according to their diameter may be performed during the manufacturing process using known processes: for example, by cyclonic separators, by sieving using air suction or by sieving in aqueous medium. In practice, it is sufficient if more than 70% of the microspheres have diameters between 70 and 130% of a specified diameter. If necessary, the ideal dissolution curve, determined by the proposed application, may be approached by mixing batches with suitable different diameters. Moreover, particles that do not comply with the specifications may be recycled.

The mechanism by which substances in a solid state crystallize in the presence of vapors containing at least one solvent has not yet been established. The crystallization process may well conform, as regards the effect of the solvents, to the traditional principles that apply in saturated solutions and in molecular mobility.

It is possible that some molecular rotational or transference movement occurs, which seems to depend on the particular type of solvent used and to the temperature of vaporization [19].

.It is clear, however, that the temperatures at which the crystallization is obtained are well below vitreous transition temperatures and are in fact only in accordance with that required for the solvents' vapor pressure. Without wishing to be bound by any theory, we contemplate that the vapor molecules of the solvent or five solvents might form microcondensations and minute accumulations of solvent on the surface of the particles to be crystallized, thus bringing sufficient energy for the surface molecules of the solid particles to form organized structures (e.g., crystalline domains). By the same token, if present in the vapor, water molecules become available for the formation of hydrates, when required for stable polymorphs.

Once the organizational and/or water-absorbing process starts at the surface, it is possible that the crystallization process gradually spreads into the interior of the particle without the need for contact with or dissolution within the solvent. If this is correct, there are two facts, which seem to indicate that these microcondensations or molecular agglomerations, are extremely minute. Firstly, if enough solvent condensation occurred on the surface of the particle, the solvent would at least partially dissolve it and modify its shape. To avoid any partial dissolution, the amounts deposited by the vapor must be extremely minute.

Secondly, during exposure to solvent vapors, the particles, because of their small size and large quantity, inevitably come in contact with one another. If there is any surface dissolution of the particles, as would occur if the substantial quantities of amounts of deposited vapor were not very minute, the particles would tend to stick to each other and form lumps or agglomerates. Under the conditions described herein, this does not occur.

3. Examples

3.1 Example 1: microspheres of 17-beta-estradiol

The spherical 17-beta-estradiol is prepared by spraying at 210°C and solidified at −50°C (spray/congealing method). In this stage, the ME are formed by amorphous solids (**Figure 1**), which when put in contact with water at 40°C, are deformed by the growth of crystals on their surface. In order to obtain the microspheres with

the thermodynamically more stable crystalline form, a hemihydrate, the spheres are stored in an atmosphere saturated with vapors of a mixture of ethanol: water (50:50), for 4 h at a temperature of 20 and 25°C. Finally, the microspheres are stored at 40°C to remove the solvent (**Figure 2**). At the end of the process, the spheres are stable, since no change in their morphology is observed after being stored in water at 40°C for more than 9 months.

3.2 Example 2: testosterone microspheres

Two polymorphs and two testosterone hydrates are published in the literature, hydrates being thermodynamically more stable. The preparation of the testosterone microspheres has the same preparation steps that were used for the estradiol microspheres. The stabilization or crystallization was made by storing the microspheres in a saturated atmosphere of vapors of a solution of acetone: water (80:20). The physical stability was evaluated by microscopic observation of storage microspheres in water at 40°C; the freshly prepared testosterone microspheres (uncrystallized) were unstable, while the crystallized microspheres were stable for more than 40 days.

3.3 Example 3: progesterone microspheres

The unstable or metastable spherical solids of progesterone are obtained with the same method (spray/congealing) described in the previous examples. Progesterone has two polymorphic forms, form I have a melting temperature of 131°C and form II with a melting temperature of 123°C, of which form I has a thermodynamically most stable structure.

To obtain the microspheres in the most stable crystalline form, they were stored in an atmosphere saturated with vapors of an ethanol solution: water (50:50) at room temperature for 4 h. The crystallization kinetics of the microspheres was studied by thermal analysis (DSC), where it was observed that polymorph II is the crystalline form that is first formed and later transformed into polymorph I, by the vapors of the solvent mixture.

In the crystallized microspheres, no change in their surface was observed after 6 months of storage in water at 40°C.

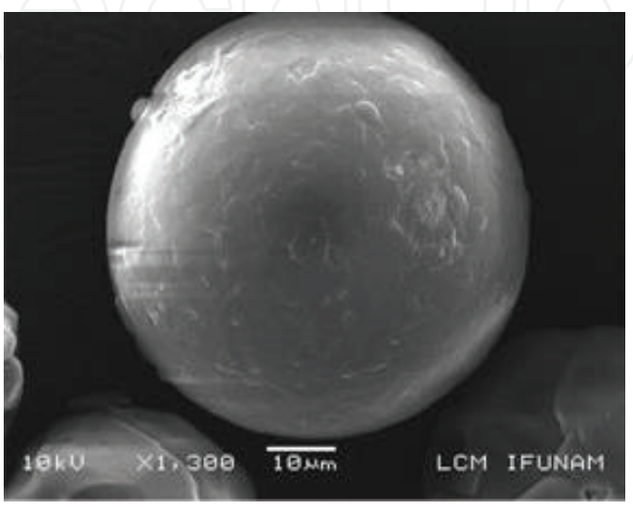

Figure 2.
Estradiol microsphere: estradiol/cholesterol 1:1.

3.4 Example 4: astemizole microspheres

In the same way that the microspheres formed by spheroidal molecules (spray/congealing) are obtained, the microspheres of astemizole are prepared, crystallized with vapors of ethyl acetate or acetone. Its physical stability was confirmed after observing under the microscope spheres stored in water at 40°C for more than 2 months.

3.5 Example 5: astemizole pellets

Pellets obtained in a conventional manner were stabilized with the following crystallization method. When the microspheres were placed in a recipient of approximately 7 L and exposed for 24 h at 20–25°C to the vapors of 2 mL of ethanol kept in a porous cellulose material, the initially amorphous microspheres crystallized completely in the presence of the vapors.

The microspheres were later dried at 60°C in a vacuum for 24 h, and the residual ethanol present in the microspheres was less than 0.01%.

3.6 Example 6: cholesterol microspheres

Cholesterol microspheres were prepared by the spray/congealing method, through microscopic observations of microspheres stored in water, it was concluded that the spheres obtained are formed by amorphous solids.

The crystallization of the spheres is possible by storage in atmospheres of acetic acid at 30°C for 8 h.

3.6.1 Crystallization of substance mixture

The obtaining of spherical particles from mixtures of materials is particularly interesting in the pharmaceutical industry since the end products can have different kinetic (dissolution rates), and chemical (stability) properties to the materials separately. The possibility of stabilizing spheres of material mixtures with this technology greatly increases its application in health areas. To prepare stable spheres formed by mixing of substances, it is important that the materials are thermally stable and chemically compatible.

3.7 Example 7: microspheres of 17-beta-estradiol: cholesterol (40:60)

The microspheres of the mixture were obtained by the spray/congealing method. The stabilization by the crystallization method with solvent atmospheres was possible with the use of 96% by weight ethanol at a temperature of 30°C for 24 h. To remove the solvent, the microspheres were stored in an oven at 60°C. To study the physical stability, the microspheres are stored in water at 40°C and observed under a microscope, after 82 days, the crystallized microspheres retain their spherical shape while those that were not crystallized are deformed by crystals on the surface.

3.7.1 Stability in vivo

In the case of slow release injected or implanted medicinal drugs, the physical integrity of the particles after their administration to the patient is essential to assure the desired rates of delivery and the reproducibility of effect. Thus, the stability in vivo of the microspheres described in the previous example was checked in New Zealand male rabbits.

Microscopy photographs was taken 1, 4, 7 and 14, 10 days after intramuscular injection showed that the microspheres remain whole, until they have finally dissolved. For comparison, microspheres that had not been crystallized were also injected. Their microscopy photographs showed that these microspheres changed into nonspherical shapes.

3.8 Example 8: microspheres of a mixture of 10% 17-beta-estradiol and 90% cholesterol

As for the previous example, the microspheres of this mixture were obtained by melting together the components, sprayed into droplets and congealed into microspheres. Initially, they showed a high amorphous content.

When the microspheres were placed in a recipient of approximately 7.0 L and exposed for 24 h at 0°C, to the vapors of 8 mL of ethanol kept in a porous cellulose material, the initially amorphous microspheres crystallized completely in the presence of the vapors.

The microspheres were later dried at 60°C in a vacuum for 24 h and the residual ethanol present in the microspheres was less than 0.01%.

To evaluate stability of the crystallized microspheres, they were placed in aqueous solution at 40°C and observed by optical microscopy after 141 days.

3.9 Example 9: microspheres of a mixture of 95.2% progesterone and 4.8% 17-beta-estradiol

As for the previous examples, the microspheres of this mixture were obtained by melting together the components, sprayed into droplets, and congealed into microspheres. Initially, they showed a high amorphous content.

When the microspheres were placed in a recipient of approximately 7 L and exposed for 24 h at 20–25°C to the vapors of 2 mL of ethanol kept in a porous cellulose material, the initially amorphous microspheres crystallized completely in the presence of the vapors.

The microspheres were later dried at 60°C in a vacuum for 24 h, and the residual ethanol present in the microspheres was less than 0.01%.

3.10 Example 10: microspheres of a mixture of 60% progesterone and 40% cholesterol

As for the previous examples, the microspheres of this mixture were obtained by melting together the components, sprayed into droplets and congealed into microspheres. They initially showed a high amorphous content.

When the microspheres were placed in a recipient of approximately 7 L and exposed for 24 h at 30°C to the vapors of 2 mL of ethanol kept in a porous cellulose material, the initially amorphous microspheres crystallized completely in the presence of the vapors.

The microspheres were later dried at 60°C in a vacuum for 24 h and the residual ethanol present in the microspheres was less than 0.01%.

4. Conclusion

This technology is widely applicable in forming stable, crystallized particles, microspheres, and pellets of a variety of organic compounds and mixtures that

maintain their shape in aqueous solution. Hence, the present method should find significant utility in the manufacture of pharmaceuticals and pharmaceutical compositions, particularly where treatment calls for administration of the pharmaceutical in a slow release formulation. This can be translated into low doses, more secure treatment alternatives, with less frequent side effects, and as an alternative route of administration.

Author details

Salvador Espino y Sosa[1*], Myriam Cortés Fuentes[2], Jacobo Alejandro Gómez Rico[2] and Manuel Cortés Bonilla[3]

1 Clinical Research Branch, Instituto Nacional de Perinatología, Mexico City, Mexico

2 Centro A.F. de Estudios Tecnológicos (CAFET), Mexico City, Mexico

3 Department of Information Management, Instituto Nacional de Perinatología, Mexico City, Mexico

*Address all correspondence to: manuel.cortes@inper.gob.mx

References

[1] Newburger J, Goldzieher JW. Pharmacokinetics of ethynyl estradiol: A current view. Contraception. 1985;**32**(1):33-44

[2] New guidelines for the treatment of menopausal symptoms, and the prevention of osteoporosis in women with menopausal symptoms, have been released. Inpharma Weekly. 2004;**1460**:4. https://doi.org/10.2165/00128413-200414600-00006

[3] Stevenson JC. Type and route of estrogen administration. Climacteric. 2009;**12**(sup1):86-90

[4] Cortés-Bonilla M, Bernardo-Escudero R, Alonso-Campero R, Francisco-Doce MT, Hernández-Valencia M, Celis-González C, et al. Treatment of menopausal symptoms with three low-dose continuous sequential 17β-estradiol/progesterone parenteral monthly formulations using novel non-polymeric microsphere technology. Gynecological Endocrinology. 2015;**31**(7):552-559

[5] Li CI, Malone KE, Porter PL, Weiss NS, Tang M-TC, Cushing-Haugen KL, et al. Relationship between long durations and different regimens of hormone therapy and risk of breast cancer. Journal of the American Medical Association. 2003;**289**(24):3254-3263

[6] Nelson HD, Vesco KK, Haney E, Fu R, Nedrow A, Miller J, et al. Nonhormonal therapies for menopausal hot flashes: Systematic review and meta-analysis. Journal of the American Medical Association. 2006;**295**(17):2057-2071

[7] Nahas EAP, Nahas-Neto J, Orsatti FL, Carvalho EP, Oliveira MLCS, Dias R. Efficacy and safety of a soy isoflavone extract in postmenopausal women: A randomized, double-blind, and placebo-controlled study. Maturitas. 2007;**58**(3):249-258

[8] Basaria S, Wisniewski A, Dupree K, Bruno T, Song M-Y, Yao F, et al. Effect of high-dose isoflavones on cognition, quality of life, androgens, and lipoprotein in post-menopausal women. Journal of Endocrinological Investigation. 2009;**32**(2):150-155

[9] The NAMS. 2017 hormone therapy position statement advisory panel. The 2017 hormone therapy position statement of The North American Menopause Society. Menopause. 2017;**24**(7):728-753

[10] Cortés-Bonilla M, Alonso-Campero R, Bernardo-Escudero R, Francisco-Doce MT, Chavarín-González J, Pérez-Cuevas R, et al. Improvement of quality of life and menopausal symptoms in climacteric women treated with low-dose monthly parenteral formulations of non-polymeric microspheres of 17β-estradiol/progesterone. Gynecological Endocrinology. 2016;**32**(10):831-834

[11] Capes JS, Cameron RE. Contact line crystallization to obtain metastable polymorphs. Crystal Growth & Design. 2007;**7**(1):108-112

[12] Yu L. Inferring thermodynamic stability relationship of polymorphs from melting data. Journal of Pharmaceutical Sciences. 1995;**84**(8):966-974

[13] Haleblian JK, Koda RT, Biles JA. Isolation and characterization of some solid phases of fluprednisolone. Journal of Pharmaceutical Sciences. 1971;**60**(10):1485-1488

[14] Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y, Furuyama S. Characterization of polymorphs

of tranilast anhydrate and tranilast monohydrate when crystallized by two solvent change spherical crystallization techniques. Journal of Pharmaceutical Sciences. 1991;**80**(5):472-478

[15] Matsuda Y, Kawaguchi S, Kobayashi H, Nishijo J. Physicochemical characterization of spray-dried phenylbutazone polymorphs. Journal of Pharmaceutical Sciences. 1984;**73**(2):173-179

[16] Carstensen JT. Stability of solids and solid dosage forms. Journal of Pharmaceutical Sciences. 1974;**63**(1):1-14

[17] Tang F, Zhu C, Gan F. Effect of solvent vapor on optical properties of Pr4VOPc in polymethylmethacrylate. Journal of Applied Physics. 1995;**78**(10):5884-5887

[18] Rayne S. Comment on group contribution-based method for determination of solubility parameter of nonelectrolyte organic compounds and solubility parameters of nonelectrolyte organic compounds: Determination using quantitative structure-property relationship strategy. Industrial and Engineering Chemistry Research. 2013;**52**(10):3947-3948

[19] Hancock BC, Zografi G. Characteristics and significance of the amorphous state in pharmaceutical systems. Journal of Pharmaceutical Sciences. 1997;**86**(1):1-12