# Noninvasive *in Vivo* Percutaneous Absorption Measurements Using X-Ray Fluorescence

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X-ray fluorescence (XRF) has been used to determine *in vivo* the percutaneous absorption of 5-iodouracil (5IU) in dimethyl sulfoxide (DMSO) on female Sprague-Dawley rats. An average absorption rate constant of 122 ± 34 μg/cm²-hr was obtained from the XRF measurements on four rats. A comparative study was performed with radiolabeled ($^{125}$I) 5IU in which the absorption rate constant was determined to be 126 ± 20 μg/cm²-hr. The XRF system described provides a simple, noninvasive means of measuring the percutaneous absorption rate of select compounds by the surface disappearance method.

KEY WORDS: percutaneous absorption; topical delivery; X-ray fluorescence spectrometry; 5-iodouracil.

## INTRODUCTION

*In vivo* percutaneous absorption can be determined by the indirect method of measuring the amount of radioactivity in the blood, feces, or urine following the topical and intravenous application of a radiolabeled compound (1). However, the radiotracer method has been limited to a few studies (2) because of difficulties and expense encountered in the use of radiolabeled compounds *in vivo*.

Several other methods for studying *in vivo* percutaneous absorption include monitoring a specific pharmacologic response to a topically applied compound (3); a single-point or periodic measurement of the disappearance of a "compound specific" signal from the skin surface (4); the direct measurement of the drug concentration in the blood following topical application (5); and the stripping method which predicts, by linear extrapolation, the percutaneous absorption of a compound by measuring its concentration in the stratum corneum at the end of a short application time (6). A detailed discussion of the relative merits of these techniques is given in Ref. 7.

In this paper we introduce the use of X-ray fluorescence (XRF) as a simple, noninvasive technique for monitoring percutaneous absorption *in vivo* by the surface disappearance method. XRF is an elemental analysis technique that has been applied to a variety of medical tracer studies (8) and has been used to determine the iodine distribution in the thyroid gland. In XRF, the elements in a sample are excited to emit their characteristic X rays by exposing the sample to a source of low-energy ionizing radiation. In our case, the 60-keV gamma rays and 17-keV neptunium (Np) L X-rays from a 241-americium isotopic source were used as the excitation source. The energy of the emitted X ray identifies the element, and the number of X rays of a given energy is a measure of the concentration of that element in the sample matrix. Ideally, XRF could be used to measure the absorption rate of any compound that contains elements that are found in only trace levels in the skin. Practically, the skin matrix is such that XRF is well suited for measuring the percutaneous absorption of compounds that contain (or can be labeled with) elements with an atomic number greater than or equal to 20. In this work we have demonstrated the viability of using XRF by measuring the percutaneous absorption of 5-iodouracil (5IU) in albino female Sprague-Dawley rats. The surface disappearance measurements were performed by monitoring the area of the fluoresced iodine $K_\alpha$ peak from 5IU as a function of time.

## MATERIALS AND METHODS

### Feasibility Study

Paraffin wax (Boyle-Midway, New York) was used as a phantom model of human skin to test the feasibility of using XRF for measuring percutaneous absorption. Various amounts of 5-chloro-7-iodo-8-hydroxyquinoline (Sigma, St. Louis, MO) were added to melted wax in a glass beaker. Appropriate volumes of the resulting mixtures were poured into 15-cm² flat, plastic petri dishes and allowed to cool overnight to produce 0.5-, 1-, 2-, and 3-mm-thick paraffin/drug samples. A membrane cutter was then used to prepare 3-cm-diameter samples from areas in the large disks that displayed no air bubbles. Uniform thickness of the 3-cm disks was confirmed by measurement with calipers. For each thickness, a set of eight disks was prepared with drug concentrations of 3, 15, 30, 150, 300, 500, 750, and 1000 μg/cm².

The 60-keV gamma rays from a sealed (stainless-steel window) 200-mCi $^{241}$Am point source (Amersham, Arlington Heights, IL) were used as the fluorescence source. A 30-mm² Si(Li) X-ray detector (Canberra, Meriden, CT) with a full-width half-maximum (FWHM) resolution of 160 eV (Mn $K_\alpha$) was used to measure fluoresced X-rays. Each phantom disk was secured to a cardboard sample holder with cellophane tape. The point source and X-ray detector were each placed 3 cm from the sample holder at an angle of +75° and −75° with respect to the phantom disk surface. A 2-mm-thick Pb shield was placed between the detector and the XRF excitation source. The 30–1000 μg/cm² samples were fluoresced for 5 min, while the 3 and 15 μg/cm² samples were fluoresced for 20 min. The peak areas in the XRF spectra were determined using the ASAP basic spectroscopy package (Canberra, Meriden, CT).

### *In Vivo* Study

A schematic diagram of the XRF system employed in the *in vivo* absorption studies is shown in Fig. 1. The 60-keV gamma rays and 17-keV Np L X rays from a sealed (Be

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window) 500-mCi $^{241}$Am annular source (Amersham, Arlington Heights, IL) were used as the fluorescence source. A 30-mm$^2$ Si(Li) X-ray detector (Princeton Gamma-Tech, Princeton, NJ) with a FWHM resolution of 165 eV (Mn $K_\alpha$) was used for the XRF measurements. The detector is mounted vertically in a tripod assembly with the end cap pointing down; the end of the detector is 20 cm from the bottom of the tripod. The lead annular source holder, which is attached to the end cap of the detector (Fig. 1), is designed in such a way that the XRF source irradiates an area of 3.1 cm$^2$ on a sample that is placed 2.0 cm from the holder. The radiation dose from the $^{241}$Am source at this distance is 100 mrem/hr.

5-Iodouracil (5IU) (Aldrich, Milwaukee, WI) was dissolved in dimethyl sulfoxide (DMSO) (Aldrich, Milwaukee, WI). Three concentrations were used in the study: 82, 157, and 220 mg of 5IU in 1 ml of DMSO. Female Sprague–Dawley albino rats (Harlan, Indianapolis, IN) were anesthetized with 1 ml of a 1:10 Nembutal:saline solution i.p. and 0.25 ml Ketoset i.m. Each rat was then prepared by shaving a small area around the scapular region, first with electric clippers and then with a disposable razor. A 2-cm-diameter, 7-mm-thick plastic ring was applied to this area with surgical adhesive (Stomahesive, Squibb, Princeton, NJ). The rat was then placed on a plastic platform, secured with cling gauze wrap, and placed with the prepared site 2 cm from the front surface of the XRF source holder (Fig. 1). A 5-min XRF spectrum was collected for background subtraction. The rat was then removed from the XRF system and a second plastic ring (1 cm diameter, 8 mm thick) was centered inside the first and secured with adhesive. Forty microliters of the 5IU drug solution was pipetted into the smaller ring and allowed to absorb for 15 min. The smaller ring was then removed, the rat repositioned in the XRF system, and a 5-min fluorescence spectrum was accumulated.

The absorption of the 5IU solution was followed by periodically accumulating a 5-min XRF spectrum of the applied site over a maximum period of 48 hr. Between each XRF measurement, the rat was removed from the XRF system and the plastic platform and allowed to recover in the cage. During this time, the drug site was protected by wrapping the site with cling gauze and covering with tape. After the initial XRF measurement, the rat was reanesthetized before being placed back in the XRF system.

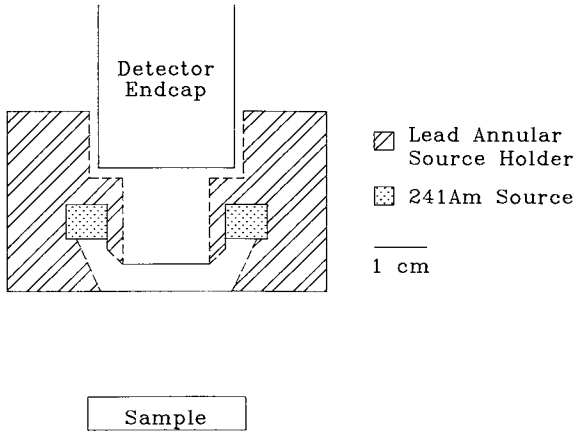


Fig. 1. The XRF system used in the *in vivo* percutaneous absorption measurements.

### *In Vivo* Gamma Camera Study

$^{125}$I-Radiolabeled iodouracil was prepared by dissolving 1 mg of uracil (Sigma, St. Louis, MO) in 1 ml of a pH 7.4 phosphate buffer solution (PBS). One hundred microliters of this solution was diluted with 900 μl of PBS. Three hundred microcuries of $^{125}$I and 3 iodobeads (Pierce, Rockford, IL) were then added to the dilute solution and the resulting mixture was agitated for 10 min. Three extractions of the solution with 10 ml of ethyl acetate were then performed. A 5-hr interval was allotted between each extraction. The ethyl acetate extracts were combined and the ethyl acetate was evaporated under nitrogen gas over a 24-hr period. The resulting product was dissolved in 1 ml of DMSO and confirmed by thin-layer chromatography. The radiolabeled iodouracil was recollected from the silica plate by extraction with ethyl acetate.

The absorption of $^{125}$I-5IU was determined by applying 40 μl (25 μCi) of a $^{125}$I-5IU solution (62 mg 5IU/ml DMSO) to the shaved back of a rat prepared as previously described. Following administration of the $^{125}$I-5IU solution, the rat was positioned beneath the head of a Siemans Digitrac 37 Gamma Camera (Siemans Gammasonics, Des Plaines, IL) that had been fitted with a low-energy parallel-hole collimator. Five-minute static images were accumulated at 0, 24, and 36 hr postadministration using the cardiac Medical Systems ACE data acquisition program. In order to determine the rate of absorption, a region of interest was selected around the site of administration, and the counts in that region were determined for each time point after background subtraction.

## RESULTS AND DISCUSSION

### Feasibility Study

The iodine $K_\alpha$ fluorescence yield from the 3-mm-thick paraffin phantoms is plotted against the areal concentration of the 5-chloro-7-iodo-8-hydroxyquinoline in Fig. 2. The fluorescence yield is simply the area of the X-ray peak divided by the count time. Similar results were obtained for the other phantom series. The results for all four thicknesses are summarized in Table I. It is clear from these data that the iodine fluorescence yield provides a signal that is linear over the areal concentration and thickness ranges examined. The thickness range of the phantoms was chosen to be representative of the range expected for skin and the upper areal concentration of 1 mg/cm$^2$ was chosen to be representative of the typical initial concentration of a topically applied compound.

The increase in fluorescence yield (slopes in Table I) with decreasing phantom thickness can be attributed chiefly to the decreased attenuation of the iodine $K_\alpha$ X rays in the thinner paraffin samples. This variation demonstrates that the XRF technique is not a "true" surface analysis technique; i.e., the signal does not disappear when the compound leaves the skin surface. Instead, the decrease in the fluorescence yield with time will be due to both the attenuation of the X-ray yield as the compound and/or marker atom

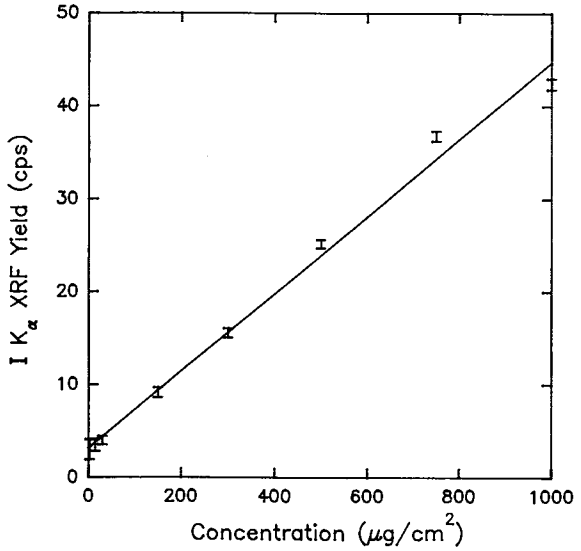


Fig. 2. The iodine $K_\alpha$ fluorescence yield (cps) from 5-chloro-7-iodo-8-hydroxyquinoline in the 3-mm-thick paraffin phantoms.

metabolite(s) migrates through the skin and the decrease in the X-ray yield as the compound and/or marker atom metabolite(s) is taken up by the blood and lymph vessels. If absorption is defined as the combination of permeation and resorption, this difference will have no affect upon absorption rate measurements at a given site. The difference does, however, imply that caution should be exercised when comparing absorption rates from different sites as any variation may result simply from a change in fluorescence yield with skin thickness. The magnitude of any such site-to-site variations will increase as the energy of the X ray, or corresponding atomic number of the marker atom, decreases.

### *In Vivo* Study

The *in vivo* experiments were performed with 5IU because of the slow absorption rate of 5-chloro-7-iodo-8-hydroxyquinoline (9). An example of an XRF spectrum of the skin area immediately following application of the 5IU solution is given in Fig. 3A. The large background, which is present in all the XRF spectra, is due to the coherent and incoherent scattering of the excitation photons from the rat. It is corrected for by subtracting the initial "background" spectrum of the subject from each subsequent fluorescence spectrum. The corresponding background corrected spectrum is shown in Fig. 3B. No dead-time correction is required in the background subtraction procedure, as the dead time of the XRF counting system for a given animal remained constant.

**Table I.** The Iodine $K_\alpha$ Fluorescence Yields from 5-Chloro-7-iodo-8-hydroxyquinoline in Paraffin Phantoms[a]

| Thickness (mm) | Slope [cps/($\mu g/cm^2$)] | Intercept (cps) | Correlation coefficient | Norm |
|---|---|---|---|---|
| 3 | 41 ± 1 | 3.0 ± 0.5 | 0.996 | 2.0 |
| 2 | 42 ± 3 | 3.5 ± 1.8 | 0.991 | 3.1 |
| 1 | 48 ± 2 | 1.6 ± 1.1 | 0.993 | 2.8 |
| 0.5 | 56 ± 3 | 2.3 ± 1.4 | 0.991 | 3.3 |

[a] The lines were fit to a plot of the fluorescence yield (cps) versus areal concentration of the drug.

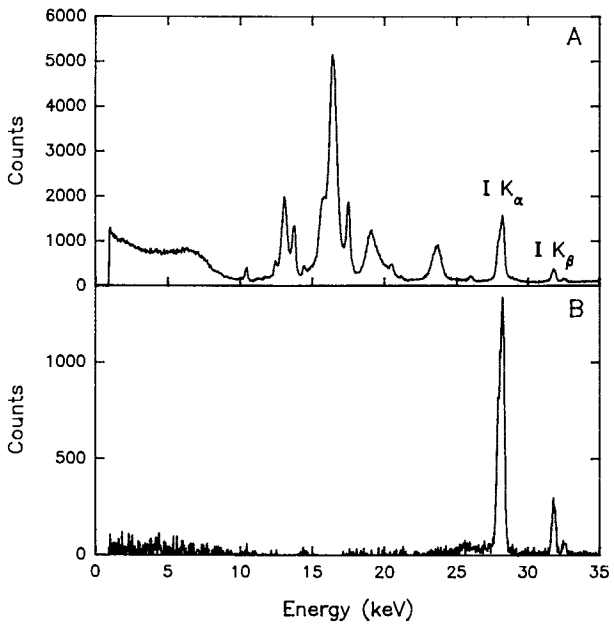


Fig. 3. The original (A) and background-subtracted (B) XRF spectra of the skin of a rat immediately following the topical application of 11.2 mg/cm$^2$ of 5-iodouracil.

The iodine $K_\alpha$ fluorescence yield in the background-corrected spectra from rat number 2 is plotted against time in Fig. 4. This animal received an initial topical application of 4.2 mg/cm$^2$ of 5IU. The data in Fig. 4 indicate that the absorption of the 5IU/DMSO solution follows zero-order kinetics with an absorption rate constant, $k_0$, of 152 $\mu g/cm^2$-hr. The rate constant is obtained from

$$k_0 = \left(\frac{A_0}{FY_0}\right) * m$$

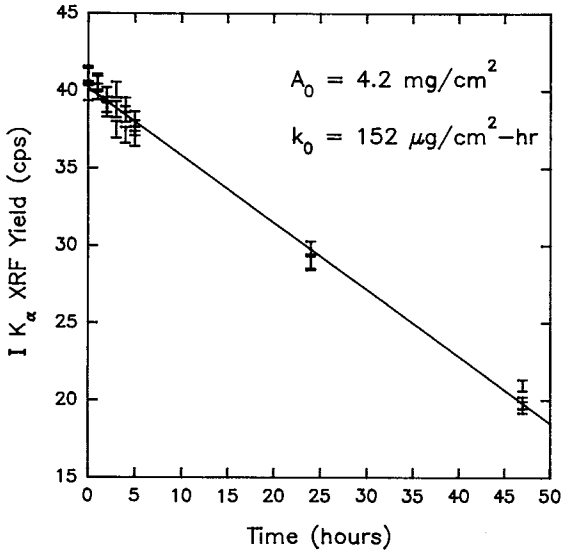


Fig. 4. The variation of the iodine $K_\alpha$ fluorescence yield with time from rat number 2.

where $A_0$ is the initial areal concentration of 5IU, $FY_0$ is the iodine $K_\alpha$ fluorescence yield at time $t = 0$, and $m$ is the slope of the line fit to the data.

The absorption rates obtained from four rats by the XRF method are summarized in Table II. These results yield an average absorption rate of 122 ± 34 μg/cm²-hr for 5IU in DMSO. Because the variation in the measured absorption rates is comparable to that observed in other multiple subject percutaneous absorption studies (10), we attribute the spread in $k_0$ to interanimal variations.

### *In Vivo* Gamma Camera Study

Because no literature value for the percutaneous absorption of 5IU in DMSO could be located, the absorption of 5IU was determined using an alternate technique. A gamma camera was used to follow the surface disappearance of radiolabeled 5IU. The absorption rate was determined by monitoring the decrease in the intensity (counts in the region of interest) of the 35-keV gamma ray with time. The results of the experiment yield a rate constant ($k_0$) of 126 ± 20 μg/cm²-hr. This value is in good agreement with the XRF results.

In addition to checking the XRF absorption rate, the gamma camera experiment was also used to investigate the lateral diffusion of 5IU on the skin. Erroneous results would be obtained in the XRF measurements if the drug and/or marker atom metabolites moved laterally out of the 3.1-cm² area subtended by the XRF fluorescence system. No radioactivity was detected above background outside this area during the 36-hr measurement.

## CONCLUSION

The viability of using XRF as a simple, noninvasive means to measure percutaneous absorption by the surface disappearance method has been demonstrated. The chief advantage of the technique is that it does not rely upon the use of radiolabeled compounds. It does, however, require that the subject be exposed to an external localized radiation dose. In our system, this dose amounts to 8 mrem per each 5-min XRF measurement. For perspective, an individual receives 200 to 1000 mrem for a single dental X ray and the recommended occupational exposure limit for the skin is 15 rem per year (11).

The practical application of the method will depend upon the concentration-dependent fluorescence yields of the XRF system employed. An estimate of the XRF yields for our system is given in Table III. Because the fluorescence yields are directly proportional to the areal concentration, the values given in Table III can be used to determine the concentration ranges that can be examined with the XRF system. For example, consider a compound that contains bromine as the marker atom. The initial fluorescence yield $FY_0$ in our system for this compound would be equal to

$$FY_0 = W * A_0 * 240$$

where $W$ is the weight percentage of bromide in the compound and $A_0$ is the initial areal concentration of the compound applied to the skin. Because the statistical uncertainty of the X-ray fluorescence yield measurement is given by

$$\sigma FY = \sqrt{\frac{FY}{t}}$$

the precision of the yield measurement at low concentrations can be improved by increasing the counting time $t$. An increase in counting time will, naturally, result in a larger radiation dose for the subject. Other compounds that could be readily investigated with our system include zinc oxide, bromoestrogen, or halogenated peptides. And finally, types of studies to which the noninvasive XRF measurements would be well suited include investigating the effects of enhancers on percutaneous absorption and monitoring the absorption and/or disappearance rate of compounds applied to damaged skin.

Table II. The Absorption Rates of 5-Iodouracil in DMSO

| Rat no. | Initial concentration (mg/cm²) | Absorption rate (μg/cm²-hr) | Correlation coefficient | Norm |
|---|---|---|---|---|
| 1 | 8.0 | 86 ± 2 | 0.994 | 1.8 |
| 2 | 4.2 | 152 ± 8 | 0.993 | 0.6 |
| 3 | 4.2 | 100 ± 6 | 0.993 | 0.3 |
| 4 | 11.2 | 150 ± 5 | 0.966 | 6.7 |

Table III. An Estimate of the Initial Fluorescence Yields that Would Be Obtained with Our XRF System for Other Elements Applied to the Skin at a Concentration of 1 mg/cm²[a]

| Element | Fluorescence yield (cps) |
|---|---|
| I[b] | 11 |
| Ag | 11 |
| Br | 240 |
| Zn | 220 |
| Cl | 8 |

[a] No correction was made for variation in background with X-ray energy in the calculations. Note that the concentration of 1 mg/cm² is the elemental areal concentration.

[b] Measured fluorescence yield.

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