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The Effect of Probe Path Length Calibration on Dissolution Tests Performed with a Fiber-Optic In Situ Dissolution Test System

e-mail: lxx6668@163.com

Pingping Xu, Xinxia Li*, Li Li, Yang Liu, Bin Kong, Kun Nie, and Jian Chen

School of Pharmacy, Xinjiang Medical University, No.393 Xinyi Road, Xinjiang, Urumqi 830011, China

ABSTRACT

The purpose of this research was to provide a method for calibrating the path length of probes used for dissolution tests performed with a fiber-optic dissolution test system (FODT). Methods found in pharmacopeia use the absorptivity of a 60.0 µg/mL potassium dichromate solution for calibrating 10-mm cuvettes. This absorptivity should only be employed to calibrate probes with a 5-mm gap where the total path length is 10 mm, since the calibration will be inaccurate if this absorptivity is used with different path length probes. In this article, we establish a calibration method using the absorptivities of potassium dichromate solutions at concentrations of 120.0, 300.0, and 600.0 µg/mL with 5-, 2-, and 1-mm cuvettes to calibrate probes with 2.0-, 1.0-, and 0.5-mm gaps, respectively. The calibration method established here for FODT minimizes calibration inaccuracies observed with calibration of probes using the method in Appendix IVA of the *Chinese Pharmacopeia*.

INTRODUCTION

Determination of the release rate of active ingredients from drug preparations is an important procedure in pharmaceutical laboratories. Traditionally, the technique is time consuming and labor intensive as it requires the removal of samples at specified time points followed by analysis of the samples with UV–vis spectrophotometry or high performance liquid chromatography (HPLC). Fiber-optic dissolution test systems (FODT), first introduced in 1988 (*1*), make dissolution testing less time consuming and labor intensive; no sample withdrawal is required because measurements are carried out in situ using a fiber-optic probe inserted directly into each dissolution vessel. The use of FODT in the pharmaceutical industry is increasing because it is a more efficient approach for dissolution testing.

Several research groups (*2–4*) have worked to develop fiber-optic techniques for dissolution testing. Real time or rapid data collection is possible with FODT and provides a wealth of information including complete drug release profiles from dissolution tests. FODT is especially useful in the development of new drugs and new dosage regimes where a large number of drugs require dissolution screening. The principles of dissolution testing with FODT have been described in detail in previous reports (*5, 6*).

In FODT, the sample "volume" is simply the liquid present in the probe gap at the time of measurement. The path length is twice the length of this gap because there is a mirror at the probe tip that reflects the light back to the detector (*5*). By selecting a replaceable probe with an appropriate spacing, the path length can be varied. The path length of the probes must be calibrated before an FODT is used for dissolution testing. The calibration of an FODT is analogous to the calibration of a UV spectrophotometer. The absorptivity of a standard solution of potassium dichromate with a concentration of 60 µg/mL can be used to calibrate a 10-mm cuvette (*7*) or the path length of a 5-mm FODT probe. If the standard absorptivity is used to calibrate probes with different path lengths, inaccuracies can be introduced. In this study, probes with different path lengths were first calibrated using the potassium dichromate standard absorptivity cited in Appendix IVA of the *Chinese Pharmacopeia* (*ChP*). We defined this method as the Single-Parameter Method (SPM). The probes with different path lengths were then calibrated using the absorptivities of potassium dichromate obtained using a UV spectrophotometer with different standard concentrations and different path length cuvettes. We defined this method as the Multi-Parameter Method (MPM). After the probes were calibrated with these two methods, dissolution tests were performed with carbamazepine and hydrochlorothiazide tablets. Results from both calibration approaches were compared to determine the errors and validate calibration of the probes with the MPM.

The *ChP* is not the only pharmacopeia to employ absorptivity for calculating the accumulative dissolution rates for certain drugs. This approach is also utilized by the *British Pharmacopoeia* (*BP*) for dissolution tests of phenylbutazone, chlortetracycline, and tylosin tablets, as well as for nonpharmacopeial methods (*8, 9*). The advantages of this approach are that it consumes less chemical standard and takes less time. However, the absorptivity is based on a specified concentration and a path length of 10 mm. In this study, we employed an FODT to determine dissolution rates where detection was performed

*Corresponding author.

in situ and no sample dilution was required. Varying the path length to ensure that the absorbance is in the linear range is an approach that has been used in previous studies (*10–12*). When the absorbance of a sample exceeded the range of 0.2–0.8 using a 10-mm path length probe, we altered the probe path length according to the Beer–Lambert law so that the absorbance was within the linear range.

MATERIALS AND METHODS

Materials

A fiber-optic drug dissolution in situ test system (FODT) was developed by Jian et al. (*2*) of the Xinjiang Medical University and Xinjiang FOCS Biotech Development Co., Ltd. A UV spectrophotometer (Cintra 40, GBC Scientific Equipment Pty. Ltd., Australia) was used for the offline control experiment.

Potassium dichromate standard reagents and carbamazepine tablets (100 and 200 mg) were purchased from Jiangsu Sihuan Bioengineering Co., Ltd. (Lot 0803041) and Beijing Nuohua Pharmaceutical Co., Ltd. (Lot x0303), respectively. Hydrochlorothiazide tablets (50 mg) were purchased from Shandong Renhetang Yaoye Co., Ltd. (25 mg, Lot 080205). Carbamazepine (Lot 100142–199503) and hydrochlorothiazide reference standards (Lot 100309–200702) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China. Hydrochloric acid and sulfuric acid were all of analytical reagent grade and were obtained from Fuchen Chemical Reagents (Tianjin, China). Purified water was produced in the laboratory and degassed before use.

Methods

Calibration of Different Path Length Probes with Potassium Dichromate

A stock solution of potassium dichromate was prepared by dissolving 60 mg of dried standard with 0.005 mol/L sulfuric acid. Potassium dichromate solutions at 600.0, 300.0, 120.0, and 60.0 µg/mL were then prepared from the stock solution. The absorbances of the potassium dichromate solutions were determined using cuvettes with path lengths of 1, 2, 5, and 10 mm.

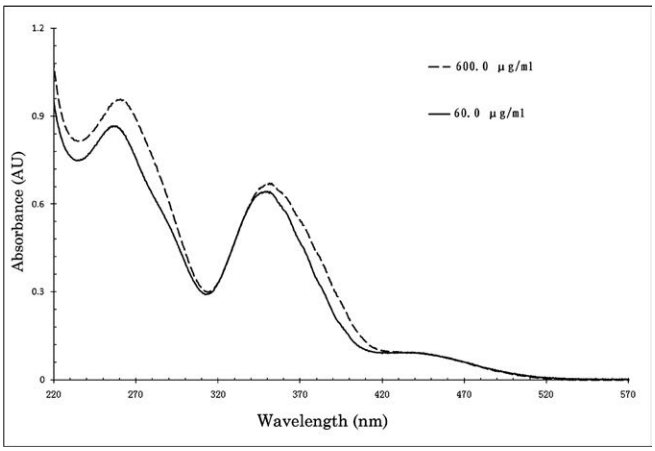

Figure 1. Absorption spectra of potassium dichromate at different concentrations.

Absorption Spectra of Potassium Dichromate Solutions

The absorption spectra of 60.0 and 600.0 µg/mL potassium dichromate solutions were determined from 220 to 440 nm using a UV spectrophotometer with 10-mm and 1-mm cuvettes, respectively.

Dissolution Test of Carbamazepine Tablets

Comparison of Errors after Calibration with SPM and MPM

Dissolution was carried out using Apparatus 2 (paddle) of the *ChP* at 150 rpm and a constant temperature of 37 ± 0.5 °C using 1000 mL of diluted hydrochloric acid. The detection wavelength was 285 nm, and the absorptivity was 518. Using FODT, probe gaps of 0.5 and 1.0 mm were used for carbamazepine tablets with label strengths of 200 and 100 mg, respectively. Sampling was performed at specified times. A control experiment was conducted using a UV spectrophotometer and the *ChP* method. The probe path lengths were calibrated before the dissolution testing using the SPM and MPM.

Table 1. Absorptivities of Different Concentrations of Potassium Dichromate at Different Wavelengths with Different Path Length Cuvettes (n = 6)

	$E^{1\%}_{10mm}$ (60.0 µg/mL)[a]		$E^{1\%}_{5mm}$ ± %RSD (120.0 µg/mL)		$E^{1\%}_{2mm}$ ± %RSD (300.0 µg/mL)		$E^{1\%}_{1mm}$ ± %RSD (600.0 µg/mL)	
Wavelength (nm)	**Average**	**Range**	**Average**	**Range**	**Average**	**Range**	**Average**	**Range**
235	124.5	123.0–126.0	126.9 ± 0.09	126.5–127.3	134.0 ± 0.13	133.3–134.7	135.9 ± 0.16	135.2–136.6
257	144.0	142.8–146.2	147.3 ± 0.07	147.0–147.6	156.0 ± 0.09	155.4–156.6	158.4 ± 0.21	157.6–159.2
313	48.6	47.0–50.3	48.9 ± 0.13	48.7–49.1	50.7 ± 0.51	50.4–51.0	50.2 ± 0.46	49.9–50.5
350	106.8	105.5–108.5	108.2 ± 0.10	108.0–108.4	112.4 ± 0.10	112.1–112.7	111.0 ± 0.18	109.5–110.5

[a] Cited from *ChP*

Table 2. Carbamazepine Tablets (100- and 200-mg) with FODT and Control Experiment (UV) Dissolution Data

	Single Parameter Method (SPM)			Multi-Parameter Method (MPM)		
	Dissolution (%) (n=6)		$\triangle\bar{x}^b$	Dissolution (%) (n=6)		$\triangle\bar{x}$
Label Strength (mg)	FODT[a]	Control		FODT	Control	
100	79.7 ± 4.1	84.5 ± 3.8	4.8	83.7 ± 1.9	83.7 ± 1.9	0
200	74.1 ± 2.3	82.0 ± 0.4	7.9	80.9 ± 2.0	82.9 ± 1.0	2.0

[a] Path length was 2 mm for 100-mg label strength and 1 mm for 200-mg label strength.
[b] Absolute difference between the means

Absorptivities of Carbamazepine Solutions at Different Concentrations

Carbamazepine solutions of 19.9, 39.8, 99.6, and 195.2 µg/mL were prepared, and their absorbances were determined using 10-, 5-, 2-, and 1-mm cuvettes, respectively, with a UV spectrophotometer.

Dissolution Test of Hydrochlorothiazide Tablets

Comparison of Errors after Calibration with SPM and MPM

Dissolution was carried out using Apparatus 1 (basket) of the *ChP 2005* at 150 rpm and a constant temperature of 37 ± 0.5 °C using 1000 mL of diluted hydrochloric acid. The detection wavelength was 272 nm, and the absorptivity (1 cm, 1%) of hydrochlorothiazide was 640. Probes with 2.0-mm gaps were selected, and the dissolution test was carried out for 30 min. At the end of the test, the control experiment was carried out according to the *ChP* method.

Hydrochlorothiazide Absorptivities at Different Concentrations

Hydrochlorothiazide solutions of 10.8, 21.6, 54.0, and 108.0 µg/mL were prepared, and the absorbances were measured using 10-, 5-, 2-, and 1-mm cuvettes, respectively, with a UV spectrophotometer.

RESULTS

Potassium Dichromate Absorptivities and Absorption Spectra

The absorptivities of the potassium dichromate solutions at different concentrations and wavelengths are listed in Table 1 and are significantly different at different concentrations with different cuvette path lengths ($p < 0.05$). The absorption spectra of 60.0 and 600.0 µg/mL potassium dichromate solutions (Figure 1) show that the absorbance maxima and minima are different at these two concentrations.

Dissolution Testing of Carbamazepine Tablets

Dissolution Errors after Calibration with SPM and MPM

The dissolution test results for 60 min are shown in Table 2. The standard deviations (SD) for carbamazepine tablets of 100 mg and 200 mg were 4.8 and 7.9, respectively, when the probes were calibrated with SPM. When the probes were calibrated with MPM, the SD decreased significantly to 0.0 and 2.0 for carbamazepine tablets of 100 mg and 200 mg, respectively ($p < 0.05$).

Table 3. Absorptivities of Carbamazepine Solutions with Different Concentrations

Concentration (µg/mL)	Path Length (mm)	Absorptivity
19.9	10.0	517.2
39.8	5.0	519.6
99.6	2.0	539.1
195.2	1.0	559.3

Table 4. Actual (C_1) and Calculated (C_2) Concentrations[a] with Different Cell Path Lengths

C_1 (µg/mL)	Path Length, L (mm)	$C_1 \times L$	A	C_2 (µg/mL)
9.95	10	99.5	0.5146	9.93
19.91	5	99.55	0.5173	19.97
49.8	2	99.6	0.5365	51.8
97.6	1	97.6	0.5459	105.4

[a] Absorptivity (518) given in the *ChP* for carbamazepine was used.

Absorptivities of Carbamazepine Solutions at Different Concentrations

The absorptivities of carbamazepine solutions at concentrations of 19.9, 39.8, 99.6, and 195.2 µg/mL were calculated according to the Beer–Lambert law (Table 3). In dissolution tests with FODT, the final concentrations for carbamazepine tablets with label strengths of 100 and 200 mg were 100 and 200 µg/mL, respectively. Therefore, based on the results in Table 3, absorptivities of 539.1 and 559.3 should be employed to calculate the dissolution rate of carbamazepine 100- and 200-mg tablets, respectively. The results were inaccurate when the absorptivity for carbamazepine cited in the *ChP* (518) was used to calculate the dissolution rates for the carbamazepine 100- and 200-mg tablets (Table 4). If the absorptivity is constant, the absorbance should remain constant when the concentration is increased and the path length is decreased. However, the absorbance was not constant (Table 4), indicating that the absorptivity is not constant.

Table 5. Dissolution Data for Hydrochlorothiazide Tablets with FODT (path length 4 mm) and Control Method (UV)

Single Parameter Method (SPM)			Multi -Parameter Method (MPM)		
Dissolution (%) ($n = 6$)		$\triangle\bar{x}^a$	Dissolution (%) ($n = 6$)		$\triangle\bar{x}$
FODT	Control		FODT	control	
91.5 ± 1.9	94.8 ± 1.8	3.3	91.0 ± 6.8	92.4 ± 6.5	1.6

[a] Absolute difference between the means

Table 6. Absorptivities of Hydrochlorothiazide Solutions at Different Concentrations

Concentration (µg/mL)	Path Length (mm)	Absorptivity
10.8	10.0	644
21.6	5.0	650
54.0	2.0	666
108.0	1.0	670

Table 7. Actual (C_1) and Calculated (C_2) Concentrations[a] with Different Cell Path Lengths

C_1 (µg/mL)	Path Length, L (mm)	$C_1 \times L$	A	C_2 (µg/mL)
10.8	10	108	0.6957	10.87
21.6	5	108	0.7019	21.93
54.0	2	108	0.7196	56.22
102.0	1	102	0.6951	108.6

[a] Absorptivity cited in the *ChP* for hydrochlorothiazide was used.

Dissolution Test of Hydrochlorothiazide Tablets

Dissolution Errors after Calibration with SPM and MPM

A control experiment was carried out according to the *ChP* method. The dissolution test results for 30 min are listed in Table 5. The SDs between the control and FODT dissolutions with calibration of the probes carried out by SPM and MPM were 3.3 and 1.6, respectively.

Absorptivities of Hydrochlorothiazide Solutions at Different Concentrations

The absorptivities of hydrochlorothiazide solutions with concentrations of 10.8, 21.6, 54.0, and 108.0 µg/mL were calculated according to the Beer–Lambert law (Table 6). For the dissolution test of 25-mg hydrochlorothiazide tablets, the final concentration was 25 µg/mL. Therefore, according to the absorptivity results, an absorptivity of 650 should be used to calculate solution concentrations. However, the absorptivity was not constant when the concentration was increased and the path length was decreased correspondingly. Inaccurate results would be generated if the dissolution concentrations were calculated with an absorptivity of 640 as cited in the *ChP* method (Table 7).

DISCUSSION

Chemical deviations from Beer's law can occur when the absorbing species are involved in an equilibrium reaction. In potassium dichromate solutions, the following equilibrium reaction occurs:

$$Cr_2O_7^{2-} + H_2O \Leftrightarrow 2H^+ + 2CrO_4^{2-}$$

At low potassium dichromate concentrations, the primary ion is $HCrO_4^-$, with approximately 1–4% of the $Cr_2O_7^{2-}$ ion formed by dimerization where the amount of $Cr_2O_7^{2-}$ depends on the chromium concentration (*13*). The absorptivities of the $HCrO_4^-$ ion ($E_{1cm}^{1\%}$) are 124.5, 144.0, 48.6, and 106.6 at 235, 257, 313, and 350 nm, respectively. When the concentration of the solution of potassium dichromate is high, the dimer $Cr_2O_7^{2-}$ is formed. The spectral absorptivity of this $Cr_2O_7^{2-}$ dimer is similar to that of $HCrO_4^-$, but its overall spectral shape is significantly different, as shown in Figure 1 (*14*).

The Beer–Lambert law is valid only for low concentrations of analyte. This is because absorbances will change as the refractive index varies with analyte concentration. For sufficiently low concentrations of analyte, the refractive index remains constant, hence the Beer–Lambert law is valid.

The accumulative dissolution rates (%) of different drugs as determined with probes calibrated using the Single Parameter and Multi-Parameter methods show that the results from the probes calibrated by the Single Parameter method had larger mean absolute errors than the results from the probes calibrated by the Multi-Parameter method. Furthermore, the mean absolute errors decreased as the probe path length increased. Therefore, we suggest that when employing FODT, the probe path length should be calibrated using the absorptivities of potassium dichromate at the corresponding concentrations. When drug concentrations exceed a specified range where the Lambert-Beer law is valid, the absorptivity should not be employed to calculate the accumulated dissolution. In this case, calibration with a reference substance should be employed to ensure generation of accurate data.

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