Research Article

An *In Vitro* Method of Evaluating Tolnaftate Release from Topical Powder

Tacey X. Viegas,[1,2] Arthur H. Kibbe,[3] Ahmed H. Hikal,[1] Robert W. Cleary,[1] and Alan B. Jones[1]

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A dissolution apparatus was constructed to evaluate tolnaftate release from topical powders. It consisted of a mesh unit to support the powder, a receptor phase, and a sink. This report describes three parameters that were used to evaluate this technique. First, three different areas of contact were examined using 52-, 41-, or 30-μm mesh supports. Second, the effect of the pH on the dissolution rate was studied, using aqueous buffers of pH 3, 5, 7, or 8 as the receptor phase. Finally, different topical powder formulations containing different amounts of tolnaftate were tested. The results obtained showed that the percentage of tolnaftate released from topical powders increased at low pH levels and with the larger mesh support. The percentage released was greater in a starch–talc preparation than in a talc-only preparation. The mesh was replaced by a semipermeable membrane (2.5- to 4 nm pore size) to function as an *in vitro* model for intradermal diffusion. The results showed that a cream initially released more drug than powder formulations.

KEY WORDS: topical; powder; tolnaftate; dissolution.

INTRODUCTION

The biological activity of drugs, following topical administration, is effected in three steps:

(a) topical bioavailability—release of the drug from its dosage form;
(b) pharmacokinetics—diffusion of the drug through the skin, followed by distribution, metabolism, and excretion; and
(c) pharmacodynamics—interaction of the drug with target cells.

Previous studies have demonstrated different techniques and apparatuses used in evaluating these stages, some specifically dealing with bioavailability (1–7). Similar studies on the efficacy of topical powders were not conducted, although a few microbiological assays have been developed (8–10).

The aim of this study was to explain *in vitro* the topical bioavailability and pharmacokinetics of a medicated topical powder. The drug selected was tolnaftate, chemically known as *O*-2-naphthyl-*N*-methyl-*m*-tolyl thiocarbamate (Fig. 1). It is a synthetic antifungal used primarily against athlete's foot, jock itch, and ringworm. *In vitro*, tolnaftate inhibits the growth of *Trichophyton mentagrophytes* at concentrations of 7.5 to 75 ng/ml (11).

[1] Department of Pharmaceutics, School of Pharmacy, University of Mississippi, University, Mississippi 38677.
[2] To whom correspondence should be addressed.
[3] Client Services, Bioresearch Laboratories, 87 Senneville Road, Senneville, Quebec, Canada, H9X3R3.

MATERIALS AND METHODS

Dissolution Apparatus

A binary-phase system consisting of a sink (lower layer) and a receptor phase (upper layer) was set up in a 1000-ml beaker. This sink design of the dissolution apparatus was employed to accommodate the inherent low aqueous solubility of the drug. Even though the data reflect the appearance of drug in the sink, after having passed through the aqueous buffer, such data reflect the release of drug from the formulation on the support. The sink was 100 ml chloroform (UV Grade, Fisher Scientific, Springfield, N.J.), and the receptor phase was 80 ml aqueous buffer of either pH 3, pH 5 (both 0.1 *M* citrate buffers), pH 7, or pH 8 (both 0.1 *M* phosphate buffers). Tween 20, a hydrophilic surfactant, was added to the buffer at a concentration of 0.03% (v/v) to improve its wetting capacity. A glass tube (1 cm in diameter) was inserted through the receptor layer to allow the withdrawal of chloroform from the sink (Fig. 2). This sink was stirred at 60 to 70 rpm with the aid of a 15-mm-long magnetic bar and a stirrer-hot plate (Cole Palmer Instrument Co., Ill.). Higher speeds were avoided, since they produced a vortex

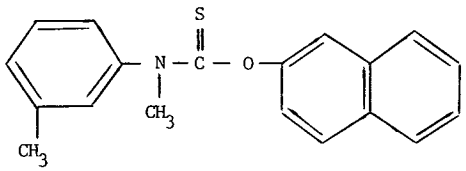

Fig. 1. Structure of tolnaftate.

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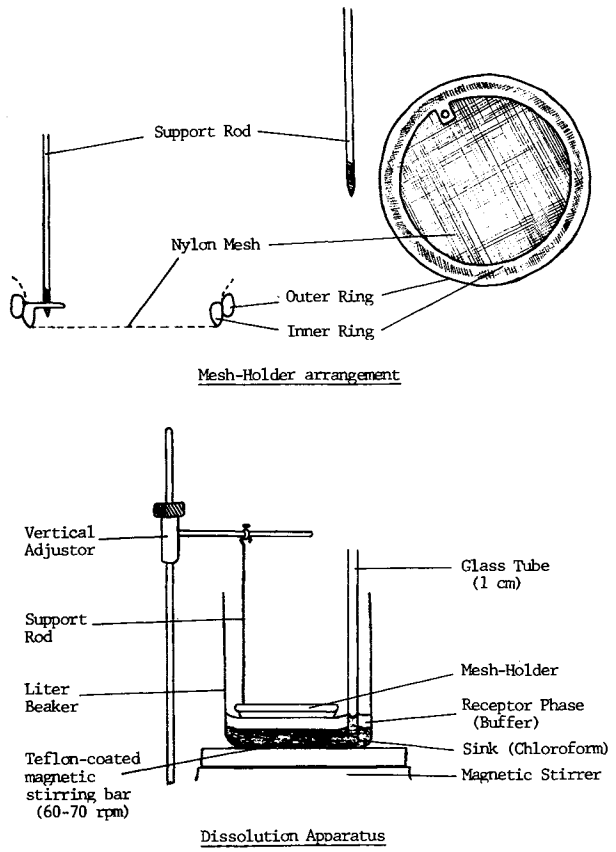

Fig. 2. Dissolution apparatus and mesh-holder arrangement used in the study.

in the system, with microemulsion formation at the chloroform–buffer interface.

The nylon mesh (52, 41, or 30 μm; SpectraMesh; Spectrum Medical Industries, Calif.) was held firmly between the inner and the outer rings of a plastic circular holder (New Berlin Plastics, Wis.). This holder (7-cm outer diameter and 6.67-cm inner diameter) provided a total internal area of 34.9 cm^2. The 52- and 41-μm meshes (33% open area) thus have open areas of 11.5 cm^2, while the 30-μm mesh (21% open area) has an open area of 7.3 cm^2. The mesh-holder arrangement was fitted to a support rod, which was in turn held to a stand by a vertical adjustor and hence could then be raised or lowered in the beaker. For experiments using the semi-

Table I. Formulations Used in the Present Study

Concentration of tolnaftate (%)	Official/trade name	Diluent(s)
100	Tolnaftate USP	None
10[a]	—	Talc
5[a]	—	Talc
1	Tinactin powder	Starch and talc
1	Aftate powder	Talc
1[a]	—	10% corn starch in talc
1[a]	—	20% corn starch in talc
1	Tinactin cream	PEG 400 and propylene glycol

[a] Powders were prepared for this study by geometric dilution and with a glass mortar and pestle.

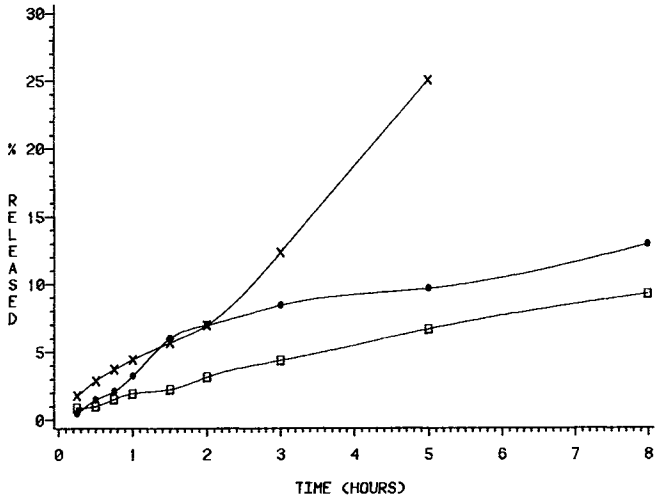

Fig. 3. Effect of mesh size on tolnaftate release from Tinactin powder at pH 3. (×) 52 μm; (●) 41 μm; (□) 30 μm.

permeable membrane (SpectraPor/2; Spectrum Medical Industries, Calif.), the membrane simply replaced the mesh in the apparatus. All experiments were conducted at room temperature.

Procedures

Drug Analysis. The amount of drug appearing in the sink at any given time was determined by transferring a 3.0-ml aliquot of the chloroform to a tube containing anhydrous sodium sulfate crystals to remove any water. The absorbance of this solution was measured (Beckman Acta III spectrophotomer) at 258 nm in 1.0-cm-path length cells with chloroform as the reference. Calibration standards were prepared in a similar manner.

Drug Solubility. The solubility of tolnaftate in buffer (pH 5, 7, or 8) was determined by adding excess drug (4 to 5 mg) to 50.0 ml buffer. These were mixed overnight to allow saturation to occur. The solutions were then filtered (Whatman No. 1 paper), and the filtrates extracted with five 1.0-ml portions of chloroform. The combined chloroform extracts were analyzed as above.

Release of Tolnaftate from Powder on Mesh Supports. A topical powder sample was spread over the available area of mesh within the holder using a spatula and minimal force to prevent sifting of the powder. The lower side of the mesh was then wiped in a single stroke with a laboratory tissue. The difference in weight between the loaded mesh and the previously weighed empty mesh-holder arrangement gave the weight of the powder retained between the interstices of the mesh. The apparatus was then fixed to the stand and lowered into the beaker so that the lower surface of the mesh just touched the surface of the buffer. This placement was noted as zero time. At time intervals of 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 5.0, and 8.0 hr, 3.0-ml aliquots of chloroform were removed from the sink and assayed for drug. This procedure was carried out using buffers of pH 3, pH 5, pH 7, or pH 8; mesh sizes of 30, 41, or 52 μm; and selected topical powder formulations listed in Table I. Each experiment was performed in duplicate and data are presented as mean values.

Table IIa. Effect of pH on Percentage of Tolnaftate Released from a Pure Tolnaftate Powder Sample (Mean Percentage of Two Trials)

Time interval (hr)	Percentage released at pH			
	3.0	5.0	7.0	8.0
0.25	0.88	0.22	0.07	0.96
0.5	2.79	1.17	0.15	3.89
0.75	5.58	2.97	0.10	6.05
1.0	7.52	4.75	0.11	8.29
1.5	10.16	8.73	0.12	11.22
2.0	11.89	10.28	0.23	13.49
3.0	13.90	14.22	0.20	14.52
5.0	18.35	15.49	0.19	16.77
8.0	31.72	21.26	0.27	20.39

Table IIb. Effect of pH on Percentage of Tolnaftate Released from 10% Tolnaftate in Talc (Mean Percentage of Two Trials)

Time interval (hr)	Percentage released at pH		
	3.0	5.0	8.0
0.25	1.12	0.40	0.33
0.50	3.25	1.07	0.895
0.75	4.88	1.88	1.27
1.0	6.47	2.58	1.76
1.5	7.91	3.12	2.46
2.0	10.11	3.61	2.55
3.0	13.76	5.04	3.97
5.0	22.32	9.23	5.06
8.0	24.39	15.13	6.99

Table IIc. Effect of pH on Percentage of Tolnaftate Released from 5% Tolnaftate in Talc (Mean Percentage of Two Trials)

Time interval (hr)	Percentage released at pH		
	3.0	5.0	8.0
0.25	1.04	0.853	0.45
0.50	2.27	2.47	1.19
0.75	3.34	3.903	1.85
1.0	4.78	4.43	2.05
1.5	6.07	5.29	2.63
2.0	6.83	6.12	2.99
3.0	9.01	8.91	4.13
5.0	17.11	11.645	5.21
8.0	24.95	15.51	6.66

Release of Tolnaftate from Formulations Through a Semipermeable Membrane. The mesh was replaced with a semipermeable membrane and onto it was placed a sample of tolnaftate USP, Tinactin powder, or Tinactin cream. Only buffers of pH 3, pH 5, or pH 8 were examined. Aliquots (3.0 ml) of the chloroform sink were removed at intervals of 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, and 24.0 hr and assayed for drug as above.

RESULTS AND DISCUSSION

Solubility of Tolnaftate

The results of the preliminary solubility test showed that tolnaftate dissolves best at low pH levels. The amounts dissolved as micrograms per milliter of buffer were 2.15 at pH 5, 1.21 at pH 7, and 1.67 at pH 8.

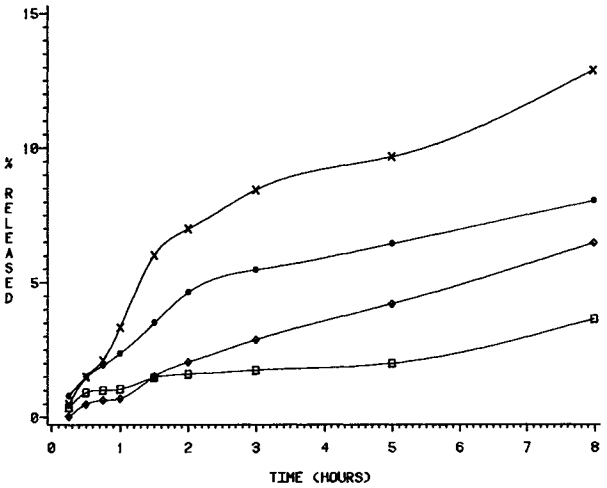

Fig. 4. Percentage of tolnaftate released from Tinactin powder in different pH buffers. (×) pH 3; (●) pH 5; (□) pH 7; (◇) pH 8.

Effect of Mesh Size on Release of Tolnaftate

To evaluate the effect of mesh size, the release of tolnaftate from Tinactin powder into a receptor phase of pH 3 or 5 was examined. The results of the pH 3 experiments are shown in Fig. 3. Similar results were obtained with pH 5 buffer. The sharp increase in the percentage released from the 52-μm mesh support after 2 hr was attributed to the dislodging of powder particles which fell through the receptor phase into the sink. The difference between the 30-μm and the 41-μm mesh data was due to the fact that the intersticial area of the 30-μm support is only two-thirds that of the 41-μm support. Thus, upon examination of these data it was decided that the 41-μm mesh support would be used for subsequent studies.

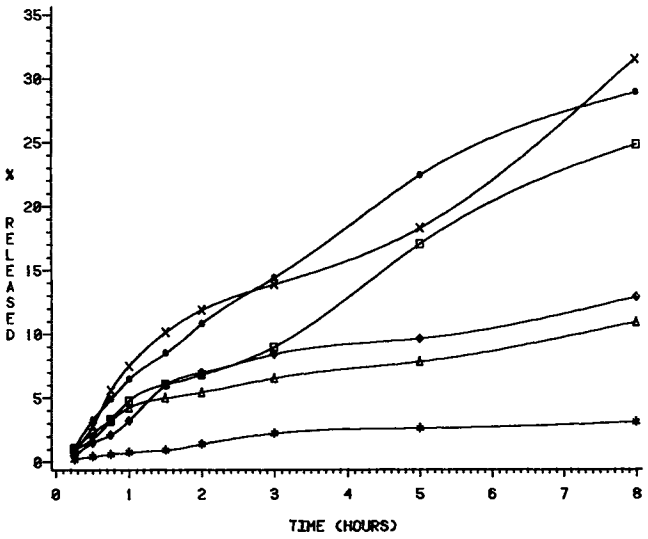

Fig. 5. Percentage of tolnaftate released from different powder formulations at pH 3. (×) Pure tolnaftate; (●) 10% in talc; (□) 5% in talc; (◇) Tinactin powder; (△) Aftate powder; (*) Tinactin cream.

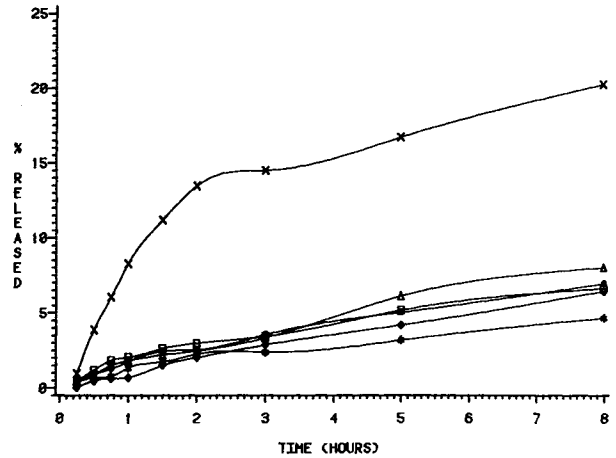

Fig. 6. Percentage of tolnaftate released from different powder formulations at pH 8. (×) Pure tolnaftate; (●) 10% in talc; (□) 5% in talc; (◇) Tinactin powder; (△) Aftate powder; (*) Tinactin cream.

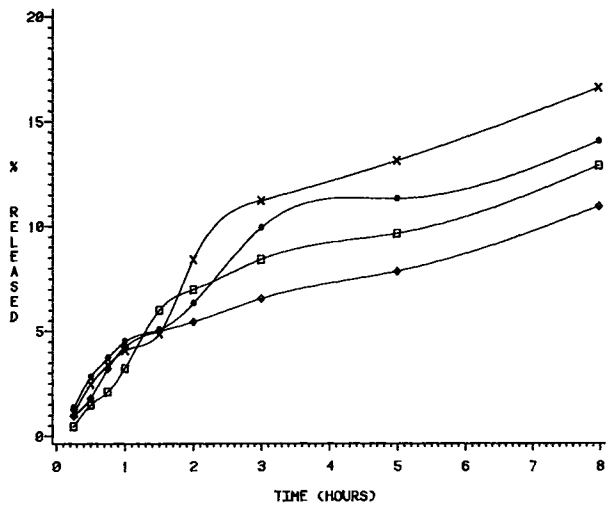

Fig. 7. Percentage of tolnaftate released at pH 3 from 1% tolnaftate formulations containing different amounts of starch. (◇) 0% Aftate powder; (□) unknown % Tinactin powder; (●) 10%; (×) 20%.

Effect of pH on the Release of Tolnaftate

Tables IIa, b, and c show the percentage of tolnaftate released against time for pure tolnaftate and 10 and 5% tolnaftate in talc preparations. The release pattern was identical at pH 3, 5, and 8. This is probably because the high amount of initial drug loaded on the mesh saturated the buffer. The partitioning of the drug from the receptor layer into the sink could be compared to a zero-order release. In the case of the other two formulations, the greatest percentages of drug were released in the pH 3 buffer, followed by the pH 5 and pH 8 buffers. The plot of the percentage released from Tinactin powder (Fig. 4) also verifies this observation. Structurally, tolnaftate possesses a basic nitrogen atom which can form soluble salts in acidic and basic media (12); hence the enhaced solubility at a very high or a very low pH. On the other hand, the dissolution rate at pH 7 was the lowest value in all cases (see Table IIa and Fig. 4).

Comparison of the Tolnaftate Formulations

Plots of percentage released versus time were made for pH 3 and 8 data (Figs. 5 and 6). Table III contains data at pH 5. The release pattern of the pure drug was the same at all three pH values, as previously explained. When comparing the other powder formulations, it was noticed that as we went from pH 3 to pH 8, the differences in release patterns were reduced. This is due to the better solubility of the drug at low pH levels. The release patterns are otherwise uniform, as seen in the pH 8 plot, suggesting that the diluents, talc and starch, play a role in controlling drug release from topical powder. The poor release from Tinactin cream could be due to the higher partitioning of tolnaftate in cream base than in the buffer. These results were supported by a two-way analysis of variance (ANOVA) (SPSS program on an IBM 4341 computer) [F = 18.24 at pH 3 and F = 18.43 at pH 5 (Tabulated F = 3.63)].

Powder formulations containing equal concentrations of tolnaftate (1%) and varying concentrations of starch (0–20%) were compared (see Table I). Starch is more hydrophilic than talc, and this explains why more drug was released from formulations containing increasing amounts of corn starch, as seen in Fig. 7.

Release from Formlations on a Semipermeable Membrane

It was observed that the cream absorbed nearly twice its weight of water from the receptor phase, and it in turn released more drug than the powder in the first 4 to 5 hr, as

Table III. Comparison of Different Tolnaftate Formulations (Mean Percentage of Two Trials)

	Percentage of tolnaftate released at pH 5					
Time (hr)	Pure tolnaftate	10% in talc	5% in talc	Tinactin 1% powder	Aftate 1% powder	Tinactin 1% cream
0.25	0.22	0.40	0.85	0.78	0.65	0.44
0.50	1.17	1.07	2.47	1.49	0.84	0.35
0.75	2.97	1.88	3.90	1.92	1.60	0.43
1.0	4.75	2.58	4.43	2.36	1.85	0.42
1.5	8.73	3.12	5.29	3.50	2.15	0.43
2.0	10.28	3.61	6.12	4.63	3.31	0.51
3.0	13.21	5.04	8.91	5.46	4.01	1.07
5.0	15.49	9.23	11.65	6.44	5.65	2.03
8.0	20.49	15.04	15.51	8.06	8.49	2.44

Table IV. Diffusion of Tolnaftate Through Membrane Using Tinactin Powder and Cream (Mean Percentage of Two Trials)

Time (hr)	Percentage of tolnaftate released					
	pH 3		pH 5		pH 8	
	Powder	Cream	Powder	Cream	Powder	Cream
0.5	0.00	0.00	0.28	0.17	0.00	0.00
1.0	0.00	0.07	0.46	0.32	0.00	0.00
1.5	0.00	0.06	0.39	0.32	0.12	0.00
2.0	0.00	0.22	0.36	0.47	0.43	0.00
3.0	0.00	0.26	0.54	0.60	0.68	0.00
5.0	0.55	0.38	1.15	0.68	0.89	0.11
8.0	0.49	0.87	1.35	0.85	2.23	0.72
24.0	3.06	2.04	3.16	1.73	6.61	2.69[a]

[a] Twenty-three-hour sample.

seen in Table IV. This was due to its water-soluble base. Because the total percentage of tolnaftate that diffused through the membrane was small (less than 10%), no definite conclusion can be drawn as to the release characteristics of the powder and cream. A two-way ANOVA on these data showed nonsignificance.

The dissolution apparatus designed was useful in evaluating topical powders. For further research it can be modified. A single aqueous receptor medium can be used if the active ingredient is water soluble. Mesh sizes smaller than 30 μm can be employed for ultrafine powders.

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