

Fiber optic dissolution analysis in quality control

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1 Experimental

A manual and a semiautomatic fiber optic quantification method was developed for monitoring the dissolution of soft gelatin capsules, containing 200 mg of active substance dissolved in a mono- and diglyceride filling. While performing the dissolution test in apparatus 2 [1] (Sotax AT 7, Sotax AG, Allschwil, Switzerland) stirred with 100 rpm at $37.0^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ the medium (900 ml aqueous citrate buffer solution, pH 3.0, with 12% Cremophor[®] EL) becomes turbid owing to the metal oxide pigments from the capsule shell. For the soft gelatin capsules a Q-value of 85% after 20 min is specified.

The spectroscopic equipment used can be seen in Table 1.

Table 1: Spectroscopic equipment used.

Manual method	Semiautomatic method
<ul style="list-style-type: none">• 1 Varian Cary 50 spectrometer (Varian International AG, Zug, Switzerland)• 1 fiber optic coupling device (Varian International AG, Zug, Switzerland)• 1 fiber optic probe (Ultra Mini TS 10 mm + 2 LL UV Li/SMA 974725/18, Hellma GmbH & Co., Müllheim/Baden, Germany) with a fixed pathlength of 10 mm	<ul style="list-style-type: none">• 1 Varian Cary 50 spectrometer (Varian, Inc., Walnut Creek, USA)• 1 Cassini multiplexer (C Technologies Inc., Cedar Knolls, USA)• 7 replaceable tip absorption probes, type FA-CT1101-APOY with the pathlength of 10 mm (C Technologies Inc., Cedar Knolls, USA)

As shown in Figure 1, the absorption readings — always acquired with an averaging time of 1 s — with the fiber optic probe were taken in the vessel.

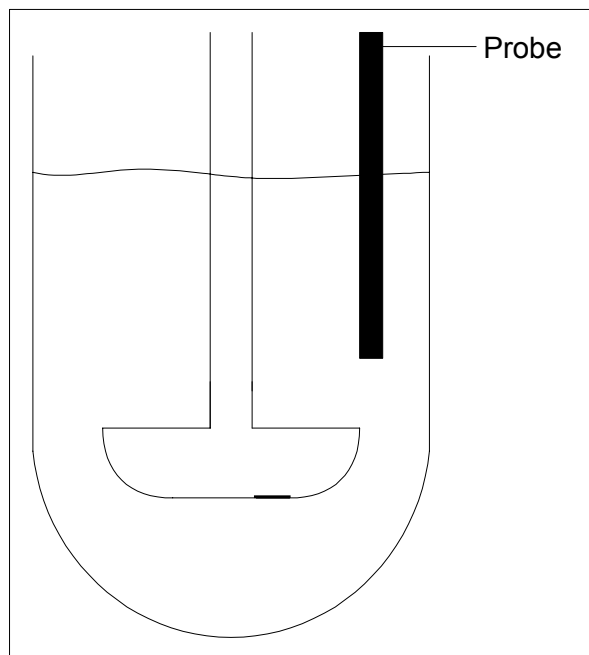


Figure 1: Experimental arrangement for taking absorption readings using a fiber optic probe.

1.1 Preliminary Experiments

For method development spectra with the drug product, the active substance, and the placebo formulation in dissolution medium were performed in order to establish a suitable wavelength for the turbidity correction.

1.2 Validation of the fiber optic methods

Using the manual fiber optic method, the absorptions at 315 (A_{315}) and 380 nm (A_{380}) were measured directly in the vessel. The amount of dissolved active substance in percent ($\%_{\text{dissolved manual}}$) was calculated using formula 1, where 53.0 is the $A(1\%, 1 \text{ cm})$ value of the active substance in placebo-spiked dissolution medium at $37.0^\circ \pm 0.5^\circ\text{C}$ and 4'500 is a conversion factor.

Formula 1: $\%_{\text{dissolved manual}} = (A_{315} - A_{380}) * 4,500 / 53.0$

Again the absorptions at 315 ($A_{315 \text{ sample}}$) and 380 nm ($A_{380 \text{ sample}}$) were measured directly in the vessel in case of the semiautomatic fiber optic method. The amount of dissolved active substance in percent ($\%_{\text{dissolved manual}}$) was calculated using formula 2 with a single point calibration by means of a standard solution, which corresponded to 100% of dissolved active substance (C_{standard}). Again 450 is a conversion factor.

Formula 2: $\%_{\text{dissolved semiautomatic}} = (A_{315 \text{ sample}} - A_{380 \text{ sample}}) * 450 * C_{\text{standard}} / (A_{315 \text{ standard}} - A_{380 \text{ standard}})$

The conducted validation experiments are presented in Table 2.

Table 2: Performed validation experiments for the manual and the semiautomatic method.

Parameter	Manual method	Semiautomatic method
Linearity, precision, and internal recovery	5 to 120% of dissolved active substance (0.011 – 0.267 mg/ml) in sextuplicate	5 to 120% of dissolved active substance (0.011 – 0.267 mg/ml) in triplicate
External recovery	-	80, 100, and 120% of dissolved active substance (0.178, 0.222, and 0.267 mg/ml) in triplicate
Method comparison	7 lots, 6 capsules per lot	4 placebo-spiked model solutions

The method comparison was performed with the reference method, which included manual sampling and filtration with a pore width of 0.45 μm (GyroDisc-PES 25, Orange Scientific, Waterloo, Belgium).

The different quantification methods — quantification by A(1%, 1 cm) vs single point calibration — are responsible for the different designs and the different y-intercept criterion in the validation experiments [2, 3]. All the acceptance criteria are shown in Table 3.

Table 3 Applied acceptance limits according to Refs. [2, 3] in the program VoAM 3.0 [4].

Criterion	Acceptance limit
Coefficient of correlation	is greater than 0.99.
y-intercept	lays within the 95% confidence interval of 2.00% (manual), respectively 5.00% (semiautomatic) of the reference x-value corresponding to the sample concentration of 100%.
Relative repeatability standard deviation	is equal or smaller than 2.00%.
Recovery	is between 98.00 and 102.00%.
Method equivalence	if the 95% confidence interval of the mean of the fiber optic method is entirely within the acceptance interval of 2.00% around the mean of the reference method.

The placebo response was determined with placebo capsules in order to gain information about the specificity.

The robustness of the analytical method for determining the amount of active substance dissolved was examined on the basis of the manual method in terms of temperature, paddle speed, and the immersion depth of the fiber optic probe window. The repeatability of the Cassini multiplexer was tested by measuring solutions of potassium dichromate (spectroscopy grade, Fluka, Buchs, Switzerland) in 0.01 N sulphuric acid [5] 70 times over one hour at 259 nm.

In order to give an overview of the dissolution process, profiles over 20 min were measured with six soft gelatin capsules.

2 Results

2.1 Method development

From Figure 2 it can be seen that the active substance does not show any absorption above 365 nm. The absorption of the placebo capsule is 0.050 over the entire wavelength range, owing to the turbidity. Above 365 nm the soft gelatin capsule absorption curve matches the turbidity line.

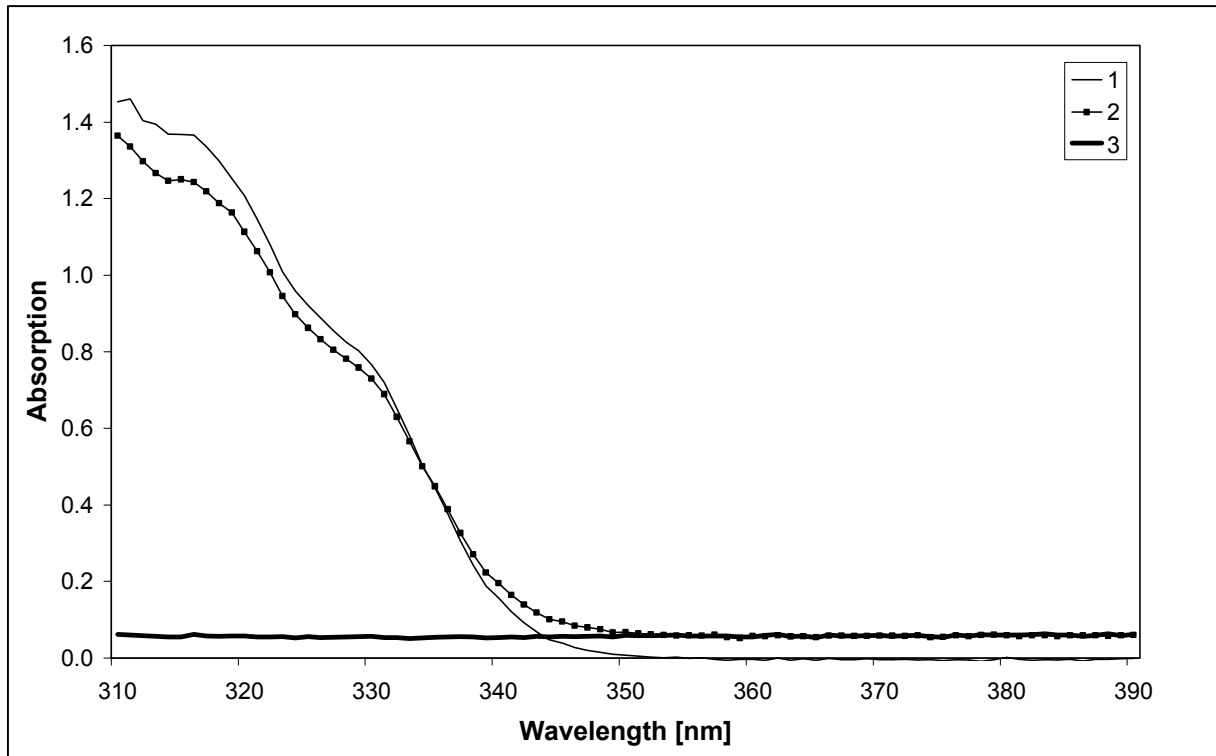


Figure 2: Absorption spectra of 0.26 mg/ml active substance (1), a soft gelatin capsule (2), and a placebo capsule (3) in 900 ml dissolution medium. The dissolved soft gelatin capsule resulted in a concentration of active substance of 0.22 mg/ml.

For the new fiber optic methods the shoulder of the active substance at 315 nm was chosen for quantification, just as in the registered model with manual sampling and filtration. The wavelength of 380 nm was chosen for the turbidity correction.

2.2 Validation of the fiber optic method

For the $A(1\%, 1\text{ cm})$ calculation for the manual method the six linearity assays were evaluated separately. The mean of the six $A(1\%, 1\text{ cm})$ values was $52.96\text{ cm}^{-1}\ \%^{-1}$ with a relative standard deviation of 0.51%. The other results of the manual method validation are presented in Table 4.

Table 4: Validation results of the manual method.

Parameter	Manual method
Coefficient of correlation	0.99968
y-intercept	-0.00155
Relative repeatability standard deviation [%]	0.44
Internal recovery [%]	98.87

The results of semiautomatic method validation are shown in Table 5.

Table 5: Validation results of the semiautomatic method.

Legend: r: coefficient of correlation
 $s_{rel. rep.}$: relative repeatability standard deviation

Parameter	Semiautomatic method						
	Probe 1	Probe 2	Probe 3	Probe 4	Probe 5	Probe 6	Probe 7
r	0.99976	0.99975	0.99973	0.99984	0.99983	0.99982	0.99972
y-intercept	0.03569	0.03562	0.03678	0.03452	0.03327	0.03502	0.03690
$s_{rel. rep.}$ [%]	0.94	0.97	1.00	0.77	0.80	0.82	1.03
Internal recovery [%]	100.41	100.41	100.44	100.33	100.31	100.32	100.43
External recovery [%]	98.86	99.41	99.06	98.93	98.92	99.12	98.96

The results in Table 4 and Table 5 all met the acceptance criteria for linearity, precision, and accuracy. The method comparisons (reference method with manual sampling and filtration) data showed no significant difference ($p = 95\%$) neither for the manual nor for the semiautomatic method.

The placebo response was -0.17% for the manual method and -0.19% for semiautomatic method.

2.3 Robustness

Table 6 demonstrates that the fiber optic detection method is unaffected by temperature ($p = 95\%$).

Table 6: Influence of temperature on the detected percentage of dissolved active substance of a placebo-spiked solution (0.222 mg/ml).

Temperature [°C]	Dissolution [%]
25	100.1
30	100.0
35	100.1
40	100.1
45	100.0

Figure 3 shows the influence of paddle speed on the detected dissolution.

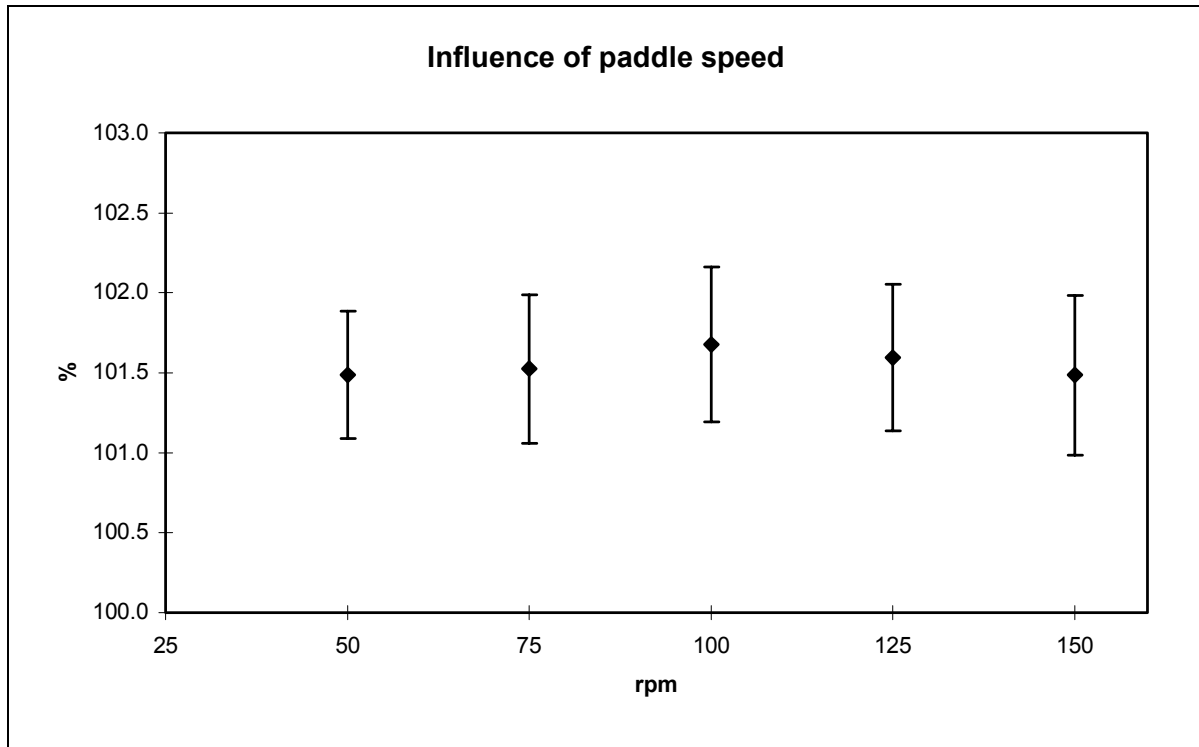


Figure 3: Influence of paddle speed on the fiber optic quantification method. The dots correspond to the mean of twelve soft gelatin capsules and the vertical lines represent ± 1 standard deviation.

With the equipment used here the radial distance between the probe and the paddle could not be varied and was within the requirements of Refs. [1, 5]. Findings for probe window immersion depths of 0.5, 2.0, 3.0 and 4.5 cm are presented in Table 7.

Table 7: Dissolution values [%] obtained at various probe immersion depths within the vessels. The depth of 3.0 cm was used for all other measurements.

Assay	Vessel	Immersion depth [cm]			
		0.5	2.0	3.0	4.5
1	1	102.0	102.2	102.1	102.1
	2	102.1	102.1	102.1	102.1
	3	101.6	101.9	101.5	101.9
	4	102.1	102.2	102.1	102.2
	5	101.8	101.9	102.0	101.7
	6	102.0	101.9	102.1	102.3
2	1	99.7	99.8	99.7	99.5
	2	99.4	99.3	99.5	99.4
	3	99.0	98.9	98.8	98.9
	4	101.0	101.0	101.1	100.8
	5	99.6	99.5	99.5	99.2
	6	99.0	99.0	98.9	99.0

A statistical comparison of the depths 0.5 and 4.5 cm did not show any significant differences ($p = 95\%$).

Furthermore, the mean absorption (sixfold determination) was measured in one vessel at six different immersion depths at 315 and at 380 nm. The mean absorption readings at both wavelengths as well as their differences are presented with the corresponding standard deviations in Table 8.

Table 8: The means of six absorption readings at 315 (A_{315}) and 380 nm (A_{380}) as well as the difference between the two (ΔA) are shown for different immersion depths in the same vessel. All obtained values are given with the corresponding standard deviations.

Immersion depth [cm]	A_{315} [AU]	A_{380} [AU]	ΔA [AU]
1.0	1.2474 ± 0.0012	0.0757 ± 0.0003	1.1717 ± 0.0013
2.0	1.2474 ± 0.0012	0.0759 ± 0.0001	1.1715 ± 0.0012
3.0	1.2468 ± 0.0009	0.0757 ± 0.0002	1.1711 ± 0.0009
4.0	1.2472 ± 0.0017	0.0761 ± 0.0003	1.1711 ± 0.0017
5.0	1.2470 ± 0.0012	0.0755 ± 0.0003	1.1715 ± 0.0011
6.0	1.2471 ± 0.0005	0.0754 ± 0.0002	1.1716 ± 0.0005

These results show that the turbidity as well the concentration of the active substance is homogenous in the examined region of the vessel because the different immersion depths do not lead to significantly different results ($p = 95\%$). Although the turbidity is measured during each quantification, further evidence about the robustness of the method is given owing to the small turbidity differences.

The statistical parameters of the repeatability experiment with the multiplexer consisting of a data set of 70 repetitive measurements over one hour are presented in Table 9.

Table 9: Statistical parameters of the 70 repetitive measurements over one hour on the multiplexer.

Legend: s: standard deviation
 s_{rel.}: relative standard deviation
 Max. maximum value
 Min. minimum value
 R: range

Parameter	Probe 1	Probe 2	Probe 3	Probe 4	Probe 5	Probe 6	Probe 7
mean	1.1513	1.1579	1.1504	1.1830	1.2115	1.1585	1.1499
s	0.0008	0.0018	0.0012	0.0011	0.0012	0.0015	0.0010
s _{rel.} [%]	0.07	0.15	0.11	0.09	0.10	0.13	0.09
Max.	1.1535	1.1638	1.1536	1.1850	1.2134	1.1605	1.1517
Min.	1.1493	1.1558	1.1461	1.1801	1.2083	1.1538	1.1470
R	0.0042	0.0080	0.0075	0.0049	0.0051	0.0066	0.0047

The results confirm that the system performs accurate over the time of a typical dissolution run.

2.4 Dissolution profile

As can be seen in Figure 4, the mean dissolution profile has a sigmoid shape, with high variability between 6 and 15 min. The acceleration at around 6 min corresponds to the rupture of the capsule shell. After 15 min the entire liquid content of the soft gelatin capsule has escaped and the 100% plateau is reached.

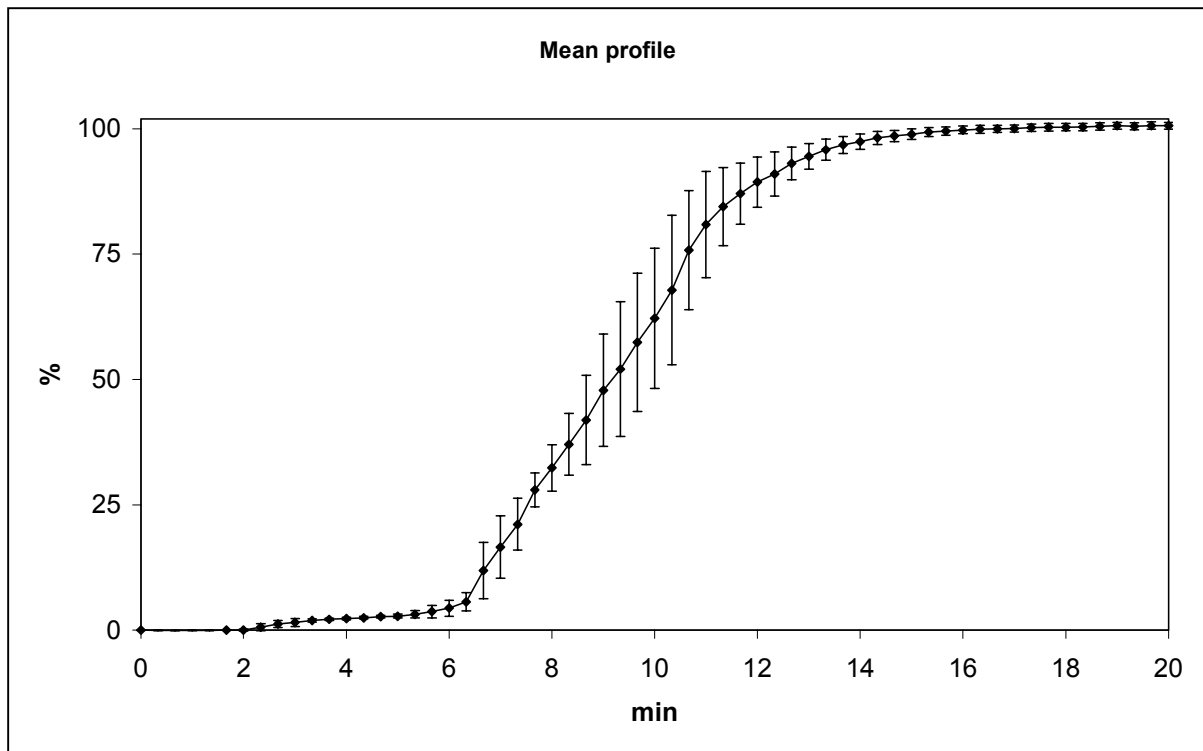


Figure 4: Mean dissolution profile of six soft gelatin capsules. The vertical lines represent ± 1 standard deviation.

3 Discussion

The experiments for the semiautomatic method were performed with the fiber optic immersion probes staying in the vessel. However, there are no validation experiments necessary owing to the hollow shaft measuring principle applied in routine analysis. And as can be seen in the robustness results there is very strong evidence, that the placing of fiber optic probes has no effect on the determined extent of dissolution [6].

4 Conclusions

The validated methods are suitable for pharmaceutical quality control in terms of accuracy, precision, reproducibility, reliability, and robustness. It could also be demonstrated that using a multiplexer has no effect with regard to method validation [2, 3].

5 References

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