

Technical Report:

Effect of Particle Size on the Bioaccessibility of Progesterone from LoxOral® in an *In Vitro* Dynamic Gastrointestinal System



Abstract:

The oral dosing of progesterone has limitations due to poor absorption (low bioavailability) and short half-life of the drug. Progesterone must be bioavailable in order to be effective for hormonal replacement therapy (HRT). For the first time, testing was done to study the bioaccessibility (estimated bioavailability) of oral prolonged release compounded special micronized progesterone in comparison to oral prolonged release compounded milled (non-micronized) progesterone. Testing performed utilized the dynamic *in vitro* TIM-1 gastrointestinal model, which simulates the gastrointestinal tract from the stomach to the small intestine. This TIM-1 system was used to examine a 100 mg capsule of special micronized progesterone compared to a 100 mg capsule of milled (non-micronized) progesterone. Both capsule formulations contained Methocel® E4M to create the prolonged release rate and LoxOral as the excipient base. The special micronized progesterone had a smaller particle size than the milled (non-micronized) progesterone. According to *in vitro* data that estimates bioavailability, results for the special micronized progesterone showed a significantly greater overall bioavailability and a decreased residue deposition in the TIM-1 simulated gastrointestinal model. These results suggest that compounded special micronized progesterone may have significant clinical benefits in hormone replacement therapy (HRT).

Introduction:

Hormonal replacement therapy (HRT) has been used extensively to treat the symptoms caused by decreased estrogen production following menopause, which can include vasomotor symptoms, urogenital atrophy, and mood or sleep disturbances (Ryan & Rosner, 2001). Estrogen therapy is frequently used in HRT and its pro-proliferative effects on the endometrium are counterbalanced by the use of progesterone, which is known to modulate the growth-stimulatory effects of estrogen (Yang *et al.*, 2011). Results from the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial recommend that oral micronized progesterone be the first choice for estradiol opposing therapy in postmenopausal women (Miller *et al.*, 1995; Barret-Connor *et al.*, 1997).

Progesterone is a drug with poor aqueous solubility and therefore has a limited dissolution rate. It can have its absorption and bioavailability significantly altered depending on the physical characteristics of the drug and the vehicle used for oral administration (Hargrove *et al.*, 1989). Several reports have demonstrated that micronization of progesterone facilitates aqueous dissolution in the small intestine (Bolaji *et al.*, 1993), and that the absorption and serum concentration of progesterone are enhanced by micronization (Hargrove *et al.*, 1989). Special micronized progesterone has a smaller particle size than milled (non-micronized) progesterone.

The goal of this study was to determine the bioaccessibility (estimated bioavailability) of compounded oral special micronized progesterone when compared to compounded oral milled (non-micronized) progesterone in a simulated *in vitro* model of the human upper GI tract.

Methodology:

Materials: Special Micronized Progesterone (Lot number C158636, C159277 and C148624) was obtained from PCCA (Houston, TX, USA), as well as the excipients Methocel E4M Premium CR (hypromellose USP, Lot number C155691) and LoxOral (Lot number 1309189). Milled (non-micronized) progesterone (Lot number 89043/B) was obtained from MEDISCA, Inc. (Plattsburgh, NY, USA).

Particle Size Testing: Particle size distribution testing of special micronized progesterone was performed by Intertek Allentown (Allentown, PA, USA) using a Horiba LA-910 laser diffraction instrument. Three lots of special micronized progesterone were analyzed and the mean and median particle size distributions were determined using laser diffraction volume-based calculations. One sample of milled (non-micronized) progesterone was analyzed for particle size distribution by Micrometrics Pharmaceutical Services (Norcross, GA, USA) using a Saturn Digsizer II instrument.

Assessment of Progesterone Bioaccessibility using the TIM-1 System: The bioaccessibility of special micronized progesterone and milled (non-micronized) progesterone was examined using the dynamic, multi-compartmental *in vitro* TIM-1 gastrointestinal model (TNO, Zeist, The Netherlands), which simulates the gastrointestinal (GI) tract from the stomach to the small intestine (Figure 1). The system reproduced very closely the successive GI dynamic conditions of healthy human adults in a fasting state with normal gastric and intestinal secretions. The analysis was performed in duplicate (n=2) at 37°C, as described previously (Dickison *et al.*, 2012). A 100 mg capsule of special micronized progesterone and a 100 mg capsule of milled (non-micronized) progesterone were added to the TIM-1 system per run. Both capsule formulations contained Methocel E4M to create the prolonged release rate and LoxOral as the excipient base. Samples were collected from the stomach, duodenum, jejunum, ileum compartments at 60, 120, 180, 240 and 300 minutes using a semipermeable Spectrum membrane filtration unit with a cut-off of 0.05 µm. The ileum effluent, which simulates fluid containing drug that would enter the colon and would not be available for absorption, was collected each hour for a total of 5 samples per run and was diluted 1:1 with ethanol to dissolve any undissolved progesterone. Upon completion of the experiment, residues remaining in the stomach, duodenum, jejunum and ileum compartments were collected, the compartments washed with ethanol, and the wash fluid was also collected and pooled. Progesterone concentrations in each sample were measured using LC MS and the total amount of progesterone recovered from each compartment was calculated with correction for recovery.

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Results and Discussion:

The bioavailability of progesterone is highly dependent upon particle size, vehicle, and route of administration (Hargrove *et al.*, 1989; Fitzpatrick *et al.*, 1999). Micronization of progesterone is known to increase the accessible surface area of the active pharmaceutical ingredient, allowing for greater absorption within the GI tract (Bolaji *et al.*, 1993), and subsequently greater serum progesterone concentrations and potential enhanced efficacy. The particle size was tested for both forms of progesterone and results confirmed that PCCA special micronized progesterone has an inherently smaller particle size than milled (non-micronized) progesterone (Table 1). The recovery profiles (%) of special micronized progesterone and milled (non-micronized) progesterone in the jejunum and ileum compartments of the TIM-1 system were combined and shown in Figure 2. The overall bioaccessibility of special micronized progesterone was 21.8%, and was significantly higher than that of milled (non-micronized) progesterone, which had an overall bioaccessibility of 1.9% ($P < 0.001$). Both special micronized progesterone and milled (non-micronized) progesterone formulations demonstrated similar levels of progesterone remaining after transit through the TIM-1 system (30 mg and 37 mg progesterone, respectively). However, the total amount of progesterone residue was slightly higher for milled (non-micronized) progesterone (86.7%) than for special micronized progesterone (72.1%) (Figure 3).

The considerably smaller particle size led to a significant increase in the bioaccessibility of special micronized progesterone in a simulated GI tract model in both the jejunum and ileum compartments, as well as significantly increased overall bioaccessibility when compared to the larger particle size of milled (non-micronized) progesterone. Bioaccessibility (the fraction of drug potentially available for small intestinal absorption) can be used to estimate the bioavailability of a drug. This data suggests that special micronized progesterone has a greater bioavailability, and therefore, a greater potential for efficacy, than milled (non-micronized) progesterone.

Table 1: Comparison of Particle Size Between Special Micronized Progesterone and Milled (Non-Micronized) Progesterone

Sample	Mean Particle Size (µm)	D10	D50	D90
Special Micronized Progesterone	5.38	1.92	4.68	9.75
Milled (non-micronized) Progesterone	92.31	34.54	89.87	153.83

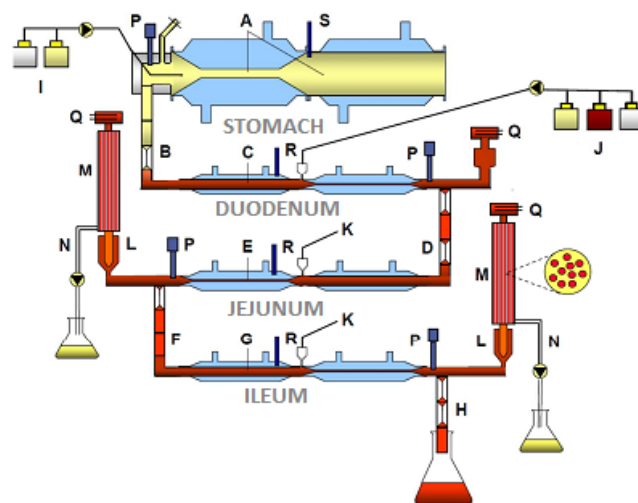


Figure 1. Schematic diagram of the dynamic, multi-compartmental model of the stomach and small intestine (TIM-1): A. stomach compartment; B. pyloric sphincter; C. duodenum compartment; D. peristaltic valve; E. jejunum compartment; F. peristaltic valve; G. ileum compartment; H. ileo-caecal sphincter; I. stomach electrolytes, HCl and enzyme secretion; J. bile, pancreatic juice and bicarbonate secretion; K. jejunum/ ileum electrolyte secretion; L. pre-filter; M. hollow fibre membrane; N. filtrate pump; P. pH electrodes; Q. level sensor; R. temperature sensor; S. pressure sensor.

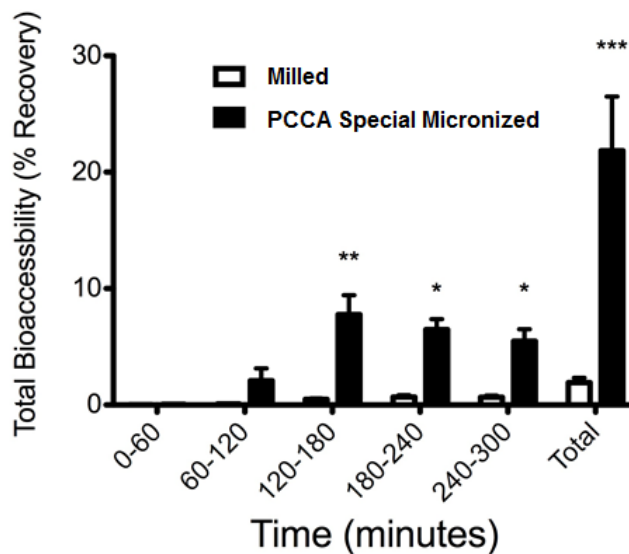


Figure 2. Overall bioaccessibility of oral special micronized progesterone and oral milled (non-micronized) progesterone over time in the TIM-1 simulated GI tract model system. Each of the 100 mg SR progesterone capsules were subjected to the stomach compartment (60 minutes), the duodenum compartment (10 minutes), the jejunum compartment (80 minutes) and the ileum compartment (150 minutes). Samples were withdrawn at 60, 120, 180, 240, 300, and 360 minutes and were assayed for progesterone concentration. The amount of progesterone recovered from each compartment was corrected for recovery and is displayed as mean \pm SD. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

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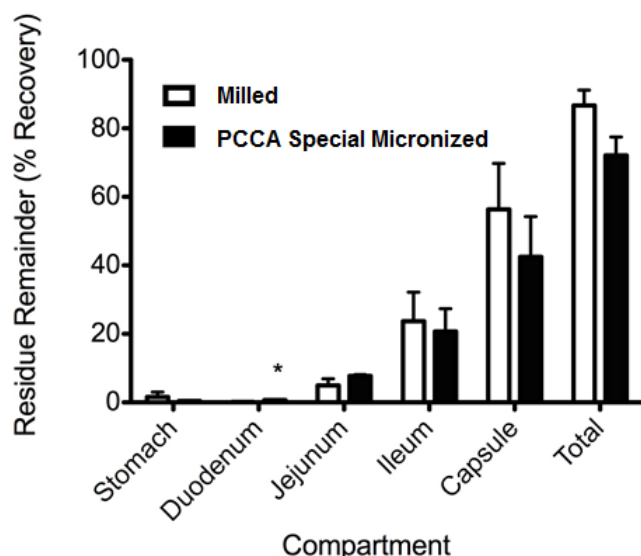


Figure 3. Special micronized progesterone residue and milled (non-micronized) progesterone residue present in specific compartments of the TIM-1 simulated GI tract system after completed TIM-1 run. Residues from each compartment were collected separately upon completion of each TIM-1 run. The amount of remaining undissolved progesterone was measured and was corrected for recovery and is displayed as mean \pm SD. * $P < 0.05$.

Conclusions:

This study provides the first assessment of progesterone bioaccessibility (estimated bioavailability) from a compounded formulation using the dynamic gastrointestinal TIM-1 system. Overall, the results of this study suggest that the ultrafine particle size of PCCA's special micronized progesterone shows an enhanced bioaccessibility profile when compared to that of milled (non-micronized) progesterone in a simulated model of the upper GI tract. These results confirm that oral compounded special micronized progesterone using Methocel E4M to create the prolonged release rate and LoxOral as the excipient is a valuable option for hormone replacement therapy (HRT).

Financial Disclosure: PCCA contracted TNO Triskelion by (Zeist, The Netherlands) to conduct this study. TNO has no proprietary or financial interests in the test products, or equity interest in PCCA, the sponsor of the study.

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