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Novel, dynamic on-line analytical separation system for dissolution of drugs from poly(lactic acid) nanoparticles

Anne Helle [a,1], Samuli Hirsjärvi [b], Leena Peltonen [b], Jouni Hirvonen [b], Susanne K. Wiedmer [a,*], Tuulia Hyötyläinen [c,**]

[a] *Laboratory of Analytical Chemistry, Department of Chemistry, P.O. Box 55, University of Helsinki, 00014 Helsinki, Finland*
[b] *Division of Pharmaceutical Technology, Faculty of Pharmacy, P.O. Box 56, University of Helsinki, 00014, Finland*
[c] *VTT Technical Research Centre of Finland, Quantitative Biology and Bioinformatics, P.O. Box 1000, 02044 VTT, Finland*

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ABSTRACT

A novel method for investigating drug release in a dynamic manner from nanoparticles including, but not limited to, biodegradable poly(lactic acid) (PLA) is reported. The PLA nanoparticles were prepared by the nanoprecipitation method. Two poorly soluble drugs, beclomethasone dipropionate (BDP) and indomethacin, were encapsulated into PLA nanoparticles, and their dissolution from the nanoparticles were followed in a dynamic way. The on-line method comprised a short column (vessel) packed with the PLA nanoparticles, on-line connected to an analytical liquid chromatographic column via a multiport switching valve equipped with two loops. The system allowed monitoring of the drug release profiles in real time, and the conditions for the drug release could be precisely controlled and easily changed. The effects of solvent composition and temperature on the rate of dissolution of the drugs from the PLA nanoparticles were investigated. The system proved to be linear for the drugs tested over the concentration range 10–3000 ng ($n = 6$, $R^2 = 0.999$ and 0.997 for indomethacin and beclomethasone, respectively) and repeatable (RSD of peak areas <0.5%). The recoveries of the dissolution study were quantitative (120 and 103% for indomethacin and beclomethasone, respectively).

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1. Introduction

Poly(lactic acid) (PLA) is widely used in nanoparticulate drug delivery systems because of its biocompatibility and biodegradation properties [1]. Biocompatible nanoparticles as drug delivery vehicles provide several advantages, including protection of the encapsulated pharmaceutical substance, improved efficacy, fewer adverse effects, controlled release and drug targeting. In order to create a successful nanoparticle formulation, the drug release profile should be known and evaluated.

Drug release from nanoparticles may occur by diffusion through the particle, by desorption from the surface, or after degradation of the nanoparticle-forming matrix. Although the drug release environment *in vivo* is complex and may be difficult to simulate, important information can be collected by *in vitro* release protocols. Nanoparticles can be stirred in a receptor medium and, at predetermined intervals, samples are withdrawn and ultracentrifugated [2–7]. Subsequently, the drug content is analyzed from the supernatant (e.g. by UV spectroscopy or HPLC). Similarly, the withdrawn sample can be treated by ultrafiltration [8,9] or by diffusion cells separated with membranes. In a dialysis setup, nanoparticles are placed either in small dialysis bags in a stirred receptor medium [10–12] or in a medium containing drug-free dialysis bags (reverse dialysis) [13–15]. Because the nanoparticles do not permeate the dialysis bags, the released drug is quantified from the compartment not containing the particles. In addition to dialysis bags, diffusion cells separated with a membrane provide the possibility to monitor drug diffusion (excluding the particles) from one compartment to another [16,17].

All of the above mentioned techniques require sample withdrawal, often sample pretreatment, and analysis with separate equipment. All extra steps increase the risk of sample contamination and alteration, and characterization of the drug release in a dynamic manner is not possible. In addition, forces created by ultracentrifugation or ultrafiltration might cause unwanted particle destruction and drug release in the medium. In our previous study, an off-line system utilizing capillary electromigration techniques for the determination of the total amount of drug in PLA nanoparticles was developed [18]. In that study, both charged and hydrophilic drugs were studied, including beclomethasone dipropionate (BDP). The method proved to be a good starting point for the

* Corresponding author. Tel.: +358 919150264; fax: +358 919150253.
** Corresponding author. Tel.: +358 400574331; fax: +358 207227071.
E-mail addresses: susanne.wiedmer@helsinki.fi (S.K. Wiedmer), tuulia.hyotylainen@vtt.fi (T. Hyötyläinen).
[1] Deceased.

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development on an *in vitro* drug release protocol. However, such an off-line system suffers from some drawbacks, of which one is that we can only detect the total amount of drugs inside the particles. It would be possible to take several subsamples in the off-line dissolution system to obtain more information on the dynamics of the dissolution. This is, however, very difficult and laborious in practice. An on-line system, which would allow studying drug release in a closed dynamic way, offers several advantages in comparison with off-line studies. The system gives information of the dynamics of the release process, which is very difficult to obtain with off-line studies. Thus, the system provides more accurate information, and it is also easy to accurately control and adjust the release conditions, such as temperature and solvent, which makes the mimicking of *in vivo* conditions straightforward. Moreover, in a closed system, the contamination and alteration due to atmospheric air or moisture or even UV light is avoided.

In this work a novel on-line system for the study of drug release from nanoparticles in a dynamic manner is presented. The system is based on a similar principle as utilized in comprehensive two-dimensional liquid chromatography. The nanoparticles are packed into a small empty vessel, which is connected to a multiport modulation valve and to an analytical HPLC system. The modulation valve is equipped with two loops, and the eluent flow from the vessel packed with nanoparticles is continuously collected and transferred to the analytical LC column for separation of analytes. Fast LC separation (<2 min) is utilized in the LC step and, thus, the release process can be monitored in very short intervals. Moreover, possible degradation can simultaneously be monitored due to efficient separation. If necessary, mass spectrometric detection can easily be utilized after the LC separation. This is clearly beneficial for studies of nanoparticles with certain types of drugs, such as peptide drugs, which are more prone to degradation than small chemical molecules. Two model drugs were selected for the studies of dissolution with the novel system: indomethacin (a non-steroidal anti-inflammatory drug) and beclomethasone dipropionate (BDP) (a corticosteroid). These drugs were selected because they are practically insoluble in water, which makes the encapsulation and, on the other hand, desorption from the PLA nanoparticles by model solvents (e.g. alcohols), easier.

2. Experimental

2.1. Chemicals

PURASORB® PDL 02A poly(D,L-lactic acid) (a donation from PURAC Biomaterials, Gorinchem, The Netherlands) (IV 0.20 dL/g) formed the nanoparticulate matrix. Other chemicals used in the nanoparticle preparation were acetone (Riedel-de Haën, Seelze, Germany) and ultrapurified water (Millipore, Molsheim, France). Beclomethasone dipropionate and indomethacin (Fig. 1) were purchased from Sigma (St. Louis, USA). 11 μm paper filters (Whatman, Brentford, UK) were used for purification of the nanoparticle dispersions. BDP is uncharged (pKa 13.05) while indomethacin is charged (pKa 3.96) under the conditions used in this study. [The pKa values were calculated using the Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris, 1994–2008 ACD/Labs.]

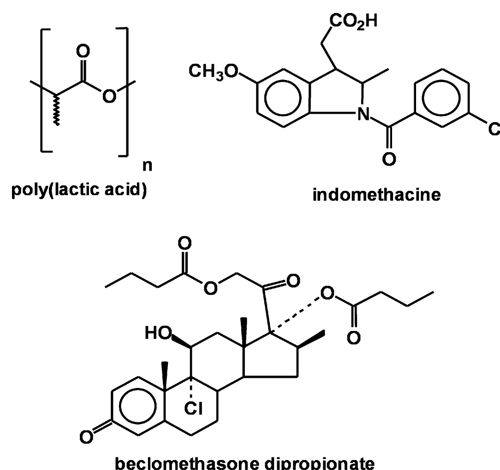

Fig. 1. Structures of PLA and the drugs investigated.

2.2. Nanoparticles

PLA nanoparticles were prepared by the nanoprecipitation method [19] as described in our previous publication [18]. Briefly, PLA and BDP or indomethacin were dissolved in acetone and precipitated as nanoparticles in water. After evaporation of the organic solvent, the nanoparticle dispersion was diluted with water to 20 mL and filtered (paper filter) to remove possible aggregates. Size distributions of the nanoparticles were determined with a Malvern Zetasizer 3000HS (Malvern, Worcestershire, UK). Particle sizing was based on photon correlation spectroscopy (dynamic light scattering); the results were analyzed by CONTIN algorithm and the sizes presented based on the intensity distributions.

2.3. Dynamic on-line LC system

The constructed apparatus is presented in Fig. 2. The vessel to be filled with PLA nanoparticles comprised a 5-cm long PEEK column with an i.d. of 2.1 mm and a double polycarbonate membrane with a pore size of 0.1 μm, supported by metal frits. First the vessel was partially filled with purified sea sand, followed by injection of 1 mL of 1 mg/mL PLA nanoparticle dispersion. After this the vessel was further filled with sea sand, and the column was tapped gently to ensure efficient filling of the voids. The vessel containing the nanoparticles was flushed with water before the dissolution studies. The water flow pushed the nanoparticles towards the outlet of the vessel where they were stopped by the filter. A Jasco PU-

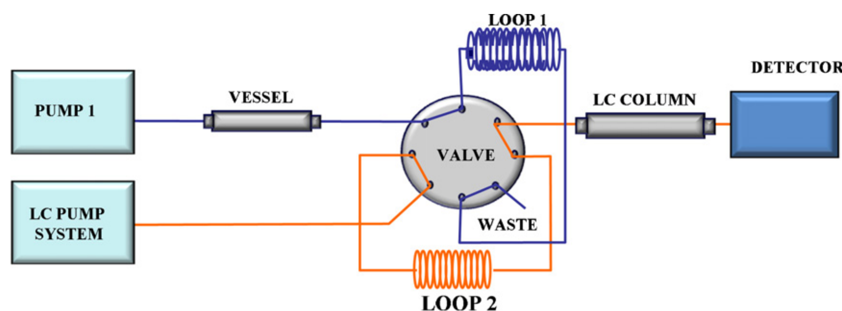

Fig. 2. Configuration of the on-line system.

980 (Tokyo, Japan) pump was used at a flow rate of 0.1 mL/min for release of the drug from the drug-loaded PLA particles in the PEEK vessel. The temperature of the vessel containing the nanoparticles was controlled with a water bath. The HPLC separation was performed with a Hewlett-Packard 1100 liquid chromatograph and a diode array detector at ambient temperature. A C18 column (XBridge C18, 75 mm × 3 mm i.d., 2.5 μm, 137 Å) was used. The isocratic eluent for the LC analysis was acetic acid (0.05%)–acetonitrile (21:79, v/v) at a flow rate of 1.6 mL/min.

The two systems were interfaced with a 10-port high-pressure two-position interfacing valve (C2-1000EP, VICI Valco, Houston, TX, USA). The valve was controlled by a laboratory-made program receiving a start-up signal from a Hewlett-Packard Chemstation. The loop size for the fraction collection was 200 μL. Modulation times between 90 and 120 s were tested.

The valve has two positions (load, inject), which are controlled by a computer program. In the load position the eluent from the second pump passes through loop number one, pushing the sample fraction into the second column. At the same time loop number two is filled with effluent from the vessel packed with nanoparticles. When the valve is in the inject position the situation is reversed. The vessel containing the nanoparticles (vessel) was then connected to a modulation valve, which is a ten-port switching valve equipped with two identical loops (Fig. 2). A short HPLC column was also connected to the valve and it was used for the separation of the released drugs (Fig. 2). Vessel is flushed with desorption solvent and small volume fractions of the effluent are transferred via the modulation valve into the LC column for HPLC separation. The effluent fractions are collected into one loop while the previous effluent fraction contained in the second loop is being transferred to the HPLC separation column for separation within the time the first loop is being filled with a new effluent fraction. The system is operated continuously, as the two loops are alternately switched between collection and injection periods.

3. Results and discussion

The aim of the study was to develop a simple system that allows the study of the dynamics of the release of drugs from PLA nanoparticles. The sizes of the PLA nanoparticles manufactured for this study and containing one of the two model drugs, namely indomethacin and BDP, were approximately 210 nm with low polydispersity indices (below 0.1) indicating that the nanoparticle preparation was successful. The structures of PLA and the drugs investigated are shown in Fig. 1. The two drugs were chosen as model substances, because of their widespread therapeutical use. Conventional dissolution testing is difficult to perform on nanoparticles containing either indomethacin or BDP because of their poor aqueous solubility properties. Indomethacin as a weak carboxylic acid is charged during the dissolution testing, but still poorly soluble in water, while BDP is practically insoluble in aqueous media. Due to the poor solubility, a physiological dissolution medium could not be used in this study. Instead, a mixture of water and organic solvent was used. The use of non-physiological solvents for dissolution is a rather standard procedure when dissolution tests for poorly soluble compounds are conducted. It is common that even the Pharmacopoeial dissolution methods for poorly soluble drugs contain non-physiological solvents as components of the dissolution media. This gives more reliable prediction of the *in vivo* dissolution behavior of the drug formulations.

3.1. Instrumental setup

The idea of the novel instrumentation for on-line dissolution studies is simple and straightforward. In this system, the studied nanoparticles are packed into a vessel, which is flushed with the dissolution solvent. The effluent flow from the vessel in continuously sampled with a modulation valve, and the collected fractions are injected to an HPLC system, where the dissolved compounds are separated and detected. The system was first optimized off-line, *i.e.* the packing procedure of the nanoparticles, and preliminary conditions of the dissolution were studied separately. Also the HPLC separation was developed first for the off-line mode. Then, fine-tuning of the final conditions was performed in on-line mode.

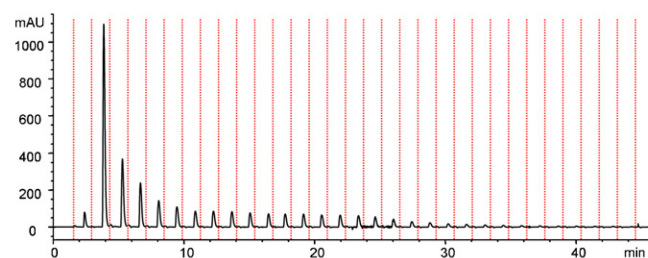

Fig. 3. Series of HPLC chromatograms obtained with the on-line system. Indomethacin from PLA, dissolution flow rate 0.15 mL/min, fractionation time 1.7 min.

First, the nanoparticles containing either indomethacin or beclomethasone were packed into a small empty vessel. Several packing procedures were tested, and the best results were obtained when the vessel was first partially filled with the supporting material (sea sand), then the suspension of nanoparticles was injected to the packing and the void was filled with sea sand. The backpressure of the vessel was very low, varying from 1 to 2 bars, depending on the flow rate and eluent composition. Before on-line combination, off-line optimization of the dissolution solvent, flow rate and time required for the dissolution studies were carried out. These results were compared with dissolution of drugs in a test-tube and results obtained with the two methods were in good agreement with each other.

As a result of a dynamic on-line dissolution analysis, a series of HPLC chromatograms are obtained, as shown in Fig. 3. Each single chromatogram corresponds one cycle of the dissolution procedure. Typically, the dissolution study took in total 60–120 min, thus leading to 30–60 chromatograms in series. The amount of dissolved/released drug is then obtained by integration of each peak.

Preliminary optimization of the HPLC separation was first done off-line to search for good separation in a short time, *i.e.* in less than 2 min. Only minor fine-tuning of the conditions was required for the on-line system. For dissolution of the drugs from the nanoparticles, pure water, acetone, acetonitrile, methanol, and various organic solvent–water mixtures were tested. Due to the poor solubilities of the model substances, especially in the case of BDP, the use of physiological buffer solutions or biorelevant dissolution media was not possible. Methanol proved to be the best choice for organic modifier and, thus, it was chosen for further studies. The drugs studied had strong retention in the HPLC column and, therefore, even relatively large volumes could be transferred from the column without significant peak broadening. The flow rate of the desorption solvent is critical in the on-line connection, because the size of the desorbed fraction and the time required for drug release (cycle time) are both dependent on it. Too high flow rate will result in large fraction volumes, which can cause severe band broadening in the HPLC separation. On the other hand, a very low flow rate of the desorption solvent increases the time required for the desorption. Here, desorption flow rates between 0.1 and 0.2 mL/min were used as they still resulted in a reasonable fraction volume (<150 μL) and the time of desorption was not excessive. The sizes of the loops were varied according to the tested fraction volume which was determined by the flow rate and cycle time. The best results were obtained when

the volume of the loop was clearly larger than the volume of the fraction because then each fraction was diluted with the LC eluent present in the loop.

For HPLC separation the eluent composition and the flow rate were optimized for fast and efficient separation of the studied drugs. To keep the fraction volume of the dissolved drugs constant when the flow rate of desorption solvent was increased, the modulation time and, thus, the HPLC analysis time was also decreased by increasing the HPLC flow rate. Therefore, in all the studies the fraction volume was kept in the range of 150–250 μL. Because a highly efficient HPLC column, packed with small particles (2.5 μm), was utilized, it would be feasible also to separate possible degradation products from the parent drug. However, in this study, no degradation of the parent drugs was noticed.

3.2. Quantitative results

To test the quantitativeness and ruggedness of the system, calibration curves for indomethacin and BDP were constructed in the LC separation (amount, m = 10–3000 ng, n = 6) and the method proved to be linear (R^2 = 0.999 and 0.997 for indomethacin and BDP, respectively). The system also proved to be very repeatable with RSD values of peak areas of 0.48 and 0.26% for indomethacin and BDP, respectively. Also the repeatability of retention times was good; <0.2% for standards and <1.3% for dissolution studies. The calibration curves were used for calculation of the total amount of desorbed drug.

The efficiency of the on-line system for the dissolution was also evaluated by comparison with off-line dissolution of the nanoparticles with methanol, acetonitrile and acetone in a test-tube followed by off-line HPLC analysis. On-line testing of the recovery was not a feasible option, as injection of standards directly to the vessel would not give realistic results, because the standards would be directly eluted from the vessel. In our previous studies, off-line dissolution was proven to give quantitative results [18]. Assuming full dissolution by the off-line method, quantitative dissolution was obtained (103 and 120%) with the dynamic system utilizing fully organic solvent (100% methanol), for BDP and indomethacin, respectively. With lower volume of the organic modifier, dissolution was only partial, particularly for BDP, for which the dissolution recovery was 47.2% with 80% of methanol and 14.6% with 60% of methanol. For indomethacin, relatively high recovery was obtained also with 80% methanol (87.1%). The repeatability of dissolution studies was satisfactory (RSDs of the summed peak areas <18%). The variation was caused by the form of the nanoparticles, *i.e.* the suspension of nanoparticles in solution. Even though the suspension was carefully mixed before an aliquot was taken, there may have been variation in the final amount of nanoparticles packed into the vessel. With dry particles, the repeatability would probably be better. However, in general, the drying process of polymeric nanoparticles is very demanding and may alter the properties of the particles. Also stabilizers are often needed during the drying, which also change the powder properties. As the aim of this study was to show the feasibility of the new dissolution method, the drug-loaded PLA nanoparticles were used as a suspension straight from the particle manufacture.

3.3. Effect of dissolution solvent and flow rate

The release and detection of the drug from nanoparticles in this type of system requires two steps: first, the compound must be desorbed from its binding site in (or on) the nanoparticles, generally modeled by rate processes such as diffusion, and then the compound must be eluted from the sample in a manner analogous to frontal elution chromatography. The latter is controlled by the thermodynamic partitioning coefficient, K_D. In the case of drugs in

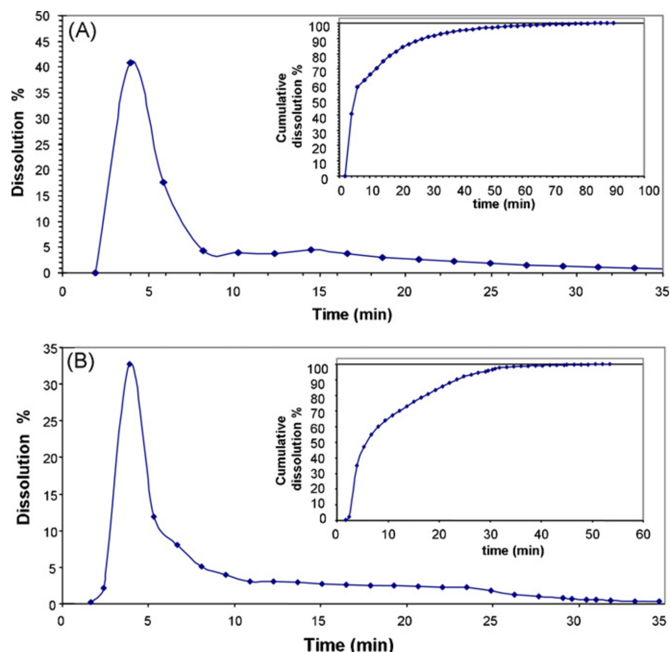

Fig. 4. Dissolution of (A) BDP and (B) indomethacin from PLA particles with 100% methanol. Absolute and accumulative amounts.

nanoparticles, analyte solubility largely affects the release rate by its effect on the K_D and, thus, either the desorption or elution steps (or a combination of these) may limit the release rate. To study whether the process is limited primarily by analyte solubility and possible retention of the analyte on matrix active sites (*i.e.* the solubility/elution process) or by the kinetics of the initial transport of bound analytes from the matrix into the elution solvent (*i.e.* the desorption/kinetic process), the effects of the desorption solvent, its flow rate, and the temperature were studied.

First, the effect of desorption solvent was studied. In our previous studies with off-line methodologies [18], it has been noticed that the drugs can be released with purely organic solvents, such as acetone (particles were broken up) or methanol. Here, methanol was chosen as the organic solvent and desorption with varying amounts of methanol (0–100%) in water was studied. The desorption profiles were studied by using two kinds of curves, namely actual and accumulative amounts of released drug versus time (Fig. 4). The first type of curve shows the behavior of the desorption more clearly. Both the studied drugs had very similar dissolution behavior (Fig. 4). With purely organic solvent, the desorption of both drugs was complete, as confirmed by comparison with quantitative results and off-line performed release of the drugs. It can be seen in Fig. 4 that for both drugs the desorption takes place in two or three steps; obviously the drugs bound to the surfaces of the particles are released first, followed by release from the inner parts of the particles. The desorption of BDP is faster than the desorption of indomethacin. When the amount of organic modifier is reduced, the efficiency of the desorption is also reduced, as can be seen in Fig. 5A–C. Particularly, the release of drugs from the polymeric matrix of the nanoparticles is much less efficient already with 80% of methanol (Fig. 5B), and with 60% of methanol (Fig. 5C) only the drugs on the surfaces can be (partially) desorbed. With pure water, the drugs were not desorbed from the nanoparticles. When the desorption solvent was changed to 100% methanol, after dissolution with 60% methanol, the remaining part of the drugs could be desorbed, as shown in Fig. 6. Interestingly, the desorption profile was then different: the final desorption took place in three clearly separate steps. These results clearly demonstrate the potential of this novel dynamic system for the characterization of drug release

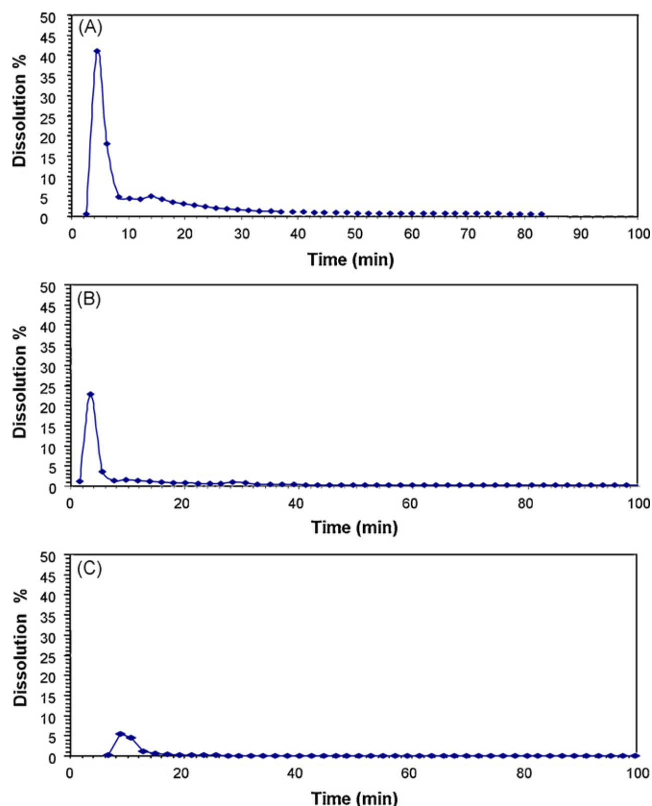

Fig. 5. Effect of concentration of methanol on the dissolution of BDP from PLA nanoparticles. The amount of methanol was (A) 100%, (B) 80%, and (C) 60%.

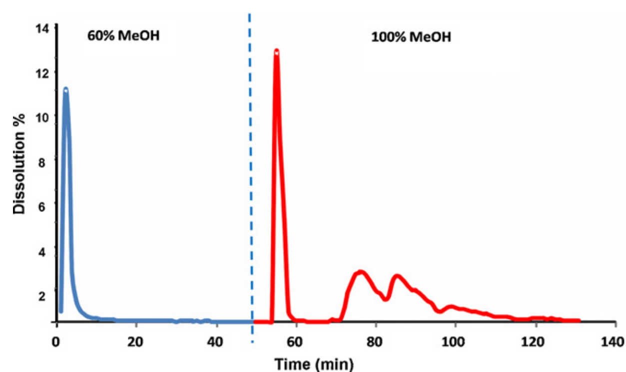

Fig. 6. Dissolution of BDP from BDP-filled nanoparticles with 100% MeOH, immediately after disssolution with 60% MeOH.

dynamics. Methanol was chosen as a dissolution medium because of the system development and poor aqueous solubility of the model substances. According to the European Pharmacopoeia, for testing of preparations containing poorly aqueous-soluble active substances, modification of the dissolution medium may be necessary. For example, a low concentration of surfactant may be added, or, although not recommended, organic solvents may be used. However, in future studies, with other types of nanoparticles and drugs, the developed methodology will be tested also with biorelevant solutions. In this study, methanol was used as a dissolution solvent also because the PLA nanoparticles do not dissolve in simple alcohols. Thus, the drug release is mostly due to diffusion through the polymeric matrix of the nanoparticles.

Next, the effect of the flow rate in the range of 0.1–0.2 mL/min on drug release was studied. The release was not significantly affected by the flow rate and, thus, it could be concluded that the release is primarily controlled by the kinetics of the initial desorption step.

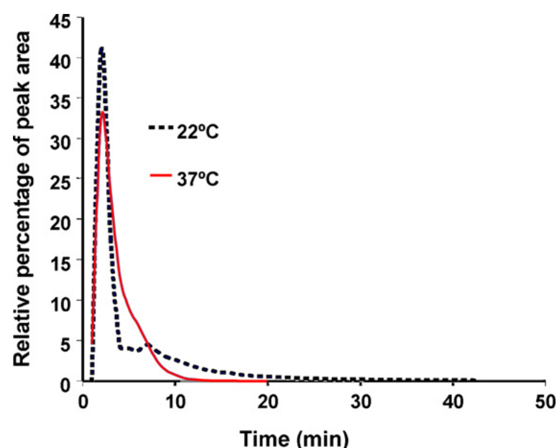

Fig. 7. Effect of temperature on the dissolution of BDP from PLA nanoparticles.

3.4. Kinetics and the effect of temperature

The effect of temperature on the desorption of drugs from the PLA nanoparticles was studied as well. The profile of the desorption changes when the temperature is increased (Fig. 7). At ambient temperature (22 °C), three modes of desorption can be observed, while at 37 °C only two modes, which are partially overlapping, are observed. Obviously, the increase in temperature increases the diffusion rates of the analytes and makes desorption faster, particularly from the inner parts of the particles. The structure of these PLA nanoparticles should not be affected by the temperature increase to 37 °C, because the glass transition temperature (T_g) of the polymer (above which the mobility of the polymer chains increases) is around 45 °C (data not shown).

4. Conclusion

A novel on-line LC system that allows dynamic determination of dissolution/release of drugs from nanoparticles was developed. The system proved to be simple and easy to use. The applicability of the system was demonstrated with PLA nanoparticles with encapsulated indomethacin and beclomethasone dipropionate. The drug release from the particles was followed by changing the composition of the dissolution solvent and by changing the temperature. With the system the conditions of the dissolution can be adjusted and changed in a simple and straightforward way, providing a good starting point for the development of *in vitro* drug release protocols with dissolution solutions closer to physiological conditions. Moreover, the system can be easily coupled with mass spectrometric detection as well.

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