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An Approach to Developing Dissolution Standards for Turmeric Capsules: Paddle Rotating Method

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Abstract A simple dissolution test and standard was developed for turmeric capsules to replace the less discriminating disintegration test stated in the Supplement to Thai Herbal Pharmacopoeia 2004. A previous investigation showed that the rotating device basket was not effective enough for stirring the liquid at the bottom of the vessel. A coning effect due to dense materials in the formulation was observed thus causing variability in the results. Therefore the further study was performed for establishing the standards with paddle instead in 2008. Consequently, the proposed test used the paddle at 75 rpm and dissolution medium composed of 0.05 M hydrochloric acid and sodium lauryl sulfate at 0.6% w/v. The acceptable Q-value was proposed as 75 per cent in 60 minutes.

Key words: **dissolution, paddle method, curcumin, turmeric capsules**

Introduction

Turmeric capsules are included in Supplement to Thai Herbal Pharmacopoeia (THP) 2004 under the title "Khamin Chan Capsules". Its claimed category is stomachic and carminative[1]. The available strength stated in the Pharmacopoeia is 250 mg of powdered form per capsule. THP Tumeric is the dried rhizome of Cucuma longa L (Family Zingiberaceae)[1,2]. The major chemical constituents are volatile oil, and curcuminoids, 50-60 per cent of which are a mixture of curcumin, monodesmethoxycurcumin and bisdesmethoxycurcumin[1]. Its principal use is for the treatment of dyspepsia and stomachic[1,3,4]. The tests for weight variation and disintegration are required in this THP monograph. Generally, the disintegration testing does not assure dissolution of the active ingre-

dients as the dissolution testing does. So a previous investigation has been made in establishing dissolution specifications utilizing curcumin as a marker substance with a rotating basket device[5]. However, the result showed that the basket was not effective enough for stirring the liquid at the bottom of the vessel. The coning effect due to dense materials in formulation was observed thus causing variability in the results[6]. It was, therefore, the purpose of this study to further investigate an alternative way of dissolution method by replacing a rotating basket device with a paddle device.

Methodology

Chemicals and apparatus

Curcumin, Lot 453335/1, was from Fluka, Germany. Water was purified water. All chemical reagents were analytical-reagent grade and were used as purchased. A Spectronic 1001 Plus UV-Visible double-beam spectrophotometer was from Milton Roy Co., USA. A dissolution apparatus Model 10-1500 was from Vankel Industries Inc., USA. The paddles were used through this study. A helix (sinker) was made of non-reactive metal (stainless steel). The apparatuses were calibrated as scheduled according to the Quality Control Manual of Bureau of Drug and Narcotic, Department of Medical Sciences. All experiments were carried out at ambient temperatures.

Capsule selection

This study was carried out in 2008. Five different lots (two boxes each) of turmeric capsules with label claims 0.024 g of curcuminoids were purchased from a retail pharmacy. Each box contained 100 capsules. All the capsules were at least one year away from their expiration dates at the time of testing.

Before subjecting to the dissolution testing, each product was tested for contents of curcuminoids, water, uniformity of weight, and disintegration. The official requirements of THP 2004 was followed[1].

Determination of curcuminoid content

The procedure described in THP 2004 was followed[1]. The standard curcumin curve was plotted at the concentrations of 0.8, 1.6, 2.0, 2.4 and 3.2 µg/mL. For sample preparation, the contents of 20 curcumin capsules were weighed and mixed. An accurately weighed portion of the capsule contents, containing about 300 mg of curcumin, was transferred to a 10-mL volumetric flask. Tetrahydrofuran was added to volume and was mixed. It was set aside at room temperature for 24 hours. A 1.0-mL portion of the clear supernatant liquid was diluted with methanol to produce 25.0 mL. A 1.0 mL-portion of this solution was transferred into a 50-mL of volumetric flask. It was diluted to volume with methanol and was mixed well. Then the absorbance of the sample preparation was measured at about 420 nm, using methanol as the blank. By the reference to the standard curcumin curve, the content of curcumin in the portion of the capsule taken was calculated on the anhydrous basis. The intra-day coefficient of variation ranged between 1.95 and 2.85 percent.

Determination of water

The water content in the sample was determined by using Azeotropic Distillation Method described in THP 2004 (1). The introduction of 200 mL of toluene and 2 mL of water into a 500-mL round bottom flask was performed. It was distilled for about 2 hours and allowed to cool to room temperature. The water volume was read. Then the accurately weighed quantity of powdered content from turmeric capsules, containing about 10 g, was placed in the flask and was distilled. When the water was distilled over, the inside of the condenser tube was rinsed with toluene. The distillation was continued for 5 minutes. Then the heat

was removed and the receiving tube was allowed to cool to room temperature. When the water and toluene were completely separated, the volume of water was read and the percentage of water present in the sample was calculated.

Determination of uniformity of dosage units

Ten turmeric capsules in each lot were weighed individually. One capsule was opened without losing any part of the shell and the content was removed as completely as possible. Then the shell was cleaned with a small brush and weighed. The weight of the contents in each capsule was the difference between the weighings. The weight of the contents in each capsule was compared with the average weight for the capsules. To meet the requirement of uniformity of dosage units, not more than two of the individual weights deviated from the average weight by more than the percentage deviation of 10 per cent and none deviated by more than twice that percentage[1].

Disintegration test

The procedure of disintegration test for the turmeric capsule was described in THP 2004[1] and TP I. Six turmeric capsules were used in each test, using water as the immersion fluid. To meet the requirement, all capsules disintegrated within 30 minutes.

Dissolution procedure

Preparation of various dissolution media

To prepare pH 4.0 buffer, a 2.86 mL aliquot of glacial acetic acid was added to 900 mL of water in a 1000 mL volumetric flask. An aliquot of a 50% w/v solution of sodium hydroxide was added to this solution to adjust pH to 4.0 ± 0.05, before water was added to make up the final volume. This solution was deaerated by heating to about 45 ˚C and slowly vacuum-degassing for about 5 minutes. A 900 mL portion was transferred to a 1,000 mL dissolution vessel and placed into the dissolution tester's water bath. The medium was equilibrated to 37 ˚C ± 0.5 ˚C for about 30 minutes prior to starting the dissolution test.

To prepare sodium lauryl sulfate (SLS) 0.2% in 0.01 M hydrochloric acid (pH 2.2), a 2.0 g portion of SLS was transferred to a 1000 mL volumetric flask and dissolved into approximately 300 mL of water. A 0.85 mL aliquot of concentrated hydrochloric acid (~12.1M) was added to the solution and then diluted to volume with water. This solution was deaerated under vacuum and a 900 mL portion was transferred to a 1000 mL dissolution vessel and placed into the dissolution tester's water-bath for equilibration at 37 ˚C ± 0.5 ˚C for about 30 minutes.

To prepare SLS 0.3% in 0.05 M hydrochloric acid (pH 1.4), a 3.0 g portion of SLS was transferred to a 1000 mL volumetric flask and dissolved into approximately 300 mL of water. A 4.25 mL aliquot of concentrated hydrochloric acid (~12.1M) was added to the solution and then diluted to volume with water. After the deaeration, 900 mL of the medium was used and equilibrated similar to that described for 0.2% SLS in 0.01 M hydrochloric acid.

To prepare SLS 0.4% and 0.6% in 0.05 M hydrochloric acid (pH 1.4), proceed as directed in the preparation of 0.3% SLS in 0.05 M hydrochloric acid but using 4.0 g and 6.0 g portions of SLS instead of a 3.0 g portion of SLS.

Standard curcumin curve and curcumin determination

A 400 µg/mL solution of curcumin in methanol was prepared. Aliquots of this solution were diluted with methanol to obtain solutions containing 0.4, 0.8, 1.6, 2.0, 2.4, and 3.2 µg/mL of curcumin. Measure the absorbances of the curcumin solutions relative to the blank at 420 nm, using a 1 cm cell. A 10 mL portion of the dissolution medium was withdrawn from the vessels at 30, 45, and 60 minutes. The dissolution medium withdrawn was not replaced. The sample was

filtered using a Whatman filter paper No. 1. The first few mL was discarded. Five mL of the filtrate of the 6 individual capsules withdrawn were combined to obtain the pooled sample which was then diluted to 50.0 mL with methanol. It was further diluted five-fold with methanol and the absorbances were measured, using methanol as the blank. The average amount of curcuminoids, expressed as curcumin, dissolved at different time was determined by using the standard curcumin curve and calculated on the anhydrous basis.

Statistical analysis of data.

The analysis of variance (ANOVA) and the test for normal distribution of data (Kolmogorov-Smirnov One -sample Test) were performed. The significance level (α) was set at 0.05.

Results

Curcuminoid and water contents, weight variation and disintegration tests

Five lots of marketed turmeric capsules bearing the curcuminoid content on the label were collected. The tested products met the THP 2004 standards regarding the curcuminoid content, calculated as curcumin, and the water content. The contents of curcuminoids were from 8.0 to 10.0 per cent w/w; they were within THP 2004 officially allowable limit (not less than 5.0 per cent w/w). The tested products passed the specified THP 2004 tests for weight variation and disintegration. The amount of water found in all products ranged between 6.0 per cent and 7.0 per cent v/w; they also complied with the THP 2004 standard (not more than 10.0 per cent v/w).

Dissolution results

Following the procedure stated in THP 2004, the standard curcumin curve was created with one more concentration of 0.4 µg/mL. The curve showed high degree of correlation with coefficient of determination ($r^2 = 0.9998$). In determining curcumin in the dissolution aliquots, it demonstrated that the capsule shells did not interfere with the analytical results.

The dissolution profiles of turmeric capsules in different dissolution media using the paddle method at 75 rpm are shown in Fig. 1.

An increasing degree of dissolution was obtained with the introduction of sodium lauryl sulfate (SLS). The lowest amount of surfactant necessary to achieve 75-85 percent release of curcuminoids in 60 minutes was 0.6% SLS in 0.05 M hydrochloric acid. The rate of agitation of 75 rpm was proved to provide adequate agitation for dissolution. In setting the test time, it is depicted in Fig. 2 that a 60-minute collection time point was about at the plateau of the profile thus providing less variable results. Detailed observations during the dissolution tests including approximate capsule disintegration time, shape formed by the disintegrated particles (cone-shaped piles or circular-shaped piles) are shown and illustrated in Table 2 and Fig. 3.

Table 1 Summary of the testing results for weight variation, curcuminoid content, water and disintegration of tumeric capsules

Lot	Average weight (mg), SD (n=10)	Weight variation	Curcuminoid content (%w/w)	Water (%v/w)	Disintegration
A	303.6,1.7	pass	8.8	6.5	pass
B	302.2,1.9	pass	8.4	6.3	pass
C	304.3,1.5	pass	10.0	7.0	pass
D	305.4,1.4	pass	8.0	6.0	pass
E	301.9,1.6	pass	7.4	6.4	pass

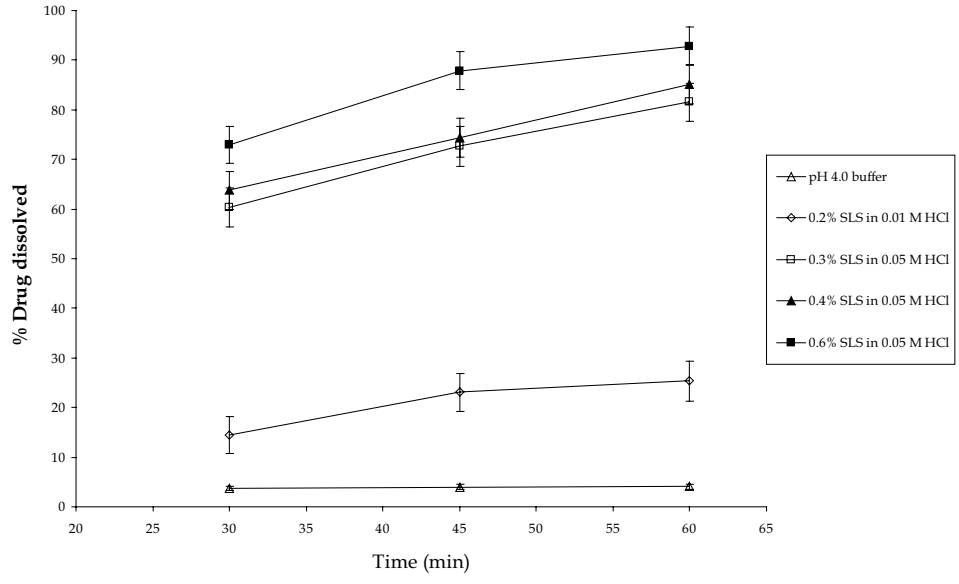

Figure 1 Dissolution profiles of turmeric capsules containing 0.024 g curcuminoids using the paddle method at 75 rpm and 900 mL of various media at 37° ± 0.5°C (n=3)

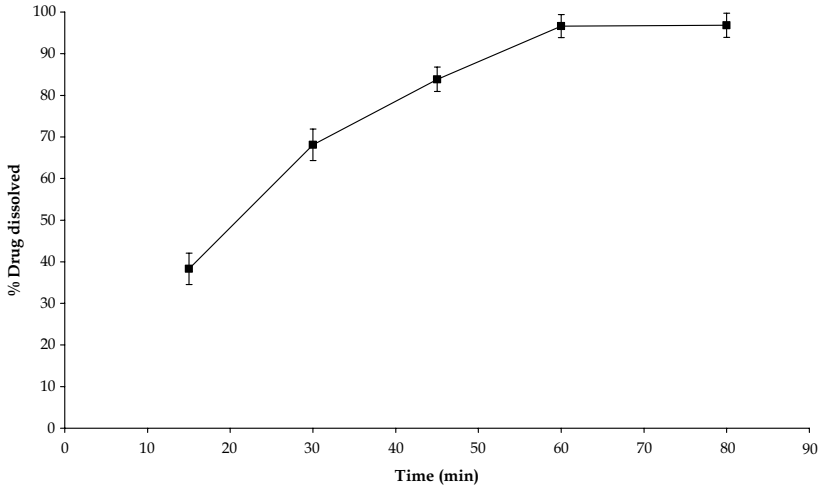

Figure 2 Dissolution profiles of turmeric capsules containing 0.024 g curcuminoids using the paddle method at 75 rpm and 900 mL of 0.6% SLS in 0.05 M hydrochloric acid at 37° ± 0.5°C.

Establishment of acceptable "Q" value

Table 3 shows a comprehensive dissolution profile obtaining from each lot of turmeric capsules using 0.6% SLS in 0.05 M hydrochloric acid and paddle at 75 rpm.

The sampling time points were 30, 45 and 60 minutes. The data in Table 3 demonstrates that the distribution of all values was normal ($p > 0.05$).

The acceptance criteria for dissolution of botanical dosage forms was adopted from the United States Pharmacopeia (USP)[7] since they have not yet been included in THP. Table 3 defines that the first stage of dissolution test consists of testing 6 dosage units. If the average amount dissolved is greater than or equal to Q + 10%, then the dissolution test criteria are met and the test is passed.

Table 2 Observations made during dissolution of turmeric capsules (Paddle: 75 rpm; Temp : 37° ± 0.5°C)

Medium	Observations*
pH 4.0 buffer	Disintegrated within 2 min. into swirling cone-shaped piles of fine particles.
0.2% SLS in 0.01 M HCl	Disintegrated within 2-3 min. into swirling cone-shaped piles of fine particles centered under the paddle on the bottom of the vessel.
0.3% SLS in 0.05 M HCl	same as above
0.4% SLS in 0.05 M HCl	same as above
0.6% SLS in 0.05 M HCl	same as above

**In some batches of products, parts of the disintegrated capsule shells stick to the helixes.*

Fig. 3 Particles from contents of Turmeric capsules centered under the paddle in cone-shaped piles.

In order to establish an acceptable Q value, the statistics indicating predicted frequency of Stage 2 testing is illustrated in Table 4[8,9]. The data depicted in the table are on the assumption that Q value of 75 or 80 is the proposed dissolution specification at one or two candidate time points[7,9,10]. It is to be noted that the calculations for the per cent of average amount dissolved less than Q + 10% have been adjusted to the per cent of average amount dissolved less than Q + 9.5%. This was done to account for the rounding aspect of the dissolution test when comparing the aver-

Table 3 Dissolution profile of five lots of turmeric capsules (Dissolution medium: 0.6% sodium lauryl sulfate in 0.05 M hydrochloric acid; Paddle: 75 rpm; temperature : 37° ± 0.5°C)

	Percentage		
	30	**45**	**60**
Lot A	78.54	88.73	92.35
Lot A	76.02	84.86	94.56
Lot B	75.82	79.28	84.42
Lot B	76.60	78.52	92.78
Lot C	71.55	85.77	92.81
Lot C	68.63	80.93	91.77
Lot D	66.49	82.06	86.25
Lot D	70.96	83.07	87.45
Lot E	70.18	87.87	89.58
Lot E	75.63	84.09	94.73
Mean,SD	**73.04,3.99**	**83.52,3.42**	**90.67,3.57**

Table 4 USP dissolution acceptance criteria for a pooled sample[8]

Stage	Number tested	Acceptance Criteria
S_1	6	Average amount dissolved is not less than Q + 10%
S_2	6	Average amount dissolved (S_1 + S_2) is equal to or greater than Q + 5%
S_3	12	Average amount dissolved (S_1 + S_2 + S_3) is equal to or greater than Q

Table 5 Summary statistics for potential Q-value

Collection time point (minutes)	Number of results	Average (standard deviation)	Potential Q-value	% of Average amount dissolved predicted to be less than Q + 9.5%	Predicted frequency of Stage 2 testing
45	10	83.52(3.42)	75	0.6141	3.63 %
45	10	83.52(3.42)	80	0.9599	3.62 %
60	10	90.67(3.57)	75	0.0418	0.25 %
60	10	90.67(3.57)	80	0.3707	2.20 %

Table 6 Q-Value to yield 20 percent Stage 2 testing for a pooled sample

Collection Time Point (minutes)	Number of Results	Average (Standard Deviation)	Q-Value to Achieve Stage 2 Testing Frequency of 20%
45	10	83.52(3.42)	68.04
60	10	90.67(3.57)	74.92

age amount dissolved to the acceptance criteria[7,8]. Referring to Table 5, the manufacturer will likely propose a specification of Q = 70 percent while a specification of Q = 80 percent would lead to extend Stage 2 testing.

According to the methodology to set potential dissolution specifications, the allowable per cent Stage 2 testing would be proposed[8]. Supposing that 20 percent Stage 2 testing be desirable. This requires that no more than 4 per cent of the average amount dissolved is less than Q + 10% (or Q + 9.5%, taking into account the normal rounding procedures). This Stage 2 testing frequency would be achieved if the true mean were 1.75 σ above Q + 10% (or Q + 9.5%, taking into account the normal rounding procedures). Thus, the Q-value to achieve this Stage 2 testing could be obtained by subtracting the quantity "10 + 1.75 σ" (or the quantity "9.5 + 1.75 σ") from the overall average.

Table 6 summarizes the Q value to yield 20 percent Stage 2 testing when the aboved methodology was applied. It was convinced clearly that the specification that could perhaps be agreed upon is Q = 75 percent at 60 minutes since it leads to an acceptable frequency of Stage 2 testing (0.25%) as illustrated in Table 5.

Discussion

As proposed by P. Kucera[11], the basket-stirring apparatus with the stirring speed of 100 rpm should be tried on capsules of botanical preparations. The previous study on the dissolution of five lots of commercial turmeric capsules utilizing basket rotating method have shown the dissolution behaviour of curcumin, a marker substance that about 90 per cent of curcumin dissolved in 0.8% SLS in 0.05 M hydrochloric acid in 60 minutes[2]. To produce universally accepted public standards on dissolution testing of Turmeric capsules, an alternative method such as the rotating paddle method was experimented. Consequently, the proposed dissolution test used the paddle device (with helix) at 75 rpm and required a release (Q) of 75 percent in 60 minutes.

However, comparable to the basket method[5], the coning effect still appeared. The use of a helix (sinker) in the test, which prevents the capsule from floating on medium surface during the dissolution procedure, probably caused additional agitation beyond the expected stirring of the paddle. It might be a cause of variability in the results.

In this study, water was not included as the testing media since it has been demonstrated in the previous study that curcumin dissolved very poorly in water (less than 1 per cent)[5]. For the preparation of medium, it is recommended that the SLS-containing media be used within 12 hours. This is because the percentage dissolved of curcumin was found to be about 20 per cent less if the SLS-containing media used was deaerated and stood overnight. Sodium lauryl sulfate (SLS), a commonly used surfactant in dissolution media for poorly water soluble drugs, was used to increase the dissolution of curcumin. In this study, to reach the same 75-85 percent release of curcuminoids the proposed dissolution medium (0.6% w/v) contained slightly lower concentration of SLS than the medium (0.8% w/v) in the previous study[5].

However, the dissolution standards for turmeric capsules with both devices can replace the less discriminating disintegration test stated in the Supplement to Thai Herbal Pharmacopoeia 2004

Conclusion

From this investigation, the proposed dissolution test uses the paddle device (with helix) at 75 rpm and requires a release (Q) of 75 percent in 60 minutes. The proposed dissolution medium is composed of 0.05 M hydrochloric acid with sodium lauryl sulfate at 0.6% w/v. Therefore, both devices, basket and paddle, offer comparatively reliable specification for dissolution of Turmeric Capsules.

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บทคัดย่อ **การพัฒนามาตรฐานการละลายของยาแคปซูลขมิ้นชัน: วิธีอุปกรณ์ใบพาย**

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ได้พัฒนาวิธีทดสอบการละลายและมาตรฐานสำหรับยาแคปซูลขมิ้นชันเพื่อนำไปใช้แทนวิธีทดสอบการแตกตัวที่ปรากฏในตำรามาตรฐานยาสมุนไพรฉบับเพิ่มเติม พ.ศ. 2547 การศึกษาที่ผ่านมาได้แสดงให้เห็นว่าวิธีการใช้อุปกรณ์ตะกร้าที่เสนอไว้นั้นมีประสิทธิผลไม่เพียงพอที่จะคนของเหลวที่ส่วนล่างของภาชนะ จากการสังเกตเกิดปรากฏการรูปกรวยเนื่องจากการรวมกลุ่มกันแน่นของผงยา ดังนั้นใน พ.ศ. 2551 จึงได้มีการศึกษาเพื่อจัดทำมาตรฐานข้างต้นโดยใช้อุปกรณ์ใบพายแทน วิธีการที่เสนอไว้ใช้อุปกรณ์ใบพายที่ความเร็ว 75 รอบต่อนาที และตัวกลางการละลายประกอบด้วยกรดไฮโดรคลอริกเข้มข้น 0.05 โมลาร์โดยมีโซเดียมลอริลซัลเฟตละลายอยู่ในความเข้มข้นร้อยละ 0.6 น้ำหนักต่อปริมาตร ค่า Q ที่ยอมรับได้เสนอไว้เท่ากับ 75% ในเวลา 60 นาที

คำสำคัญ: **การละลาย, วิธีใบพาย, เคอร์คูมิน, ยาแคปซูลขมิ้นชัน**