Investigation of the Release Test Method for the Topical Application of Pharmaceutical Preparations: Release Test of Cataplasm Including Nonsteroidal Anti-inflammatory Drugs Using Artificial Sweat

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A simple procedure for determining the *in vitro* release profile of a cataplasm for use in a quality control procedure has been developed. Since the disk assembly in the USP for patch dosage forms was unsuited for use in a release test due to penetration of the dissolution medium into the cataplasm from the screw part of the device and the cataplasm swelled, new holders were designed. In the new holder, a cataplasm is held in position by sandwiching it between a stainless-steel O-ring and a silicon O-ring on the stainless steel board, 2 acrylic boards hold the O-rings and the stainless steal board, and the entire assembly is placed at the bottom of the dissolution vessel. The release profile was determined using the "Paddle over Disk" method in USP26. Furthermore, in order to prevent the swelling of the cataplasm, artificial sweat was used as the dissolution medium. The release profiles of the nine marketed brands of cataplasm containing indomethacin, ketoprofen, and flurbiprofen, respectively, were determined over a 12-h period. By adjusting the ion concentration and volume of the media, and the release surface-area of the cataplasm exposed to each medium, the procedure was found to be reproducible for *in vitro* release characterization of nine marketed brands. This shows that this technique can be used as a quality control tool for assuring product uniformity.

Key words release test; cataplasm; swelling; artificial sweat

A method for testing the release and percutaneous absorption from a dosage form is very important as a means of quality control for assuring product uniformity.[1)] Furthermore, if adequate conditions are established for the release test method, it should be possible to determine compliance with drug-release requirement. For example, the tolerances in the *Official Monographs* for the USP relate the amount of drug released from a Nicotine Transdermal System to the amount of dose absorbed *in vivo*. On the other hand, it has been stated in the general rules for preparation in JP14 that "Functions which control the releasing rate of objective drugs from preparation may be added to official preparations for the purpose of controlling efficacy revelation time or decreasing side-effect. Unless otherwise specified, however, the function added preparations must meet the correspondent drug-release requirement." However the release test for percutaneous absorption of a dosage forms has not yet been established as an "official" method. In the light of this, it is readily apparent that a standard release procedure is needed which can be used for percutaneous absorption dosage forms. Thus, the authors examined the application of the release test method to a nonsteroidal anti-inflammatory drug (NSAID) containing the cataplasm which is a patch most frequently used in Japan for lumbago or bruising.[2)] Important advantages of the method are simplicity and reproducibility as well as the availability of suitable equipment.[3—6)] In addition, an aqueous solution should be used as the dissolution medium to minimize any environmental pollution, although release test results using organic solvents have been reported.[6,7)] One of the major problems encountered during the development of the release method using aqueous solution is the cataplasm swelling due to the penetration of water, because the cataplasm consists of water-soluble polymers. Since it has been reported that this swelling of water-soluble polymer is suppressed by some metal ions,[8,9)] the use of artificial sweat (AS) was investigated as a dissolution medium in this paper. Furthermore, the authors carried out the release test on representative products containing indomethacin (IM), ketoprofen (KP) and flurbiprofen (FP), respectively, and obtained the *in vitro* release profiles of these products.

Experimental

Materials The cataplasms used in this study are four products containing IM (A, B, C, D), two products containing KP (E, F) and three products containing FP (G, H, I). These were provided from the respective manufacturing and marketing companies. IM was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). KP and FP were kindly supplied by Nissan Chemical Industries, Ltd. (Tokyo, Japan) and Kaken Pharmaceutical Co., Ltd. (Chiba, Japan), respectively. These materials were recrystallized from ethanol. Melting points were measured and compared with literature data.[10)] All other chemicals and solvents were of reagent grade.

Release Test Apparatus: The release test was carried out using a six-spindle JP Apparatus 5 (NTR-6100, Toyama Industrials Co., Ltd., Toyama, Japan) employing glass vessels, a paddle speed of 100 rpm and 700 or 1000 ml degased AS. The disk assembly (TDOD-047, Hanson Research Corp., U.S.A.) or holders (Fig. 1) were used to hold the cataplasm. Cuprophan Flat Membrane™ (Cut-off MW 10000, Medicell International Ltd., U.K.) was used for the cataplasm swelling suppression test.

Release Experiment: A distance of 3.0 cm between the paddle blade and the release surface was initially set. The vessel was covered with plastic plates during the test to minimize evaporation. A cataplasm circle was cut to fit a 3.0 or 1.0 cm diameter O-ring (Fig. 1), and the protective layer was carefully removed from the cataplasm. The drug containing cataplasm was placed between the stainless steel O-ring and the silicon O-ring with the exposed drug side of the cataplasm facing the steel O-ring. The steel–cataplasm–silicon O-ring sandwich held together was placed on the stainless steel board, and fixed with the acrylic board. This assembly was placed at the bottom of a dissolution vessel containing 700 or 1000 ml AS at 32 °C. Small aliquots (generally 500 μl and 2 ml when the small holder was used), were collected at 0.5-, 1-, 2-, 4-, 6-, 8-, and 12-h, and the content of each drug was analyzed by HPLC. The same amount of fresh dissolution medium was added to keep the volume constant throughout the experiment.

Preparation of Artificial Sweat The composition of human sweat is shown in Table 1.[11)] Some salt mixtures containing representative cations

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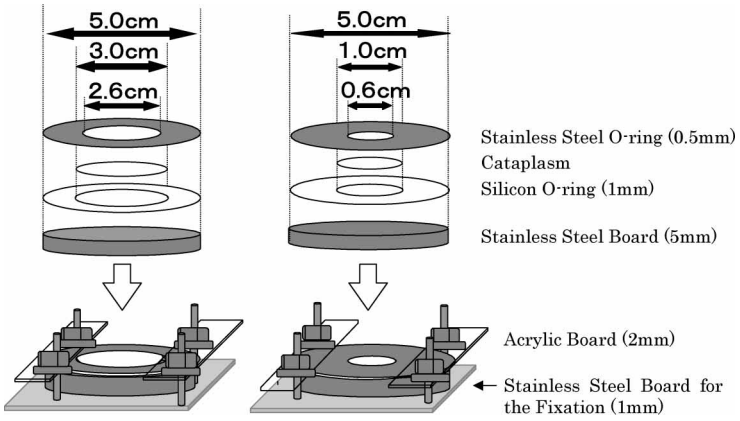

Fig. 1. Schematic Diagram of Holder Assemblies Designed for the Cataplasms

Left and right panels indicate large and small release area assemblies, respectively. Numbers in parentheses indicate thickness.

Table 1. Composition of Human Sweat

Na	9.7—94.1	Cl	0—65.1
Ca	0.2—6	P	0.003—0.014
Mg	0.03—4	S	0.022—0.231
Fe	0.022—0.068	Br	0.002—0.006
Cu	0.002	F	0.011—0.095
Mn	0.001—0.003	I	0.043—0.096
Zn	0.016—0.052	K	4.3—10.7
	etc.		

Units: mEq/l. Quoted from ref. 1.

Table 2. Composition of Artificial Sweat (AS)

Salt	AS(3)	AS(60)	AS(120)	AS(240)
NaCl	2.92	5.49	5.49	5.49
$CaCl_2$	0.166	3.32	6.64	13.28
$MgSO_4$	0.12	0.24	0.24	0.24
KH_2PO_4	1.02	1.36	1.36	1.36
pH	5.4	4.5	4.5	4.5

Units: g/l. Numbers in parentheses indicate milliequivalents of calcium ion. Each ion concentration and pH in AS(3) was adjusted to the median values of the human sweat in Table 1. For AS(60), AS(120) and AS(240), each ion concentration except for calcium was adjusted to the upper limit and the pH to lower limit of human sweat.

and anions in human sweat were prepared as AS. AS(3) (the number in parenthesis represents milliequivalents of calcium ion: mEq/l) was prepared so that the concentration of the positive ions was the same as the median value of the cation-concentrations in human sweat. In addition, the pH was adjusted to the median value (5.4) of the pH (4.5—6.3) of human sweat using 0.1 N NaOH. Furthermore, AS(60), AS(90), AS(120), AS(150) and AS(240) which increased the calcium ion concentration were prepared (Table 2). In these AS, NaCl, $MgSO_4$ and KH_2PO_4 were adjusted to concentrations of 5.49 g/l (94 mEq/l), 0.24 g/l (4 mEq/l) and 1.36 g/l (10 mEq/l), respectively, which is the upper limit of the concentration of each cation in human sweat. In addition, the pH was adjusted to 4.5 (lower limit of the pH of human sweat) using 0.1 N NaOH.

Measurement of Cataplasm Swelling The rates of cataplasm swelling in various AS(S) were studied by weighing the increase in water content in the cataplasm samples. Cataplasm disks (3.0 cm diameter) were weighed, mounted in the holder (large) and immersed in 700 ml of various AS at 32 °C. After 12 h, the cataplasm was removed and weighed after gentle surface wiping using absorbent paper. The degree of swelling was calculated using the following equation:

$$\text{degree of swelling} = \frac{W_2}{W_1}$$

where W_1 is the initial weight of the cataplasm and W_2 is the swollen weight of the same cataplasm after immersion in various AS.

Table 3. HPLC Conditions

Drug (medium)	Mobile phase 0.1% phosphoric acid : acetonitrile	Detection	Internal standard
Indomethacin (water)	50 : 50	265 nm	—
Indomethacin (AS)	45 : 55	265 nm	—
Ketoprofen (water, AS)	60 : 40	259 nm	4-Hydroxybenzoic acid *n*-amyl ester
Flurbiprofen (water, AS)	55 : 45	259 nm	4-Hydroxybenzoic acid *n*-hexyl ester

—: Not utilized. Absolute calibration method was used.

Drug Solubility The solubility of each drug in the dissolution media used in this study was determined as follows: excess drug was added to test medium, and the suspension was stirred in a water bath at 32 °C. After equilibrium, part of the solution was withdrawn and passed quickly through a MILLEX™-HV (0.45 mm, Millipore corp., U.S.A.) filter. The filtrates were diluted with the same media, followed by HPLC analysis.

Analytical Method The concentrations of IM, KP and FP in each dissolution medium were determined by HPLC. The HPLC conditions were as follows: pump, LC-10AS; detector, SPD-10A; system controller, SCL-10A; auto injector, SIL-10A_{XL}; chromatopack, C-R5A; column oven, CTO-10A (Shimadzu Co. Ltd., Kyoto, Japan); column, Inertsil C8 (4.6×250 mm, 5 μm, GL Science Inc. Ltd., Tokyo, Japan); column temp., 60 °C; flow rate, 1.5 ml/min. The other determination conditions are shown in Table 3.

Results

Preliminary Examination of the Device and Artificial Sweat When the combination of Cuprophan Flat Membrane™ and the disk assembly adopted in the USP were used in the release test of cataplasms, the dissolution medium penetrated into the cataplasm from the release surface and screw part of the assembly, and the drug was released not only from release surface but also from the side face. In order to inhibit the penetration of water through the side face, a new holder shown in Fig. 1 was designed. Swelling of the cataplasm was observed as shown in Fig. 2A. It was obvious that the swelling occurred due to the penetration of the water from the release face covered with Cuprophan Flat Membrane™ (Fig. 2B). Since it has been reported that the swelling of water-soluble polymer is suppressed in the presence of cations (especially Ca^{2+}) and at a low pH,[8] the use of AS(3) as dissolution medium was examined. The swelling of cataplasm in AS(3) was considerably reduced compared with that in water (Fig. 2C). From this result, it was thought that a high

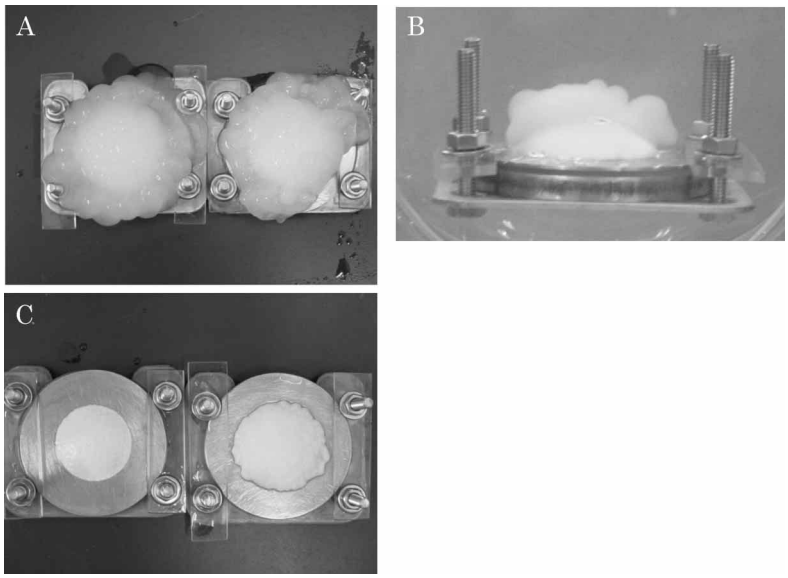

Fig. 2. Picture Images of Cataplasms under the Drug-Release Test in Various Dissolution Mediums

A: Cataplasms were immersed in water (left: sample A, right: sample B). B: Cataplasms covered with Cuprophan Flat Membrane™ were immersed in water. C: Cataplasms were immersed in AS(3) (left: sample C, right: sample D).

Table 4. Degree of Swelling of Each Product Immersed in AS and AS(S)

Dissolution medium	Products								
	A	B	C	D	E	F	G	H	I
AS(3)	3.51	3.02	2.68	4.69	3.90	3.18	Collapse	5.74	5.78
AS(60)	0.98	0.94	0.70	1.32	0.84	0.91	0.80	1.45	0.99
AS(90)	—	—	—	1.24	—	—	—	1.12	—
AS(120)	—	—	—	1.16	—	—	—	0.97	—
AS(150)	—	—	—	1.05	—	—	—	—	—
AS(240)	—	—	—	0.99	—	—	—	—	—
AS(S)	(60)	(60)	(60)	(240)	(60)	(60)	(60)	(120)	(60)

—: Not determined. Numbers in parentheses indicate milliequivalents of calcium ion. Each ion concentration and pH in AS(3) were adjusted to the median values of the human sweat in Table 1. For AS(60), AS(120) and AS(240), each ion concentration except for calcium ion was adjusted to the upper limit and pH to lower limit of the human sweat. AS(S) represents the dissolution medium suppressing the swelling of the product.

cation concentration may be more effective as far as reducing the swelling of the cataplasm was concerned. Thus, swelling was controlled mainly by adjusting the Ca^{2+} concentration of AS.

Dissolution Medium for the Cataplasm Based on the above results, the composition of the dissolution medium (AS) for each cataplasm was determined. Table 4 shows the degree of swelling of products A—I in various AS. The degree of swelling was anticipated to change with variations in the cation concentration in AS. From these results, the AS(S) for each cataplasm in which the swelling was completely suppressed was selected as the dissolution medium.

Volume of Dissolution Medium In USP 26 *Dissolution and Drug Release Testing*, it states that the quantity of release medium used should be not less than 3 times that required to form a saturated solution of the drug substance. The drug solubility in AS(S) was determined and compared with the calculated concentration based on the assumption that the drug is completely released from the cataplasm (Table 5). From these results, the volume of dissolution medium was set at 700 ml for 8 cataplasms except for sample D. However, in the case of IM product (sample D) the use of 700 ml dissolution medium, AS(240), did not fit the USP criteria (sink condition), even if the small holder was used. If 1000 ml is used as the dissolution medium instead of 700 ml, the ratio of the calculated concentration for the solubility will change from 0.35 to 0.25. Thus, the volume of the dissolution medium was set at 1000 ml for sample D.

Table 5. The Solubility and Drug Concentration in Dissolution Medium

Drug	Dissolution medium	Solubility, Soly (μg/ml)	Drug concentration[a], Cd (μg/ml)	Cd/Soly
Large holder				
IM	AS(60)	2.57±0.36	5.08±0.11	1.98
	AS(240)	1.57±0.14		3.24
KP	AS(60)	132.9±2.53	2.17±0.06	0.02
FP	AS(60)	22.6±3.90	3.03±0.14	0.13
	AS(120)	18.1±1.49		0.17
Small holder				
IM	AS(60)	2.57±0.36	0.55±0.03	0.21
	AS(240)	1.57±0.14		0.35

Solubility was determined at 32 °C. *a*) Estimated concentration of drugs in each dissolution medium assuming complete drug release from the cataplasm.

Release Test The release profiles of the nine marketed

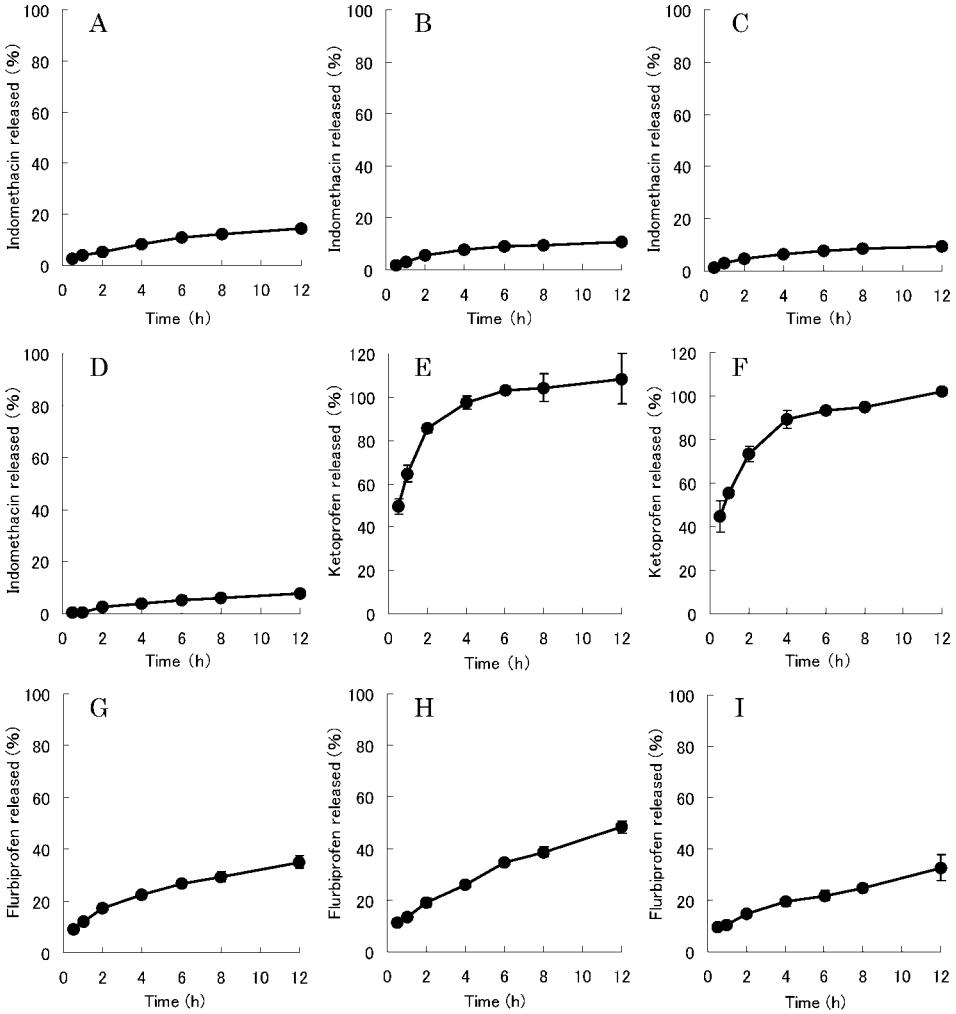

Fig. 3. Release Profiles of Drugs from Various Cataplasm Products

A, B, C and D, IM products; E and F, KP products; G, H and I, FP products. AS(S) shown in Table 4 was applied as a dissolution medium for each product.

brands of IM, KP and FP cataplasms in AS(S) are shown in Fig. 3. The IM-containing cataplasms (A, B, C, D) released about 10% of the loading dose of IM in AS(S) over a 12-h period. The KP-containing cataplasms (E, F) released almost 100% of the KP in AS(S). The FP-containing cataplasms released 35% of the drug in G, 48% in H and 33% in I in AS(S).

Discussion

In the release test (or dissolution test) of the drug from pharmaceutical formulations including transdermal patches, it is important to keep the release-surface area constant throughout the experiment. However, the cataplasm swelled in water when using a disk assembly and the smooth surface changed to be a "bee-hive" one. The cataplasm swelling seems to be due to the penetration of water from the side face and the release surface. In order to suppress penetration from the side face, we devised new holders instead of the disk assembly designed for transdermal patches in the USP. Although this device was used, swelling occurred owing to penetration from the release surface.

It is thought that the penetration of water into the cataplasm, *i.e.* swelling, is dependent on the difference in osmotic pressure between the water layer in the cataplasm and the dissolution medium, and the osmotic pressure of the cataplasm is dependent on the content of water-soluble polymer in the cataplasm. As mentioned above, it has been reported that a highly concentrated ionic solution suppresses the swelling of water-soluble polymers.[8] Thus, artificial sweat was selected as a highly concentrated ionic solution. It is clear from Table 4 that the swelling of 9 kinds of cataplasm can be suppressed by changing the ion concentration in the dissolution medium. As the formulation of cataplasm varies from manufacturer to manufacturer,[6] AS of different ionic concentration is necessary for each cataplasm. Although it is specified in the USP that the sink condition was maintained throughout the experiment, when using the large holder IM-containing products did not satisfy this condition. Then, using the small holder instead of the large one and increasing the volume of dissolution medium from 700 to 1000 ml, the IM cataplasm was able to satisfy this condition.

The comparative release profile of IM-, KP- or FP-containing cataplasms shows similar *in vitro* release among IM products and among KP products, but a distinctly different release among FP products (Fig. 3). This observed difference in release characteristics is attributed to different cataplasm characteristics. With the variety of sizes and NSAID products in cataplasms, it is essential that one standard method be

used for the release test so that variables may be kept to a minimum. The procedure used in this paper is able to accomplish this, working well with all marketed NSAID-containing cataplasms. These results suggest that it can be used widely as a quality control tool for assuring the product uniformity of a cataplasm.

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