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COMMUNICATION

# Ketoprofen Suppository Dosage Forms: In Vitro Release and In Vivo Absorption Studies in Rabbits

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**ABSTRACT**

*In vitro release of ketoprofen from suppository bases and in vivo absorption in rabbits were studied. Suppositories containing 50 mg of ketoprofen were prepared using theobroma oil, esterified ($c_{10}$–$c_{18}$) fatty acids, and polyethylene glycol 1000 bases. The displacement values of the drug were determined and found to be of the order of theobroma oil > esterified ($c_{10}$–$c_{18}$) fatty acids and polyethylene glycol 1000 bases. The suppository hardness data revealed that the theobroma oil base produced relatively brittle suppositories. Using the USP dissolution method, the release of ketoprofen was observed to be greatest from polyethylene glycol 1000 suppositories. With the dialysis technique, the maximum release of drug was obtained from theobroma oil suppository containing polysorbate 40 at a 6% level. Selected suppository formulations were evaluated for rectal absorption studies in rabbits. The in vivo data showed that the optimum drug absorption took place from the polyethylene glycol 1000 base and theobroma oil formulation containing 6% polysorbate 40.*

## INTRODUCTION

Ketoprofen, a nonsteroidal drug, was first synthesized in 1967 (1). It has been clinically proven to be an effective and potent anti-inflammatory agent with analgesic and antipyretic properties (2,3). It is being used for symptomatic relief of acute and long-term rheumatoid arthritis (4–7), alkylosing spondylitis, and acute gouty arthritis (8–10). However, like other nonsteroidal anti-inflammatory drugs, the oral administration of ketoprofen carries the risks of gastrointestinal irritation, bleeding, nausea and vomiting, and the like (11–17). To minimize these complications, one of the alternate ways of administering such a drug could be the rectal route.

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The previous work (18–21) on the rectal administration of ketoprofen has provided little information as to the effects of the suppository vehicle, the hardness, the melting point range, and the influence of surfactants on the in vitro and in vivo drug release. In this study, ketoprofen suppository dosage forms were investigated using three different bases. These included theobroma oil, esterified ($c_{10}$–$c_{18}$) fatty acids, and polyethylene glycol 1000. Also, synthetic surfactants were incorporated in the suppository formulations to evaluate their effects on the in vitro drug release and in vivo absorption in rabbits.

## EXPERIMENTAL

### Materials

The following materials were obtained from commercial sources: Ketoprofen (material I) (Wyeth Laboratories, Philadelphia, PA), sorbitan monooleate (material II) (ICI Atlas Chemical Corp., Wilmington, DE), polysorbate 40 (material III) (ICI Atlas Chemical Corp., Wilmington, DE), theobroma oil (base A) (Ruger Chemical Company, Irvington, NJ), esterified ($c_{10}$–$c_{18}$) fatty acids (base B) (Hulis American, Piscataway, NJ), polyethylene glycol 1000 (base C) (J. T. Baker Chemical, Phillipsburg, NJ). All other reagents were analytical or reagent grade.

### Equipment

Equipment used included USP dissolution apparatus (Van-Kel Industries, Edison, NJ), USP disintegration apparatus (Erweka, Chemical and Pharmaceutical Co., New York, NY), fracture point testing apparatus (Erweka, Chemical and Pharmaceutical Co., New York, NY), spectrophotometer (Perkin-Elmer, Lambda 4B, UV/VIS Moel, S. Plainfield, NJ), and high-performance liquid chromatography (HPLC) (Water Associate, Model 440, Milford, MA).

### Preparation of Suppositories

Suppository formulations of material I are exhibited in Table 1. These were prepared by the fusion method using a metal mold (22). The displacement values in bases A, B, and C were determined according to a published procedure (23), and the amount of material I required for each suppository formulation was calculated.

### Weight Variation Test

From each formulation series, 20 randomly selected suppository samples were weighed individually, and the average weight and the percentage deviation values were calculated.

### Content Uniformity

Although the USP does not specify the content uniformity test for suppositories, the relative potency of each group of samples was determined. Only samples with a drug content of 100% ± 10% were included in this study.

### Melting Point Range

A narrow melting point range is important for maintaining the shape of the suppository at the ambient storage conditions and for controlling the melting time after insertion into the rectum (24). Each suppository sample was placed in a dialysis tube (15 cm long) and then tied from both ends with a cotton thread. A thermometer was placed in contact with the mass of the sample in each tube and placed in a water bath maintained at 38°C ± 0.5°C. The time required for each sample to melt completely with respect to the temperature was determined.

### Breaking Test (Hardness)

The breaking test was designed to measure the hardness of the suppositories. This was determined by weight in kilograms under which a sample collapsed at a specific temperature by utilizing the fracture point testing apparatus (Erweka, Chemical and Pharmaceutical Co., New York, NY).

### Analytical Methods

#### In Vitro Assay Procedure

Plots of absorbance versus wavelength for solutions of material l in water, methanol, and phosphate buffer (pH 8) solution were developed. The maximum absorbance values were observed at 260 nm for water and phosphate buffer (pH 8) solutions and at 257 nm for methanol solution. Beer's law was followed for 1–20 μg/ml concentrations. The stability of material I in phosphate buffer solution was determined. After 24 hr at 37°C, no loss in potency was noted.

#### In Vivo Assay Procedure

The blood serum samples were analyzed according to a published HPLC method (25).

***Table 1***

*Formulations*

| | % w/w | | | | | | |
|---|---|---|---|---|---|---|---|
| Sample | A | A-IIa | A-IIb | A-IIc | A-IIIa | A-IIIb | A-IIIc |
| A series (theobroma oil) | | | | | | | |
| Ketoprofen | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Sorbitan monooleate | — | 2.0 | 4.0 | 6.0 | — | — | — |
| Polysorbate 40 | — | — | — | — | 2.0 | 4.0 | 6.0 |
| Theobroma oil | 97.5 | 95.5 | 93.5 | 91.5 | 95.5 | 93.5 | 91.5 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | % w/w | | | | | | |
| Sample | B | B-IIa | B-IIb | B-IIc | B-IIIa | B-IIIb | B-IIIc |
| B series esterified ($C_{10}$–$C_{18}$) fatty acids | | | | | | | |
| Ketoprofen | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Sorbitan monooleate | — | 2.0 | 4.0 | 6.0 | — | — | — |
| Polysorbate 40 | — | — | — | — | 2.0 | 4.0 | 6.0 |
| Esterified ($C_{10}$–$C_{18}$) fatty acids | 97.5 | 95.5 | 93.5 | 91.5 | 95.5 | 93.5 | 91.5 |
| Total | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Sample | C (% w/w) | | | | | | |
| Ketoprofen | 2.5 | | | | | | |
| Polyethylene glycol 1000 | 97.5 | | | | | | |
| Total | 100.0 | | | | | | |

## Drug Release Studies

Two different methods were employed for studying the in vitro release of material I from suppository samples. For method A, the USP rotating basket dissolution apparatus was used for the determination of release rates of material I by this method. Each suppository sample was placed in a wire basket and lowered into a beaker containing 900 ml of phosphate (pH 8) buffer solution previously maintained at 37°C ± 0.5°C. The basket was rotated at 100 rpm, and 5-ml samples were withdrawn from the dissolution medium at appropriate time intervals. These were replaced by equal volumes of phosphate buffer solution to compensate for sampling. Each solution was then filtered and assayed for the concentration of material I spectrophotometrically at 260 nm.

For method B, using a dialysis technique, the dialyzing tubes were prepared using dialysis tubing soaked overnight in phosphate buffer (pH 8) solution. After rinsing the tubes, the suppository sample was placed in each tube and tied from both ends with a cotton thread. This was then suspended vertically in a 1000-ml beaker containing 900 ml of phosphate buffer (pH 8) solution maintained at 37°C ± 0.5°C. The diffusion medium was constantly stirred throughout the period of study. At appropriate time intervals, 5-ml samples were withdrawn and replaced with equal volumes of phosphate buffer solution to compensate for sampling. Each solution was then filtered and assayed for the concentration of material I spectrophotometrically at 260 nm.

## In Vivo Studies

Nine healthy white male New Zealand rabbits (2–3 pounds) were randomly divided into three groups. Five different suppository samples, each containing 50 mg of material I, were used rectally in this study. At time zero, each rabbit received the suppository samples of material I as follows: group 1, theobroma oil (sample A) suppository; group 2, polyethylene glycol 1000 (sample C) suppository; and group 3, esterified ($c_{10}$–$c_{18}$) fatty acids (sample B) suppository. Retention of the suppository samples in the rectum was ensured by the use of an appropriate size styrofoam disk taped to the rectum after insertion. After 1 week of washout period, group 1 received sample A-IIIc, and group 2 orally received 50 mg of material I

suspended in water as a control. The blood samples (2 ml) were withdrawn by cardiac puncture at various time intervals up to 6 hr.

## RESULTS AND DISCUSSION

During the manufacture of suppositories, some difficulty is experienced in achieving the exact dosage. This is because the volume of suppositories from a particular mold is uniform, but its weight may vary because the density of the drug usually differs from the density of the base with which the mold is calibrated. Therefore, the displacement values of material I in bases A, B, and C were determined and were found to be $1.06 \pm 0.02$, $1.12 \pm 0.04$ and $0.9 \pm 0.08$, respectively.

The weight variations of 20 randomly selected suppositories containing 50 mg of material I in three different bases were determined and were found to be 1.91 to 2.06 g for base A, 1.90 to 2.05 g for base B, and 1.92 to 2.04 g for base C. All samples met the acceptable limits of the German, Russian, and Nordic pharmacopeias (26). Although the USP does not specify the content uniformity standards for suppositories, these were determined and found to be within $100\% \pm 10\%$ of the labeled amount of material I in all cases.

The appropriate melting point of a suppository ensures its handling and release of the drug after administration in the rectum. This was determined using the USP disintegration apparatus. From the data, it was observed that the time required to disperse suppositories at the temperature 35°C–37°C for sample B was twice as much as with sample A or C.

The breaking load of suppositories of material I in three different bases as a function of temperature was determined. These data were plotted, and almost straight lines were obtained, except for sample A, which gave a steeper curve. This indicates that the sample A suppository was relatively more brittle than that of samples B and C. Also, from the curves, it was possible to view the degree of deformation for each sample.

Using the USP dissolution apparatus, the in vitro release of drug from the suppository samples was determined. The data reveal that the time required to release 100% of the drug from sample C was significantly less than for all other samples evaluated. This could be due to the solubilization of material I in the water-soluble glycol 1000 base. Also, a significant increase in the drug release was observed when synthetic surfactants were included in the fatty bases A and B. In both cases, surfactant III had a more-profound effect on the drug release than surfactant II. This may be attributed to the higher HLB value of surfactant III and its effectiveness in lowering the surface tension during the drug release from these hydrophobic vehicles. Interestingly enough, the diffusion rate constant value was found to be the highest ($27.36 \times 10$ mg%/min) for sample C and the lowest ($0.81 \times 10$ mg%/min) for sample B with the minimum in vitro release of drug. Since sample C is made from a water-soluble base, the USP dissolution method without the diffusion membrane may not give the appropriate drug release profile. Therefore, the dialysis (method B) was employed to study the release of material I from these suppository samples. Using this method, the maximum release of material I was observed with sample A-IIIc compared to all other samples studied. Here again, the presence of surfactant III strongly influenced the drug release and allowed more drug to diffuse through the membrane.

### In Vivo Absorption Studies

Based on the in vitro data, samples A, B, C, and A-IIIc were selected for the in vivo rectal absorption studies in rabbits. An equal oral dose of material I was used as a control. The plasma concentrations of material I versus time were determined for up to 360 min after each dose. From the in vivo data, it was observed that the highest peak of the curve was obtained with the control at 4.13 μg/ml, followed by sample C at 3.88 μg/ml, sample A-IIIc at 3.71 μg/ml, and sample A at 3.16 μg/ml. The time for the peak value was found to be 60 min for all samples except sample B, which was observed to be 120 min. The area under the curve (AUC) values for material I were calculated as 923.70 μg/ml for the control, 663.45 μg/ml for sample A, 584.70 μg/ml for sample B, 886.05 μg/ml for sample C, and 728.25 μg/ml for sample A-IIIc. Also, the average concentrations of material I in rabbits were found to be 71.83%, 63.30%, 95.92%, and 78.84% from samples A, B, C, and A-IIIc, respectively. These data indicate that sufficiently high systemic absorption of material I took place via suppository samples C and A-IIIc, and it remained minimum from sample B.

The peak plasma concentration, peak time, and the AUC data were statistically analyzed using the *t* test, and the results are exhibited in Table 2. From this, it is observed that there are no significant differences in the peak concentrations of the suppository samples and the control. However, the times for the peak plasma concentration data show a significant difference between suppository sample B and the control. For the AUC, no significant differences between samples C and the control or between sample A-IIIc and the control were observed.

***Table 2***

*Statistical Evaluation of the Serum Data Using Student* t *Test*

| Sample | Average of Peak Serum Concentration Versus Time Curve (μg/ml) | Time of Peak Value of Serum Concentration Versus Time Curve (min) | Average of AUC (0–360 min) (μg/ml) |
|---|---|---|---|
| A and control | n.s. | n.s. | s |
| | $p > .025$ | $p > .025$ | $p > .025$ |
| B and control | n.s. | s | s |
| | $p > .025$ | $p > .025$ | $p > .025$ |
| C and control | n.s. | n.s. | n.s. |
| | $p > .025$ | $p > .025$ | $p > .025$ |
| A and A-IIIc | n.s. | n.s. | n.s. |
| | $p > .025$ | $p > .025$ | $p > .025$ |

s = significantly different at .05 level; n.s. = not significantly different at .05 level; AUC = area under the curve.

The results of this investigation indicate that ketoprofen was well absorbed rectally in rabbits from samples C and A-IIIc. The in vitro data served well in the selection of samples for in vivo evaluations. Although a clear advantage was observed using polyethylene glycol 1000 as a suppository base, a combination of theobroma oil and polysorbate 40 (sample A-IIIc) may be a suitable choice when a fatty vehicle is desirable.

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