Dissolution testing for prolonged-release solid dosage form containing drug substances degrading in test media. Application to polymeric delivery systems of paracetamol

A A Rakina, T I Spriridonova and S I Tverdokhlebov

National Research Tomsk Polytechnic University, 30, Lenin Ave., Tomsk, 634050, Russia

E-mail: tverd@tpu.ru

Abstract. Synthetic biodegradable polymers are widely used as materials for the targeted drug delivery and controlled release devices. In order to control the rate of drug release from polymer scaffolds surface ordinary dissolution testing is performed. It includes placing a device into testing media, taking probes at determined time and estimating the drug concentration. As for prolonged release the issue is the drug degradation in test media what leads to false interpretation of results. This study was focused on the investigation of paracetamol degradation in phosphate buffer saline media for three days and its effect on the results of polymeric drug delivery system dissolution test. It was shown, that relative error in the released drug concentration evaluation can rise up to 34% depending on the exposure time and the initial probe concentration. An approach of experimental results correction is proposed.

1. Introduction

Advanced drug delivery and controlled release are now one of the most important themes in modern pharmaceutics. Especially, solid dosage forms for prolonged release. Among all technologies biodegradable polymeric drug delivery systems are considered truly perspective because of their biocompatibility and non-invasive dissolution in the body what makes further surgical intervention for de-implantation not necessary. In order to study the drug release profile from any drug dosage form a dissolution testing should be performed.

Dissolution testing (DT) is required for most solid oral dosage forms [1]. The main aim of this analytical test is to detect the release of an active pharmaceutical ingredient (API) from the formulated product. For targeted drug delivery dosage forms to be efficacious, the API(s) must be absorbed in the space near the site of activity. This process characterizes the drug substance bioavailability and involves two stages: dissolution and absorption. Dissolution is the process of extracting the API out of the dosage form solid-state matrix into body fluids. Absorption is the process of transporting the drug substance to the target through diffusion. In the dissolution testing at set time points, aliquots of filtered medium are removed and analyzed for API content by UV-vis or HPLC methods. Common issue is the API degradation: if the drug degrades in the DT media, the results of the content analysis can represent smaller value of released API in comparison to real value. Detecting the API degradation and estimation of its kinetics is crucial in DT data interpretation [2].

Paracetamol is a commonly used anti-inflammatory drug. Moreover, it is a highly suitable model drug for testing new drug delivery systems. When incorporated, paracetamol can mimic other same

average molecular weight molecules release. However most DT protocols of paracetamol dosage forms include not more than one-day testing. For prolonged release dosage forms DT has to be corrected due to paracetamol degradation to products such as hydroquinone, p-aminophenol, p-nitrophenol, 1,4-benzoquinone and, NAPQI (N-acetyl-benzoquinone imine) [3].

This study aims determining the degradation kinetics of paracetamol in phosphate buffer saline media during three days. Also the application of presented data to real DT of eletrospun polycarolatone nanofibers incorporated with paracetamol substance is presented.

2. Materials and methods

2.1. Preparation of paracetamol solutions

First, phosphate buffer saline (PBS, pH=7,2-7,4) was prepared by dissolving a tablet (Biolot, Russia) in distilled water according to the manufacturer instructions. Pure pharmaceutic substance of paracetamol was received from Shandong Xinhia Pharmaceutical (China). Substance was dissolved in PBS to get a highest in the experiment concentration – 875 μg/ml. Next fifteen points were prepared by double dissolution of the previous point. All experiments were performed in triplets. As a result, 16 solutions with different concentrations were prepared.

2.2. Study of paracetamol degradation

The amount of paracetamol in each probe was determined using UV-vis spectrometry (Specord 250 Plus, Analytik Jena AG, Germany), λ=245 nm (for concentations 0.21 – 27.4 μg/ml) and 300 nm (27.4 – 875 μg/ml). Analytical signal – absorption (peak height). In this study dependence of concentration drop in the solution on the exposure time (0, 1 and 3 days) and on the initial solution concentration (from 0.21 to 875 μg/ml) was studied. So, 16 solutions were prepared for every exposure time type. All experiments were performed three times.

2.3. Preparation of PCL fibers by electrospinning

Polycaprolactone (PCL) Mw~70–90 kDa (Sigma–Aldrich, Germany) was dissolved in hexafluoroisopropanole (HFIP) (Ekos-1, Russia) at a concentration of 7 wt.%. For the preparation of 2 wt./wt % and 32 wt./wt % (drug substance/dry polymer weight) paracetamol-loaded PCL solutions, previously dissolved in HFIP paracetamol powders were added to PCL granules and then refilled with the rest of the solvent. Mixtures were left for 30 hours at the room temperature in sealed glass containers until full homogenization. Before electrospinning solutions were stirred on the magnetic stirrer for 30 min.

Electrospinning of nanofibers was carried out on NANON-01 (MECC CO., Japan) with a 200 mm diameter drum collector. The process parameters used in the current study are shown in Table 1.

Table 1. The process parameters optimized for preparation of PCL nanofibers.

Characteristics	Value
Voltage, kV	20
Feed rate, ml/h	5
Collector rotation speed, rpm	50
Needle	G 21

After fibers formation scaffolds were removed and placed into a custom made vacuum camera for 24 hours (5×10^{-3} Pa) to remove residual solvents.

2.4. Drug release study

Untreated and e-beam treated electrospun PCL scaffolds, both with the incorporated drug, were immersed in Phosphate Buffer Saline (PBS, pH 7.4) at 25ºC with three replicates for each type of scaffold. The experiments were run in 2 ml of PBS without stirring, and the drug release results were

found to be within one standard deviation to each other. At predetermined time points, a 1 mL aliquot was withdrawn for further analysis and replaced with an identical volume of the fresh medium. The amount of released paracetamol was determined using UV-vis spectrometry (Specord 250 Plus, Analytik Jena AG, Germany), λ=245 nm and 300 nm.

3. Results and discussion

It was shown in the experiment, that UV-vis absorption spectra of paracetamol in water solution has two characteristic peaks – at 245 nm and at 300 nm. Absorption at 245 nm is a suitable analytical signal for analysing solutions with paracetamol concentrations from 0.1 to 16 μg/ml, higher concentrations are better characterised by absoption at 300 nm. Figure 1 represents the difference in solutions' UV-vis spectra.

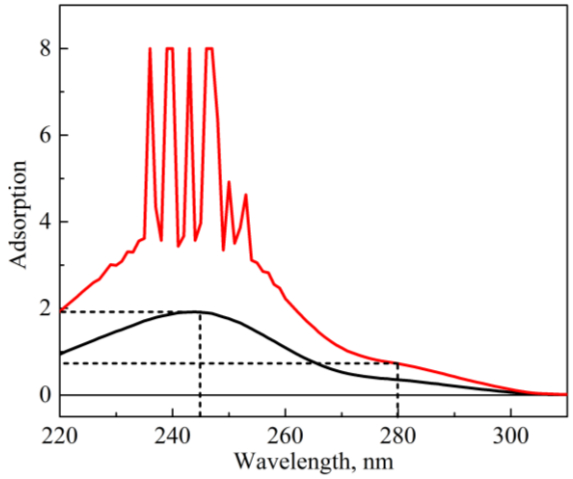

Figure 1. An example of absorption spectrum for 218 μg/ml and 14 μg/ml paracelamol solutions in PBS.

Thus, a study of the absorption value dependence on the initial concentration of paracetamol in solutions and on the exposure time were calculated to paracetamol concentration using two calibration curves (245 nm and 300 nm "0 day"). Results of all experiments are summarized in Figure 2.

It is shown that the observed concentration of drug significantly decreases after exposure for all initial concentrations and exposure times. Moreover, the drop of the absorption values are most noticeable for larger initial paracetamol concentrations and one day of drug exposure in the PBS. In some probes further growth of the absorption of the solutions is observed. We assume that the reason of this phenomenon may be due to the accumulation of the paracetamol degradation products in probes/ Literature data confirm that this products in water solutions have absorption area close to pure paracetamol solution [4].

Based on the obtained absorption data, calibration curves for wavelengths of 245 nm (0.21 - 13.67 μg/ml) and 300 nm 27.34 -875 μg/ml) were built. Results are presented in Figure 3.

All experimental points for each wavelength and storage time are approximated with high accuracy by a linear function. It means that paracetamol solutions with any initial concentration from studied range degrades with the same relative speed and it is possible to use the same tangent of calibration curve k for calculation of all paracetamol concentrations for probes taken in one day. As it was mentioned above, the values of absorption substantially decrease after the one day of the exposure and slightly increase after three days. However, this increase is almost absent for probes with lower concentrations, while the other curve is more sensitive to the storage time.

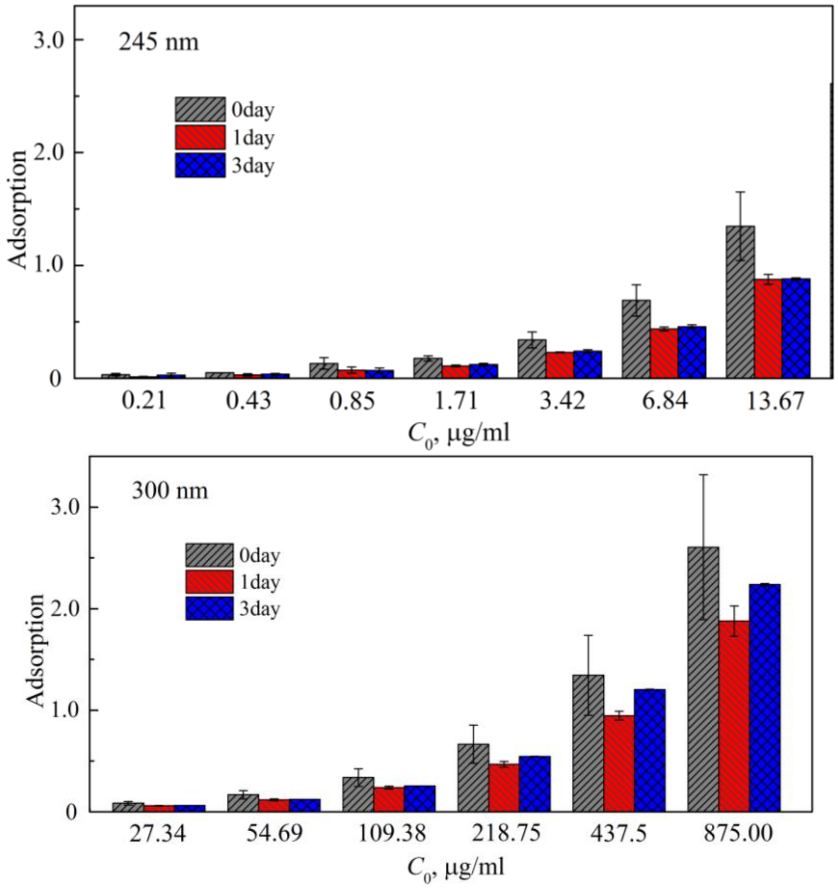

Figure 2. The dependence of absorption on the initial concentration of paracetamol and the time of storage in the PBS for 245 nm (at the top) and 300 nm (at the bottom).

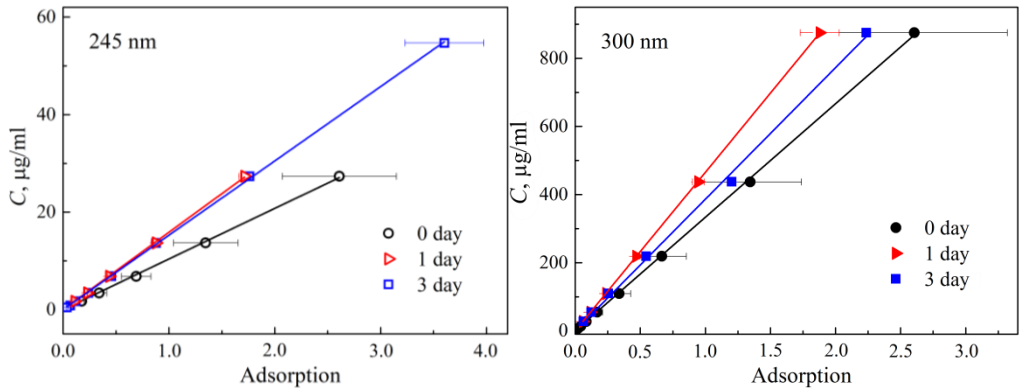

Figure 3. Calibration curves for the different storage time of paracetamol in the buffer.

In order to assess the effect of the paracetamol in-reactor degradation on the results of the drug release experiments, the relative difference in calculating the amount of the released drug (ΔC) was calculated as:

$$\Delta C = \frac{C(t)-C_0}{C(t)} \cdot 100\%, \quad (1)$$

where C_0 is the initial paracetamol concentration, $C(t)$ is the concentration after the period of time (1 or 3 days). The relative differences for each time of storage and wavelength as well as parameters of calibration curves for experiment processing are presented in Table 2.

Table 2. The accuracy of the approximation of the experimental data for the calibration curves by a linear function (R^2), the tangent of the slope of the calibration lines (k) and the relative errors in the concentrations determination (ΔC) .

	245 nm			300 nm		
	0 day	1 day	3 day	0 day	1 day	3 day
R^2	0.99	0.99	0.99	0.99	0.99	0.99
k	10.37	15.84	15.24	333.23	465.25	386.21
ΔC, %	-	34.53	21.22	-	22.95	13.72

In order to show how the correction in concentration calculation helps to make a right interpretation of dissolution testing the results of drug release from three paracetamol-loaded polycaprolactone scaffolds were analyzed. One of scaffolds (2p) had a low concentration of paracetamol (2 wt./wt %) and probes taken from the reactor were exposed in the PBS for one day. The second sample contained a relatively high concentration of paracetamol (32 wt./wt %) and probes were exposed in the PBS for three days. The drug release profiles without taking into account paracetamol degradation are shown in Figure 4a. The largest amount of drug was released from the scaffold with the highest paracetamol concentration, while the amount of drug released from the sample with only 2 wt./wt % of paracetamol was around 10% smaller. Wherein, even the highest value of amount of released drug does not exceed 70%.

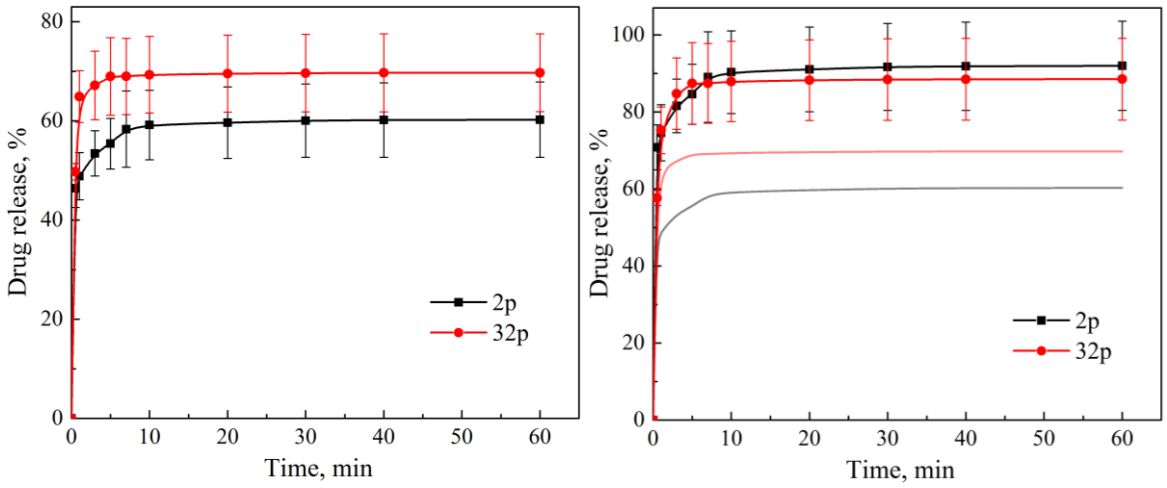

Figure 4. The paracetamol release profiles for different concentrations without any corrections (a) and after correction in comparison to the uncorrected ones (translucent lines) (b).

The paracetamol release profiles after taking into account the degradation process are presented in Figure 4b. Those profiles were obtained by using the corrected calibration curves instead of incorrected for 0 day. It is clearly seen that the corrected amount of released drug is much higher than uncorrected one. The relative difference for each time is already had been presented in the Table 2. In accordance with the previously obtained data, difference between profiles for sample 32 which was kept in the PBS for three days is smaller than for sample 2p which was kept only one day despite the higher drug concentration.

4. Conclusion

It was shown that for relatively long periods (one day or more) of conducting the dissolution testing and experiments of the release of paracetamol from polymers it is especially important to take into account

the paracetamol degradation. The technique for the dissolution testing correction of paracetamol-loaded prolonged release drug dosage forms was proposed. By using the proposed corrected calibration curves results for tests on materials with concentration range between 0.21 and 875 μg/ml within three days of exposure can be corrected. This technique is easy to use and allows to significantly increase the accuracy of the experimental results. Also, proposed approach can be transferred to other systems with degrading drug.

Acknowledgements
This work was financially supported by the Ministry of Science and Higher Education of the Russian Federation, Federal Target Program (agreement #14.575.21.0140, unique identifier RFMEFI57517X0140).

References
[1] Azarmi S, Roa W, & Löbenberg R 2007 *International Journal of pharmaceutics* **328 (1)** 12
[2] Higuchi T 1963 *Journal of pharmaceutical sciences* **52 (12)** 1145
[3] Trovo A G, Nogueira R F P, Agüera A., Fernandez-Alba A R and Malato S 2012 *Water research* **46 (16)** 5374
[4] Moctezuma E et al. 2012 *Journal of hazardous materials* **243** 130-138