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# Setting accelerated dissolution test for PLGA microspheres containing peptide, investigation of critical parameters affecting drug release rate and mechanism

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ABSTRACT

The objective of this study was development of accelerated in vitro release method for peptide loaded PLGA microspheres using flow-through apparatus and assessment of the effect of dissolution parameters (pH, temperature, medium composition) on drug release rate and mechanism. Accelerated release conditions were set as pH 2 and 45 °C, in phosphate buffer saline (PBS) 0.02 M. When the pH was changed from 2 to 4, diffusion controlled phases (burst and lag) were not affected, while release rate during erosion phase decreased two-fold due to slower ester bonds hydrolyses. Decreasing temperature from 45 °C to 40 °C, release rate showed three-fold deceleration without significant change in release mechanism. Effect of medium composition on drug release was tested in PBS 0.01 M (200 mOsm/kg) and PBS 0.01 M with glucose (380 mOsm/kg). Buffer concentration significantly affected drug release rate and mechanism due to the change in osmotic pressure, while ionic strength did not have any effect on peptide release. Furthermore, dialysis sac and sample-and-separate techniques were used, in order to evaluate significance of dissolution technique choice on the release process. After fitting obtained data to different mathematical models, flow-through method was confirmed as the most appropriate for accelerated in vitro dissolution testing for a given formulation.



## 1. Introduction

The development of controlled drug delivery systems for parenteral application, such as microspheres, liposomes, or implants, became of significant importance in the last few decades. Advantages of such systems are numerous: (i) better control of drug release and minimized fluctuation in systemic drug concentration, (ii) optimized exposure with reduction in side effects, (iii) reduced administration frequency leading to better patient compliance (Rawat et al., 2012; Shen and Burgess, 2012). Parenteral microspheres, based on biodegradable polymer Poly (lactide-*co*-glycolide) (PLGA), have been successfully used as controlled drug delivery systems (Rawat and Burgess, 2011) and are commercially available for products such as anticancer drugs (Decapeptyl® SR (Reslow et al., 2002)), antipsychotics (Risperdal® Consta (Ramstack et al., 1997)), hormone analogues (Sandostatin® LAR (Bodmer et al., 1995), Somatuline® Depot (Pellet and Roume, 1999) etc. Drug release from PLGA microspheres is controlled by diffusion, polymer erosion, or a combination thereof (Faisant et al., 2002). Typically, drug release follows tri-phasic profile: (i) burst phase – release of immediately available drug substance (non-encapsulated and pore related), (ii) lag phase – time for polymer chains to degrade to soluble oligomers, (iii) erosion phase – fast release of approximately zero order release kinetics (Zolnik et al., 2006).

The development of an appropriate in vitro release testing method for PLGA microspheres is mandatory as a quality control method for batch release. In addition, the dissolution test is essential for formulation and/or manufacturing process optimization, or as a biorelevant method for development of in vitro-in vivo correlation (Rawat and Burgess, 2011). This test, if linked with in vivo behavior of the drug, ensures optimal safety and efficacy of the formulation. Currently, release method development for

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parenteral drug delivery systems is hindered by lack of guidance; thus, it is based on recommendations and guidelines for oral extended release formulations. Nevertheless, in last few years, the Food and Drug Administration (FDA) has published recommendations for 13 products for parenteral application.

Various dissolution methods have been used and can be categorized into three main groups: (i) sample-and-separate, (ii) continuous flow and (iii) dialysis method (Zolnik and Burgess, 2008; Faisant et al., 2006; Delplace et al., 2012). Sample-and-separate is a widely used method for microspheres due to its simplicity. Microspheres are introduced into a vessel containing release media under agitation (mainly USP apparatus 2), and the determination of the released amount is performed after sampling and separation of the microspheres, either by centrifugation or filtration. The disadvantages of this method are microsphere aggregation, use of large volumes and inaccuracies due to sample loss. The dialysis method requires the use of dialysis sac of a porous cellulose membrane of various molecular weight cut offs (MWCO). Microspheres are suspended in a dialysis sac, and the released concentration is monitored in the recipient chamber. The disadvantages of this method are associated with microsphere aggregation due to lack of agitation, and the violation of sink conditions is possible when the drug release from microspheres is relatively rapid compared to the drug diffusion through the membrane. The continuous flow method utilizes USP apparatus 4. The test medium is continuously pumped through the microsphere bed placed in the flow-through cells. This method is often used for simulation of dynamic in vivo environment (Shen and Burgess, 2012). Disadvantages of this method are possible filter clogging and back pressure (Zolnik and Burgess, 2008; Kastellorizios and Burgess, 2012).

For 13 injectable formulations listed on the FDA web-site for the dissolution testing recommendations, 8 are based on USP 4 method (flow-through cell), while others are using the sample-and-separate method (USP 2 or vials) or are still under development.

The general outcome of many workshops on drug release method development for injectable formulations concludes that USP 4 apparatus is the most appropriate (Burgess et al., 2002, 2004). It shows several advantages over other techniques, such as the possibility to use large as well as small volumes without violating sink conditions, a constant perfusion of sample simulating in vivo environment, and the minimization of aggregation risk or sample (microspheres) floating.

Drug release from PLGA microspheres can range from days to months; therefore, the focus is placed on shortening the time-span and on developing accelerated in vitro release methods (Zolnik and Burgess, 2007), by choosing the most appropriate release technique and by defining the optimal testing conditions, which play an important role. Accelerated drug release can be achieved by altering many parameters. Among those parameters are the most studied ones: temperature, pH, test medium composition, surfactants, and agitation rate. Very often accelerated release tests can require extreme conditions that can lead to a change in drug release mechanism. Therefore, it is essential to investigate and understand how parameters employed in accelerated testing may affect the drug release mechanism and, thus estimate the predictability of the dissolution test versus factors found to be critical in vivo (Shen and Burgess, 2012).

The objective of this paper is to study in vitro release technique for testing PLGA microspheres loaded with a model peptide. In vivo release of the peptide lasts for 70 days after intramuscular application (internal clinical data, not published); therefore, the focus is on the development of an accelerated in vitro dissolution method that will enable complete drug release in several days, as well as on the investigation and understanding of the influence of dissolution parameters on drug release rate and mechanism.

## 2. Material and Methods

### 2.1. Materials

Peptide, a cyclic somatostatin analog (MW ≈ 1 kDa) in a form of salt, was encapsulated in microspheres of 50% branched poly(DL-lactide-*co*-glycolide glucose polymer) (PLGA) (MW ≈ 52 kDa) and 50% linear PLGA (MW ≈ 12 kDa), consisting of 50% lactic acid and 50% glycolic acid. Both, peptide and PLGA were from Novartis Pharma AG, Basel, Switzerland. All other used chemicals were of an analytical reagent grade.

### 2.2. Peptide solubility

The solubility of the salt form of the peptide was studied in different media: (i) phosphate buffer saline (PBS 0.02 M) of different pH (pH 2, pH 3, pH 4, pH 5, pH 6, pH 7, pH 7.4, pH 8), (ii) 0.9% solution of sodium chloride (NaCl), (iii) lactic acid solution. The lactic acid solution was used in order to mimic the muscle environment. The solubility was determined by incubating an excess of peptide in 1 ml of the medium at room temperature.

### 2.3. Preparation of microspheres

Peptide loaded PLGA microspheres were prepared using an oil-in-water (O/W) solvent extraction technique. A mixture of star and linear PLGA was dissolved in methylene chloride and filtered. The peptide was homogeneously dispersed in the prepared polymer solution. The solution was then injected in aqueous solution of PVA under homogenization. The microspheres were solidified under stirring at a higher temperature to allow the evaporation of methylene chloride. The solidified microspheres were filtered and washed by water for injection and then dried in a vacuum dryer.

### 2.4. Characterization of the microspheres

#### 2.4.1. High performance liquid chromatography

The peptide assay was performed using HPLC system (Agilent 1100) at 230 nm. The HPLC system consisted of a gradient pump, an automatic sample injector and a UV absorbance detector. The mobile phase A consisted of water/acetonitrile/tetrahydrofurane/trifluoroacetic acid (900 ml/50 ml/50 ml/0.93 ml) and mobile phase B of water/acetonitrile/tetrahydrofurane/trifluoroacetic acid (50 ml/900 ml/50 ml/0.83 ml). The analytical column used was a Reversed Phase C18, e.g. YMC-Pack, ODS-AQ, 3 μm, 150 × 3 mm (Stagroma). The flow rate was set at 0.8 ml/min, with retention time at 16.5 min and the evaluation was done by Chromeleon Chromatography Data System (Version 6.80, Dionex, California, USA).

#### 2.4.2. Determination of initial drug loading

Approximately 20 mg peptide-loaded microspheres were dissolved in 10 mL of acetonitrile:methanol (3:2) mixture. The samples were centrifuged at 3500 rpm for 10 min, and the supernatant was analyzed using the HPLC system described above.

#### 2.4.3. Differential scanning calorimeter (DSC)

The samples were analyzed using TA Instruments 2920 DSC. The samples were heated up to 80 °C and cooled down to 5 °C at a rate of 10 °C/min; the second cycle was used to determine the glass transition temperature (Tg). The samples were analyzed in aluminum pans with pinhole lids.

#### 2.4.4. Particle size analysis

The particle size of the microspheres was determined using a laser particle size analyzer (Sympatec HELOS, Sympatec GmbH,

Germany). One hundred milligrams of microspheres were suspended in 6 mL of distilled water and subjected to vortex mixing for 10 s before the analysis.

### 2.5. In vitro release studies

In vitro release tests were performed using USP apparatus 4 (Sotax CE7 smart, Sotax, Aesch, Switzerland) with flow-through cells (22.6 mm diameter) packed with 9 g of glass beads (1 mm) to prevent microsphere agglomeration in a closed system mode. Twenty milligrams of microspheres were dispersed in the flow-through cells, while 500 ml of 0.02 M PBS (pH 2) was circulated through a fiberglass filter (1.6 μm). A flow rate of 16 ml/min was used. At pre-determined time points, 1 ml samples were withdrawn and analyzed by HPLC. Drug release was studied under various test conditions:

- Osmolality and ionic strength: in phosphate buffer of 50, 200, 380 mOsm/kg, at pH 2 and 45 °C (see Section 3.1 for explanation). Osmolality above 50 mOsm/kg was adjusted using NaCl or Glucose in order to additionally investigate the effect of ionic strength on drug release.
- pH: phosphate buffer saline (0.02 M PBS) pH 2 and pH 4; ortho-phosphoric acid was used to adjust the pH. Tests were performed at 45 °C. The range of pH was selected based on the peptide stability and solubility (see 3.1. for explanation).
- Temperature: 45 °C and 40 °C in 0.02 M PBS, pH 2.

After investigating different test conditions and defining those that give accelerated drug release, other types of experimental setups were compared to USP 4 system:

- Sample-and-separate: USP apparatus 2 (Sotax AT7 smart, Sotax, Aesch, Switzerland) was used under rotation speed 50 rpm, 45 °C. Forty milligrams of microspheres were suspended in 1000 ml of 0.02 M PBS, pH 2. At pre-determined time points, 1 ml samples were separated by filtration through a fiberglass filter (1 μm) and then analyzed by HPLC (as explained above).
- Dialysis bag: 4 mg of microspheres were suspended in a Float-A-Lyzer (MWCO 8–10 kD) using 1 ml of 0.02 M PBS, pH 2. Float-A-Lyzer was placed in flat-bottomed vials containing 50 ml of PBS in a shaker water bath (GFL m.b.H. & Co., Burgwedel, Germany) at 80 rpm, at 45 °C. At known time points, 1 ml samples were withdrawn and replenished by fresh medium. The samples were then analyzed using HPLC method.

Each experiment was conducted in triplicate (mean values ± S. D. are indicated in figures).

### 2.6. Data analysis

The Weibull function (Eq. (1)) was used to model in vitro release data:

$$X_t/X_{inf} = 1 - \exp\left[-t^{\beta}/\alpha\right] \quad (1)$$

Where: $X_t$ is the percentage of drug release at time t, and $X_{inf}$ denotes 100% release; α is the scale factor referring to the apparent rate constant; β describes the shape of the curve as exponential (β = 1), sigmoidal (β > 1) or parabolic (β < 1).

Weibull is a descriptive function useful for comparing dissolution profiles.

For the calculation of scale and shape factors, the least-squares minimization technique was used (Li et al., 2007).

In order to analyze the release mechanism, several mathematical models were used:

a) Zero order release (Eq. (2)):

$$Q_t = Q_0 + K_0 t \quad (2)$$

Where: $Q_t$ is the amount of drug dissolved at time t, $Q_0$ is the initial amount of drug in the solution and $K_0$ is the zero order release constant.

This model can be used for modified release dosage forms with constant release kinetics independent of drug concentration.

a) Higuchi model (Eq. (3))

$$Q_t = K_H t^{1/2} \quad (3)$$

Where: $Q_t$ is the amount of drug released at time t; $K_H$ is the Higuchi dissolution constant.

This model can be used for several modified release systems where diffusion is the dominant release mechanism.

a) Korsmeyer-Peppas model (Eq. (4)):

$$X_t/X_{inf} = Kt^n \quad (4)$$

Where: K is the release rate constant; n is the release exponent, indicator of the drug release mechanism. For spherical swellable controlled release systems, the release exponent $n \leq 0.43$ corresponds to Fickian diffusion, $0.43 < n < 0.85$ to anomalous (non-Fickian) transport, n = 0.85 to case II transport defined by polymer swelling and drug release controlled by relaxation and diffusion, and n > 0.85 to super case II transport (Costa and Sousa Lobo, 2001; Ritger and Peppas, 1987; Peppas, 1985).

## 3. Results and discussion

### 3.1. Peptide solubility

Peptide solubility determined in 0.02 M PBS at 37 °C showed pH dependency (Table 1). The solubility in PBS medium pH 7.4 was relatively poor and identical to results observed in 0.9% NaCl solution, probably due to neutral pH. Very good solubility was observed in acidic range of pH 2, and the results obtained in lactic acid solution were comparable. This finding indicates that the solubility is not dependent on the medium composition but rather on the pH value. Nevertheless, the peptide is not stable in the lactic acid solution since degradation products were observed; thus, PBS of pH 2 was selected as a dissolution medium.

**Table 1**
Peptide solubility in different test media.

| Medium | 0.9% NaCl | PBS pH 7.4 | PBS pH 7 | PBS pH 6 | PBS pH 5 | PBS pH 4 | PBS pH 3 | PBS pH 2 | Lactic acid |
|---|---|---|---|---|---|---|---|---|---|
| Solubility (mg/ml) | 0.01 | 0.01 | 0.03 | 0.1 | 0.1 | 0.2 | 0.7 | 1.7 | 1.8 |

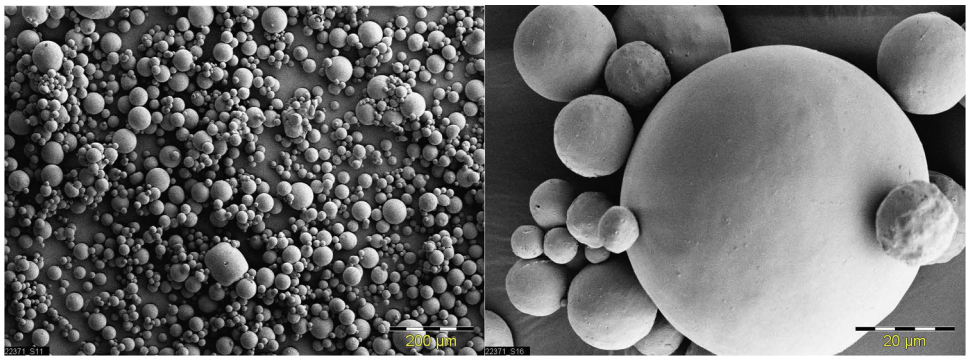


**Fig. 1.** SEM micrographs of PLGA microspheres loaded with peptide.

### 3.2. Microspheres characteristics and glass transition temperature

The peptide loading was 25% (w/w). The particle size analyses showed a unimodal distribution with diameters between 18 μm and 76 μm with a mean diameter of about 40 μm (Fig. 1). The glass transition temperature of the microspheres was determined to be 42 °C in dry state and below 30 °C in wet state (in distilled water).

### 3.3. In vitro release

#### 3.3.1. Effect of testing conditions on drug release rate and mechanism

*3.3.1.1. Effect of pH.* The pH of the dissolution medium is a very important factor, as it is known that acidic and basic pH conditions can enhance the hydrolysis of ester bonds of the PLGA, affecting the

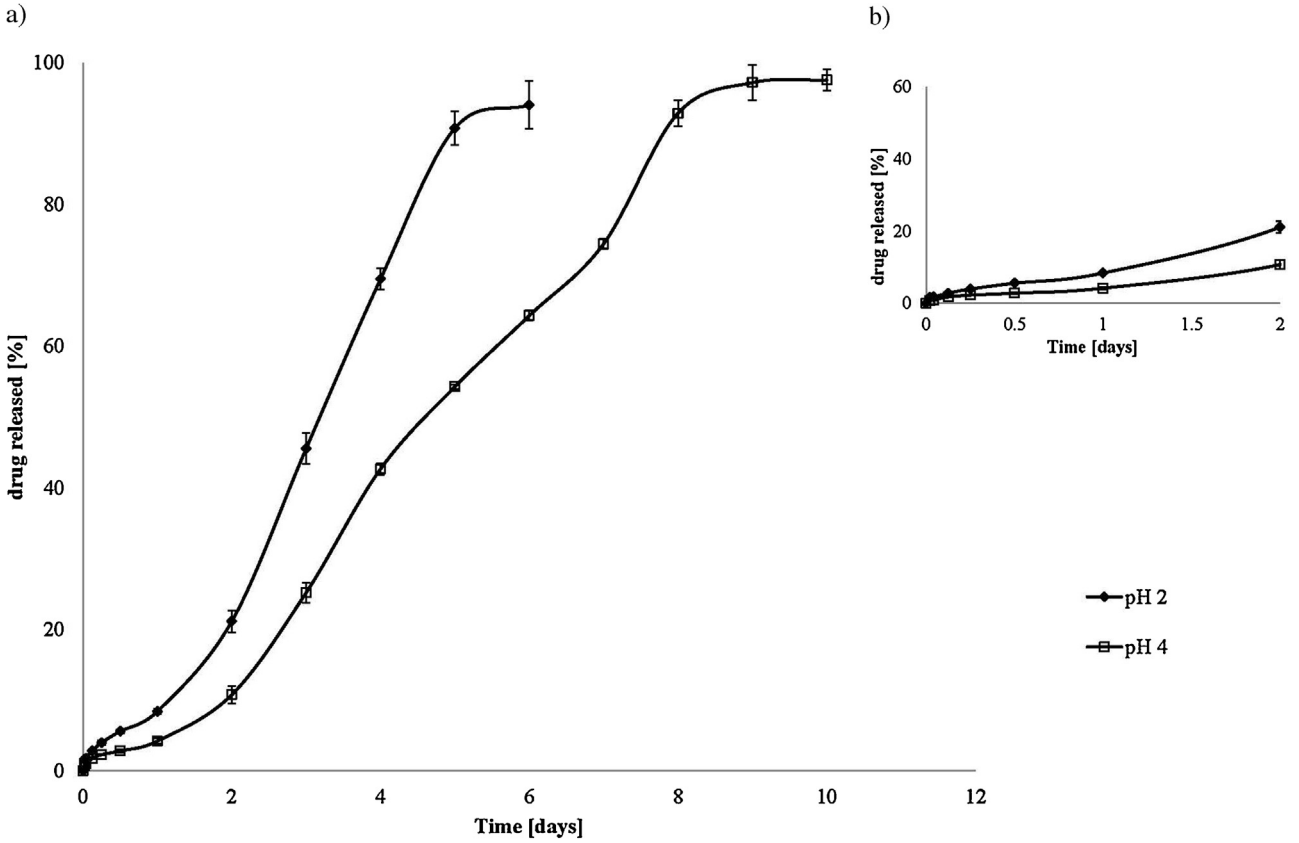


**Fig. 2.** a) Peptide release from PLGA microspheres at pH 2 and pH 4 in PBS buffer 0.02 M (45 °C), using USP 4 method. b) Extended scale of a) covering first 2 days of peptide release from PLGA microspheres.

drug release kinetics (Shen and Burgess, 2012). In order to investigate the effect of pH on drug release rate, experiments were performed in PBS 0.02 M at pH 2 and pH 4, due to the good solubility and stability of the peptide in the acidic range. Results are shown in Fig. 2.

It is known that the rate-controlling mechanism during the burst and lag phases is diffusion (Zolnik and Burgess, 2007; D'souza et al., 2005). When dissolution data obtained up to 1 day were fitted to Korsmeyer-Peppas model, n parameters were 0.48 at pH 2 and 0.43 at pH 4. It should be kept in mind that this model was established on a monodispersed system (microspheres of one size); therefore, for a sample showing distribution of particle size, variations in n value can be expected (Ritger and Peppas, 1987). Taking this into consideration, it can be concluded that both values are indicator of diffusion as rate-controlling mechanism.

The percentage released during the burst phase was very similar, 1.6 and 0.8% at pH 2 and pH 4, respectively; the lag phases were comparable as well (Fig. 2b). A similar trend in the drug release, during burst and lag phases at pH 2 and pH 4, indicates that the release kinetics during diffusion controlled phases is not dependent on the pH.

The release rate during the erosion phase was faster at pH 2 and was completed by day 6 compared to the release rate at pH 4, which ended by day 10. This observation was confirmed by Weibull scale factor $\alpha$ of 3.717 at pH 2 vs 6.224 at pH 4. The shape factors $\beta$ were similar (2.256 at pH 2 and 1.839 at pH 4), demonstrating a similarity of dissolution curves. The obtained results indicate that the change of dissolution medium pH from 2 to 4 does not affect the release mechanism, but rather only the rate, as a consequence of a slower hydrolysis of the ester bonds (Rawat et al., 2012).

For further experiments, the value of pH 2 was chosen as it enables the acceleration of the drug release without any significant change in the drug release mechanism.

*3.3.1.2. Effect of temperature.* Temperature is one of the parameters that are widely used to accelerate drug release from PLGA-based delivery systems as it increases polymer mobility. The mechanism of drug release acceleration can be different: within the first two phases (burst and lag), the drug release is accelerated by diffusion as a higher temperature softens polymer chains and increases their mobility (Shameem et al., 1999), while during the third phase, such a temperature enhances hydration and polymer erosion (Shen and Burgess, 2012).

Fig. 3 shows the release from microspheres in PBS at pH 2, at 40 °C and 45 °C. Even though both tested temperatures are higher than Tg of the polymer (below 30 °C) and the polymer is in a rubbery state, a difference in drug release is observed as the mobility of the polymer and the morphology of the micropsheres are changing with increasing temperature.

The time to achieve complete peptide release was reduced from 18 to 6 days by increasing the temperature from 40 °C to 45 °C. The Weibull scale factor was 8.220 at 40 °C (vs 3.717 at 45 °C). Interestingly, the burst release was higher at 40 °C (4.7% vs 1.6% at 45 °C). This might be due to morphology changes and structural reorganization within the microspheres. At higher temperatures, polymer mobility is enhanced and can lead to pore closing, which can result in decreased burst release (Zolnik et al., 2006).

Diffusion of drug substance during the lag phase was somehow slower at 40 °C, which was confirmed by the Korsmeyer-Peppas model giving the n value of 0.15 (vs. 0.48 at 45 °C). A slower release

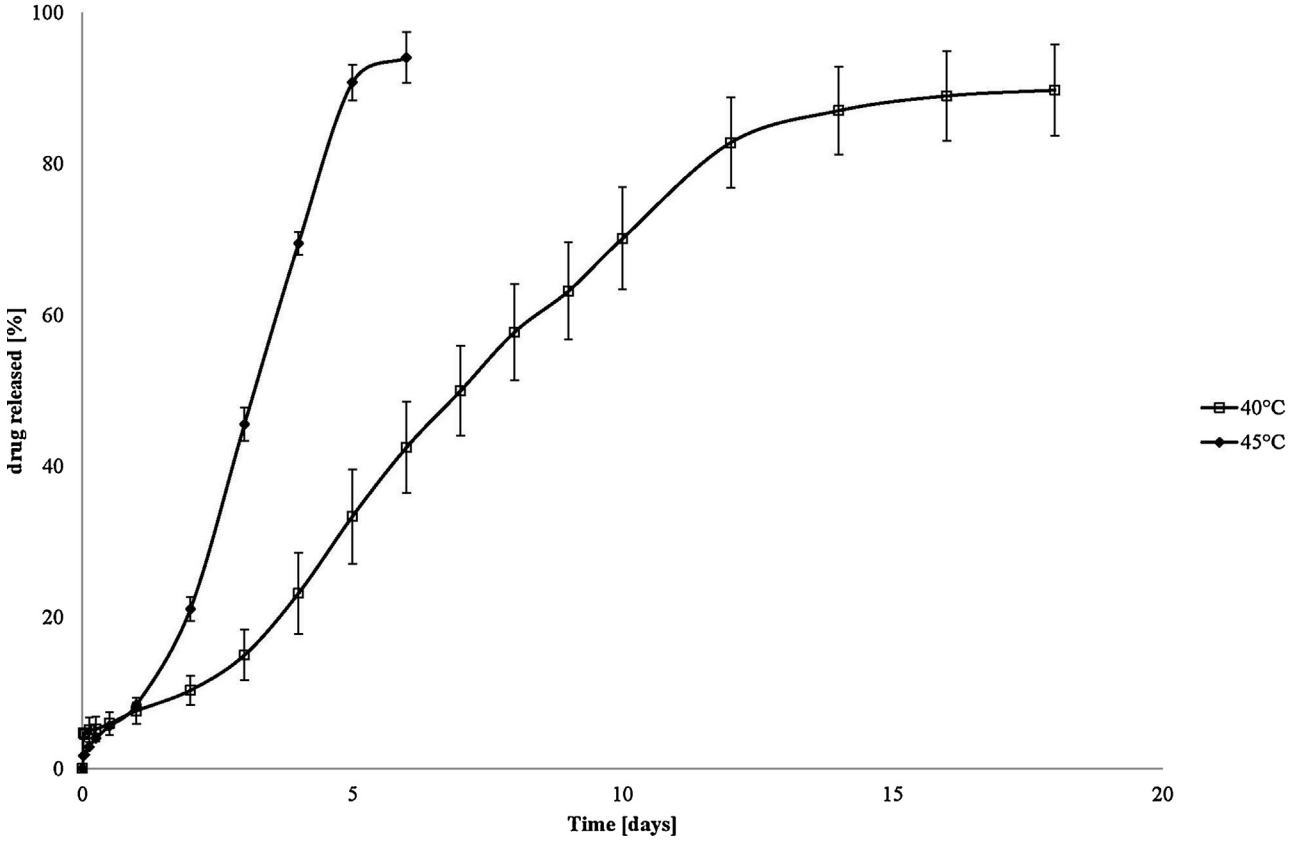


**Fig. 3.** Peptide release from PLGA microspheres at 40 °C and 45 °C in PBS buffer 0.02 M (pH 2), using USP 4 method.

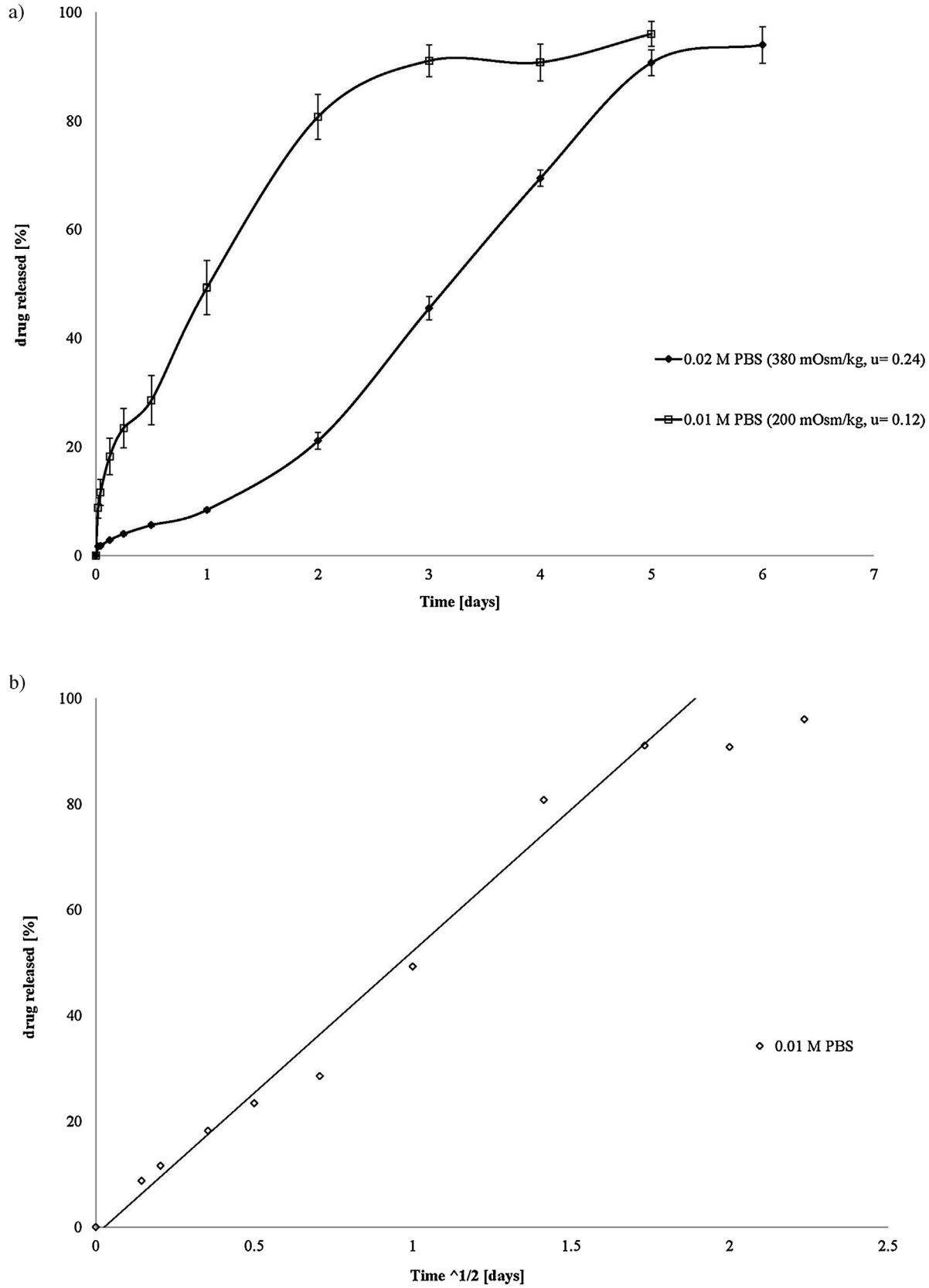


**Fig. 4.** a) Peptide release from PLGA microspheres in PBS 0.02 M and 0.01 M (pH 2, 45 °C), and b) Cumulative release in PBS 0.01 M (pH 2, 45 °C) vs square root of time using USP 4 method.

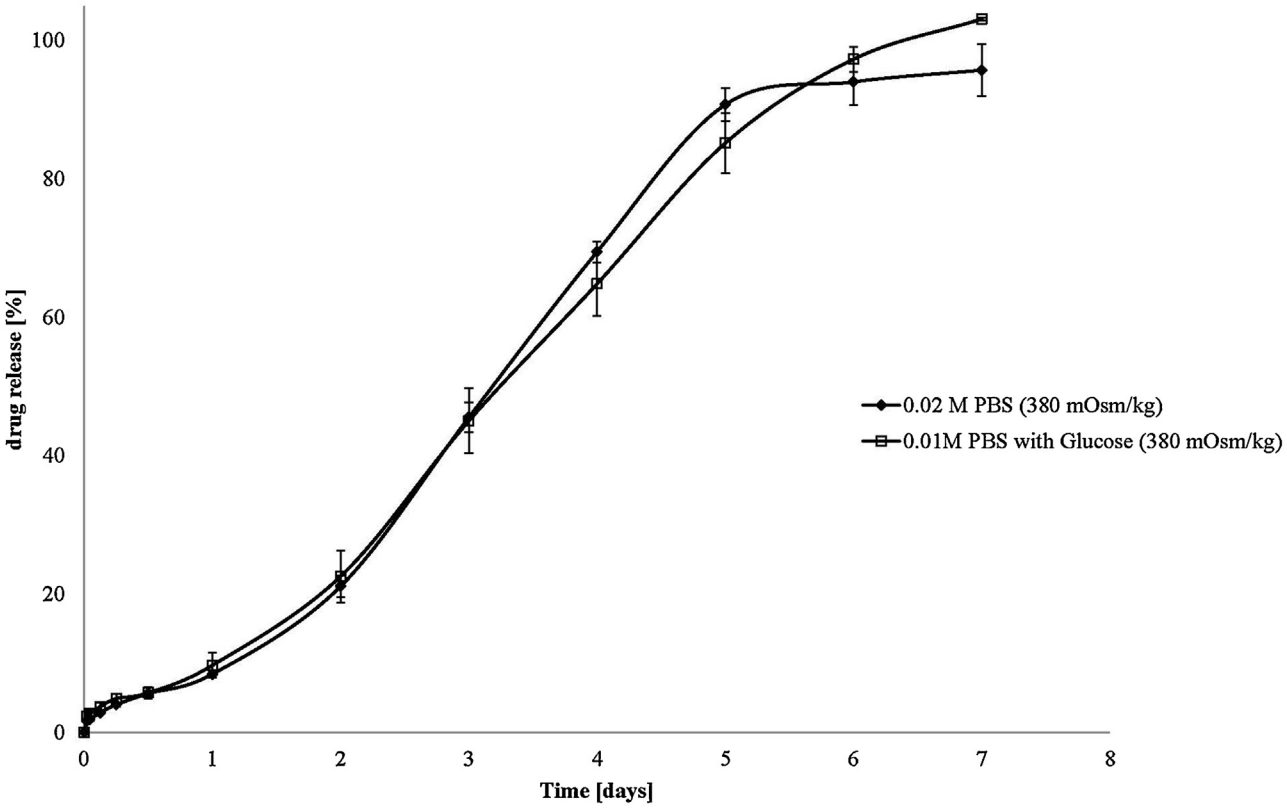


**Fig. 5.** Peptide release from PLGA microspheres in PBS 0.02 M ($\mu$ = 0.24, 380 mOsm/kg) and PBS 0.01 M with Glucose ($\mu$ = 0.12, 380 mOsm/kg), at pH 2 and 45 °C, using USP 4 method.

at 40 °C was probably the consequence of a decreased mobility of the drug molecules.

The shape factor β was slightly different (1.651 at 40 °C versus 2.256 at 45 °C), but it still defined the sigmoidal shape of the release curve.

Due to a significantly longer drug release at 40 °C, a temperature of 45 °C was chosen as the optimal one for accelerated dissolution testing.

*3.3.1.3. Effect of buffer concentration and osmotic pressure.* As shown in Fig. 4a, the effect of buffer concentration was tested in PBS. When the buffer concentration was decreased from 0.02 M ($\mu$ = 0.24) to 0.01 M ($\mu$ = 0.12), a significant increase in drug release rate was observed. The release mechanism was also changed from tri-phasic to bi-phasic, and the calculated Weibull β factor was 0.764, which is a characteristic of an exponential shape of the dissolution curves. After plotting the fraction of the released drug versus the square root of the time (Higuchi model), a good linearity was obtained up to day 3 when 90% of the drug was released (Fig. 4b). This observation indicates that the drug release in 0.01 M PBS as test medium is mainly controlled by diffusion. Additionally, the Korsmeyer-Peppas exponent n for the entire profile was 0.42, confirming diffusion as the rate-controlling mechanism.

In order to investigate whether this change in drug release mechanism is triggered by the ionic strength of the test medium or by the osmotic pressure, an experiment was performed in 0.01 M PBS in addition of glucose to adjust osmolality.

As shown in Fig. 5, when 0.01 M PBS with glucose of ionic strength $\mu$ = 0.12 and osmolality 380 mOsm/kg was used as a release medium, no significant differences in drug release were observed compared to 0.02 M PBS with the same osmolality (380 mOsm/kg), but ionic strength of $\mu$ = 0.24. After fitting the data obtained in 0.01 M PBS with glucose to the Weibull function, a scale factor α was found to be 4.372, while a shape factor β was 1.833 (vs α = 3.717 and β = 2.256 in 0.02 M PBS). The calculated values can be considered similar, indicating that the ionic strength does not have a significant influence on the peptide release from PLGA microspheres. Thus, the main driving force of the release mechanism is osmotic pressure.

The effect of osmotic pressure on drug release from PLGA microspheres has already been described (Faisant et al., 2006; Heya et al., 1994). After the insertion of microspheres in an aqueous medium, water penetrates quickly into the microspheres dissolving the incorporated drug substance while the hydrophobic polymer remains undissolved. The established gradient in osmotic pressure forces the degradation of the polymer as well as the diffusion of the dissolved drug substance. Consequently, the rate of drug release is increased.

In this study, a decrease in osmotic pressure also leads to a change in the drug release mechanism (Fig. 4a and b). The observed change might be due to the fact that the degradation of the polymer is very rapid in the medium of lower osmotic pressure; thus, the diffusion becomes the rate controlling factor.

*3.3.2. Comparison of dissolution methods*

In order to achieve accelerated drug release with distinguished three phases after an in depth investigation of various parameters, the following optimal conditions were selected: PBS 0.02 M of pH 2 at 45 °C. The results obtained using USP 4 method were compared to two other techniques described in the literature (dialysis method and USP 2), and the results are shown in Fig. 6. All the profiles had a sigmoidal shape, and the complete drug release was

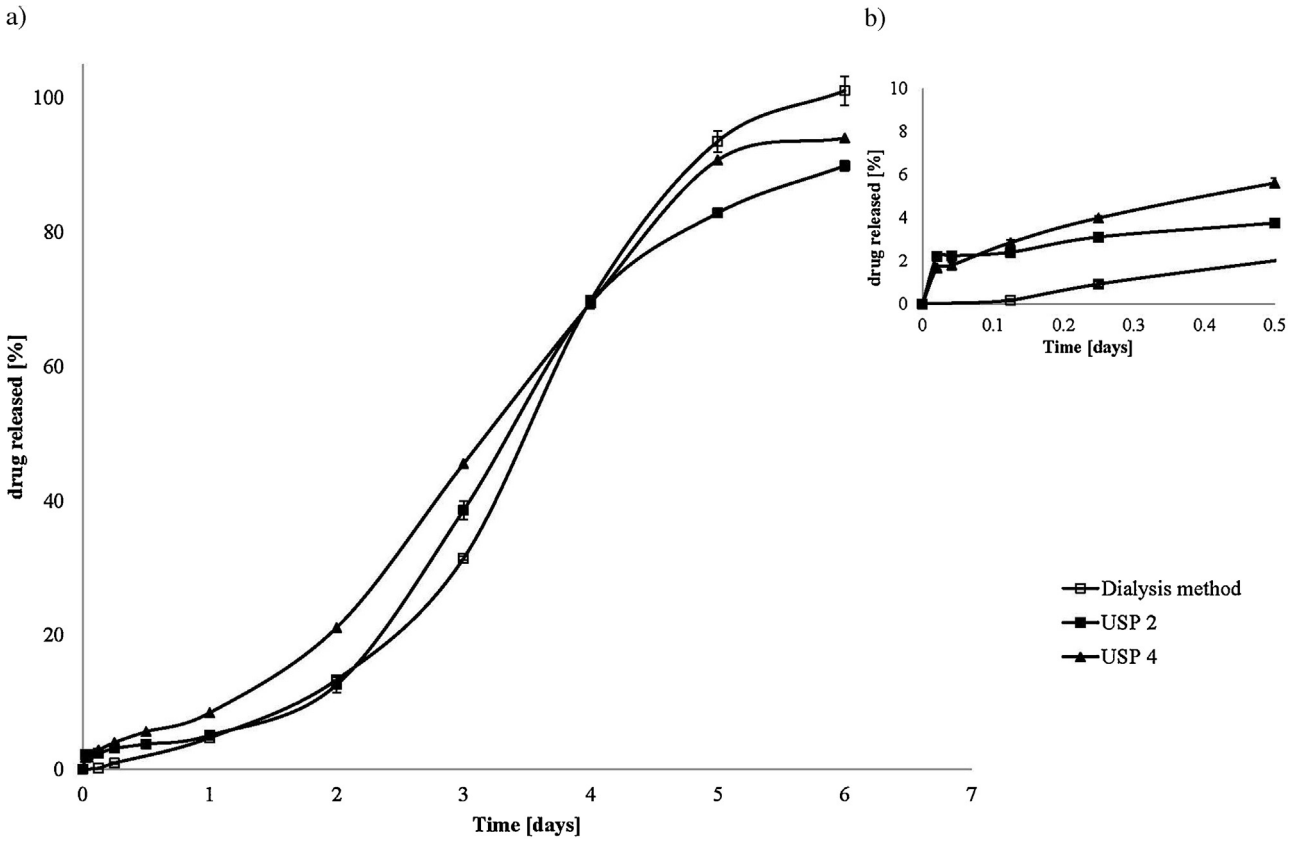


**Fig. 6.** a) Peptide release from PLGA microspheres in PBS (0.02 M, pH 2, 45 °C) using Dialysis method, USP 2, and USP 4 methods. b) Extended scale of a) covering first 0.5 day of peptide release.

achieved after 6 days for all methods, giving a 12-fold acceleration compared to in vivo release (over 70 days). Measured burst concentrations for dialysis method, USP 2 and USP 4 were 0.17%, 2.2% and 1.7%, respectively. The burst release was somehow lower when the dialysis method was used, which might be due to the presence of the membrane.

Plots of fractional release versus the square root of time (Higuchi model) showed a nonlinear relation, confirming the complex release mechanism (Bodmer et al., 1992). Additionally, the obtained results were fitted using the Korsmeyer-Peppas model (Fig. 7). The burst release was presented by the first sampling point and was thus excluded. As imposed by the model, a fraction <60% was taken into consideration for data fitting (Ritger and Peppas, 1987; Peppas, 1985; Chime et al., 2013).

For USP 2 and USP 4 methods, a lag of up to 1 day and erosion phase could be distinguished. The n values for the lag phase were 0.35 and 0.48 for USP 2 and USP 4, respectively. This finding suggests that the driving mechanism of the drug release during the lag phase is diffusion, as a value of 0.48 is very close to the border value of 0.43. For the erosion phase, n values were above 1, corresponding to super case II transport. Additionally, a cumulative release during the erosion phase was fitted to the zero order model, and the calculated correlation coefficients were $R^2 = 0.998$ for USP 2 and $R^2 = 0.999$ for USP 4 method. These findings indicate that the drug release during the last phase is controlled by a combination of polymer erosion and drug diffusion with the zero order release kinetics.

In the case of the dialysis method, the lag phase could not be distinguished from the erosion phase based on the Korsmeyer-Peppas model. This change in the release mechanism for the dialysis method might be due to a slower diffusion of the peptide through the membrane compared to the diffusion from microspheres. Besides, the presence of the additional membrane for drug diffusion might be the reason for the unsuccessful fitting of the drug release kinetics to the applied models.

To further compare dissolution profiles, the data were fitted to the Weibull function, as it can best describe the sigmoidal shape curves (D'souza et al., 2005).

After fitting the in vitro profile, the obtained values for the α parameter were 3.892, 3.455, and 3.717 for dialysis, USP 2, and USP 4, respectively. The obtained values for the scale factors were very similar for all methods, leading to the conclusion that the rate of drug release is not dependent on the method choice. For the shape factor β, the following values were obtained: 3.418 (dialysis), 3.116 (USP 2) and 2.256 (USP 4). In all cases β is above 1 which confirms sigmoidal shape of the curve.

Even though the Weibull parameters indicate a shape similarity between all profiles, it has been demonstrated by the Korsmeyer-Peppas model that the drug release mechanism is somehow different when the dialysis sac method is used. On the other hand, USP 2 and USP 4 methods gave very similar dissolution profiles with the three phases: burst, lag, and erosion.

This observation indicates that existing compendial methods might be successfully used for the development of an accelerated dissolution test for slow release injectable dosage forms with complex release mechanism. Nevertheless, the authors would give a slight advantage to USP 4 dissolution apparatus for the following reasons:

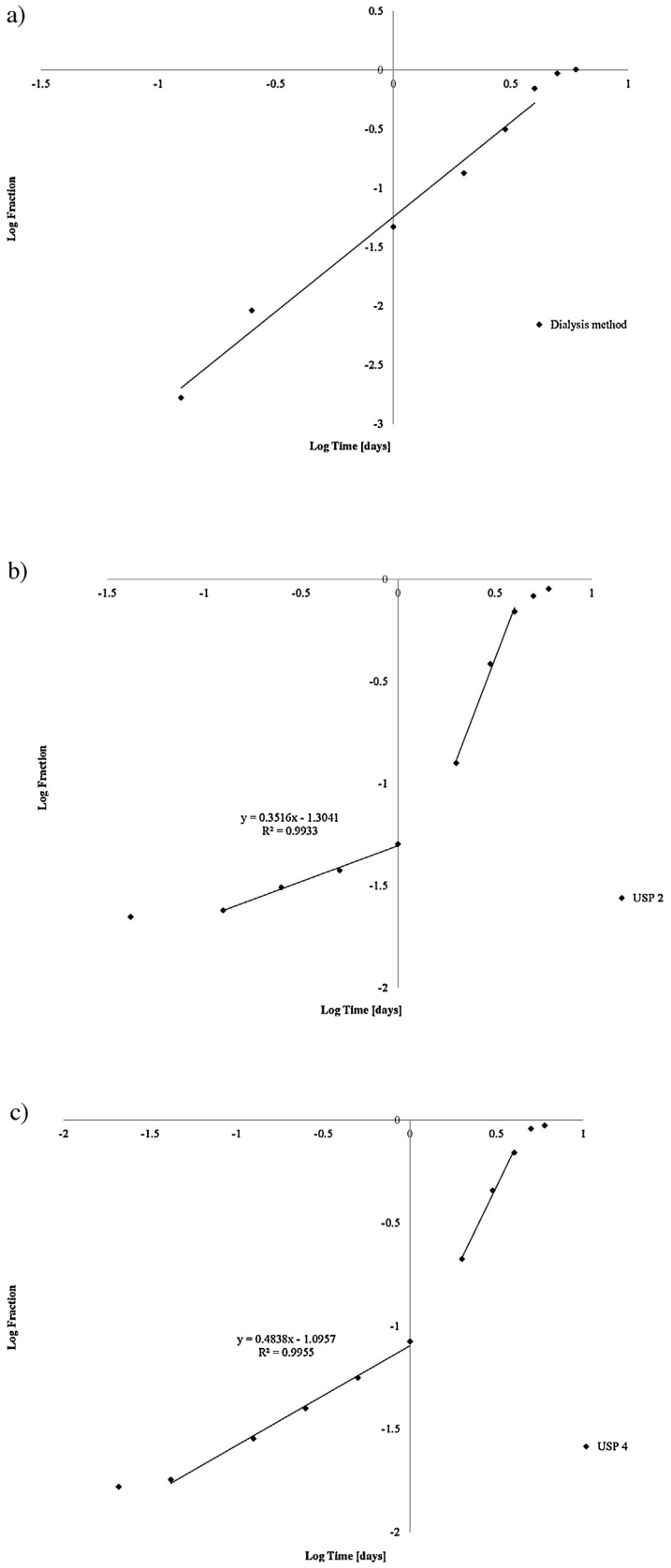


**Fig. 7.** Korsmeyer-Peppas model for peptide release in PBS (0.02 M, pH 2, 45 °C) using a) Dialysis method, b) USP 2, c) USP 4.

- an easy handling of the microspheres without a risk of aggregation or floating,
- a limited evaporation of the test medium even at high temperatures, as the system operates in a closed loop mode,
- a better simulation of in vivo conditions due to the sample being exposed to a constant perfusion by the test medium; thus, this method has a higher biorelevant potential.

For the tested somatostatin analog, it can be concluded that the choice of the in vitro release test medium and of the dissolution technique is of high importance for the development of an accelerated in vitro method that will not lead to an alteration of the release mechanism. Nevertheless, this aspect needs to be tested for any drug encapsulated in microspheres.

## 4. Conclusion

Drug release testing is an important tool for assuring performance and safety of controlled-release injectable dosage forms. Accelerating drug release without changing the release mechanism is an additional challenge in the development of biorelevant methods.

In this study, it has been shown that, by altering the temperature, the pH or the composition of the release medium, it is possible to achieve a significant acceleration of the drug release from PLGA-based microspheres. It has also been demonstrated that a change of the pH and the testing temperature (above glass transition temperature) does not significantly affect the drug release mechanism, while the composition of the test medium can change the mechanism from erosion to a diffusion-controlled one due to the osmotic effect. For a PLGA-based formulation showing a complex release mechanism (burst, lag, and erosion phase) for an encapsulated somatostatin analog, a significant acceleration in the drug release can be achieved using PBS as a dissolution medium at pH 2 and 45 °C.

After comparing different dissolution techniques, USP 4 (flow-through) was chosen as the most appropriate method for the tested PLGA formulation, as compared to the dialysis sac method and USP 2. This finding is in agreement with a general recommendation by the authorities (Martinez et al., 2010; Burgess et al., 2002, 2004).

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