

PARENTERAL PHARMACEUTICAL FORMULATION IN SUSPENSION, HAVING
SUSTAINED RELEASE, IN LOW AND ULTRALOW DOSAGE, IN HORMONAL
THERAPY IN THE CLIMACTERIC SYNDROME

FIELD OF THE INVENTION

5 A parenteral pharmaceutical formulation in suspension,
of sustained release, in low and ultra-low dose, for
therapeutic use of hormonal therapy during climacteric
syndrome.

Prior Art

10 Climacteric syndrome or menopause is a physiological
event that marks the end of reproductive life in women, it
occurs universally to all women that reach their middle age.
Globally (men and women), this represented in 1990, 10% of
the planet's population (5-8% in underdeveloped countries and
15 near to 15% in industrialized countries. Hill, K. 1996. The
demography of menopause. Maturitas. Vol. 23, No.2: 113-127).
Between 1990 and 2000, some 24.5 million women went through
menopause each year, in China only; they were near to 5
million per year. In the year 2020 this amount will be
20 doubled, with half of those women in Asia. The median of the
age at which menopause is reached in Europe and the United
States is 50 years (Ginsberg, J. 1991. What determines the
age at the menopause? Br. Med. J. Vol. 302, No. 6788:1288-

1289), while in India and the Philippines it is reached at the median age of 44 years (Goodman, M.J., et al. 1985. Menarche, pregnancy, birth spacing and menopause among the Agta women foragers of Cagayan province, Luzon, the Philippines. Ann. Hum. Biol. Vol.12, No. 2: 169-177; Sharma, V.K., & Saxena, M. S. 1987. Climacteric symptoms: A study in the Indian context. Maturitas. Vol. 3, No. 1:11-20), in the United Arab Emirates is 48 years (Rizk, D.E., et al. 1998. The age and symptomatology of natural menopause among United Arab Emirates women. Maturitas Vol. 29, No.3: 197-202), the same as Turkey, Israel, and most part of Asia, Mexico, Nigeria and Ghana (Khwak, K.T. 1992, Epidemiology of the menopause, Br. Med. Bull. Vol. 48: 249-261). Menopause is defined as the permanent cessation of menstruation due to the loss of follicular ovarian function, there are many symptoms associated to it, of variable frequency and severity, among them, vasomotor symptoms, hot flashes, night perspiration, vaginal dryness, bad humor, irritability, anxiety, depression, insomnia, headaches, urinary disorders, pain in articulations and fatigue (Ringa, V. 2000. Menopause and treatments. Quality of life research. Vol. 9: 695-707). Menopause corresponds to the last menstrual period that occurs due to the loss of ovarian activity and is identified

when 12 months of amenorrhea have elapsed (Malacara, J.M. 2003. Menopausia: Nuevas evidencias, nuevos enigmas. Rev. Endocrinol y Nutr. Abr.-Jun. Vol. 11, No.2: 61-72). It only occurs in the human species, although in some mammals such as lions or baboons, ovarian function decreases (Packer, C., et al. 1998. Reproductive cessation in female animals. Nature. Vol. 392: 807-811).

In the treatment of the symptomatology and discomfort associated with climacteric, hormonal replacement has been used, nevertheless, such treatments have been correlated with the appearing of some forms of cancer (Zárate A., & Hernández Valencia, M. 2002. Terapia de reemplazo hormonal en mujeres menopáusicas tratadas por cancer mamario. Rev Med. IMSS Vol. 40, No. 5: 369-371; Cóppola, Francisco, et al. 2004. La terapia hormonal en la post-menopausia y las promesas incumplidas. Rev. Méd. Uruguay. Vol. 20, No. 2: 130-135; Schairer, C., et al. 2000, Menopausal estrogen ad estrogen-progestin replacement therapy and breast cancer risk. JAMA. Vol. 283, No.4: 485-491), while other therapies have demonstrated to be much less effective. The aim of an effective compound or formulation for the intended purposes is to achieve estradiol balance (a molecule that has an effective action in the uterus, making it to continue with

its function, however, it causes an increase in the risk of suffering uterine cancer), with a uterus protector such as progesterone (nevertheless, it seems to increase the possibility of suffering from breast cancer) on this account,
5 we thought of using low and ultra-low doses in replacement therapy.

Low and ultra-low dose estrogen. In the current medical literature , this expression covers any product made from estrogen, natural or synthetic, containing half or less the
10 amount that is usually used for replacement hormone therapy either oral or transdermal during climacteric or post-menopause, while ultra-low dose is equivalent to the fourth part of the standard dose, that is, 1.0mg of estradiol and 20.0mg of progesterone for a low dose and, between 0.25 to
15 0.50 mg of estradiol and 15.0 mg of progesterone for an ultra-low dose (Velasco-Murillo V. 2006. Estrógenos a dosis bajas y estrógenos de síntesis. ¿Opciones para el remplazo hormonal en el climaterio? Rev Med Inst Mex Seguro Soc 2007; Vol. 45, No. 4: 381-387. Carranza Lira, S. 2008. Dosis baja
20 de terapia hormonal durante el climaterio. Ginecol. Obstet. Mex. Vol. 76, No. 5: 267-274). Hormonal doses of 1mg of estradiol and 20mg of progesterone, or 0.5mg of estradiol and
15 mg of progesterone for intramuscular monthly

administration, proposed in this invention, comply with the criteria for low doses for use in hormonal replacement therapy during climacteric, for both types of hormones. The benefits from the treatment with estrogen at low doses are appreciated with the results in table 1 below:

Table 1. Effectiveness and side effects of estrogen at standard and low doses.

	Standard Dose	Low Dose
Effectiveness		
Suppression of vasomotor disorders	80 to 90%	60 to 70%
Time for maximum effectiveness	4 weeks	8 to 12 weeks
Remission of vaginal atrophy	Favorable effect	Favorable effect
Increase in bone mineral density	Favorable effect	Favorable effect
Side effects		
Incidence of Irregular bleeding	23%	12%
Incidence of Spotting or licking	44%	22%
Incidence of endometrial hyperplasia with single estrogen	27.3%	3.2%

There are several options to treat and cure this problem, for instance, US patent 3 733 407 discloses a method for a hormonal replacement method to relieve or prevent the symptoms of menopause, with the disadvantage that the daily
5 dose causes the patient to leave treatment.

US patent 4 425 339 discloses a treatment method to relieve menopause symptoms administering estrogen and progestogen in a four-phase sequence, for a period of 100 days, with the disadvantage of favoring treatment
10 abandonment.

US patent 4 900 734 discloses pharmaceutical compounds containing estradiol and progesterone for their oral administration, estradiol is contained in a dosing solution containing a micronized progesterone suspension administered
15 in one capsule.

US patent 4 945 103 discloses a treatment method for women with premenstrual syndrome, through the administration of melatonin and progestogen, with the disadvantage that it has to be administered on a daily basis which favors
20 treatment abandonment.

US patent 5 514 382 discloses a mineral and vitamin supplement of daily intake containing vitamin a-, β -carotene, niacin, ribloflavin, pantothenic acid, pyridoxine, biotin,

aminobenzoic acid, inositol, iodine, iron, magnesium, manganese, molybdenum, selenium, zinc and bioflavonoids to relieve the symptoms of menopause, it has to be administered everyday with the disadvantage of favoring treatment
5 abandonment.

The object of this invention is to provide an effective pharmaceutical compound or formulation, for the therapeutic use of hormonal therapy in women with uterus to mitigate the symptomatology and discomfort associated with the climacteric
10 syndrome.

Another object of this invention is to provide an effective pharmaceutical formulation, for the hormonal therapeutic use in women with uterus, during the treatment of the symptomatology and to mitigate discomfort associated with
15 clicmateric syndrome.

Another object of this invention is to provide an effective pharmaceutical formulation, for hormonal therapy in women with uterus, for climacteric syndrome, with a dosage form that allows the compliance of the treatment, for
20 instance, monthly in the case of transdermal application.

Another object of this invention is to provide an effective pharmaceutical formulation for hormonal therapy in women with uterus, in the treatment of the symptomatology and

discomfort associated with climacteric syndrome, in a low dose in order to minimize the risk of appearance of side effects, such as breast cancer.

Another object of the invention is to provide an effective pharmaceutical formulation for hormonal therapy in women with uterus to treat the symptomatology and discomfort associated with climacteric syndrome, in ultra-low doses in order to minimize the risk of undesirable effects such as breast cancer.

An additional object of this invention is to provide a pharmaceutical compound or formulation that can be applied parenterally, that is to say, in an intramuscular or subcutaneous manner.

Summary

In one aspect, there is provided a parenteral pharmaceutical formulation in suspension, of sustained release, containing suspended estradiol and progesterone particles for hormonal replacement in female mammals in low and ultra-low dose, in the form of an injectable suspension formed by estradiol particles, progesterone particles, a surfactant agent, an isosmotic agent, a viscous increasing agent and one or more preservatives.

Brief description of the Drawings

Figure 1 is a graphic of an evaluation of hot flashes per month in a period of six months.

Figure 2 is a relative graphic of the evolution of
5 hot flashes during the 6 months of the analysis (severe hot flashes).

Figure 3 is a graphic related to the evolution of hot flashes during the 6 months of the analysis (moderate hot flashes).

DETAILED DESCRIPTION OF THE INVENTION

The present invention consists of a parenteral pharmaceutical compound or formulation that can be administered intramuscularly in a suspension, of sustained
5 release, of suspended estradiol particles and progesterone for hormonal replacement in female mammals, in low and ultra-low doses. The formulation contains estradiol particles at an interval of 5 to 100 micrometers, progesterone particles at 5 to 100 micrometers, a surfactant agent, an isosmotic agent, a
10 viscosity increasing agent and one or more preservatives.

Any surfactant agent capable of modifying the surface tension is selected in order to moisturize the hydrophobic particles of estradiol and progesterone.

The isosmotic agent is chosen only if it has the
15 capacity of providing the same isotonicity as cells.

A viscosity increasing agent is chosen only if it is capable of modifying the viscosity of the vehicle for the particles to be able to suspend and re-suspend.

A preservative is chosen only if is capable of
20 inhibit microbial growth.

The agent to adjust pH is chosen only if it equilibrates the pH of the compound in physiological conditions.

The pharmaceutical compound can be injected and it
5 is released in a sustained manner for a month in virtue of which the dose might be monthly.

The compound is achieved through the use of micro-particles such as those prepared in accordance with US patent 2005/0025827, however, in this case this is a monthly
10 therapy, through an injectable compound in which the size of the estradiol and stable progesterone is from 5 to 100 micrometers which is attained through.

Below, in an expository manner, but not limited to, we give a series of examples focused in preparing the
15 product, the form of obtaining of the estradiol and progesterone particles is provided for the formulation as well as the obtaining of the injectable.

Examples to prepare estradiol and progesterone particles.

Obtaining of estradiol particles.

20 Estradiol is melted and atomized by centrifugation in an atomizer by freezing at a temperature between 165 to 190°C. In the same equipment, formed micro drops are frozen at a temperature between -20 to -5°C.

The obtained solid particles are crystallized in accordance with the proceeding described in patent application US 2005/0025827, achieving stable forms from this
5 active principle.

Afterwards, the obtained solid particles are classified in accordance with their size through an ultrasonic sieve, considering as adequate, those that have size between 1 and 100 micrometers.

10 The particles obtained from the previous step, are sterilized with ethylene oxide.

Obtaining of progesterone particles.

Progesterone is melted and atomized by centrifugation, in an atomizer by freezing, at a temperature between 130 and
15 170°C. In the same equipment, the formed micro drops are frozen at a temperature between 60 and -20°C.

The obtained solid particles are classified in accordance with their size through an ultrasonic sieve, considering as adequate, those that have size between 1 and
20 100 micrometers.

Afterwards, the particles of interest are crystallized in an oven, at a temperature between 50 and 105°C, achieving stable forms of this active principle.

The particles obtained from the previous step, are
5 sterilized with ethylene oxide.

In order to achieve the final product, these stable estradiol and progesterone particles are mixed with surfactant agent, an isosmotic agent and a preservative, in a preferred modality, as illustrated in the following examples.

10 Used components might be:

Surfactant Agent	Polysorbate 20, polysorbate 80, Dioctyl sodium sulfosuccinate, polyoxyethylene resin oil.
Isosmotic Agent	Sodium chloride, lactose, trehalose, mannitol, glycerin, sucrose
Viscosity Increasing agent	Sodium Carboxymethyl Cellulose, polyethylene glycol 300, polyethylene glycol 400, polyethylene glycol 3350
Preservative	Methylparaben, Propylparaben, Phenol, Thiomersal, m-Cresol, chlorobutanol, benzalkonium chloride, Benzyl alcohol, 2-Phenoxyethanol.
pH adjusting agent	Hydrochloric Acid, Phosphoric Acid, Sodium Hydroxide, Sulfuric Acid

Formulation Examples Including Estradiol and Progesterone

Example 1.

Syringe with powder:

0.25 mg of sterile estradiol particles and 1.50 mg of sterile progesterone particles obtained in accordance with the previous proceeding, mixed aseptically in a V mixer, upon obtaining of a homogeneous mixture. The obtained product is aseptically deposited in syringes, using a filling - stopper machine for syringes.

Aqueous Vehicle

10 Is made from the following components:

Component	Unitary Formula
Methylparaben	0.69 mg
Propylparaben	0.08 mg
Mannitol	24.00 mg
Sodium Carboxymethyl Cellulose	0.38 mg
Polysorbate 80	0.10 mg
Water for injectables	0.50 ml
Hydrochloric Acid 1.0 N	To adjust pH, if needed

All of the raw materials are of pharmaceutical degree.

Parabens are dissolved in water for injectables, hot and in the same solution Mannitol and Sodium Carboxymethyl Cellulose are dissolved.

15 Separately, polysorbate is dissolved in water for injectables and both solutions are incorporated.

The pH of the solution is adjusted to a value between 5.0 and 6.5. This solution is sterilized by filtration and is filled aseptically with a vial in a machine for this purpose.

The product, a dispersion powder, is composed of the
5 syringe with the powder and the vial with the vehicle. These components are incorporated into a sole product at the moment of its administration.

The obtaining of a stable form is achieved, which means an injectable compound, intramuscular or subcutaneous, of
10 sole dose that maintains its physical, physical-chemical and microbiological characteristics for at least two years, in such a manner that therapeutic effects are not affected.

Example 2.

Syringe with powder: 0.50mg of sterile estradiol particles
15 and 15.0mg of sterile progesterone particles obtained in accordance with the previous proceeding, they are mixed aseptically in a V mixer, upon obtaining of a homogeneous mixture. The obtained product is aseptically deposited in syringes, using a filling - stopper machine for syringes.

20 Aqueous vehicle.

The following components are used:

Component	Unitary Formula
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Methylparaben	0.69 mg
Propylparaben	0.08 mg
Mannitol	24.00 mg
Sodium Carboxymethyl Cellulose	0.38 mg
Polysorbate 80	0.10 mg
Water for injectables	0.50 ml
Hydrochloric Acid 1.0 N	To adjust pH, if needed

All of the raw materials are of pharmaceutical degree.

Parabens are dissolved in water for injectables, hot and in the same solution Mannitol and Sodium Carboxymethyl Cellulose are dissolved.

- 5 Separately, polysorbate is dissolved in water for injectables and both solutions are incorporated.

The pH of the solution is adjusted to a value between 5.0 and 6.5. This solution is sterilized by filtration and is filled aseptically with a vial in a machine for this purpose.

- 10 The product, a dispersion powder, is composed of the syringe with the powder and the vial with the vehicle. These components are incorporated into a sole product at the moment of its administration.

The obtaining of a stable form is achieved, which means an
15 injectable compound, intramuscular or subcutaneous, of sole dose that maintains its physical, physical-chemical and microbiological characteristics for at least two years, in such a manner that therapeutic effects are not affected.

Example 3.

Syringe with powder:

1.00mg of sterile estradiol particles and 20.0mg of
5 sterile progesterone particles obtained in accordance with
the previous proceeding, they are mixed aseptically in a V
mixer, upon obtaining of a homogeneous mixture. The obtained
product is aseptically deposited in syringes, using a filling
- stopper machine for syringes.

10 Aqueous vehicle.

The following components are used:

Component	Unitary Formula
Methylparaben	1.37 mg
Propylparaben	0.15 mg
Mannitol	48.00 mg
Sodium Carboxymethyl Cellulose	0.75 mg
Polysorbate 80	0.20 mg
Water for injectables	1.0 ml
Hydrochloric Acid 1.0 N	To adjust pH, if needed

All of the raw materials are of pharmaceutical degree.

Parabens are dissolved in water for injectables, hot and
in the same solution Mannitol and Sodium Carboxymethyl
15 Cellulose are dissolved.

Separately, polysorbate is dissolved in water for injectables and both solutions are incorporated.

The pH of the solution is adjusted to a value between 5.0 and 6.5. This solution is sterilized by filtration and is
5 filled aseptically with a vial in a machine for this purpose.

The product, a dispersion powder, is composed of the syringe with the powder and the vial with the vehicle. These components are incorporated into a sole product at the moment of its administration.

10 The obtaining of a stable form is achieved, which means an injectable compound, intramuscular or subcutaneous, of sole dose that maintains its physical, physical-chemical and microbiological characteristics for at least two years, in such a manner that therapeutic effects are not affected.

15 Example 4.

Syringe with powder:

0.25mg of sterile estradiol particles and 15.0mg of sterile progesterone particles obtained in accordance with the previous proceeding, they are mixed aseptically in a V
20 mixer, upon obtaining of a homogeneous mixture. The obtained product is aseptically deposited in syringes, using a filling - stopper machine for syringes.

Aqueous vehicle.

The following components are used:

Component	Unitary Formula
Polyethylene glycol 400	200 mg
Polysorbate 80	2.41 mg
Mannitol	16.0 mg
Water for injectables	1.0 ml
Hydrochloric Acid 1.0 N	To adjust pH, if needed

All of the raw materials are of pharmaceutical degree.

Polyethylene Glycol, mannitol and polysorbate are
5 dissolved in water for injectables.

The pH of the solution is adjusted to a value between 5.0 and 6.5. This solution is sterilized by filtration and is filled aseptically with a vial in a machine for this purpose.

The product, a dispersion powder, is composed of the
10 syringe with the powder and the vial with the vehicle. These components are incorporated into a sole product at the moment of its administration.

The obtaining of a stable form is achieved, which means an injectable compound, intramuscular or subcutaneous, of sole
15 dose that maintains its physical, physical-chemical and microbiological characteristics for at least two years, in such a manner that therapeutic effects are not affected.

Example 5.

Syringe with powder:

1.00mg of sterile estradiol particles and 20.0mg of sterile progesterone particles obtained in accordance with the previous proceeding, they are mixed aseptically in a V mixer, upon obtaining of a homogeneous mixture. The obtained product is aseptically deposited in syringes, using a filling - stopper machine for syringes.

Aqueous vehicle.

The following components are used:

Component	Unitary Formula
Polyethylene glycol 3350	28.9 mg
Polysorbate 80	2.41 mg
Mannitol	8.68 mg
Water for injectables	1.0 ml
Hydrochloric Acid 1.0 N	To adjust pH, if needed

10 All of the raw materials are of pharmaceutical degree.

Polyethylene Glycol, mannitol and polysorbate are dissolved in water for injectables.

The pH of the solution is adjusted to a value between 5.0 and 6.5. This solution is sterilized by filtration and is filled aseptically with a vial in a machine for this purpose.

The product, a dispersion powder, is composed of the syringe with the powder and the vial with the vehicle. These components are incorporated into a sole product at the moment of its administration.

The obtaining of a stable form is achieved, which means an injectable compound, intramuscular or subcutaneous, of sole dose that maintains its physical, physical-chemical and microbiological characteristics for at least two years, in
5 such a manner that therapeutic effects are not affected.

CLINICAL STUDIES

Effectiveness

An Effectiveness and security clinical study was carried out in 103 peri and post-menopausal women with vasomotor and
10 vulvovaginal symptomatology, administering three different suspension doses of Estradiol: Cholesterol/Progesterone Microspheres, administered every 30 ± 3 days, for 6 consecutive months:

0.5 mg of Estradiol/ 15 mg of Progesterone

15 1.0 mg of Estradiol/ 20 mg of Progesterone

1.0 mg of Estradiol/ 30 mg of Progesterone

Effectiveness and security results are presented below.

Effect on vasomotor symptoms.

The Effectiveness of the three dose levels of Estradiol
20 (E2)/Progesterone (P4): 0.5mg of E2/15 mg of P4, 1 mg of E2/20 mg of P4, 1 mg of E2/30 mg of P4, administered every 30 ± 3 days, in the relieving of vasomotor symptoms in frequency and intensity (moderate and severe) in peri and post-

menopausal women between 40 and 65 years old, was evaluated in a 6 month study (n= 103). The three evaluated dose levels were not presented among them, statistically significant differences in the decrease in the. number of hot flashes at the third and sixth administration *, ** ($p > 0.05$).

Table 2. Number, average and percentage in the decrease of total hot flashes per treatment and administered dose.

Type of Treatment	Analysis	Evaluations (monthly)							p Value
		Basal	1	2	3*	4	5	6**	
A 0.5 mg E ₂ / 15mg P ₄	n	38	38	38	37	35	34	34	$p < 0.05$
	Average (DE)	165.84 (112.95)	104.68 (72.91)	60.34 (55.78)	49.11 (47.89)	39.97 (54.16)	39.29 (48.22)	27.09 (41.27)	
	Decrease Percentage	0%	37%	64%	70%	76%	76%	84%	-
B 1.0 mg E ₂ / 20mg P ₄	n	29	27	26	25	24	24	24	$p < 0.05$
	Average (DE)	174.76 (221.31)	102.93 (114.72)	50.73 (51.77)	23.36 (23.98)	27.46 (50.20)	23.42 (58.72)	18.54 (40.32)	
	Decrease Percentage	0%	41%	71%	87%	84%	87%	89%	-
C 1.0 mg E ₂ / 30mg P ₄	n	36	33	32	29	26	26	26	$p < 0.05$
	Average (DE)	220.19 (222.52)	124.33 (105.03)	67.28 (70.13)	39.21 (42.17)	32.88 (49.17)	21.00 (34.24)	16.23 (24.04)	
	Decrease Percentage	0%	44%	69%	82%	85%	90%	93%	-

Friedman Test ($p < 0.05$)

* $p = 0.056$ upon comparison of the three groups in dose 3.

10 ** $p = 0.478$ upon comparison of the three groups in dose 6.

The intensity of hot flashes evaluated at month 6 presented a statistically significant decrease ($p < 0.05$) at three dose levels. In the evaluation at 3 months, the

decrease of moderate hot flashes was greater in treatment B (1.0 mg of E2/20 mg of P4) when compared with treatments A and C (0.5mg of E2/15mg of P4 and 1.0mg of E2/ 30mg of P4 respectively) ($p \leq 0.05$).

5 At the end of the proceeding, there was no significant difference between the three treatments with regard to decrease proportions.

Table 3. Change in the intensity of hot flashes in basal, intermediate and final evaluations in each type of
10 treatment.

Severity of Hot Flashes	Treatments											
	A				B				C			
	Basal n=38	Int n=37	Final n=34	p Value	Basal n=29	Int n=25	Final n=24	p Value	Basal n=36	Int n=29	Final n=26	p Value
Slight	73.0	25.9	15.8	$p < 0.05$	78.8	18.7	13.7	$p < 0.05$	108.6	24.2	12.1	$p < 0.05$
Average (DE)	(65.3)	(29.0)	(27.0)		(131.9)	(20.7)	(33.3)		(118.2)	(29.7)	(22.2)	
Moderate	69.5	18.5	10.2	$p < 0.05$	64.6	3.9	4.0	$p < 0.05$	54.4	12.6	4.0	$p < 0.05$
Average (DE)	(110.9)	(24.1)	(26.7)		(72.8)	(6.8)	(9.2)		(78.4)	(22.8)	(9.0)	
Severe	23.3	4.7	1.2	$p < 0.05$	31.4	0.7	0.9	$p < 0.05$	57.3	2.4	0.1	$p < 0.05$
Average (DE)	(50.3)	(12.2)	(4.1)		(62.8)	(3.6)	(3.7)		(107.1)	(8.7)	(0.4)	

Basal (-1 month), intermediate (3rd month) and final (6th month); Friedman Test ($p \leq 0.05$)

Effect on vulvovaginal symptoms

Vulvovaginal symptoms decreased considerably in the
15 three groups ($p < 0.05$).

Dysuria disappeared in the group of 1mg of Estradiol/ 30 of Progesterone (P4)m although it was not statistically relevant in the group of 1mg of Estradiol (E2)/ 20mg of Progesterone, probably because in this group, from the basal stage, the proportion was lower than groups A and C.

Dyspareunia had an important decrease in group B (1.0 mg of E2/20mg of P4) ($p < 0.05$).

The proportion of volunteers with post-coital bleeding was low in relation to the rest of vulvovaginal symptoms in the basal stage and disappeared totally in the three treatment groups.

Table 4. Modifications in vulvovaginal atrophy symptoms.

Symptoms and signs	Treatments											
	(A)				(B)				C			
	0.5mg E ₂ and 15mg P ₄				1.0mg E ₂ and 20mg P ₄				1.0mg E ₂ and 30mg P ₄			
	Basal n=38 %	Int n=37 %	Final n=34 %	p Value	Basal n=29 %	Int n=25 %	Final n=24 %	p Value	Basal n=36 %	Int n=29 %	Final n=26 %	p Value
Vaginal Dryness	50.0	24.3	17.6	$p < 0.05$	58.6	12.0	8.3	$p < 0.05$	36.1	31.0	23.1	$p > 0.05$
Vulvar and vaginal irritation and pruritus	42.1	24.3	14.7	$p < 0.05$	37.9	16.0	4.2	$p < 0.05$	30.6	13.8	15.4	$p < 0.05$
Dysuria	28.9	2.7	2.9	$p < 0.05$	10.3	4.0	0.0	$p < 0.05$	19.4	10.3	0.0	$p < 0.05$
Dyspareunia	18.4	5.4	2.9	$p > 0.05$	17.2	4.0	0.0	$p < 0.05$	8.3	0.0	3.8	$p > 0.05$
Post-coital Bleeding	2.6	2.7	0.0	$p > 0.05$	6.9	0.0	0.0	$p > 0.05$	5.6	0.0	0.0	$p > 0.05$

Basal: -1month, int: 3months, final: 6 months. Cochran's Test
($p \leq 0.05$).

Quality of Life

The evaluation of the quality of life was made through
5 the Utian Scale. Results are presented in Table 5.

Table 5. Evaluation of Quality of Life (Utian Scale)

Domain	Average Referen ce Value	Treatments											
		(A) 0.5mg E ₂ and 15mg P ₄				(B) 1.0mg E ₂ and 20mg P ₄				(C) 1.0mg E ₂ and 30mg P ₄			
		Basal	Int	Final	p	Basal	Int	Final	p	Basal	Int	Final	p
Occupation	25	26.1	30.7	29.7	0.00	28.0	29.5	29.8	0.48	22.6	27.7	28.5	0.00
Mean		(8.0)	(5.5)	(7.0)	1	(8.0)	(6.1)	(5.6)	1	(9.6)	(6.0)	(5.6)	3
(SD)										)	)	)	
Health	21	21.3	22.9	23.1	0.67	22.0	23.3	22.7	0.47	21.8	22.0	21.6	0.67
Mean		(5.4)	(4.4)	(4.0)	1	(5.1)	(4.8)	(4.2)	1	(6.1)	(4.3)	(3.1)	4
(SD)										)	)	)	
Emotional	20	18.2	17.6	17.0	0.03	19.3	18.6	17.1	0.00	20.2	18.2	18.2	0.10
Mean		(4.7)	(3.7)	(3.3)	1	(4.3)	(3.7)	(4.1)	4	(5.2)	(4.2)	(4.3)	4
(SD)										)	)	)	
Sexual	8	9.6	10.7	10.4	0.43	9.4	10.2	10.5	0.33	9.1	10.5	10.4	0.19
Mean		(3.5)	(2.4)	(2.3)	4	(3.6)	(3.1)	(2.7)	0	(2.9)	(2.1)	(2.6)	8
(SD)										)	)	)	
Total Mean	74	75.0	81.8	80.0	0.17	78.6	81.6	80.1	0.86	73.7	78.3	78.6	0.07
(SD)		(16.8)	(10.3)	(11.9)	5	(12.2)	(10.5)	(11.1)	7	(17.7)	(9.9)	(10.1)	0

Basal (-1 month), intermediate (3rd month) and final (6th month); Friedman Test ($p \leq 0.05$)

The Quality of Life (Utian Scale) of the participants showed a significant increase ($p < 0.05$) in the occupational (treatments A and C) and emotional (treatments A and B) domains, health and sexual domains did not show a significant improvement ($p > 0.05$).

Security

In a monitoring study at 6 months carried out in 103 peri and post-menopausal women (with intact uterus) which were treated every 30 ± 3 days with an IM injection of an aqueous suspension of microspheres of Estradiol/Progesterone, endometrial security was evaluated using the following parameters: endometrial thickness identified through endovaginal ultrasound and endometrial biopsy.

Result of ultrasound studies.

Basal endometrial thickness in the peri-menopausal group was 5.4mm and the final value 4.9mm. In the post-menopausal group, basal endometrial thickness was 3.4mm and the final value 3.0mm.

Endometrial Biopsy Result

A total of 55 endometrial biopsies were carried out, in which, after 6 months of continuous treatment, no histological report of endometrial hyperplasia was found.

Table 6 presents the results of this evaluation.

Table 6. Incidence of endometrial hyperplasia after 6 months of treatment. ITT Population.

Characteristics	(A) 0.5 mg E2/15 mg P4 n=38	(B) 1.0mg E2/20 mg P4 n=29	(C) 1.0mg E2/30 mg P4 n=36
No. of volunteers with evaluable biopsies at 6 months	22	15	18
No. (%) of volunteers with hyperplasia at 6 months.	0.0	0.0	0.0

Effect on uterine bleeding or spotting pattern.

5 The effects of the injectable suspension of microspheres of Estradiol/Progesterone on the uterine bleeding or spotting, were registered for 6 consecutive months in the patient's journal and reported in the programmed visits. Results are shown in Tables 7 and 8.

10 In 58 peri-menopausal women, the duration of the spotting/bleeding days of their basal pattern, was maintain without changes throughout the six treatment cycles with the three doses.

15 Table 7. Average spotting/bleeding days in each evaluation per type of treatment in peri-menopausal women.

Variables	Analysis	Evaluation (month)						
		Basal and beginning of treatment	1 st	2 nd	3 rd	4 th	5 th	6 th
Treatment A n=22								
Spotting	n	9	11	11	10	10	9	8
	average days	3.0	3.2	5.1	3.3	2.5	2.7	2.1
	(min-max)	(1.0-6.0)	(2.0-5.0)	(2.0-18.0)	(1.0-9.0)	(1.0-4.0)	(1.0-4.0)	(1.0-4.0)
Bleeding	n	8	11	12	12	10	6	9
	average days	3.4	4.1	3.9	4.0	3.8	3.2	4.0
	(min-max)	(1.0-5.0)	(1.0-8.0)	(1.0-10.0)	(2.0-7.0)	(3.0-7.0)	(1.0-5.0)	(3.0-7.0)
Treatment B n=15								
Spotting	n	8	11	11	7	11	9	9
	average days	3.6	4.4	3.4	4.3	3.1	3.1	2.6
	(min-max)	(2.0-8.0)	(2.0-13.0)	(2.0-5.0)	(2.0-12.0)	(2.0-6.0)	(2.0-5.0)	(1.0-4.0)
Bleeding	n	8	11	9	8	10	9	11
	average days	3.0	3.5	2.9	3.3	2.9	3.0	3.8
	(min-max)	(1.0-5.0)	(1.0-7.0)	(1.0-5.0)	(2.0-4.0)	(1.0-4.0)	(1.0-7.0)	(1.0-9.0)
Treatment C n=21								
Spotting	n	7	10	11	9	9	10	10
	average days	4.9	4.5	4.2	5.6	5.2	5.3	4.0
	(min-max)	(3.0-6.0)	(1.0-14.0)	(1.0-9.0)	(1.0-14.0)	(1.0-9.0)	(1.0-21.0)	(1.0-11.0)
Bleeding	n	7	8	8	7	10	7	8
	average	3.4	3.8	3.9	3.9	3.7	2.6	2.9

	days							
	(min-max)	(1.0-5.0)	(2.0-7.0)	(3.0-7.0)	(2.0-7.0)	(1.0-6.0)	(1.0-4.0)	(1.0-7.0)

Of the 45 post-menopausal women, the group which presented the biggest proportion of spotting/bleeding episodes were those that received the treatment of 1 mg of Estradiol/30mg of Progesterone (6 out of every 15 women) with 5 duration of 15 days.

Table 8. Average spotting/bleeding days in each evaluation per type of treatment in post-menopausal women.

Variables	Analysis	Evaluation (month)						
		Basal and beginning of treatment	1 st	2 nd	3 rd	4 th	5 th	6 th
Treatment A n=16								
Spotting	n	1	1	-	1	-	-	1
	average days	2.0	1.0	-	2.0	-	-	3.0
	(min-max)	(2.0)	(1.0)	-	(2.0)	-	-	(3.0)
Bleeding	n	-	-	-	1	-	-	1
	average days	-	-	-	4.0	-	-	1.0
	(min-max)	-	-	-	(4.0)	-	-	(1.0)
Treatment B n=14								
Spotting	n	1	-	1	3	2	3	1
	average days	5.0	-	3.0	2.3	3.5	2.3	5.0
	(min-max)	(5.0)	-	(3.0)	(1.0-)	(1.0-)	(1.0-)	(5.0)

					5.0)	6.0)	4.0)	
Bleeding	n	-	-	1	1	-	1	-
	average	-	-	4.0	1.0	-	2.0	-
	days							
	(min-max)	-	-	(4.0)	(1.0)	-	(2.0)	-
Treatment C n=15								
Spotting	n	-	2	3	6	4	5	6
	average	-	2.5	2.0	4.8	3.0	3.6	3.2
	days							
	(min-max)	-	(2.0- 3.0)	(1.0- 3.0)	(3.0- 6.0)	(2.0- 4.0)	(2.0- 5.0)	(1.0- 9.0)
Bleeding	n	-	-	2	2	2	2	6
	average	-	-	3.5	1.0	3.0	3.0	2.2
	days							
	(min-max)	-	-	(2.0- 5.0)	(1.0)	(2.0- 4.0)	(3.0)	(1.0- 4.0)

Local and Systemic Security

The three treatments showed successful local and systemic tolerability after the administration of 545 monthly injections. Adverse events observed with very common frequency ($\geq 10\%$) and common ($\geq 10\%$, $<10\%$) are presented in Table 9.

Local tolerability, the pain in the injection site was the event classified as very common and inflammation in the injection site was observed as a common event in the three evaluated treatments.

In relation to the adverse systemic events, headache followed by mastalgia were the events observed as very

common; irritability, sickness, anxiety and blurry vision were observed as common events throughout the study.

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Table 9 Side effects with very common and common classification

Affected System	Events	Very Common (> 10%)			Common (> 1% to <10%)		
		A n=38	B n=29	C n=36	A n=38	B n=29	C n=36
General	Cramps	--	--	--	✓	--	--
	Headache	✓	✓	✓	--	--	--
Cardiovascular	Palpitations	--	--	--	--	✓	--
	Peripheral vascular insufficiency	--	--	--	--	--	✓
	Tachycardia	--	--	--	--	✓	--
Digestive	Hypogastric Colic	--	--	--	✓	--	--
	Anorexia	--	--	--	--	✓	--
	Increased appetite	--	--	--	--	✓	--
	Nausea	--	--	--	--	--	✓
Nutritional Metabolic	Dyslipidemia	--	--	--	✓	--	✓
	Hyperlipidemia	--	--	--	--	--	✓
Skeletal-Muscle	Arthralgia	--	--	--	✓	--	✓
	Myalgia	--	--	--	✓	--	✓
	Bone pain	--	--	--	--	--	✓
Nervous	Depression	--	--	--	✓	--	--
	Aggressiveness	--	--	--	--	--	✓
	Anguish	--	--	--	--	--	✓
	Anxiety	--	--	--	--	--	✓
	Nocturnal Phobia	--	--	--	--	--	✓
	Insomnia	--	--	--	✓	--	✓
	Irritability	--	--	--	✓	✓	✓
	Sickness	--	--	--	✓	✓	✓
	Nervousness	--	--	--	✓	✓	✓

Dermatological (in the site of application)	Burning	--	--	--	--	✓	✓
	Increase	--	--	--	✓	✓	✓
	Pain	✓	✓	✓	--	--	--
	Ecchymosis	--	--	--	--	--	✓
	Induration	--	--	--	--	--	✓
	Edema	--	--	--	✓	--	--
	Erythema	--	--	--	--	✓	--
Sense Organs	Blurry Vision	--	--	--	✓	✓	✓
	Visual Alterations	--	--	--	--	--	✓
Genitourinary	Increase of endometrial thickness	--	--	--	✓	✓	--
	Increase of menstrual bleeding	--	--	--	--	--	✓
	Menstrual Colic	--	--	--	--	✓	--
	Fibrocystic changes of breasts	--	--	--	✓	--	--
	Cystic decay	--	--	--	--	✓	--
	Pelvic Inflammation	--	✓	--	✓	--	✓
	Mastalgia	✓	--	✓	--	✓	--
	Water retention	--	--	--	✓	✓	--

EFFECTIVENESS AND SECURITY SUMMARY

Effectiveness

Results show that the three treatments were effective to reduce climacteric symptoms in an approximate period of 1 month, said reduction was maintained with a descending tendency until the end of the study.

The reduction of vasomotor symptoms at the end of the sixth month of treatment was:

84% with the dose of 0.5 mg of E2/15 mg of P4

10 89% with the dose of 1mg of E2/20 mg of P4

93% with the dose of 1mg of E2/30 mg of P4

The treatment with 1 mg of E2/20 mg of P4 maintained a better Effectiveness and security equilibrium in comparison with the other two treatments.

15 Vulvovaginal symptoms showed tendency to decrease with the 3 treatments, with significant statistical difference in the treatment with 1mg of E2/20mg of P4.

The Quality of Life (measured in UTLAN scale) of women participating in the study has a significant improvement (p<0.05) in the occupational (treatments A and C) and emotional (treatments A and B) domains and in the domain of health and sex there was no significant improvement (p>0.05).

20

Security

It was evaluated through the recording of spotting/bleeding patterns and the adverse effects reflected in tolerability; local, systemic and to change of endometrial
5 conditions.

Peri-menopausal women preserved a normal menstrual pattern with the three treatments. The frequency of the spotting/bleeding episodes decreased in the group of post-menopausal women that received the treatment with a dose of
10 0.5mg of E2/15mg of P4 and 1.0mg of E2/20 mg of P4 in comparison with the dose of 1.0 mg of E2/30 mg of P4.

The 3 treatments has acceptable local and systemic tolerability evaluated in a total of 545 intramuscular administrations.

15 In the tolerability evaluation, the most common symptom was pain in the site of application, in the treatment of 1.0mg of E2/30 mg of P4 in 6.29%, treatment 1.0mg of E2+20mg of P4 in 5.26% and in treatment 0.5mg of E2/15mg of P4 in 4.1%, but in no case was it a reason for abandonment.

20 Systemic adverse events with definite relation , 8 out of 317 total, represented 2.5 % of the total of the recorded events. In the first place of frequency were registered: Myalgia (3 of 317= 0.95%) in reference to the abdomen/pelvic

group and in second place mastalgia (2 of 317=0.63%), as the most frequent events with relation to Estradiol and Progesterone.

The events that caused abandonment of the study were
5 headache/visual disorders (4/103 volunteers) and one case of probable exacerbation of venous insufficiency.

No compromise of endometrial health was observed with the use of treatments in the analyzed sample. Endometrial security evaluation was carried out through ultrasound in a
10 sub-sample of volunteers through endometrial biopsy. The result of the ultrasound showed that the average endometrial thickness was inferior to 5mm both in peri-menopausal and post-menopausal women and endometrial biopsies reported no appearing of hyperplasia.

15 No hyperplasia observed after six months of continuous administration of the three treatments.

CLAIMS

1. A parenteral pharmaceutical formulation in suspension, of sustained release, containing suspended estradiol and progesterone particles for hormonal replacement
5 in female mammals in low and ultra-low dose, in the form of an injectable suspension formed by estradiol particles, progesterone particles, a surfactant agent, an isosmotic agent, a viscous increasing agent and one or more preservatives.
- 10 2. A parenteral pharmaceutical formulation in accordance with claim 1, in which estradiol particles are of a size between 1 and 100 micrometers.
3. A parenteral pharmaceutical formulation in accordance with claim 1, in which progesterone particles have a size
15 between 1 and 100 micrometers.
4. A parenteral pharmaceutical formulation in accordance with claim 1, in which the obtained product is a suspension.
5. A parenteral pharmaceutical formulation in accordance with claims 1 to 4, in which the product is applied in a
20 parenteral pharmaceutical form.

6. A parenteral pharmaceutical formulation in accordance with claim 5, in which the parenteral pharmaceutical form is intramuscular.

7. A parenteral pharmaceutical formulation in accordance with claim 5, in which the parenteral pharmaceutical form is subcutaneous.

8. A parenteral pharmaceutical formulation in accordance with claim 5, in which the parenteral pharmaceutical form is intradermal.

9. A parenteral pharmaceutical formulation in accordance with claim 1, in which the surfactant agents are selected from the group that consists of: polysorbate 20 polysorbate 80, dioctyl sodium sulfosuccinate and polyoxyethylene resin oil.

10. A parenteral pharmaceutical formulation in accordance with claim 1, in which the isosmotic agents are selected from the group that consists of: sodium chloride, lactose, trehalose, mannitol, glycerin and sucrose.

11. A parenteral pharmaceutical formulation in accordance with claim 1, in which preservatives are chosen from the group that consists of: Methylparaben, Propylparaben, Phenol, Thiomersal, m-Cresol, chlorobutanol, benzalkonium chloride, Benzyl alcohol and 2-Phenoxyethanol.

12. A parenteral pharmaceutical formulation in accordance with claim 1, in which the viscosity increasing are chosen from the group that consists of: Sodium Carboxymethyl Cellulose, polyethylene glycol 300, polyethylene glycol 400, polyethylene glycol 3350.

13. A parenteral pharmaceutical formulation in accordance with claim 12, in which the pH of the injectable form is between 4 and 7.

14. A parenteral pharmaceutical formulation in accordance with claim 1, in which the dose is between 0.25 to 0.50mg of estradiol and 15.0mg of progesterone for ultra-low dose.

15. A parenteral pharmaceutical formulation in accordance with claim 1, characterized because the low dose is between 0.5mg of estradiol and 15 mg of progesterone to 1.0 mg of estradiol and 20mg of progesterone.

**PARENTERAL PHARMACEUTICAL FORMULATION IN SUSPENSION,
HAVING SUSTAINED RELEASE, IN LOW AND ULTRALOW DOSAGE, IN
HORMONAL THERAPY IN THE CLIMACTERIC SYNDROME**

5

ABSTRACT

Parenteral pharmaceutical formulation or composition, in suspension, having sustained release, containing suspended particles of estradiol and progesterone for hormonal replacement in female mammals in low and ultralow dosage; the formulation consists of an injectable suspension comprising particles of estradiol, particles of progesterone, a surfactant agent, an isosmotic agent, a thickening agent, and one or more preservation agents, wherein the estradiol is in particles having a size between 1 and 100 microns and the progesterone is in particles having a size between 1 and 100 microns, for application thereof in parenteral, intramuscular, subcutaneous or intradermal pharmaceutical form.

15

Figure 1