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A Quantitative Method for the Determination of Amphiphilic Drug Release Kinetics from Nanoparticles Using a Langmuir Balance

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Amiodarone is a drug that is widely used in the treatment of heart disease. To circumvent side effects, colloidal drug carriers have been designed to deliver the drug specifically to the site of action. For the purposes of in vitro characterization of such particles, difficult test systems are employed that usually require the quantitative separation of the drug carrier from the release medium before analysis. In this work, a Langmuir balance was used to characterize amiodarone release. Drug-loaded nanoparticles were prepared from a biodegradable polyester and assayed for their drug release kinetics. Simultaneously, nanoparticles were analyzed for their drug release by a standard procedure based on dialysis tubes combined with high-performance, liquid chromatography. The results obtained by the Langmuir balance experiments were compared with those obtained from high-performance liquid chromatography and were found to correlate well. The interexperimental variation was 4.4% for the Langmuir method ($n = 4$), and the interexperimental variation for HPLC was 2.9% ($n = 3$). The major advantage of this new method is the possibility diminishing significantly the required sample amount for the experiment, allowing drug detection in the lower nanomolar range. Moreover, the avoidance of prior nanoparticle separation from the release medium provides important progress of this technique. The Langmuir balance has proven its adaptability as a new sensitive tool for the characterization of amphiphilic drug release kinetics.

Antiarrythmic drugs are widely used in heart disease treatment.[1] One candidate for such treatment is amiodarone, which has proven to be highly efficient because of its antianginal and antiarrythmic properties.[2] Side effects due to the lipophilic properties of amiodarone have been reported, on the basis of the accumulation in several tissues other than the heart combined with its long half-life, for example, its effect on the thyroid.[3] Subsequently, strategies have been followed to develop carrier systems that would allow drug delivery to the site of action for the transported molecule.[4] Among various colloidal drug delivery systems, nanoparticles (NP) represent a very promising approach to achieve this aim.[5−9]

Since an important element in the development of such drug delivery formulations consists of the release kinetics of the incorporated drug, different in vitro test systems are widely applied in the process of dosage form optimization.[10] Usually, NPs are incubated in a release medium, and samples are taken from the medium at appropriate time intervals, analyzed for their drug content, and replaced by fresh medium. In most cases, the direct determination, for example, quantifying the drug remaining in the carriers and the indirect method determining the released drug in the supernatant, correlate well. Therefore, the indirect method is preferable because of its ease of handling.[11,12] However, colloidal systems have to be separated from the release medium in order to determine the drug release kinetics, since they do not undergo quick sedimentation as a result of their relatively small size (between 150 and 500 nm[4]). A rather long centrifugation step usually has to be performed, which may impede the determination of the drug release within an adequate time interval. Even if NPs are separated from their release medium by a dialysis membrane, the whole process is still influenced by the possibly low solubility of the drug. In this case, sink conditions, which must be provided for such experiments, can be critical due to low drug concentrations in the release medium impeding the analytical procedure.

Indeed, several high performance liquid chromatography assays, as well as a capillary electrophoresis method, have been

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(1) Gill, J.; Heel, R. C.; Fitton, A. *Drugs* **1992**, *43*, 69−110.
(2) Marcus, F. I.; Fontaine, G. H.; Frank, R.; Grosgogeat, Y. *Am. Heart J.* **1981**, *101*, 480−493.
(3) Singh, B. N.; Nademanee, K. *Am. Heart J.* **1983**, *106*, 857−869.
(4) Kreuter, J. *Drugs and the Pharmaceutical Sciences*; Marcel Dekker: New York, 1994; Vol. 66.
(5) Langer, R. *Nature* **1998**, *392*, 5−10.
(6) Carino, G. P.; Jacob, J. S.; Mathiowitz, E. *J. Control. Release* **2000**, *65*, 261−269.
(7) Maincent, P.; Thouvenot, P.; Amicabile, C.; Hoffman, M.; Kreuter, J.; Couvreur, P.; Devissaguet, J. P. *Pharm. Res.* **1992**, *9*, 1534−1539.
(8) Nakada, Y.; Fattal, E.; Foulquier, M.; Couvreur, P. *Pharm. Res.* **1996**, *13*, 38−43.
(9) Lamprecht, A.; Ubrich, N.; Yamamoto, H.; Schäfer, U.; Takeuchi, H.; Maincent, P.; Kawashima, Y.; Lehr, C. M. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 775−781.
(10) U.S. Pharmacopoeia & the National Formulary. USP 23-NF18, 1995.
(11) Hombreiro Pérez, M.; Zinutti, C.; Lamprecht, A.; Ubrich, N.; Hoffman, M.; Bodmeier, R.; Maincent, P. *J. Controlled Release* **2000**, *64*, 429−438.
(12) Lamprecht, A.; Ubrich, N.; Yamamoto, H.; Schäfer, U.; Takeuchi, H.; Lehr, C. M.; Maincent, P.; Kawashima, Y. *J. Controlled Release* **2001**, *71*, 297−306.

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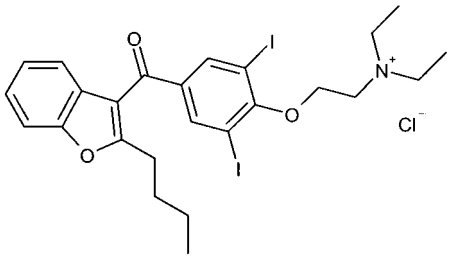

Figure 1. Molecular structure of amiodarone.

described for the determination of amiodarone.[13−19] However, the separation step between NP and the drug release medium would still be a problem that remained.

We report here on a new technique for the analysis of release kinetics occurring from such drug-loaded nanocarriers by using a Langmuir balance. On the basis of the hypothesis that the amphiphilic drug amiodarone may locate itself at the air/water interface after release from the NP, its quantification seems to be possible (Figure 1).[20] This would be a very promising alternative analytical procedure, not only for the application of amiodarone, but for all kinds of compounds exhibiting amphiphilic properties.

EXPERIMENTAL SECTION

Materials. The model drug amiodarone hydrochloride was obtained as a gift from Sanofi (Montpellier, France). The biodegradable polymer Resomer RG 502 (poly[lactic-*co*-glycolic acid] 25/50; M_w 10 000) (PLGA) was obtained from Boehringer Ingelheim AG (Ingelheim, Germany). Poly(vinyl alcohol) (Rhodoviol 4/125, 88% hydrolyzed) (PVA) was chosen as the surface active agent and was supplied by Prolabo (Fontenay s/Bois, France). Lipoïd S100 (soybean lecithin) was a kind gift from Lipoïd GmbH (Ludwigshafen, Germany). All other chemical reagents were obtained from Sigma (Steinheim, Germany) and Fisher Scientific (Elancourt, France) and were of analytical grade.

Nanoparticle Preparation and Characterization. The preparation of NPs was achieved by adjusting the simple emulsion (o/w) technique, previously reported in the literature.[12,21] Briefly, amiodarone (2 mg) was dissolved in 2 mL of methylene chloride containing 125 mg of the polymer (PLGA) under magnetic stirring. This organic solution was thereafter poured into the PVA aqueous solution (15 mL), and the emulsion was homogenized with a microson ultrasonic cell disruptor (Misonix Inc., Fermingdale, NY) at 20 W for 5 min. Thereafter, the solvent evaporation step was performed in a Büchi Rotavapor RE 121 (Büchi, Flawil, Switzerland) for 30 min, reducing the pressure stepwise down to 10−30 mbar with a diaphragm pump. The NPs were then washed twice by centrifuging them at 15000*g* at 4 °C for 20 min and redispersed in distilled water in an ultrasonic bath.

The NPs were analyzed for their size distribution by photon correlation spectroscopy using a Malvern Autosizer 4700 (Malvern Instruments S.A., Worcestershire, U.K.) at a fixed angle of 90° in the volume mode. The ζ-potential was determined with a Coulter Delsa 440 (Coulter Scientific Instruments, Amherst, MA). All batches were diluted with distilled water prior to the analysis and were analyzed in triplicate.

Determination of Drug Release Kinetics with High Performance Liquid Chromatography. The in vitro release kinetics were performed by dialysis: 1 mL of drug-loaded NP suspension was loaded into a dialysis tube and inserted into a flask with 100 mL of sodium chloride solution (0.9%, w/v) as a release medium under magnetic stirring at 250 rpm, the medium also containing empty liposomes simulating an interface. To provide sink conditions, large release volumes usually have to be used, thus impeding the drug concentrations required for the quantitative determinations. This was avoided by adding a second compartment into the release medium in the form of liposomes, which acted as a kind of acceptor phase, accumulating the drug from the release medium. At appropriate intervals, 0.5-mL samples were withdrawn and assayed for drug release and replaced by 0.5 mL of fresh sodium chloride liposomal solution. The amount of amiodarone in the release medium was determined by high performance liquid chromatography (HPLC) as described below. All measurements were performed in triplicate.

The liposome batches were prepared using a solvent evaporation method: 0.7 g of Lipoid S100 as lipid was dissolved in 4 mL of ethanol and rotary-evaporated into a thin lipid film at 60 °C and dried for a further 30 min. The lipid film was rehydrated with 6 mL of distilled water by hand-shaking at 60 °C for 10 min and by sonication, incubated at 60 °C for 20 min, and then extruded through a 100-nm polycarbonate filter (Liposofast Basic, Avestin, Ottawa, Canada) a total of 20 times. The liposomes had a mean diameter of 106.7 ± 3.2 nm and were applied to the drug release medium without further purification.

The drug content of the supernatant was analyzed by HPLC on the basis of a method described previously.[22] The setup was as follows: RP-18 column (LiChrospher 100, Merck, Darmstadt, Germany); eluent, methanol/water/ammonium hydroxide (94:5:1); flow rate, 1.5 mL/min. Amiodarone was detected by UV absorbance at 244 nm. Samples of 50 μL were injected into the column.

Langmuir Balance Experiments. The Langmuir balance experiments were generally performed as follows: samples at the air/water interface were prepared by spreading them with a microsyringe on the maximal available area (927 cm^2) of a Lauda FW2 Langmuir film balance (Lauda-Königshofen, Germany). Measurement of the surface pressure was performed by a floating barrier connected to a force transducer; accuracy was ±0.5 mN/m. The surface pressure at the beginning was lower than 0.5 mN/m. During all experiments, a sodium chloride solution at 0.9% (w/v) was used as the dispersion and release medium. This was to ensure a water-insoluble monolayer, allowing the exact determination of released amiodarone by avoiding the relocation of the monolayer into the water.[23]

(13) Hutchings, A.; Spragg, B. P.; Routledge, P. A. *J. Chromatogr.* **1986**, *382*, 389−393.
(14) Kannan, R.; Miller, S.; Perez, V.; Singh, B. N. *J. Chromatogr.* **1987**, *385*, 225−232.
(15) Ou, C. N.; Rognerud, C. L.; Duong, L. T.; Frawley, V. L. *Clin. Chem.* **1990**, *36*, 532−534.
(16) Jandreski, M. A.; Vanderslice, W. E. *Clin. Chem.* **1993**, *39*, 496−500.
(17) Zhang, C. X.; Aebi, Y.; Thormann, W. *Clin. Chem.* **1996**, *42*, 1805−1811.
(18) Myung, S. W.; Chang, Y. J.; Min, H. K.; Kim, D. H.; Kim, M.; Kang, T. K.; Yoo, E. A.; Sohn, Y. T.; Yim, Y. H. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2046−2054.
(19) Kollroser, M.; Schober, C. *J. Chromatogr. B* **2002**, *766*, 219−226.
(20) Ferreira, J.; Brasseur, R.; Chatelain, P.; Ruysschaert, J. M. *J. Pharm. Pharmacol.* **1986**, *38*, 561−566.
(21) Blanco, M. D.; Alonso, M. J. *Eur. J. Pharm. Biopharm.* **1997**, *43*, 287−294.
(22) Weir, S. J.; Ueda, C. T. *J. Pharm. Sci.* **1985**, *74*, 460−465.

Table 1. Particle Size, Polydispersity, ζ-Potential, and Encapsulation Rates of the Nanoparticle Formulations

	mean particle size nm	particle size after 48 h release nm	polydispersity index	ζ-potential mV	encaps rate %
blank NP	194.2 ± 5.9	192.1 ± 4.6	0.092 ± 0.003	−2.26 ± 0.09	
amiodarone NP	195.3 ± 8.2	195.0 ± 7.9	0.141 ± 0.008	0.63 ± 0.08	93.8 ± 1.5

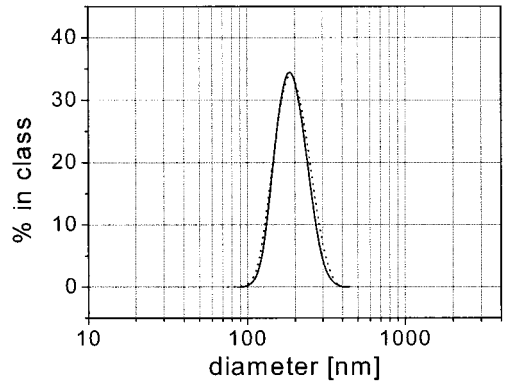

Figure 2. Size distribution of blank (····) and amiodarone-loaded (—) PLGA NP measured by dynamic laser scattering in the volume mode.

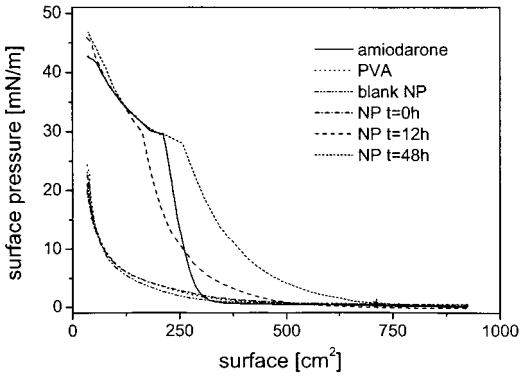

Figure 3. Surface pressure/surface area isotherms of NP at different time points during the release experiments.

1. Isotherm Characterizations. A 5-μL portion of an amiodarone−nanoparticle suspension was applied to the air/water interface, and the isotherm surface pressure/surface area plots were recorded at different time points; for example, $t = 0$, $t = 12$, and $t = 48$ h, respectively.

2. Determination of Drug Release Kinetics with the Langmuir Balance. Pure amiodarone solutions at a concentration of 2 mg/mL in chloroform were used for calibration by applying different known drug quantities to the air/water interface. Isotherm plots were performed, and at 10 mN/m, their exact air/water interface occupations were taken as a basis for the drug release calculation. We checked that all the isotherms were independent of the compression rates.

For the drug release experiments, 5-μL NP suspensions in distilled water were applied to the air/water interface and were compressed to an initial surface pressure of 10 mN/m, which was equivalent to an area of 250−300 cm^2 on the Langmuir balance. Thereafter, the Langmuir balance was run in the isobar mode, and changes of the air/water interface area were recorded at appropriate time points. Similar runs of blank or amiodarone-loaded NP were performed in triplicate.

RESULTS AND DISCUSSION

As shown in Figure 2, NPs had a submicrometer size and were relatively monodispersed. Since drug loss from the internal organic to the external aqueous phase should be kept to a minimum during the preparation procedure, including an emulsification step, the stability of the emulsion is crucial. During the solvent evaporation process, there is a gradual decrease in the dispersion volume and, consequently, a subsequent increase in the viscosity of the dispersed droplets. This affects the droplet size equilibrium, potentially involving the coalescence and the agglomeration of the droplets during the early step of solvent removal.[24,25] This problem was reduced by adding surfactants to the continuous phase and providing a thin protective layer around the droplets, hence reducing their coalescence. Generally, the applied surfactants are reported to remain at the NP surface throughout the preparation step, after which they desorb into the dispersing phase during later applications. In these experiments, PVA was used in order to provide sufficient stability to the initial emulsion. PVA is a very widely applied surfactant for micro- and nanoparticle formulation and represents a kind of standard in pharmaceutical particle technology.[26] The general characteristics of the NP formulation are reported in Table 1.

To prove the hypothesis that released amiodarone moves to the air/water interface, surface pressure/surface area isotherms were recorded. Isotherms were performed at different stages of the release experiment in the sodium chloride solution, as shown in Figure 3. PVA solutions, blank NPs, or drug-loaded NPs at $t = 0$ h exhibit similar isotherms. All curves were first dominated by the presence of PVA, whose signal was present even after the intensive washing steps at the end of the preparation procedure. The greater the amount of drug released, the more the shape of the isotherm approximated that of pure amiodarone.

On the basis of this observation, it was estimated that the Langmuir balance could be used for quantitative drug release determinations. The pure amiodarone standards exhibited characteristics in the surface pressure plots that were similar to the results reported recently.[23] All isotherms displayed a continuous shift to larger surfaces with increased drug concentrations (Figure 4). The plotting of the applied amiodarone amounts against its resulting occupation of surface area at 10 mN/m permitted the fitting of a calibration curve, which was used to quantify the amount of released amiodarone. In these isotherms, the theoretical occupied area per amiodarone molecule varied between 52 and 58 Å^2 at the fixed surface pressure of 10 mN/m, depending on

(23) Boury, F.; Gautier, J. C.; Bouligand, Y.; Proust, J. E. *Colloids Surf. B* **2001**, *20*, 219−227.
(24) Jalil, R.; Nixon, J. R. *J. Microencaps.* **1990**, *7*, 297−325.
(25) Arshady, R. *J. Controlled Release* **1991**, *17*, 1−22.
(26) Feng, S.; Huang, G. *J. Controlled Release* **2001**, *71*, 53−69.

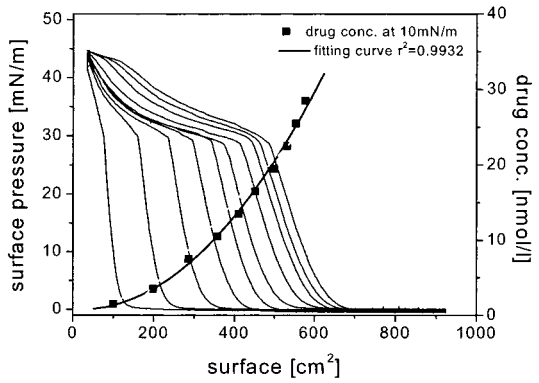

Figure 4. Calibration curve (■) resulting from isotherm surface plots of different amiodarone amounts. At 10 mN/m, the exact air/water interface occupation was taken as a basis for the drug release calculation.

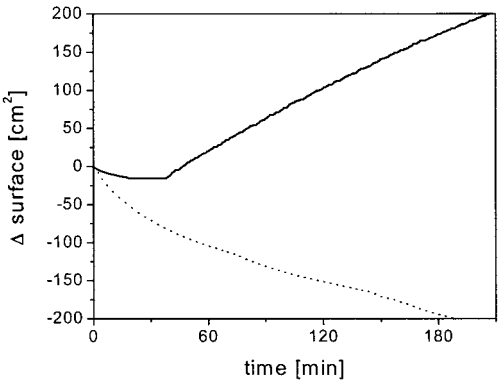

Figure 5. Change of air/water interface versus time during the release experiment by comparing amiodarone-loaded (—) with blank NP (····).

the amiodarone concentration. These results were in line with recent observations about the molecular area of amiodarone at the air/water interface.[23]

The detection limits were found at the first isotherm shown in Figure 3. The amiodarone concentration at this data point was 10 nM. An already clearer signal was obtained with a 17 nM solution. Compared to a recently published method as HPLC/MS/MS, these concentrations were found to be between the mentioned limit of detection (1.5 nmol/L) and the limit of quantification (73.4 nmol/L).[19] Thus, this Langmuir method could be rated as a relatively sensitive method. Blank NPs exhibited a decrease in surface area over time during isobar measurements at 10 mN/m which might be mainly due to the presence of PVA. In an initial state, a desorption of PVA from the NP surface may take place, followed by its movement toward the air/water interface. Afterward, a dislocation of PVA into the aqueous subphase lowers the surface area (Figure 5). For amiodarone-loaded NPs, surprisingly, at first a decrease of surface area was found that stopped after 30 min to 1 h and was followed by the estimated increase. This initial decrease of the surface area might be explained again by the very early presence of PVA at the air/water interface. After ~30 min, the release of amiodarone and the following orientation toward the interface starts to dominate after a turning point leading to an increase of surface area.

The involvement of the surfactant on the processes at the air/water interfaces made it impossible to measure drug release

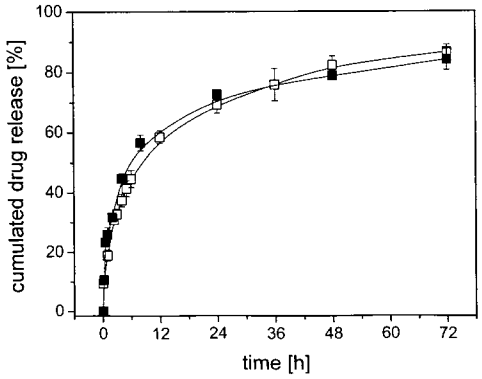

Figure 6. Comparison of the drug release kinetics determined by a standard HPLC method (■) and the Langmuir balance setup (□). A multiple regression of the two release profiles led to coefficient of $r^2 = 0.8872$.

directly using this method; however, the differences between surface area obtained with amiodarone-loaded and blank NPs at corresponding time points were estimated as the resulting amiodarone release and were used for further comparison with results from HPLC. The calculations were carried out by assuming that the surfactant and the drug behave as an ideal mixture. Since generally surfactants are applied in the preparation process of NPs, they may desorb from the NP surface and may certainly influence the determination of surface pressure. Therefore, it is concluded that a controlled release experiment with unloaded NPs is required in order to distinguish signals of the released drug from residual surfactant.

To prove the use of the Langmuir balance in the quantitative characterization of drug release kinetics, these results have been correlated with those of a standard procedure. The HPLC method was applied to quantify the drug release kinetics under comparable conditions. To ensure an equivalent release and partitioning behavior compared to the circumstances in the Langmuir trough, liposomes were prepared and dispersed in the release medium representing the concurrent interface. To allow a quantitative separation of NPs and liposomes, a dialysis membrane system was applied to separate the drug-containing NPs from the liposomes in the acceptor phase.

It was found that results from the Langmuir balance and the standard HPLC method correlated well (Figure 6). The interexperimental variation was 4.4% for the Langmuir method ($n = 4$), and interexperimental variation for HPLC was 2.9% ($n = 3$).

The avoidance of nanoparticle separation from the release medium prior to the analytical procedure might be an essential advantage of this technique. This permits the measurement of drug release by a continuous method avoiding the purification of the released drug before its quantification.

In general, the drug release occurred in two phases: a first initial burst release was followed by a more sustained release of the drug over 2 days. The first stage of drug release showed a significant initial burst effect, involving the release of almost 40% of the total encapsulated drug amount within the first 2 h. The drug release profile might be influenced by an inhomogeneous drug distribution within the NP. Thus, one reason for the predominant burst release might be based on the accumulation of amiodarone near the particle surface. This hypothesis seems to be supported by the ζ-potential measurements, which exhibited

a slight increase in the ζ-potential of amiodarone-loaded NPs compared to uncharged particles (Table 1). It is generally assumed that drugs are released by several processes, such as diffusion through the particle matrix, release by polymer degradation, and solubilization and diffusion through microchannels that are formed by erosion.[27] Coffin and McGinity[28] stated that polyester NPs are affected by polymer degradation after 50−100 days, depending on the presence of anionic or nonionic surfactants, respectively. Similar stability was reported from polyesters placed at the air/water interface.[29,30] Therefore, it can be concluded that polymer degradation did not occur and did not influence drug release in this case, especially since the NPs did not significantly change their mean diameters within the experimental period (Table 1). Thus, any significant influence of the polyester on the measurements at the interface can be excluded if drug release experiments are performed within such relatively short time periods.

(27) Washington, C. *Drug Release from Microparticulate Systems, Drugs and the Pharmaceutical Sciences*; Marcel Dekker: New York, 1996; Vol. 73, pp 155−181.
(28) Coffin, M. D.; McGinity, J. W. *Pharm. Res.* **1992**, *9*, 200−205.
(29) Malzert, A.; Boury, F.; Saulnier, P.; Benoit, J. P.; Proust, J. E. *Langmuir* **2000**, *16*, 1861−1867.
(30) Boury, F.; Ivanova, Tz.; Panaiotov, I.; Proust, J. E. *Langmuir* **1995**, *11*, 599−606.

CONCLUSIONS

The present investigations show that the use of the Langmuir balance allows the characterization of drug release kinetics from colloidal drug carriers at very low concentrations. Even if this new method might be limited in its application to amphiphilic drugs, it seems to be interesting that the required drug amount is in the nanomolar range, especially for very expensive new drugs. Another notable, distinct advantage is the possibility of avoiding the circumstantial procedure of separating the releasing carrier system from the release medium. Moreover, this technique might be interesting for structures that are difficult to detect by HPLC systems.

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