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Research Article

Physico-chemical characterization, analytical method development and solubility studies for progesterone

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ABSTRACT

Progesterone is a well known natural contraceptive, a drug of BCS class II, which was used in early centuries to protect from the side effects of estrogens like abortion. For the preformulation studies, the solubility of progesterone in various oils, solvents, surfactants and co-surfactants was determined for further formulation development. The Progesterone has been evaluated for XRD, DSC, Partition coefficient, melting point and its solubility is also checked in different solvents. Physico-chemical characterization studies showed that progesterone has a melting point of 127^0C. The solubility of drug progesterone was evaluated in methanol, and ethanol. The analytical method developed for the estimation of progesterone in methanol and ethanol showed maximum absorbance at λ_{max} of 241 nm and 247 nm respectively at pH 7.4.Linearity studies indicated that estimation of progesterone between 1.00 µg /ml to 10.00 µg /ml was found to be linear with regression equation of y = 0.0575x + 0.0041; R^2 = 0.9985 with methanol and y = 0.0442x+0.0041; R^2 = 0.9968 with ethanol. On the basis of solubility studies two oils with highest solubility (Oleic acid and Myritol), two surfactants (Tween80 and Tween20) and three co-surfactants (Ethanol, Propanol and PEG400) were selected respectively for further formulation studies. The above analytical parameters indicated that the developed UV Spectrophotometric method for progesterone was simple, accurate, precise and reproducible.

Introduction

Progesterone is a C-21 steroid which is synthesized from both tissue and circulating cholesterol. Cholesterol is transformed to pregnenolone which is then converted via a combined dehydrogenase and isomerase to progesterone. The principle production sites are the adrenals and ovaries and the placenta during pregnancy. The majority of this steroid is metabolized in the liver to pregnanediol and conjugated as a glucuronide prior to excretion by the kidneys.

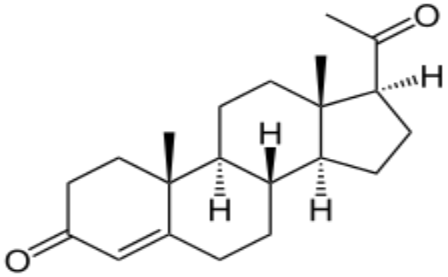

Figure 1: Structure of Progesterone

The most common symptoms heard by physicians from their female patients are problems related with weight gain, fatigue, and loss of libido, depression, headaches, joint pain and mood swings. Many physicians and scientists are becoming getting

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aware of a common link between these symptoms and diseases and that common link is an imbalance between the female sex hormones, progesterone and estrogens [1].

Natural progesterone can be given orally, topically or by injection. However, the best way is topically (transdermally). Transdermal delivery is gaining in popularity day by day as evidenced by the growing use of estrogen, testosterone, nitroglycerine (for angina) and even nicotine patches [2, 3].

According to BCS classification progesterone comes under BCS Class II which means high permeability and poor solubility [5]. For the said reason it is necessary to optimize and do the solubility studies for designing a formulation of progesterone.

Review of literature given has insight that very few spectrocolorimetric and high performance liquid chromatographic method for the analysis of progesterone. Hence the present investigation was undertaken for drug candidate physico-chemical characterization, to develop a simple and robust UV Spectrophotometric method of Progesterone.

Material and Method

Chemicals & Apparatus

Progesterone(ASG Biochem Pvt.Ltd.), Acetonitrile ACN (Fischer Scientific, Mumbai), Methanol (Fisher Scientific, Mumbai), Ethanol absolute (ChangshuYanguan Chemicals, China), n-Octanol (SDFCL, Mumbai), Ethyl oleate(Chemzone, Ahamdabad), Iso Myristyl Palmitate (Thomas baker chemicals Pvt. Ltd. Mumbai, India), Myrritol (BASF, Germany), MCT (BASF, Germany), Oleaic Acid (Molychem , Mumbai), Tween 20(Lobachem, Mumbai), Tween 60 (Lobachem, Mumbai), Cremophore RH 40(BASF, Germany), Cremophore ELP(BASF, Germany), Tween 80 (Thomas baker (chemicals) Pvt. Ltd. Mumbai, India), Kolliphor HS 15 (BASF, Germany), Propanol (Multichem, Mumbai), Propylene Glycol (Thomas baker (chemicals) Pvt. Ltd. Mumbai, India), PEG 400 (SD Fine-Chem Limited, Mumbai), PEG 200 (Maan Medex, Nagpur), Glycerine (Chemtex, Kolkata), Magnetic stirrer (Remi Scientific Instruments, Mumbai), UV spectrophotometer(Shimadzu, Japan), X'Pert PRO, Panalytical Company, Netherlands, Mettler Toledo, 822e, Switzerland.

Determination of Melting point:

For determination of melting point USP method was followed. Small quantity of progesterone was placed into a sealed capillary tube. The tube was placed in the melting point apparatus. The temperature in the apparatus was gradually increased and the observation of temperature was noted at which progesterone started to melt and the temperature when the entire drug gets melted. This method is also known as capillary method. [12]

Determination of Partition coefficient:

To determine the partition coefficient of the progesterone, the shake flask method was used; it is the classical and the most useful method of determination of partition coefficient. Briefly the procedure could be explained as excess amount of API was added in 10ml mixture of n-Octanol and water (1:1). The system was prepared in triplicate and was shaken gently in the separating funnel for 24 hours for achieving equilibrium. Then the two phases were separated and centrifuge at 8000 rpm for 20 minutes. After centrifugation, the concentration of progesterone in both phases was determined by UV spectroscopy and partition coefficient was calculated using the equation [11].

It can be determined by the formula:

$K_{o/w}$= C1/C2

Where, C1 = Conc. of solute in organic phase, C2 = Conc. of solute in aqueous phase,$K_{o/w}$= Partition coefficient

Log P = log ($K_{o/w}$)

XRD of Progesterone

PXRD patterns were recorded on a powder x-ray diffractometer (X'Pert PRO, Panalytical Company, Netherlands) using Ni-filtered, CuKα-radiation, voltage of 60 Kv and a current of 50 mA. The scanning rate employed was 1°/min over the 10° to 60° diffraction angle (2θ) range. The PXRD pattern of progesterone was recorded. (fig. 2)

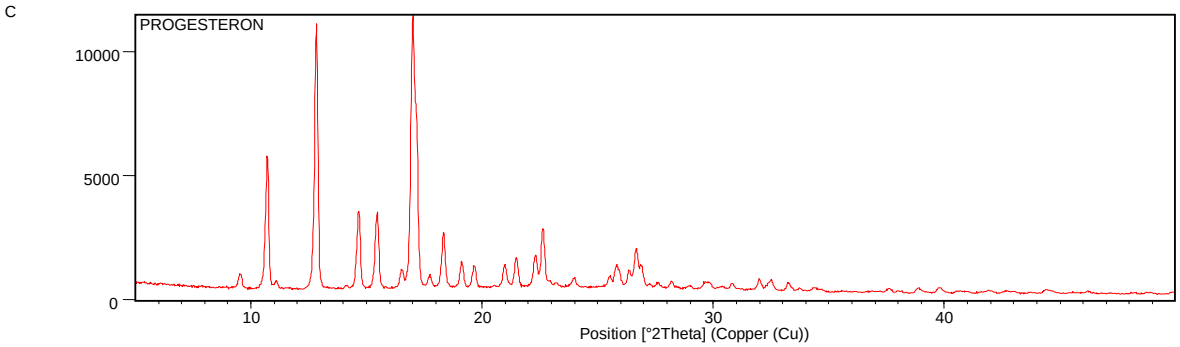

Figure 2: XRD of Progesterone

DSC of Progesterone

DSC scans were recorded for progestrone, physcisal mixture and nanoemulsion gel using Differential Scanning Calorimeter (DSC) (Mettler Toledo, 822e, Greifensee, Switzerland). The

samples were weighed and hermetically sealed in 40 µl aluminum pans, which were further heated over a temperature range of 30ºC to 300ºC at a heating and cooling rate of 10ºC/min. Similarly an empty aluminum pan was also prepared and used as reference. The liquid nitrogen was used for reestablishing the starting temperature by purging it into the equipment at a rate of 30 ml/min. Pure indium was used to calibrate the temperature and enthalpy of the instrument. The results were assessed using the accompanying STARe software. Normalization was performed on the obtained results with respect to the respective sample masses to facilitate comparison. (fig3)

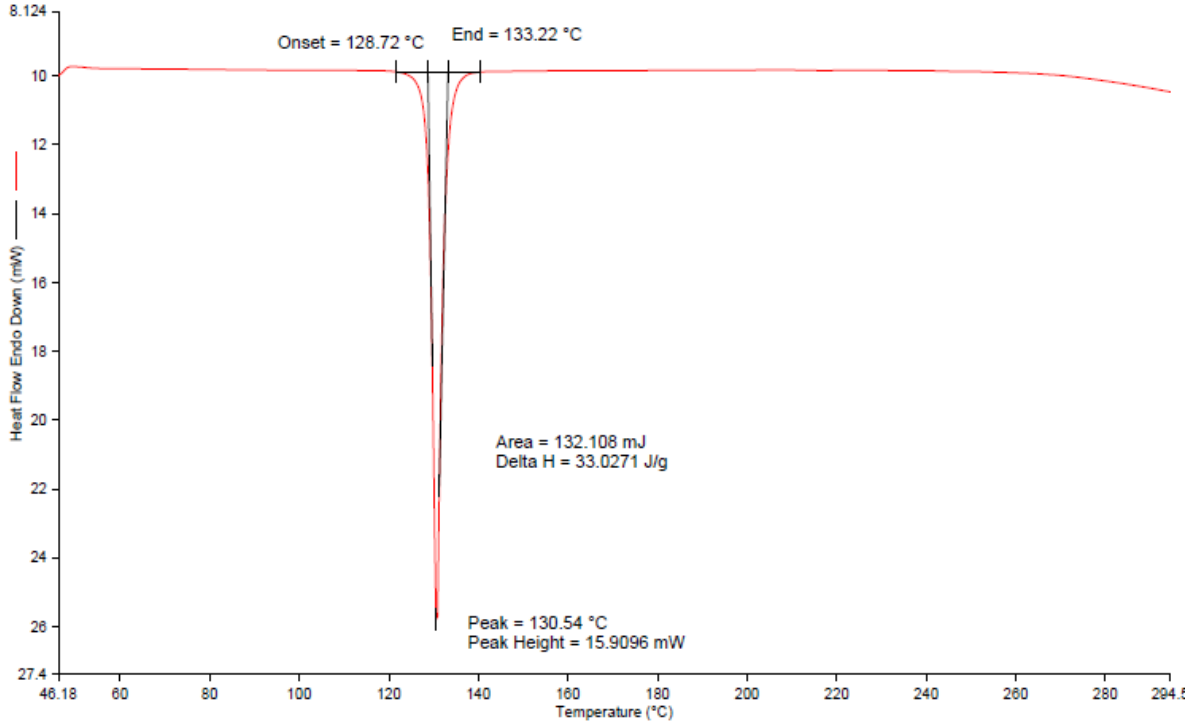

Figure 3: DSC of drug progesterone

Calibration curve of progesterone in methanol

2.5mg was dissolved in 25 ml methanol to prepare 100µg/ml stock solution. From this stock solution samples were withdrawn for the range of 1µg/ml-10µg/ml. The base line was corrected and it was scanned by using UV spectrophotometer by in the range 200-400 nm. Absorbance of drug at different concentrations was calculated and graph was plotted. (table1)

Table 1: Absorbance of different dilutions

S.No.	Concentration (µg/ml)	Absorbance at 241 nm
0	0	0
1.	1	0.051±0.004
2.	2	0.115±0.004
3.	3	0.181±0.001
4.	4	0.248±0.003
5.	5	0.3±0.003
6.	6	0.353±0.003
7.	7	0.411±0.001
8.	8	0.46±0.001
9.	9	0.515±0.002
10.	10	0.574±0.001

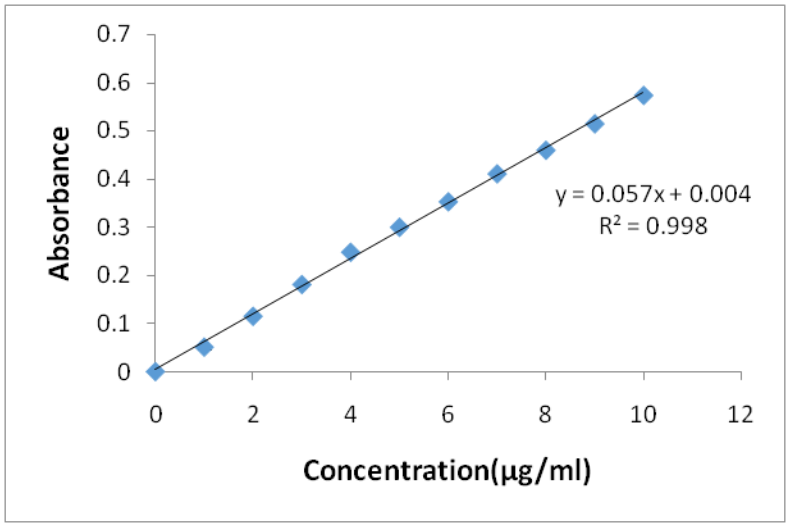

Figure 4: Calibration curve of Progesterone in methanol

Calibration curve of progesterone in 30% ethanolic buffer (pH- 7.4)

2.5mg was dissolved in 25 ml 30% Ethanolic Buffer (pH- 7.4) to prepare 100µg/ml stock solution. From this stock solution samples were withdrawn for the range of 1µg/ml-10µg/ml. The base line was corrected with 30% Ethanolic Buffer (pH-7.4) and it was scanned by using UV spectrophotometer by in the range 200-400 nm. Absorbance of drug at different concentrations was calculated and graph was plotted.

Table 2: Absorbance of different dilutions

S.No.	Concentration (µg/ml)	Absorbance at 247 nm
0	0	0
1.	1	0.044±0.002
2.	2	0.084±0.001
3.	3	0.137±0.003
4.	4	0.196±0.001
5.	5	0.225±0.004
6.	6	0.277±0.002
7.	7	0.321±0.001
8.	8	0.36±0.002
9.	9	0.402±0.001
10.	10	0.431±0.003

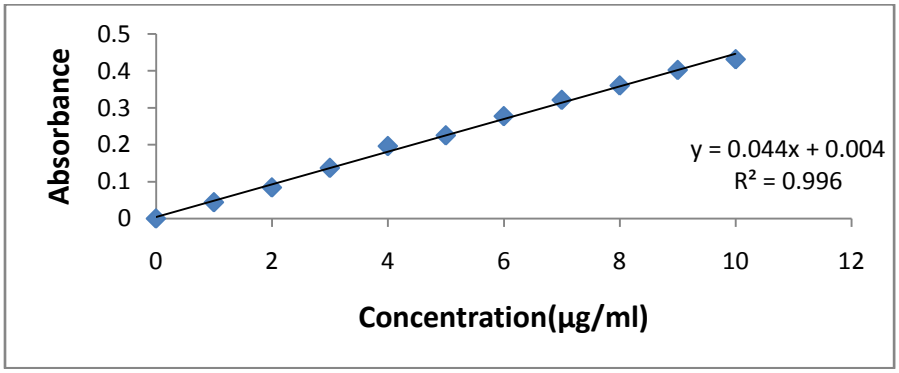

Figure 5: Calibration curve of progesterone in 30% Ethanolic Buffer (pH- 7.4)

Solubility studies

The solubility of Progesterone in various solvents was determined by dissolving excess amount of Progesterone in 3ml of each of the selected solvents in 5ml capacity Stoppard vials separately. Each glass vial was then mixed for 10 min using a vortex mixer. The mixture vials were then kept at 37±1.0 °C in a shaker bath for 72 h to get equilibrium. The equilibrated samples were removed from shaker and centrifuged at 8000 rpm for 15 min. The supernatant was taken and filtered through a 0.45µm membrane filter. The concentration of API was determined in each solvent by UV spectrophotometer by scanning from 200-400nm [6,7].

Optimisation of oil

Table 3: Solubility of progesterone in different Oils

S.No.	Oils	Amount of Progesterone (mg/ml)
1	Ethyl oleate	42.339±0.202
2	IPM	33.216±0.268
3	Myritol	58.01±0.268
4	MCT	51.813±0.202
5	Oleic acid	346.78±1.012895

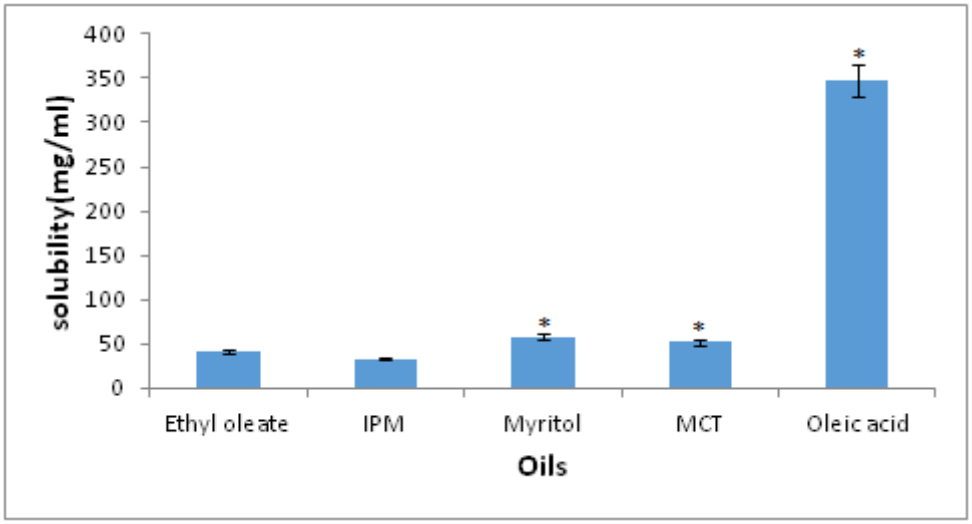

Figure 6: Solubility of Progesterone in different oils

Optimisation of surfactant

Table 4: Solubility of Progesterone in different Surfactants

S. No.	Surfactants	Amount of Progesterone (mg/ml)
1	Tween20	37.31±1.245
2	Tween60	31.052±3.561
3	Cremophor RH40	31.52±1.731
4	Cremophor ELP	11.80±0.325
5	Tween80	279.53±11.68128
6	Koliphor HS 15	53.15 ±3.56

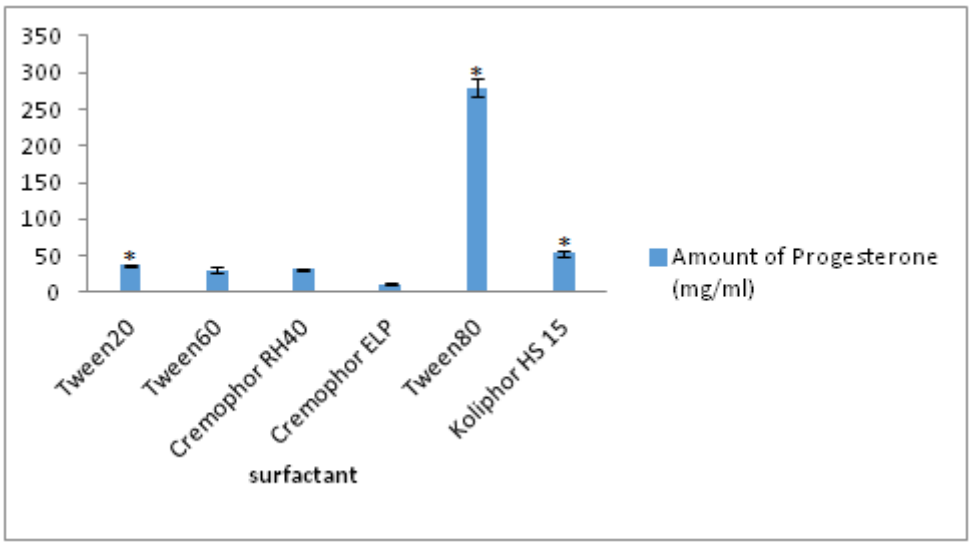

Figure 7: Solubility of Progesterone in different surfactants

Optimisation of co-surfactant

Table 5: Solubility of progesterone in different Co-Surfactants

S.No.	Surfactants	Amount of Progesterone (mg/ml)
1	Ethanol	2345.03±201.82
2	Propanol	2070.17±109.56
3	Propylene Glycol	25.85±0.61612
4	PEG 400	55.73±1.461
5	PEG 200	19.47±1.053
6	Glycerin	0.55±0.01

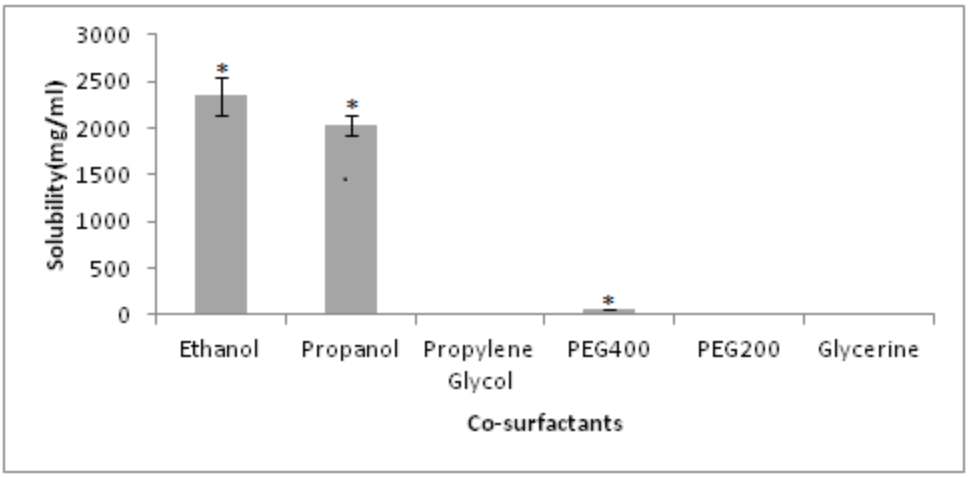

Figure 8: Solubility of Progesterone in different co-surfactants

Results

The U.V. absorption maxima of progesterone was found to be 247nm which is nearly same as reported in literature [9]. Melting point was found to be 127°C which collaborates with the literature value 126°C [10]. Results of studies on melting point and UV absorption maxima of drug suggested the values corroborating with previously reported literature values. Linearity studies indicated that estimation of progesterone between 1.00 µg /ml to 10.00 µg /ml was found to be linear

with regression equation of y = 0.0575x + 0.0041; R^2 = 0.9985 with methanol and y = 0.0442x + 0.0041; R^2 = 0.9968 with ethanol. On the basis of solubility studies two oils with highest solubility (Oleic acid and Myritol), two surfactants (Tween80 and Tween20) and three co-surfactants (Ethanol, Propanol and PEG400) were selected respectively for further formulation studies. The log p value of progesterone was found to be 3.56 which corroborates to the reported literature value 3.87 [11]. Using the proposed analytical technique, further quantization work of prospective *in-vitro* studies of RSM could be carried out for the further formulation development [4].

Discussion

The literature review encompasses the literature reports on various analytical methods of progesterone estimation useful in the study. The corroborating experimental values suggest the bulk drug sample of progesterone obtained was pure. The solubility studies of progesterone suggest that it was well dissolved in oil (Oleic acid and Myritol), two surfactants (Tween80 and Tween20) and three co-surfactants (Ethanol, Propanol and PEG400) for further formulation development [8]. The analytical method developed using UV spectrophotometer is linear and accurate with minimum variation. Although there are reports and publications of either colorimetric and HPLC methods for progesterone estimation, but there is no report or publication corresponding to the intended investigation, development and validation of UV Spectrophotometric method of Progesterone. Therefore it signifies that the proposed investigation is a novel work and the investigation would help in estimation of drug candidate spectrophotometrically in the bulk fluids and dosage forms.

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