



Influence of the dissolution medium on the release of dehydroepiandrosterone from lipophilic suppositories

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ABSTRACT

Suppositories with cocoa butter containing dehydroepiandrosterone (DHEA) without and with the addition of Span 80 and Tween 80 as surfactants with low and high HLB values were prepared. The physical properties and the drug content of all prepared suppositories were in accordance with the pharmacopoeial requirements. The release study tests in three dissolution media such as water, lactic acid solution at pH 4.2 and phosphate buffer at pH 7.4 were carried out. In acidic and alkalic media only about 10% and 27% of DHEA were released, respectively. The addition of Span 80 to the suppository mass did not improve the release process, but the addition of Tween 80 caused the increase in the amount of DHEA released in the acidic medium to about 35%. The data showed that rectal administration of suppositories with DHEA based on cocoa butter caused about 30% availability and after vaginal administration, only topical activity can be expected. By the addition of Tween 80 to the suppository mass availability of DHEA of about 35% from vaginal suppositories can be achieved.

INTRODUCTION

Dehydroepiandrosterone (DHEA) is a sex steroid precursor secreted by the adrenals in humans and other primates. It converts intracellularly to bioactive androgens and estrogens [20]. DHEA and its sulfate metabolite (DHEAS) production decreases with advancing age [21]. DHEA and DHEAS play an important role in regulating body fat and the immune system. They also influence the circulation of lipoproteins and insulin sensitivity [7,8,23]. Therefore, they may protect against diseases of aging such as atherosclerosis, osteoporosis, decreased libidinal interest and diabetes [5, 6]. There is much interest in the possible benefits of administering orally DHEA to postmenopausal women and for the purposes of maintaining sexual function, youthfulness, well-being and cognition [5,27]. The effect of intravaginal DHEA on the symptoms and signs of vaginal atrophy was also studied [27]. Symptoms such as vaginal dryness, itching, burning, irritation, and dyspareunia often occur as a result of a lack of estrogens [9,22]. Endometrial hyperplasia is the side effect observed in women receiving traditional estrogen replacement therapy that also increases the risk of carcinoma [3].

Vaginally administered DHEA may reduce symptoms of vaginal atrophy with improvements in sexual function in women who are estrogen deficient due to menopause [17-19, 26]. DHEA is an inactive precursor, which is transformed into active sex hormones (estrogens and/or androgens) only in specific cells and tissues, which possess the required enzymes [5]. Intravaginal DHEA, through local estrogen and androgen formation, causes a rapid effect without systemic exposure of other tissues, thus avoiding systemic effects of estrogens [18]. Labrie et al. [19] indicated that taking vaginal Prasterone suppositories (DHEA) daily causes the improvement in vaginal atrophy symptoms within 2 weeks.

In order to obtain both local and general actions of a drug, suppositories seem to be a convenient form of administration of the active substances [4, 19]. Cocoa butter is a frequently used lipophilic base for the preparation of lipophilic suppositories. It has a solid consistency at room temperature and it melts at body temperature. It is well tolerated and easy to obtain by various methods [25, 33]. In order to examine the pharmaceutical availability of the drug in vitro studies, the amount of the drug substance released from the dosage form and dissolved in the surrounding liquid as well as the rate at which this process occurs should be determined [28]. The quantity of a drug released from suppositories depends on many factors such as drug solubility in the base, addition

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of surfactants and solubility of a drug in a dissolution medium [1, 12, 30]. The type and quantity of a surfactant included in the suppository base cause the increase or decrease of the drug release [29, 32]. The addition of surfactants in suppository formulations is used to improve availability of poorly soluble drugs. For a poorly soluble drug, solid dispersions and eutectic mixtures of a drug with inert carriers are used to enhance the rate of dissolution and absorption [2]. When the drug is of higher solubility in the suppository base then the quantity of the released substance is smaller because the drug has a tendency to be retained in the vehicle [10]. The nonionic surfactants in the suppository base incorporate some of the drug, which prevents its release [15]. There are many reports of studies on the effect of the addition of various types of surfactants on the drug release from the suppositories with lipophilic or hydrophilic bases [12,14,15,30]. The mechanism of action of surfactants is not completely known. It is quite common that they do not have a beneficial effect on the drug release [32].

The aim of this study was to evaluate the quantity of DHEA released from lipophilic suppositories prepared with and without the addition of a nonionic surfactant. DHEA is poorly soluble so the release studies were carried out in three media at different pH values.

MATERIAL AND METHODS

Materials and Reagents

Dehydroepiandrosterone was obtained from Fagron. Tween 80 and Span 80 were purchased from Fluka, Chemika, and cocoa butter and paraffin oil from Cefarm, Lublin, Poland. All the reagents and chemicals used were of analytical grade.

Preparation of DHEA suppositories

Suppositories weighing 2 g and containing 13 mg of DHEA were prepared by hand rolling. The compositions of suppositories are presented in Table 1.

Table 1. Different suppositories compositions

Name of Component	Quantity (%) per suppository of 2 g weight		
	S1	S2	S3
DHEA	0.65	0.65	0.65
Cocoa oleum	99.35	94.35	94.35
Tween 80	-	5	-
Span 80	-	-	5

Physical evaluation of DHEA suppositories

Weight uniformity test

For each formulation, twenty suppositories were weighed and their average weight was calculated. Next, they were individually weighed using a weighing balance (Ohaus AV 513C, USA).

Softening time determination

The total deformation time was assayed by using the apparatus recommended by Polish Pharmacopoeia 9th edition [28].

Disintegration time

The disintegration time was determined by using the disintegration tester ST 30 (Erweka, Germany) according to FP 9th [28].

The data of physical parameters are given in Table 2.

Drug content analysis

The drug content was analysed spectrophotometrically using the spectrophotometer UV-VIS (model Helios Omega UV-VIS, Spectro-Lab, Thermo scientific, England) with a 10 mm matched quartz cell.

Calibration standards

A stock solution of DHEA (100 µg/ml) was prepared by dissolving DHEA in the composed mixture (methanol-water- 0.1 mol/l NaOH, 10:5:1, v/v/v). The calibration standards at a concentration of 5-50 µg/ml were obtained by the appropriate dilution of the stock solution with the composed mixture. The UV spectra of DHEA solution at a concentration of 10 µg/ml were measured spectrophotometrically. The maximum absorbance at a wavelength of 204 nm was found. The UV spectrum of DHEA at concentration of 32.5 µg/ml in mixture consisted of methanol, water and NaOH (10:5:1; v,v,v) is shown in Figure 1. Suppositories containing a small amount just 13 mg of DHEA have acceptable maximum absorbance at wavelength 204 nm. The linear regression equation of the calibration curve equals $y = 0.0148x - 0.0004$. The coefficient of determination was $R^2 = 0.9983$.

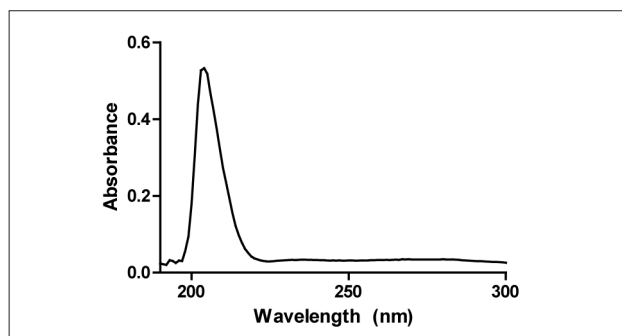


Figure 1. DHEA spectrum at concentration of 32.5 µg/ml in mixture consisting of methanol, water and NaOH (10:5:1; v,v,v)

Content uniformity

Ten randomly selected suppositories were cut into small pieces. Accurately weighed 2 g sample pieces of suppositories were transferred into a 600 ml beaker containing 160 ml of the composed mixture. The content of the beaker was blended for one hour at 37°C into a water bath and filtered through 0.22 µm membrane filter. Next, 3 ml of obtained supernatant was diluted with the composed mixture to obtain 6 ml. The absorbance of the solution was measured spectrophotometrically at 204 nm against a blank prepared using a respective suppository without the drug. The mean content of the active substance (n = 6) was calculated from the equation obtained from the calibration curve. The data are given in Table 2.

Table 2. Physical properties of suppositories prepared

Test	Results
Weight (g) mean, SD	1.99 ± 3.17
Disintegration time (min)	16.16
Softening time (min), SD	5.6 ± 0.3
Drug content (%), SD	103.57 ± 11.04

In vitro release test

In the release studies water (W), lactic acid solution at pH 4.2 (LA), phosphate buffer at pH 7.4 (PB) or mixtures of these solutions with methanol (1:1, v/v) were used as dissolution media.

Calibration curve of DHEA

The standard solutions of DHEA at concentrations 5-30 µg/ml in a mixture composed of a one of the dissolution medium (water, lactic acid at pH 4.2 or phosphate buffer at pH 7.4) and methanol in ratio 2:1 (v/v) were prepared. For each mixture the UV spectra of DHEA in concentration 10 µg/ml were determined and the maximum absorbance was found. Next, the calibration curves were prepared by measuring the absorbance of DHEA solutions spectrophotometrically at the respective wavelength. The values of the wavelengths, linear regression equations of the calibration curves and coefficients of determination are presented in Table 3.

Table 3. The values of wavelengths and parameters of the calibration curves for DHEA determination in different mixtures

The type of a mixture	Water-methanol 2:1, v/v	Lactic acid pH 4.2-methanol 2:1, v/v	Phosphate buffer pH 7.4- methanol 2:1, v/v
Wavelength (nm)	194	195	199
Linear regression equation of the calibration curve	$y = 0.0326x + 0.0325$	$y = 0.0292x + 0.0114$	$y = 0.0228x + 0.0198$
Coefficient of determination (R^2)	0.9983	0.9976	0.9957

In vitro release tests of DHEA from suppositories were carried out in the dissolution apparatus consisting of a baker and a stirrer [11] using 200 ml of a dissolution medium at a temperature of $37 \pm 0.5^\circ\text{C}$. The stirrer was rotated at the speed of 100 rpm.

A suppository was placed into the beaker containing a dissolution medium. The cloche stirrer was lowered to the height of 1-2 mm from the bottom of the beaker so that the suppository was under the stirrer. The samples, 5 ml each, were withdrawn from the dissolution medium at time intervals after 5, 10, 20, 30, 40, 50 min and filtered through a 0.22 µm millipore filter. The dissolution medium was replaced by 5 ml of a fresh solution to maintain a constant volume. The filtered samples were diluted in water or lactic acid at pH 4.2 or phosphate buffer at pH 7.4 or methanol, respectively, depending on the dissolution medium in which the test was carried out. The samples were determined spectrophotometrically at the appropriate wavelength against a blank sample (suppository without drug). DHEA concentration was calculated based on calibration curves shown in Table 3.

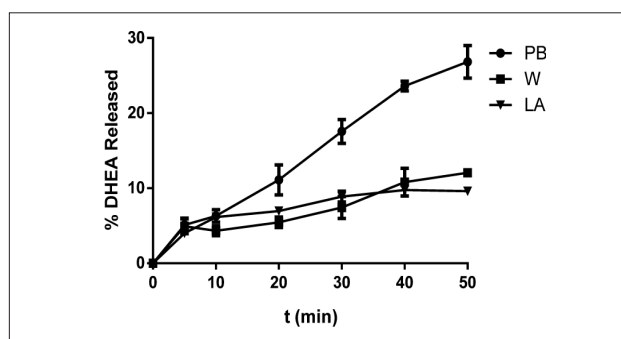
Statistical analysis

Results are expressed as the mean of six experiments (\pm SEM). For statistical evaluations, the data of release profiles of DHEA were assessed by the one way ANOVA analysis with post-hoc Turkey test, where $p < 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The data in Table 2 shows that prepared suppositories fulfilled the pharmacopoeial requirements [20] of uniformity of mass for single dose preparations (the difference relative to the average weight was $\pm 5\%$), disintegration time (up to 30 min) and softening time (up to 15 min), and the content of the active substance ranged from 85% to 115%.

The results from suppositories without surfactants presented in Figure 2 show that within 50 min only 9.63% or 12.05 % of DHEA were released in LA at pH 4.2 and water, respectively. The change of the dissolution medium to PB at pH 7.4 caused the increase of the released substance up to 26.8%, which confirmed better solubility of DHEA in an alkaline medium. These results indicated that bioavailability of DHEA from vaginal medium is poor and from a rectal medium it is only about 27%. A small amount of the released substance is a result of its lipophilicity and low solubility in the used release fluids. The dissolution media used in the research imitate the environment in which vaginal or rectal suppositories are typically placed: LA solution at pH 4.2 and W or PB at pH 7.4, respectively (16, 24, 28). The results obtained suggest that DHEA given in the vaginal form should have only a local effect, while pharmaceutical availability of the rectal form amounts to about 27%.

**Figure 2.** The amount of DHEA released from suppositories in three dissolution media ($n = 6$, $p > 0.05$)

An organic solvent or a surfactant can be added to a dissolution medium to increase the solubility of a drug. In the study performed by Sah and Saini [30] the addition of surfactant (Tween 80) into a dissolution medium at a low concentration (1%) caused a greater release rate of indomethacin from suppositories, as compared to high concentration (5%). The release rate of indomethacin was good from water-soluble suppositories bases in comparison with oil soluble bases.

In order to increase the solubility of the active substance and the possibility of comparing the dissolution profiles half of each dissolution medium was replaced by methanol. It turned out that the addition of an organic solvent to W, LA or PB caused the increase in the amount of the DHEA

released to 67.57%, 76.65% and 95.3%, respectively. The addition of methanol in the amount of 50% to all the dissolution media significantly affected the release of DHEA by increasing its solubility. The quantity of DHEA released increased significantly in each dissolution medium in the range from 56% to 68% (Fig. 3).

The additives in the suppository formulation can affect the increase or decrease of the release rate depending on the base [24]. In order to verify the effect of the surfactants to check the release process of DHEA were selected only two surfactants with different HLB values Span 80 (HLB = 4.3) and Tween 80 (HLB = 15) in a high concentration of 5%, typically added to the suppositories. In our study, the addition of Span 80 to the suppository mass did not improve the release process as the data in Figure 4 show. The quantity of DHEA released from suppositories with the addition of Span 80 decreased by about 19% in PB and by 3% in W, and in an acidic medium had no effect on the release process.

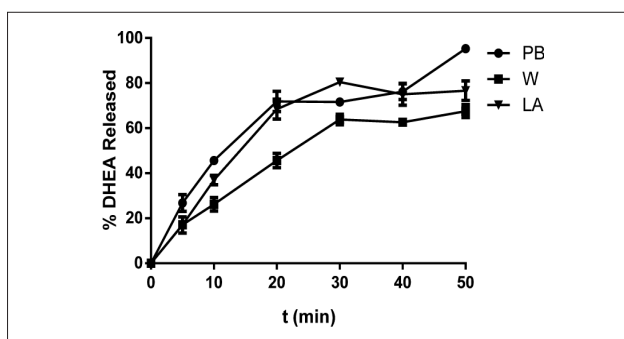


Figure 3. The amount of DHEA released from suppositories in three dissolution media with methanol ($n = 6$, $p > 0.05$)

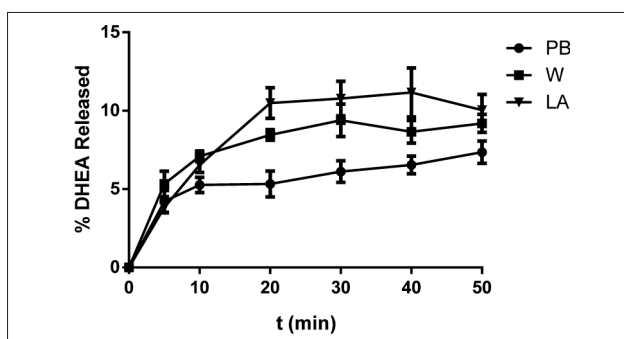


Figure 4. The amount of DHEA released from suppositories containing 5% of Span 80 in three dissolution media ($n = 6$, $p > 0.05$)

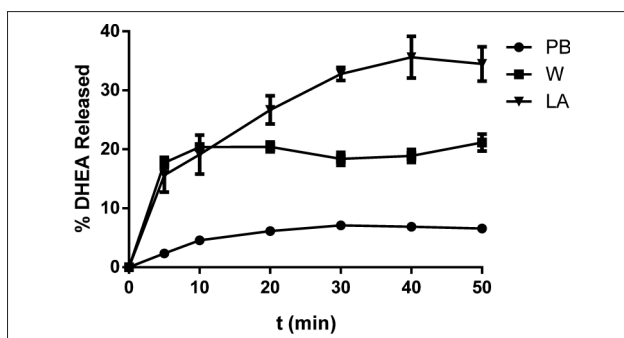


Figure 5. The amount of DHEA released from suppositories containing 5% of Tween 80 in three dissolution media ($n = 6$, $p > 0.05$)

The addition of Tween 80 to the suppository mass caused an increase in the amount of the released substance depending on the pH of the dissolution medium (Fig.5). When the pH of the dissolution medium is decreased, the quantity of DHEA released increases and amounts to 34.46%, 21.16%, 6.59% in LA, W and PB, respectively. From suppositories with Tween 80 the quantities of DHEA released in LA and W were 24% and 10% higher in LA and W, respectively, comparing with suppositories without this surfactant. In alkaline medium (PB at pH 7.4) the quantity of DHEA released amounts to only 6.59% from the suppository with the addition of Tween 80. In this medium, there was a 20% decrease in the release.

The data obtained from the dissolution studies by Dal Zorro et al. [12] showed two glucose derived surfactants which caused a decrease in piroxicam release from lipophilic and hydrophilic suppositories. A more complex surfactant, a lauroyl macrogolglyceride, showed an increase in piroxicam availability from lipophilic suppositories at higher concentrations tested (15% and 20%).

Hargoli et al. [14] proved that among the formulations containing surfactants only Witepsol H15 with 0.5% w/w of Tween 80 and Witepsol W35 with 0.5% of cetylpyridinium chloride were suitable and caused nearly a complete release of naproxen from suppositories within 30 and 60 min, respectively.

In vitro release studies carried out by Ghorab et al. [13] showed a greater release of fenoterol bromochloride from hydrophilic bases than from fatty bases. Incorporating non-ionic surfactants (Tween 60 and Span 20) into Witepsol H15 base caused an enhanced release rate of the drug at low surfactant concentrations.

In our study, as shown in Figures 6 and 7, in dissolution media with methanol, the release process from suppositories with an addition of surfactants runs worse in comparison with suppositories without surfactants. From suppositories containing Span 80 or Tween 80 in PB at pH 7.4 the quantity of DHEA released amounts to only 17.71% and 16.86%, which indicates a decrease in the amount of the substance released by about 78%. In water and LA at pH 4.2 the quantity of the released DHEA decreased in the range from 18-25% and 20-25%, respectively. Probably, the solubility of DHEA varies in acidic and alkaline media with the addition of a large amount of methanol.

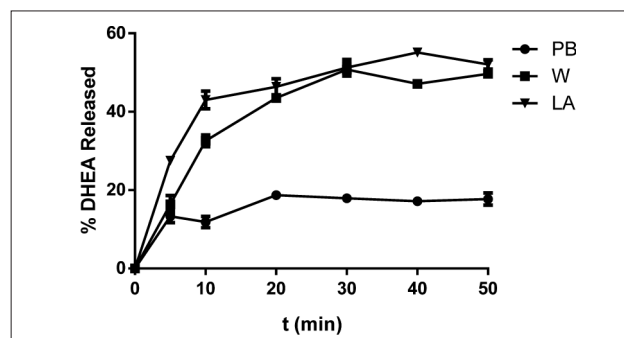


Figure 6. The amount of DHEA released from suppositories containing 5% of Span 80 in three dissolution media with methanol ($n = 6$, $p > 0.05$)

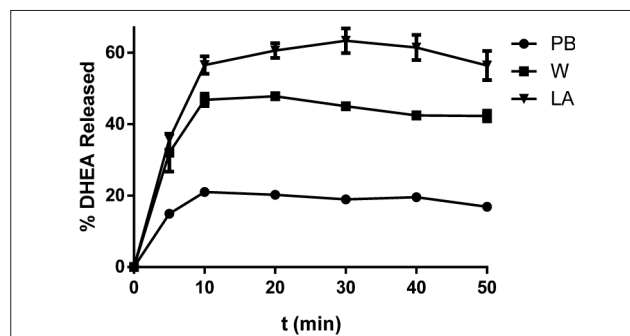


Figure 7. The amount of DHEA released from suppositories containing 5% of Tween 80 in three dissolution media with methanol (n = 6, p > 0.05)

CONCLUSION

The release of DHEA from the suppository with a cocoa butter base in acidic medium and water was poor and in the alkaline medium at pH 7.4 it was slightly better. These studies showed that DHEA administered in vaginal suppositories could be used for local effect whereas after the administration of rectal suppositories about 30% bioavailability can be achieved. With the addition of Span 80 to the suppository mass, the amount of DHEA released from suppositories did not increase, but after the addition of Tween 80 about 35% bioavailability from vaginal suppositories was obtained.

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