Evaluation of Percutaneous Absorption Performance for Human Female Sexual Steroids into Pentravan Cream

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ABSTRACT

There is a lack of studies on Pentravan cream, a widespread transdermal vehicle which is used by compounding pharmacies. The purpose of this study was to evaluate this transdermal vehicle. The permeation performance for progesterone, estradiol, and estriol in formulations containing each of those drugs separately, as well as an association of estradiol + estriol (Biest), was evaluated regarding their compounding process and their potential biological application. An excised female human skin model was used to predict the permeation and the retention of the active compounds in every skin layer in lieu of conventional tape stripping. Progesterone was the drug with the highest permeation (37.02 mcg cm^{-2} at the end of the experiment). Estradiol and estriol in Biest had permeations approximately 4-fold lower (9.44 mcg cm^{-2} for estradiol-Biest and 14.02 mcg cm^{-2} for estriol-Biest), and the profiles of estradiol in E_{emuls} and in Biest were almost the same (9.46 mcg cm^{-2} for E_{emuls}). All permeations followed pseudo-first order kinetics. For progesterone, using the percentage of permeation by dose, one can infer that a patient using the 1-g emulsion dose released by the pump containing 50 mg of progesterone will have 38.4 mg of progesterone liberated into his bloodstream, gradually and continuously for 48 hours. The results indicate that the vehicle was able to provide percutaneous absorption rates compatible with and higher than clinical treatment needs. Using the same rationale, the E_{emuls} would deliver practically the entire amount of estradiol load per dose (1.0 mg), approximately 0.5 mg of estradiol per day. As for the Biest, the dosing used would deliver almost 0.5 mg estradiol/day and 2.0 mg estriol/day. Thus, according to the results, human female sexual hormones incorporated in the oil-in-water vanishing cream base and applied topically are expected to exert their biological activities systemically with good efficacy due to their satisfactory permeation through human skin. However, one must take into account that a high quantity of drug was delivered. Thus, to avoid patient overdose, care has to be taken regarding the quantity of emulsion used.

INTRODUCTION

The transdermal delivery of drugs in semisolid dosage forms is currently a well-defined and proven-efficient pharmaceutical option.[1] Advantages include[2-5]:

- Elimination of the first-pass metabolism in the liver after the intake and also relatively low skin metabolism
- Prevention of peak and trough serum levels and enabling of gradual and constant absorption
- Less frequent dosing regimens
- Patient-friendly as it is non-invasive, which increases compliance
- Ease of discontinuation in case of adverse effects

Among the drugs that are suitable for using the skin as a therapeutic route, there are the human sexual steroids, which are commonly used as hormones for substitution therapy for the troublesome climacteric symptoms of hypogonadism.[6] These drugs include progesterone (P), 17-β-estradiol (E2), and estriol (E3). These are quite good candidates for transdermal delivery as they have physicochemical properties that are particularly suitable for diffusion through the human skin, notably their low molecular weight (P = 314.46 g mol^{-1}, E2 = 272.38 g mol^{-1}, E3 = 288.38 g mol^{-1}) and their adequate lipophilicity (log $P_{o/w}$ = 3.87 for P, 4.02 for E2, and 2.45 for E3).[7]

On the other hand, one challenge facing the semisolid formulations is that they commonly have to be applied over large surface areas to reach systemic circulation in sufficient quantity to exert their biological activities.[5] Therefore, the use of novel transdermal vehicles that are more efficient in terms of percutaneous absorption is highly encouraged. In this work, a widespread transdermal vehicle used by compounding pharmacies was evaluated. The vehicle (Pentravan) was designed to overcome the challenge referred to above as well as other practical issues of the traditional transdermal gels and creams.[8] Pentraven has already been studied for testosterone, with encouraging results.[8]

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The purpose of this work was to evaluate the permeation performance of Pentravan cream for transdermal delivery systems containing P, E2, and E3, in isolation and also in association with E2 + E3, known as bi-estrogen or Biest, a commonly and traditionally used compounded preparation in the clinical practice for menopause symptoms.[9] An excised female human skin model was used to predict the permeation and the retention of the active compounds in every skin layer using cryostat microtome in lieu of conventional tape stripping. Additionally, a manufacture process for compounding of the products was evaluated regarding the content uniformity (CU).

MATERIAL AND METHODS

Reagents, Reference Standards, Materials

The ethanol used in the preparation of the mobile phases was high-performance liquid chromatographic-grade (Lot 0000420694; Panreac, Castellar del Vallès, Barcelone, Spain) and the sodium chloride (NaCl) (Lot BCBH3643V; Sigma-Aldrich, St. Louis, Missouri), potassium chloride (KCl) (Lot 1106410; Vetec, Rio de Janeiro, RJ, Brazil), calcium chloride ($CaCl_2$) (Lot 1003396; Vetec), magnesium sulphate ($MgSO_4$) (Lot 1109865; Vetec), magnesium chloride ($MgCl_2$) (Lot 12554; Neon, São Paulo, SP, Brazil), sodium sulphate (Na_2SO_4) (Lot 1206393, Vetec), sodium bicarbonate ($NaHCO_3$) (Lot 11335; Neon) , potassium dihydrogen phosphate (KH_2PO_4) (Lot 13897; Neon), disodium hydrogen phosphate (Na_2HPO_4) (Lot 8608; Neon), acetone (Lot 13064; Neon), acetonitrile (Lot 0000449160, Panreac), chloroform (Lot 14050; Neon), ethanol (EtOH) (Lot 14756; Neon), ethyl ether (Lot DCBB1652V; Vetec), methanol (MeOH) (Lot 15960; Neon), and tetrahydrofuran (Lot 0000451253; Panreac) were all analytical grade. Ultrapure water obtained with an AquaMax-Ultra 370 Series (Young Lin, Anyang, Korea) (18.2 MΩ cm resistivity at 25°C and <10 ppb total organic carbon) was used throughout the experiments. P (Lot FT-PRO20120501#5), E2 (Lot 20120114#4), E3 (Lot ER111201#2), ethoxydiglycol (2-(2-Ethoxyethoxy) ethanol) (Lot 11H1627#4), and Pentravan (Lot 12C05-U-4-003194) for emulsion compound preparation were all kindly a gift of Fagron (São Paulo, SP, Brazil). The reference standards used were all from *United States Pharmacopeia (USP)* (Rockville, Maryland). All the mobile phases and receptor media were filtered in a 0.45-mcm filter membrane (RC-45/15 MS; Chromafil, Düren, Germany) and degassed using an ultrasonic apparatus (Model 1600A; Unique, Indaiatuba, SP, Brazil) for 30 minutes before use. The receptor medium for permeation (0.01 *M* phosphate-buffered saline (PBS), pH 7.4) was prepared using the following composition: NaCl – 138.00 m*M*, KCl – 2.70 m*M*, KH_2PO_4 – 1.43 m*M*, and Na_2HPO_4 – 8.57 m*M*. The pH was corrected when necessary using hydrochloric acid (HCl) or sodium hydroxide (NaOH), and then 0.5% of hydroxypropyl-β-cyclodextrin (HPCD) was added. All volumetric glassware used was previously calibrated.

Optimization of Semisolid Dosage Form Preparation

The transdermal emulsions used in the study were prepared following the formulations described in Table 1. Drug loads which were more commonly prescribed by physicians were chosen based on research among local compounding pharmacies.

The general process consisted of:

1. Weighing the active substances (P, E2, or E3) accurately.
2. Transferring to an agate mortar.
3. Levigating stepwise with the ethoxydiglycol.
4. Homogenizing geometrically with the vehicle.

From this initial process, some variables regarding the crucial aspects of the preparation were tested to confirm the accuracy of the semisolid dosages delivered by the single-dose packaging. For that, two different brands of airless and plunger-packaging were tested (Packaging 1 = SlimLock, Emphasys, Brazil; Packaging 2 = Pump Control, Vepakum, Brazil), using a volumetric approach (n=6 for each packaging). The densities of all products were determined, and then the packaging (volume = 30 mL) were filled with the emulsions (n=6 for each packaging) through a process that uses the so-called "force of extrusion," to optimize the micelles formation and the consequent drug encapsulation. Each packaging had three doses dispensed in beakers, and the weight was transformed to a volume using the density to verify if the packaging dispensed exactly 1 mL. Student's t-test (reference value = 1.0 mL) was used for this purpose at a 95% level of significance.

After choosing the best packaging in terms of dosing accuracy, the CU test was performed using *USP* General Chapter <905>[10] (n=10 for each substance) to determine the influence of the usage of a roll mill (Model MPC; Multipharma, Florence, Italy) to decrease particle size, and possibly increase uniformity, in the emulsions. Ten individual units for each formulation were assayed individually, and the results were expressed as the delivered dose. The acceptance value was calculated from Eq. (1):

$$|M - X| + ks \quad (1)$$

TABLE 1. Composition of the Transdermal Emulsions Containing Human Sexual Hormones.

FORMULATION	COMPONENT				
	P (mg)	E2 (mg)	E3 (mg)	Ethoxydiglycol (mL)	Pentravan (q.s.)
P_{emuls}	50.0	-	-	0.03	1 g
E_{emuls}	-	1.0	-	0.03	1 g
Biest	-	1.0	4.0	0.03	1 g

q.s. = quantity sufficient

where X is the mean of the individual contents ($\chi_1, \chi_2, ..., \chi_n$), expressed as a percentage of the label claim; $M = X$ if $98.5\% \leq X \leq 101.5\%$; or $M = 98.5\%$, if $X < 98.5\%$; or $M = 101.5\%$, if $X > 101.5\%$; k is the acceptability constant (= 2.4, for 10 units); and s is the sample standard deviation. The maximum allowed acceptance value was considered 15.[10] The processes with best results (AV acceptable) were then used throughout the analysis.

Human Skin

Abdominal human excised skin was obtained from three patients who underwent abdominoplasty (women with average age = 46, with no previous skin disease). The skins were collected immediately after the surgeries and checked visually to ensure that they were healthy and unaltered by clinical removal conditions. For transportation (less than 30 minutes), they were kept in an isotherm packaging at 4°C. At the laboratory, the skins immediately had their subcutaneous fat and connective tissue removed with bistoury, were cleaned with water and saline, wrapped in Parafilm and stored at -80°C prior to use (not more than one month).

The skins were used at full thickness. They were withdrawn from the freezer 10 minutes before use to thaw then cut into small round discs that fit the vertical diffusion cells. They were positioned over the cells in a random manner. Pre- and post-study skin barrier functionality evaluations were conducted by checking the trans-epidermal water loss (TEWL) using a vapometer (Model SWL 4261; Delfin, Kuopio, Northern Savonia, Finland).

This protocol followed The Code of Ethics of the World Medical Association (Declaration of Helsinki) and was approved by the Ethics Committee of Universidade Federal de Juiz de Fora, Brazil (Protocol No. 151.275).

Ex vivo Permeation Experiments

The experiments were designed to mimic the normal "in-use" conditions in humans. These were conducted in volumetric 7-mL static vertical diffusion cells with automatic sampling (Microette Plus, Model 60-205—40; Hanson Research, Chatsworth, California). The skin discs were positioned between the donor and the receptor compartments with the stratum corneum (SC) uppermost, ensuring that all air under the skin was removed. The available diffusion area was 1.86 cm^2 and a clamp was used to hold the compartments together. The receptor chamber was filled with PBS pH 7.4 + 0.5% HPCD, stirred at 600 rpm and maintained at 32°C ± 2°C during the whole experiment. A finite dose of the semisolid preparations was applied after the skin was allowed to equilibrate with the receptor medium for 30 minutes, using a calibrated positive displacement pipette (Model Pos-D MR-110; Rainin, Woburn, Massachusetts) on the surface of the membrane (9.3 mg for P_{emuls}, 40.0 mg for E_{emuls}, and 55.8 mg for Biest). The semisolid preparation was then evenly spread using a glass rod and applying a light force (~200 mg cm^{-2}). The rod (spreader) was checked for retention of material, thus the real applied dose was determined by subtraction of the amount of retained material from the applied dose.

Aliquots (1 mL) were withdrawn at regular time intervals (1, 2, 4, 8, 12, 16, 24, 32, 40, and 48 hour post-dosing), collected into high-performance liquid chromatography (HPLC) vials and immediately replaced with receptor medium at the same temperature. A wash of the automatic sampling tubes with 1.5 mL of the medium was performed 30 seconds prior to each collection, and the stirring was interrupted during this washing. The hormone concentrations were correspondingly corrected for the washings and replenishments. The drug concentration in each sample was determined using HPLC, and each experiment was performed in sextuplicate. The permeated amount of the drug ($Q_{real,t}$), in the time t, was calculated using Eq. (2):

$$Q_{real,t} = C_{measured,t} \cdot Vr \cdot Va \cdot \Sigma^{n-1} C_a \quad (2)$$

where $C_{measured,t}$ is the concentration measured at sampling time t, V_r is the volume of the diffusion cell, V_a is the aliquot volume and C_a is the concentration of the aliquot. Cumulative amounts of drug (mcg) penetrating per unit surface area (cm^2) were plotted as a function of time (h) for achievement of the permeation profiles. Mathematical models were applied to determine the kinetics of diffusion. Cumulative amounts of drug diffusion per unit area (mcg cm^{-2}) were plotted against time (h) for zero-order kinetics, and cumulative amounts of drug diffusion per unit area (mcg cm^{-2}) were plotted against the square-root of time (√h) for the Higuchi model, and the log of the cumulative amounts of drug diffusion per unit area (log mcg cm^{-2}) were plotted against time (h) for the first-order kinetics. The coefficients of determination (R^2) were calculated, and those with a value higher than 0.99 were considered linear. For those, the steady-state flux (J_s) was determined from the linear slope of the cumulative amount of hormone *versus* the time curve. The lag time (T_L) represented the time required to achieve the steady-state flux.

Drug Retention in the Skin

After the permeation experiments, each skin disc was withdrawn from the cells for analysis of drug retention in the skin layers. For that, the discs were immediately frozen at –30°C using tissue-embedding medium (Slee Technik, Mainz, Germany), and then 10 horizontal cuts (100 mcm) using a cryostat microtome (Model Hyrax C25; Zeiss, Oberkochen, Germany) were made. The cuts were placed into 1.5-mL conical polypropylene tubes (Eppendorf, Hamburg, Germany) containing 1 mL of methanol, and the remaining skin was placed into 10-mL conical polypropylene tubes (Eppendorf) containing 5 mL of the same solvent. All tubes were shaken mechanically, sonicated for 1 hour, filtered using 0.45-mcm filters, and then transferred to HPLC vials. They were all quantified via HPLC, and the hormone concentrations were corrected for the dilutions used.

Quantification of Hormones–High-performance Liquid Chromatography

Previously validated and published methods were employed for the quantification of the hormones.[7] The HPLC analyses were performed in a qualified and calibrated Young Lin (Korea) chromatography system composed of the following: quaternary pump (YL 9110), photodiode array detector (YL 9160), automatic injector (YL 9150), column compartment (YL 9130), and software controller (Clarity). Chromatographic separation was achieved using octadecylsilane (C18) columns – 250 × 4.6 mm, 5-mcm particle size (for P_{emuls}) and 125 × 4.6 mm and 5-mcm particle size (for E_{emuls} and Biest), all from Phenomenex (Torrance, California). The columns were connected with a pre-column (C18, 4.0 × 3.0 mm, 5 mcm) from the same manufacturer, and the temperatures were kept constant through the whole analysis (45°C for P_{emuls} and Biest, and 25°C for E_{emuls}) The analytical solutions were injected into a 20-mcL volume. The mobile phase had a flow rate of 1.2 mL min^{-1}, and the UV detection was performed at 254 nm for P and 205 nm for E2 and E3. The methods were eco-friendly to the extent that they used only ultrapure water and ethanol as reagents for the mobile phases. The composition of the phases were as follows: ethanol:water (65:35, v/v) for P_{emuls}; ethanol:water (45:55, v/v) for E_{emuls}; and ethanol:water (35:65, v/v) for Biest. The limits of detection and quantification (both in mcg mL^{-1}) for the drugs into the receptor medium were respectively set as the following: 1.68 and 5.62 for P, 0.64 and 2.15 for E2 in E_{emuls}, 3.18 and 10.60 for E2 in Biest, and 6.15 and 20.51 for E3 in Biest.

RESULTS AND DISCUSSION

Optimization of Semisolid Dosage Form Preparation

One of the harshest criticisms of semisolid preparations, hormonal or not—compounded or not, is that ignoring the efficient clinical performance, the lower incidence of skin irritation compared to patches, and the good patient compliance, the control of the applied dose is much less precise.[11] To overcome this issue, a pre-validation of the compounding process was performed, to ensure dosing reproducibility and accuracy. The first step was to ensure that the packaging used was reliable for the amount of emulsion dispensed. Two packaging from different suppliers were tested, and the emulsions were passed or not through a roll mill, and the results are expressed in Table 2. Student's *t*-test was used to ensure that the packaging dispensed the appropriate values to which they were designed.

The one-sample *t*-test returned *P*-values > 0.05 for all the four variations tested, thus any single-dose packaging could be used. Packaging 1 was chosen for the next steps because of its lower coefficients of variation, but it is interesting to note that

TABLE 2. Results from the Optimization of the Semisolid Dosage Form Preparation.

SAMPLE (mL, *n*=3, GENUINE REPLICATE)	NOT GROUND (d = 0.979214)		GROUND (d = 0.922778)	
	Packaging 1	Packaging 2	Packaging 1	Packaging 2
1	0.984	0.977	0.994	0.916
2	0.969	0.920	1.009	0.993
3	0.972	0.997	1.013	1.037
4	0.985	0.979	1.005	1.000
5	0.928	0.918	0.986	0.947
6	1.004	0.990	1.064	0.958
Mean	0.973	0.963	1.012	0.975
Standard deviation	0.026	0.035	0.027	0.043
Coefficient of variation (%)	2.647	3.672	2.701	4.436
Shapiro-Wilk normality test *p*-values	0.415	0.062	0.104	0.957
One-sample *t*-test *p*-value	0.053	0.052	0.339	0.218

CONTENT UNIFORMITY, USING PACKAGING 1

	P - P_{emuls}		E2 – E_{emuls}		E2 – Biest		E3 – Biest	
Unit (%)	Not Ground	Ground	Not Ground	Ground	Not Ground	Ground	Not Ground	Ground
1	88.94	90.55	95.17	84.76	120.00	95.65	80.24	91.09
2	88.26	88.68	97.15	78.71	74.00	91.46	99.23	91.28
3	86.19	88.54	100.27	79.39	99.48	99.44	100.42	98.50
4	83.31	91.49	100.31	81.51	101.75	94.27	86.25	93.45
5	80.34	95.81	102.96	70.63	86.56	95.52	111.89	94.35
6	90.70	96.00	98.36	81.14	112.28	98.12	100.21	95.44
7	88.27	94.22	98.64	84.04	101.67	97.23	117.25	95.62
8	84.11	94.76	107.43	82.18	94.70	99.25	92.44	97.10
9	80.73	96.98	97.53	82.99	96.70	93.32	97.42	89.85
10	89.47	88.14	101.01	84.60	95.29	91.12	94.93	92.99
Mean	86.03	92.52	99.88	81.00	98.24	95.54	98.05	93.97
Standard Deviation	3.71	3.42	3.45	4.18	12.67	3.00	10.91	2.77
Acceptance Value[a]	21.36	14.20	8.28	27.54	30.66	10.17	26.63	11.18

[a]Maximum allowed acceptance value = 15.[10]

both packaging dispensed the proper emulsion amount.

The second step was to investigate the content of uniformity of the emulsions to ensure the compounding process was generating uniform products for use. The results of these tests can also be seen in Table 2. As one can see, the formulations that were ground in the roll mill were more uniform with respect to their active substances except for the E_{emuls}, which presented the best results when not passed through the roll mill. These results indicate that the roll mill played an important role in the emulsion compound preparation process and that each formulation for each active substance had to be studied separately to verify which process produces the best usage parameters. In other words, the products in the compounded emulsion had the same potential as previously studied standard commercially produced products. The results also indicate that these compounded emulsions, dispensed in single-dose packaging are indeed reliable for use with patients with respect to the amount of the active substance they dispense.

In addition, one must note that the vehicle used was chosen because of its widespread utilization world wide and also because it has a practical advantage in that it is easier for pharmacists to prepare the final product once it is a "quasi-ready for use" emulsion, which has great physical stability and increased drug-loading capacity. Its composition also enables for drug encapsulation by an easy pharmacotechnical process called extrusion.[8] For instance, it has been previously evaluated with favorable results for use as a vehicle for transdermal testosterone,[8] and this drug is often defined as a model compound for skin permeation studies.[9]

Ex vivo Permeation Studies

Once the content uniformity issue was solved (all products were compounded using packaging 1) and they were ground with a roll mill after the incorporation of the active substance, except for the E_{emuls}), the investigation of the permeation performance of the emulsions containing P, E2, or E2 + E3 were compounded using Pentravan as the vehicle.

The clinical benefits of using such hormones transdermally have already been documented.[12-14] However, to the best of our knowledge, this report describes the first time that these hormones have been evaluated using the Pentravan vehicle, and this is the first time that the combination of estradiol + estriol (Biest) has been evaluated using *ex vivo* studies in any transdermal formulation whatsoever.

The protocol utilized was in some manner different from the standard protocol for this type of study, and one of the main differences was the duration of the permeation experiment, 48 hours rather than the conventional 24 hours. This was performed based on the experience of physicians that were part of the research group, which indicated that the currently used hormone dosages were higher than those needed by the patients. Another minor variation from the conventional protocol was the use of full-thickness skin. As the thickness of the employed skin has great influence on the results of the experiments,[15] using the skin without dermatomization helped in the mimetization of the physiological conditions, one of the main objectives of the protocol. The pre-study TEWL results also indicate that the discs were uniform in terms of their hydration levels (41.4 ± 1.2 g m^{-2} h^{-1} for P_{emuls} testing, 38.5 ± 0.8 g m^{-2} h^{-1} for E_{emuls}, and 43.6 ± 1.6 g m^{-2} h^{-1} for Biest). Additionally, post-study TEWL results (45.7 ± 2.1 g m^{-2} h^{-1} for P_{emuls} testing, 43.1 ± 2.8 g m^{-2} h^{-1} for E_{emuls}, and 49.1 ± 3.4 g m^{-2} h^{-1} for Biest) did not statistically differ from the previous ones, which is a good measure of the skin viability maintenance during the whole experiment. This could be achievable probably because the skin was used full-thickness. This assessment is an important issue once the drugs can more readily permeate the skin if it has lost its barrier function, which was not the case.

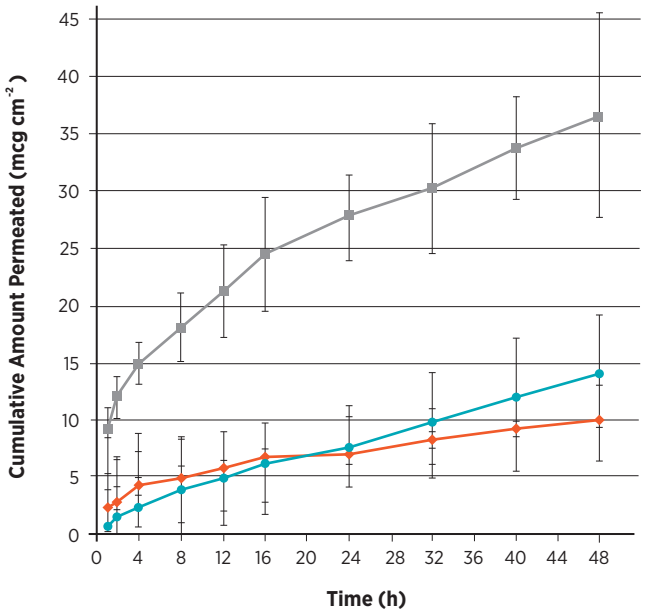
As for the receptor media used, it is known that maintaining skin conditions for water-insoluble substances is quite a challenge in permeation experiments. In a previous work from this research group,[7] the 0.01 M phosphate buffer, pH 7.4, was shown to be the most suitable for the female steroids, with a solubilizer (0.5% HPCD) added. HPCD is a chemically modified cyclodextrin that possesses high water solubility. This class of compounds possesses a lipophilic cavity and a hydrophilic exterior, therefore, they are ideal for forming complexes with non-polar molecules in relatively polar media such as PBS.[16] Besides this well-fit play in the permeation studies, HPCD was not expected to influence the intake of the drugs by the skin, as its presence was limited to the acceptor chamber (i.e., where the permeation process had already occurred).

Finally, non-occlusion leads to greater standard deviations, especially for finite dose conditions, as in the current case, but literature data indicate that the flux and the cumulative amount are not influenced by this parameter.[15] The protocol followed what appeared to be the condition that best resembled the normal conditions of *in vivo* usage (i.e., non-occlusion). The finite dose was also chosen because this condition is usually recommended for semisolid systems.[17,18] The amount of formulations used was dependent on their responses by HPLC. This was all taken into account, and the permeation experiments were performed. The permeation profiles can be seen in Figure 1, as well as the kinetic data in Table 3.

Based on the results, one can see that all studied drugs did cross the human skin when compounded using Pentravan, each one with a different flux and in a different amount. P was the drug with the highest permeation (37.02 mcg cm^{-2} at the end of the experiment), compared with the other hormones. E2 and E3 in Biest had permeations approximately 4-fold lower (9.44 mcg cm^{-2} for E2 in Biest and 14.02 mcg cm^{-2} for E3 in Biest), and the profiles of E2 in E_{emuls} and in Biest were almost the same (9.46 mcg cm^{-2} for E_{emuls}). All these data are in accordance with the drug loads of each formulation.

All formulations presented pseudo-first-order kinetics (Higuchi's model), which is in agreement with the linear correlation coefficient (R^2) higher than 0.99 for the

FIGURE 1. *Ex vivo* permeation profiles of human sexual hormones through excised human skin. Results presented as the mean ± S.D. (*n*=6).



relationship between the hormones amount permeated (mcg cm^{-2}) and the square root of time (h). Using the equation generated by this model, the permeation profiles were complemented with the determination of the hormone steady-state flux (J_s, mcg cm^{-2} h^{-1}) and the lag time (T_L, h), which varied between 1.07 (for P) to 2.00 (for E3).

Generally, the diffusion of substances across the skin is directly dependent of their interaction with the intercellular lipids, as well as their molecular weight (i.e., the greater the interaction with the lipids, the lower the diffusion through skin). Interestingly, when one compares the permeation fluxes of the formulations (range of 0.27 in E3-Biest to 4.55 in P-P_{emuls}), one can see that they cannot be understood just by these two factors. The molecular weight was inversely proportional to the flux (i.e., the heavier the molecule, the higher the flux was [E2 < E3 < P]). As for the lipophilicity, no direct relation or trend could be found.

One note that can be made is that the fluxes considerably diminished from the maximum *in vitro* release rate found in a previous work (Table 3).[7] The maximum achieved fluxes decreased from 3.7-fold in the case of E2 in E_{emuls} to 52.7-fold in the case of E3 in Biest. These *in vitro* fluxes (artificial polysulphone membrane, a passive diffusion barrier) are important for determining the release of the drug from the 3D net of the emulsion into the receptor medium, but the fluxes are expected to decrease when using biological membranes such as human skin.

It is generally assumed that the diffusional resistance of the SC is the only issue to be surmounted by transdermal drugs. However, in the case of lipophilic substances such as the steroidal hormones, the drug must also be able to escape the comfortable environment of SC into the underlying viable epidermis, which is more aqueous in nature than the first layer.[11] The viable epidermis is therefore rate-limiting because of the "partitioning out" phenomenon that affects lipophilic drugs. This phenomenon could explain, in part, the relatively low fluxes of the hormones.

The great reduction in the flux, however, is, above all, indicative that the hormone dosage used in medical routines can be higher than the necessary dose, as the transdermally applied hormones achieve circulation, though at a relatively low speed. However, using the emulsions every day can lead to hormonal overdosing.

Percutaneous Retention

The real amount of drug that permeates through the skin is represented not only by the drug quantified in the receptor medium but also by the quantity of drugs retained in the dermis. This occurs because *in vivo* the dermis vascularizes, thus the drug within it is able to reach the bloodstream.[18] As *ex vivo* skin has its microcirculation obliterated, the dermis can retain compounds that would penetrate *in vivo*,[19] and this is of particular importance for lipophilic drugs such as the hormones.[20]

The epidermis *in vivo*, for its turn, undergoes constant renewal, via outward proliferation, differentiation, and desquamation. This renewal is one of the reasons that the amount of drug observed in epidermis cannot be counted as permeated.[21] Thus, xenobiotics can be lost during *in vivo* skin via desquamation or even sebum secretion.[19] In other words, the drug in epidermis can reach the bloodstream, but reaching the bloodstream is not a certainty.

The results for the cutaneous retention experiments can be seen in Figure 2, which also offer a good

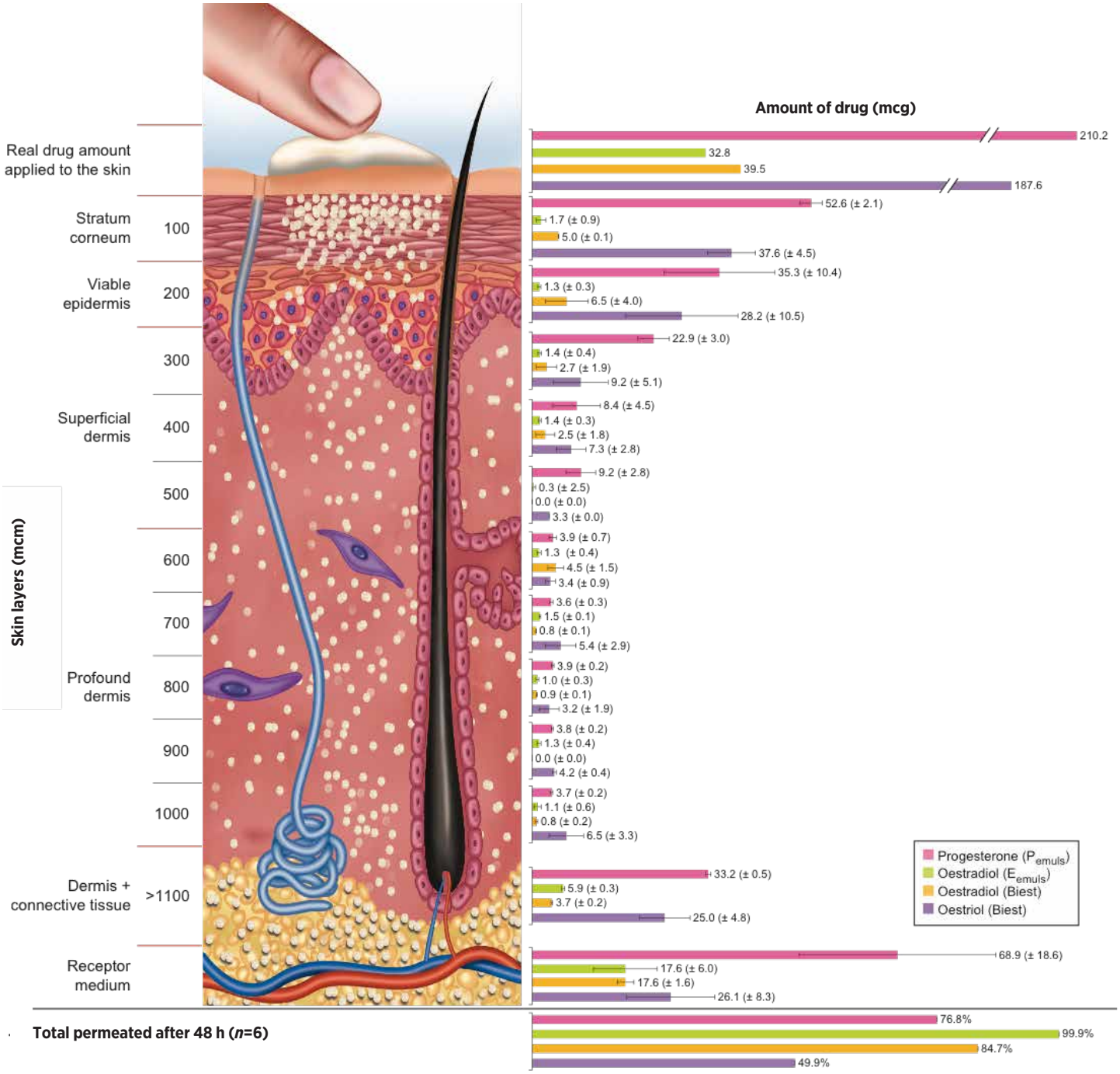
TABLE 3. Kinetic Parameters of the Transdermal Permeation of Human Sexual Hormones.

DRUG	MATHEMATICAL MODEL	EQUATION	R^2	J_S (mcg cm^{-2} h^{-1})	T_L (H)	*IN VITRO* DRUG RELEASE (mcg cm^{-2} h^{-1})[a]
P (P_{emuls})	Higuchi	y = 4.55x + 5.28	0.997	4.55	1.07	29.36
E2 (E_{emuls})	Higuchi	y = 1.15x + 1.45	0.997	1.15	1.12	4.22
E2 (Biest)	Higuchi	y = 1.13x + 1.57	0.997	1.13	1.18	5.18
E3 (Biest)	Higuchi	y = 0.27x + 1.10	0.994	0.27	2.00	14.24

[a]Maximum liberation rate[7]; J_S - steady-state flux; T_L = lag time; Results expressed as a mean of six replicates

picture of the whole permeation experiment in terms of hormone absorption. The figure shows that a relatively high amount of the hormones was retained within the SC and the other epidermal layers. It is important to cite that SC thickness can vary depending of variables such as gender, age, and body site, and then some divergence from previous works can be found.[22] Despite the retention, the drugs had good permeation. A high amount was also encountered in deeper areas of the skin, where the connec-

FIGURE 2. Permeation performance of Pentravan for human female sexual hormones, using human female skin model. Results presented as the mean ± S.D. (*n*=6).



tive tissue was present. E3-Biest presented the highest relative amount of drug retained within the SC and the epidermis. This result may be because the diffusion through the skin was directly dependent on the interaction with the intercellular lipids and also their molecular weight, and E3 presented the lowest interaction with the lipids (lower log $P_{o/w}$) and has a higher molecular weight compared with E2.[23]

Almost half of the dose applied over the skin was lost to the spreader during the application. It is possible that this would not be lost *in vivo* because the spreader in such a case would be the finger of the patient himself. However, this study considered that the patient could wash his or her hands after the application, which would result in the true loss of this amount of applied drug.

TABLE 4. Mass Balance of the Transdermal Permeation of Human Sexual Hormones.

PARAMETER	P (P_{emuls})	E2 (E_{emuls})	E2 (BIEST)	E3 (BIEST)
Theoretical drug amount applied to skin (mcg)[a]	470.0	40.0	55.8	223.2
Real drug amount applied to skin (mcg)[b]	210.2	32.8	39.5	187.6
Total permeated amount after 48 h (mcg)[c]	161.4	32.7	33.4	93.6
Retained drug in epidermis (mcg)[d]	87.8	3.0	11.5	65.7
Percentage of permeation by dose (%)[e]	76.8	99.9	84.7	49.9
Total recovery (%)[f]	109.5	107.5	109.8	87.3

[a]Total amount of cream placed in the donor chamber (5.0 mg cm^{-2} for P_{emuls}; 21.5 mg cm^{-2} for E_{emuls}; and 28.4 mg cm^{-2} for Biest)
[b]Total amount (*a*) minus the retained amount in the spreader
[c]Drug in the receptor medium + dermis
[d]Drug in the *stratum corneum* + viable epidermis
[e]Mathematical estimation of the total of drug that would permeate the skin according to the amount applied [(*c*/*b*) × 100]
[f]Mathematical estimation: Total drug quantified [in the spreader (*a* – *b*), the receptor medium (*b*), and the skin (*c*)] divided by the total amount applied (*a*). Result given as percentage (×100).

Complimentary Discussion

Figure 2 and Table 4 show the mass balance of the study and present some data that allows for some inferences about the transdermal hormones used. The acceptable recovery rate lies between 85% and 115%, and although all tests met the criterion, one can note that E3 had the lowest recovery. This is probably due to a comparatively low solubility in the receptor medium, which, for its turn, led to permeation below the limit of quantification in some collection points.

For P, using the percentage of permeation by dose (%), one can infer that a patient using the 1-g emulsion dose released by the pump containing 50 mg of P will have 38.4 mg of P liberated into his bloodstream, gradually and continuously for 48 hours. Theoretically, this would be approximately 19 mg/day, but the process follows Higuchi's kinetics, and after 24 hours of experiment, there was a cumulative 28.8 mg released (i.e., the liberation stabilized in the second day).

Progesterone Injection USP was considered the standard formulation for comparison purposes because of its bioavailability. It releases 5 to 10 mg/day of active hormone for treatment of amenorrhea and dysfunctional uterine bleeding. Within this context, a dose of 1 g of the emulsion compounded using the referred vehicle delivers even more hormone than Progesterone Injection USP, even when used every two days. That said, the use of this highly effective vehicle could lead to a paradigm change to the therapy in regards to hormone dosing. The patient could use, for instance, a quarter to half the 1 g-dose or the same emulsion quantity but with a much lower progesterone load. Such a use would be in accordance with the intended goal of lowering the applied emulsion quantity while simultaneously increasing its effectiveness.

Using the same rationale, the E_{emuls} would deliver practically the entire amount of E2 load per dose (1.0 mg), approximately 0.5 mg of E2 per day. This amount of active substance is much higher than the existing amounts currently achievable with the commercial transdermal products (0.025 to 0.1 mg/day – Climara Patch, 0.025-0.1 mg/day – Esclim Patch, 0.05 to 0.1 mg/day – Estraderm Patch) used for climacteric vasomotor symptoms or hypogonadism, although none of these products is sold in semisolid form. As for the Biest, the dosing used would deliver almost 0.5 mg E2/day and 2.0 mg E3/day. There is no transdermal product on the market that uses these two hormones in combination nor is there an E3 transdermal product available, although they are widely available in compound pharmacies. The rationale used for the delivery of E2 in E_{emuls} can be extrapolated to the delivery of the same substance in Biest.

As already noted, one question is the possibility of decreasing the quantity of the product applied, which is directly related to an increase in patient compliance. In this case, the airless plunger-packaging could be calibrated to deliver not 1 g but 300 mg or less, for example. This represents an easier application and less discomfort to the patient. This is only possible because the vehicle tested presented a high-permeation performance for the female steroids due to its ability to deliver therapeutic agents for long periods with a controlled ratio and its escape from the hepatic first-pass effect. Although not a certainty, such results are expected for other drugs loaded into the same vehicle.

CONCLUSION

According to the results, human female sexual hormones incorporated in Pentravan oil-in-water vanishing cream base and applied topically are expected to exert

their biological activities systemically with good efficacy due to their satisfactory permeation through human skin. Yet, the ground mill was a useful and necessary tool to achieve this objective, except for E_{emuls}. However, one must take into account that a high quantity of drug was delivered. Thus, care has to be taken regarding the quantity of emulsion used to avoid patient overdose.

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