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COMMUNICATIONS

A New Technique for Determining *In Vitro* Release Rates of Drugs From Creams

Keyphrases ☐ Dosage forms, topical—new technique for determining *in vitro* release rates of drugs from creams ☐ Release rate, *in vitro*—new technique for determination, drugs from creams

To the Editor:

Within about the last 10 years, dissolution testing has become recognized as one of the most useful methods for evaluating tablets and capsules. In fact, such testing has all but supplanted the traditional disintegration test. Equipment and procedures for measuring the rate and extent of drug dissolution are now becoming standardized as a result of intensive research and, necessarily, some compromises.

No such standardization appears to be occurring, however, with the equipment or procedures used for testing the release of drugs from topical dosage forms (*i.e.*, creams, ointments, gels, suppositories, *etc.*). Many investigators have done extensive research on the release of drugs from such carriers, but it seems that, almost without exception, each used a unique method for presenting the drug to some receptor phase. The reason for this may be that each was faced with unique problems with regard to the formulations.

We present here a convenient and versatile technique for the *in vitro* testing of the release of drugs from creams or ointments. This technique could be used for a wide variety of vehicles, although it was developed specifically for cream formulations which are oil in water emulsions containing 0.01% estradiol.

The ideal procedure would be one in which the sample is in direct contact with the receptor phase, because barriers used to isolate the sample from that phase have a potential leveling effect on the rate of appearance of drug (in the receptor phase). Thus, the absence of barriers should maximize the probability of measuring differences between creams which differ only slightly in their drug-release characteristics. Therefore, we first tried filling shallow cups with the cream and immersing these in water at 37° in a fashion similar to that reported previously (1). This was attempted with a variety of cups of different dimensions supported upright or inverted in the receptor phase (water). These attempts failed because the cream swelled and eventually sloughed into the water. We noted also that the creams were no longer homogeneous, *i.e.*, the first few millimeters of sample nearest the water were physically different from the bulk of the sample before the end of the test time. In addition, samples were necessarily so large (a few grams) that only a small fraction of the total estradiol was near the surface where it could be expected to be released in a reasonable length of time.

Attempts were made to isolate the cream samples from the receptor phase using semipermeable membranes. Two membranes were tested, dialysis tubing and filter paper, in procedures similar to those reported previously (2). When these barriers were used, drug appeared in the receptor phase more slowly than when they were not. Although the problems associated with sample swelling and subsequent sloughing could be alleviated by using these barriers, this approach was abandoned because of the effects on the rate of appearance of drug in the receptor phase.

With these results in mind, we developed a simple technique which allows direct contact between the cream sample and the receptor phase (water) and which eliminates or minimizes sample sloughing. Cream samples are spread into the interstices of an 80-mesh stainless steel screen. The samples prepared in this way can be submerged in gently stirred water for long periods of time. The equipment and procedure are as follows.

A number of appropriately sized pieces of stainless steel screen were first prepared by cutting 7.5 × 7.5-cm squares and removing ~1 cm from each corner (Fig. 1).

Each screen was covered on both sides along the top edge with a piece of 2.54 cm wide masking tape such that 30 cm^2

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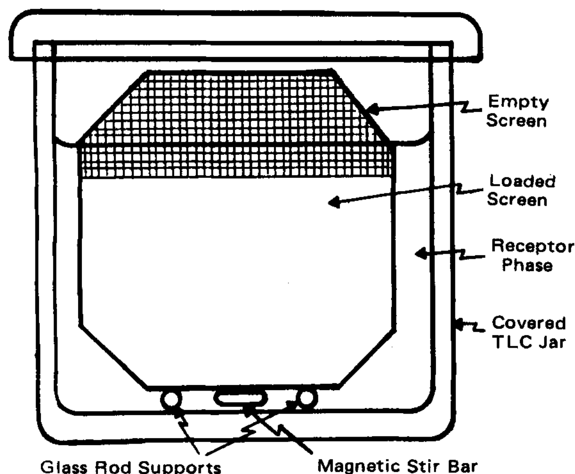

Figure 1—*The apparatus used to evaluate the* in vitro *release of estradiol from creams. The rectangular TLC developing jar normally is partially immersed in a constant temperature bath. A magnetic stirrer located beneath the constant temperature bath rotates the stir bar.*

of screen remained exposed. The taped screen was then weighed. Cream was smeared liberally into the mesh using a spatula, and excess cream was then wiped rapidly from the surface by placing the loaded screen between two disposable paper towels, pressing the towels firmly together, and pulling the screen out. After the screen with its sample was reweighed, the tape was removed and the screen was placed vertically into 120 ml of degassed water held at 37° in an 8 × 8 × 3-cm covered rectangular TLC developing jar. Small glass rods at the bottom of the jar held the screen slightly off the bottom and allowed a micromagnetic stir bar to spin fast enough (~300 rpm) just below the screen to gently mix the medium, yet not dislodge the cream from the screen. At the end of the test period, the screen with cream sample was removed from the water. The drug released into the water was recovered using a small C_{18} extraction column[1] and then analyzed by HPLC on an analytical C_{18} column using an acetonitrile–water mobile phase in a fashion similar to that reported previously (3) for dienestrol.

Figure 2 illustrates the results obtained using this procedure for a number of experimental formulations of a 0.01% estradiol cream. These formulations were alike quantitatively but differed in their processing during manufacture. These results point out a number of features of this method. First, samples can be exposed to the receptor phase for any convenient length of time; thus, a release profile can be constructed. Second, the results are easily interpreted, since they can be expressed in terms of a percentage of the total amount of drug available for release. This is possible because large (thick) samples are not necessary and the surface area of the small (~300 mg), thin sample exposed directly to the water is maximized. (The interstitial spaces comprise ~35% of the total surface area of an 80-mesh screen; thus, when both sides of the screen are taken into account, the exposed surface area of the cream is ~21 cm^2.) Third, the absence of rate-limiting or rate-influencing barriers allows subtle differences in release characteristics to be identified.

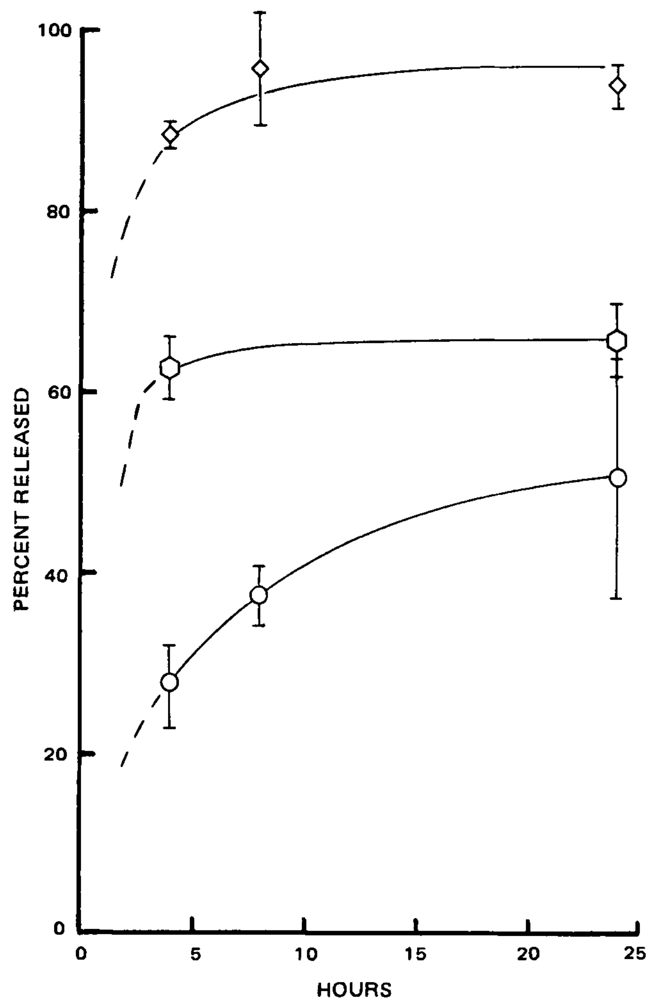

Figure 2—In vitro *release profiles for three formulations of 0.01% estradiol cream. The profile for formulation A (O) depicts the average of the data given for the three batches in Table I. The profiles for B (O) and C (◊) represent single batches, and each point is the average of three to four separate tests. The magnitude of 1 SD is also indicated at each point.*

The results illustrated in Fig. 2 are the averages of a number of trials. The individual values for three batches of one of the formulations are given in Table I. Each value

Table I—Percent Estradiol Released from Three Batches of an Experimental Formulation [a]

	Batch 1	Batch 2	Batch 3
4 hr	33.4	19.4	27.0
	30.2	28.4	28.6
	27.8	28.8	—
Average	30.5	25.5	27.8
SD	2.9	5.3	1.1
8 hr	40.7	—	40.1
	37.4	—	38.9
	31.5	—	37.1
Average	36.9	—	38.8
SD	3.9	—	1.4
24 hr	39.3	64.7	62.9
	58.1	55.8	51.6
	48.7	51.3	55.6
	46.3	54.2	55.2
	51.6	57.7	63.6
	—	—	53.2
Average	49.2	56.7	57.0
SD	6.3	5.0	5.0

[a] Formulation A in Fig. 2.

[1] Sep Pak, Waters Associates, Milford, Mass.

in this table represents the amount released from a separate test sample. The values demonstrate the typical reproducibility obtained with this procedure.

This technique could be useful for testing a wide variety of topical formulations where direct contact with the receptor phase is desired. The screen size and configuration could easily be altered to accommodate different types of vehicles, release rates, and concentrations of drugs. This technique may be unsuitable where the drug or vehicle interacts with stainless steel. Such instances are expected to be rare, however, and normally the technique should allow investigators to routinely and uniformly prepare and test small samples with high surface area to weight ratios.

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Comparison of Chromatographic and Spectrophotometric Analysis of Indocyanine Green in Plasma Following Administration of Multiple Doses to Humans

Keyphrasess ◻ High-pressure liquid chromatography—comparison with spectrophotometric analysis of indocyanine green in plasma following multiple dose administration, humans ◻ Spectrophotometry—comparison with high-pressure liquid chromatographic analysis of indocyanine green following multiple dose administration, humans ◻ Indocyanine green—comparison of spectrophotometric and high-pressure liquid chromatographic analysis following multiple dose administration, humans

To the Editor:

Following the administration of small doses (0.5 mg/kg), indocyanine green (I) is highly extracted from blood by the liver (1). Compound I has been used extensively, therefore, to evaluate hepatic function (2, 3) and to estimate hepatic blood flow in humans and laboratory animals (4, 5). Numerous reports have suggested that I is not metabolized in any species (4, 6, 7). Thus, simple spectrophotometric assays (*i.e.*, typically the determination of the absorbance at ~800 nm of plasma samples diluted with distilled water) have been used extensively to estimate the concentration of I in biological fluids. However, it has been reported recently that spectrophotometric and high-pressure liquid chromatographic (HPLC) assays yield radically different estimates of the concentration of I in plasma following the administration of a large dose (25.6 mg/kg) to the rabbit (8). Estimates of the plasma concentration of I ≥30 min postdose were found to be significantly lower using the HPLC assay (almost an order of magnitude lower at many time points). Thus, the total body clearance of I in the rabbit, calculated on the basis of plasma concentrations determined by HPLC, was much higher than that calculated from the spectrophotometric assay results. These investigators postulated that this discrepancy was due to a previously unidentified metabolite. If humans also metabolize I to a compound that interferes with the classical spectrophotometric methods, the clearance value of I based on these assays will not provide a reliable estimate of hepatic blood flow. Because of the potential implications of this assay discrepancy, we compared the spectrophotometric and HPLC assays for I using plasma samples from humans.

Two healthy male subjects (25 and 35 years of age) each received five intravenous bolus doses (0.5 mg/kg each) of I at ~1-hr intervals. Blood samples (5 ml) were collected into heparinized evacuated blood collection tubes[1] prior to and at 1, 3, 5, 7, 9, 11, and 15 min after the first, third, and fifth doses. Plasma was separated and stored at −20° until analyzed (within 36 hr). Previous studies have demonstrated the stability of I under these conditions (8), and preliminary work in our laboratory confirmed these findings.

After the addition of 1.0 μg of diazepam (internal standard) in 100 μl of methanol, proteins were precipitated with acetonitrile (1 ml plasma–1 ml acetonitrile) and the sample was centrifuged. The supernatant was then analyzed by spectrophotometric and HPLC methods. Samples were analyzed using a double beam spectrophotometer[2] equipped with a red-sensitive photomultiplier. The peak absorbance of I in the supernatant was found to occur at 786 nm, and this wavelength was chosen for analysis of all samples. The HPLC method used was that described recently (8) with the following modifications: Dual wavelength (254 and 650 nm)[3] monitoring was employed utilizing two detectors[4,5] in series. Absorbance at 650 nm was examined, since any degradation or metabolic products of I which contained an unaltered chromophore would be expected to absorb at a high wavelength similar to that of the parent compound. The high wavelength monitoring was achieved with a tungsten lamp in a variable wavelength monochromator[4]. A wavelength shorter than the maximum for absorbance by I was utilized in order to maintain an acceptable signal–noise ratio. Chromatography was performed on a reversed-phase column[6]. Of the various mobile phases studied previously (8), one composed of 0.05 *M* KH_2PO_4·Na_2HPO_4 (pH 6.0)–acetonitrile–methanol (50:47:3) was found to be most satisfactory. The peak height ratio was determined at both wavelengths relative to the diazepam peak height at 254 nm. Calibration curves were obtained in each subject's plasma for each analytical method.

[1] Vacutainer, Becton-Dickinson and Co., Rutherford, N.J.
[2] Model 25 Spectrophotometer, Beckman Instruments, Inc., Fullerton, Calif.
[3] Monitoring of column eluent was performed at 254 nm instead of 225 nm as in Ref. 8. This was advantageous since human plasma samples extracted as described above frequently contained a compound that had a retention time very similar to that of I and absorbed at 225 nm but not at 254 nm.
[4] Model 770, Schoeffel Instrument Corp., Westwood, N.J.
[5] Model 440, Waters Associates, Milford, Mass.
[6] μBondapak C_{18}, Waters Associates, Milford, Mass.

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