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Automated measurement of permeation and dissolution of propranolol HCl tablets using sequential injection analysis

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Abstract

Aim of this study was to automate sampling and quantification of the previously described apparatus for combined determination of dissolution and permeation through Caco-2 monolayer by means of sequential injection analysis (SIA). Native fluorescence of propranolol HCl in Krebs–Ringer buffer (KRB) was used for quantification. Sampling was done at three different locations within the apparatus at a high sampling frequency (approximately 60 h^{-1}). Injection volume delivered to the fluorescence detector was 50 μL for permeation monitoring and 25 μL for dissolution monitoring. Linear regression for 50 μL injection yielded a detection limit calculated as 0.04 μg mL^{-1} of propranolol HCl in KRB ($R^2 > 0.999$). However, linearity for dissolution monitoring was not given for the complete range of concentrations and first order polynomial calibration was established ($R^2 > 0.9999$). To conclude, the SIA system was able to monitor simultaneously dissolution and permeation of the immediate release propranolol HCl tablets and the authors succeeded in automating the apparatus for combined measurement of dissolution and permeation. In addition, the obtained data was consistent with data obtained by manual sampling followed by HPLC analysis.
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1. Introduction

Several apparatus for simultaneous measurement of dissolution and permeation through Caco-2 have been established in the past years [1–7], and auspicious results were obtained. Combining two *in vitro* methods to forecast behavior of solid oral dosage forms when administered *in vivo* appears to be a promising tool to obtain more vital information of the *in vivo* performance compared to deploying both assays separately, especially in regard to testing solid dosage forms containing excipients interfering with intestinal epithelia. Generally speaking, these apparatus can be divided into two classes. Ginski and Polli [8] and Kataoka et al. [6] reported on a closed dissolution and permeation system, either using the USP apparatus 2 or a specially designed dissolution vessel. In contrast, Miyazaki and coworkers [1–4] and our group [7] are using open systems for dissolution and permeation assessment. Briefly, our apparatus to be automated by means of sequential injection technique consists of a dissolution and a permeation module and it is designed to assess permeability of complete solid dosage forms. Sampling has been done manually and quantification has been done offline via a RP-HPLC method. Preliminary studies revealed plausible results using propranolol HCl immediate release tablets [7] and integrity of Caco-2 monolayer throughout the experiments. For the automation, the principles of the apparatus have not been changed, however, minor modification have been performed as it is discussed later on.

However, resulting from these preliminary studies, simultaneous analysis of dissolution and permeation turned out to be tedious and labor consumptive; not only the use of viable cells but also manual sampling and analysis with HPLC definitely limits the throughput. HPLC based quantification may be con-

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sidered as time consumptive and also as money consumptive, since expensive mobile phase, vials and further equipment have to be used. In addition, organic solvents wastage bears issues concerning environmental pollution. In most cases, however, selectivity provided by HPLC is not necessary if only one active pharmaceutical ingredient (API) is used and if sensitive and/or selective UV or fluorimetric detection are possible.

Sequential injection analysis (SIA) and its predecessor flow injection analysis (FIA), which has been developed in 1975 [9], have been continuously applied in pharmaceutical sciences and further improved [10–12]. SIA facilitates fast and reproducible on-line sample handling such as sample aspiration, sample dilution, solid phase extraction and many other applications [13]. Additionally, SIA permits – in combination with fluorescence or UV-detectors – fast and reliable quantification of many pharmaceutically applied compounds [10,11]. Beyond that, SIA is described to be a flexible, robust and a highly versatile tool for automating sampling and sample treating. For these reasons, sequential injection analysis appears to be an ideal solution for our purposes, especially when high sampling frequency and rapid online quantification is of vital interest. Several groups reported to employ SIA for automating dissolution systems using both fluorimetric [14–16] and UV [17–19] detection. Detection limit of fluorimetric quantification was comparable to quantification limits provided by HPLC based methods (30 ng mL^{-1} for ergotamine tartrate [15] and 50 ng mL^{-1} for acetylsalicylic acid [14]).

Test substance for combined measurement of dissolution and permeation was propranolol HCl. It was chosen because of its favorable biopharmaceutical properties exhibiting high solubility and high permeability [20] and is therefore assigned to the biopharmaceutical classification system class 1 [21]. Beyond that, quantification of propranolol HCl is easily accessible using either HPLC method with UV detection or fluorescence detection. Taking into account that for combined measurement of dissolution and permeation relatively high concentrations in the dissolution part and relatively low concentration in the permeation part have to be measured, fluorimetric detection would be an appropriate way to quantify in a SIA-based system dissolved and permeated propranolol HCl in the matrix Krebs–Ringer buffer (KRB).

Not only time consumptive manual sampling procedure was subjected to optimization, but also lag time and dead volumes were found to be still rather large in the previously described apparatus [7]. In order to minimize those issues, inner diameters of all tubing were chosen to be as small as possible. In addition, the volume of the acceptor compartment was decreased.

2. Materials

2.1. Materials

Transwell® permeable filter inserts (pore size 0.4 μm, 1.13 cm^2, Transwell™ type 3460) were purchased from Corning Incorp. Life Sciences (Acton, MA). Dulbecco's modified Eagle's medium (DMEM), non-essential amino acids (NeAA) and fetal bovine serum (FBS) were purchased from GIBCO (Invitrogen Corp., Carlsbad, CA). Propranolol HCl was bought from Synopharm GmbH & Co KG (Barsbüttel, Germany) and was of Ph. Eur. grade. All compounds for Krebs–Ringer buffer (KRB) were obtained from Sigma–Aldrich (St. Louis, MO) and were of cell culture tested grade. Avicel PH 102 was from Lehmann & Voss (Hamburg, Germany); Lactose EP type D20 was from J. A. Meggle Milchindustrie (Reitmehring, Germany); crosslinked PVP was from BASF (Ludwigshafen, Germany); Aerosil 200 was from Degussa (Rheinfelden, Germany); Mg-stearate was from Synopharm GmbH & Co KG (Barsbüttel, Germany). All tablet excipients were of Ph. Eur. grade.

Buffer for dissolution and permeation experiments was KRB. Composition was as follows: 1.41 mM $CaCl_2$, 3.00 mM KCl, 2.56 mM $MgCl_2$, 142.03 mM NaCl, 0.44 mM K_2HPO_4, 4.00 mM D-glucose and 10.0 mM HEPES. KRB was adjusted to pH 7.4 by means of NaOH. Carrier stream for the SIA analysis was degassed Millipore® water.

2.2. Production of tablets

Immediate release tablets containing 10 mg of propranolol HCl were manufactured using direct compression. Tablets contained 70% of Avicel PH 102 and 17% of lactose EP type D20 as filler, 1% of PVP as disintegrant, 1% of aerosil 200 and 1% of Mg-stearate as lubricants and 10% of propranolol HCl as the API. All ingredients were sieved (mesh size 315 μm), blended in a turbula mixer (TS Bachofen, Basel, Switzerland) and subsequently compressed using a Korsch EK 01 (Berlin, Germany) eccentric tablet press, gaining tablets with a mass of 100 mg and a crushing strength of 100 N (Erweka hardness tester, type TBH 30M, Erweka, Heusenstamm, Germany). For quantification, tablets were dissolved in 100.0 mL 0.1N HCl and treated with ultrasound. Quantification was done with HPLC as described in [7]. An amount of propranolol HCl of 9.70 ± 0.23 mg per tablet was determined ($n = 10$).

2.3. Cell culture

Caco-2 cells, clone C2BBe1, were purchased at passage 60 from American Tissue Culture Collection (ATCC; Manassas, VA) and used at passages 70–92. Caco-2 cells were grown to ~90% confluence in 75 cm^2 T-flasks with DMEM supplemented with 10% FBS and 1% non-essential amino acids (NeAA). Culture medium was changed every second day. The incubator temperature was set to ~37°C in an atmosphere of ~85% relative humidity and ~5% CO_2. After trypsinisation, cells were seeded on Transwells® at a density of 60,000 cells cm^{-2}. Transepithelial electrical resistance (TEER) was measured routinely and only monolayers with a TEER > 350 Ω cm^2, with background for plain filters subtracted, were used for transport studies. Subsequent to combined measurement of dissolution and permeation, integrity of the Caco-2 monolayer was confirmed by means of TEER measurement and light microscopy.

2.4. SIA system

The SIA system consisted of the FIAlab 3500 (FIAlab instruments, WA, USA) implemented with a 2.5 mL piston pump, a peristaltic pump and an eight port multiposition valve. Software was FIAlab for Windows® Version 5.9.192. Standards were aspirated by means of an autosampler (Cetac ASX 260, NE, USA). Fluorimetric detection was performed with the FIAlab fluorescence detector PMT-FL. Integration time was set to 100 ms, scan rate was adjusted to 2.0 Hz. An UV light source was purchased from Mikropack (type D-2000, Ostfildern, Germany), and the light source was linked with the fluorescence detector with a fiber optic cable. An excitation filter (λ_{exc} = 260 nm, 10 nm full width half maximum, Andover Corporation, NH, USA) and no emission filter was used.

2.5. The automated apparatus for combined dissolution and permeation measurement

The automated apparatus is schematically depicted in Fig. 1. The apparatus for combined measurement of dissolution and permeation can be divided into two modules: on the left the dissolution module with the flow through dissolution cell (USP apparatus 4, Sotax CE1, Sotax, Germany) and on the lower right side the permeation module with the flow through permeation cell, whereas both modules are connected by means of a stream splitter providing appropriate low flow rates for the permeation module. The permeation module can be subdivided into two compartments: the open apical and the closed, circulating basolateral compartment, which comprises a stirred reservoir with a volume of 1.0 mL KRB and a measurement loop to the multipositional valve. Flow rates in the dissolution and in the permeation module were adjusted to 6.5 and 1.0 mL min^{-1}, respectively. Three points of sampling within the apparatus are indicated in Fig. 1 with the capital letters D, A, and B. Samples taken at sampling port D, directly after the flow through dissolution cell, are providing the original dissolution signal. Concentrations at sampling port A, located at the apical part of the permeation module, exhibit the concentration in contact with the apical side of the Caco-2 monolayer. Concentrations measured at sampling port B allow the calculation of the amount permeated to the basolateral side of the Caco-2 monolayer (volume of the complete basolateral module 5.25 mL).

The interface between the apparatus for combined dissolution/permeation apparatus measurement and the SIA system was as follows. At sampling ports D and A, a tubing was used to directly connect the stream containing the dissolved drug with the multiposition valve. Volumes of the connecting tubing between the stream and the eight port position valve were for sampling port D and A 15 μL and 60 μL, respectively. In order to assure that correct concentrations were assessed, this connection was washed prior to each real measurement at sampling port D by aspirating 50 μL, and at sampling port A by aspirating 100 μL, respectively. Since the aspirated volumes compared to the flow rate at D (6.5 mL min^{-1}) are sufficiently low, no replenishment of the withdrawn sample was necessary. Also, at sampling port A no replenishment was required since the stream is subsequently going to waste. The basolateral vessel posing the reservoir for the closed basolateral compartment was connected to the multiposition valve by means of a measurement circuit driven by the peristaltic pump of the FIAlab 3500 system (flow = 0.9 mL min^{-1}). The dead volume of the connection between the measurement circuit and the multipositional valve was approximately 6 μL. Prior to aspiration of the real sample, 25 μL were aspirated in order to wash the T-connector and were subsequently discarded. Post-washing, 50 μL were aspirated for

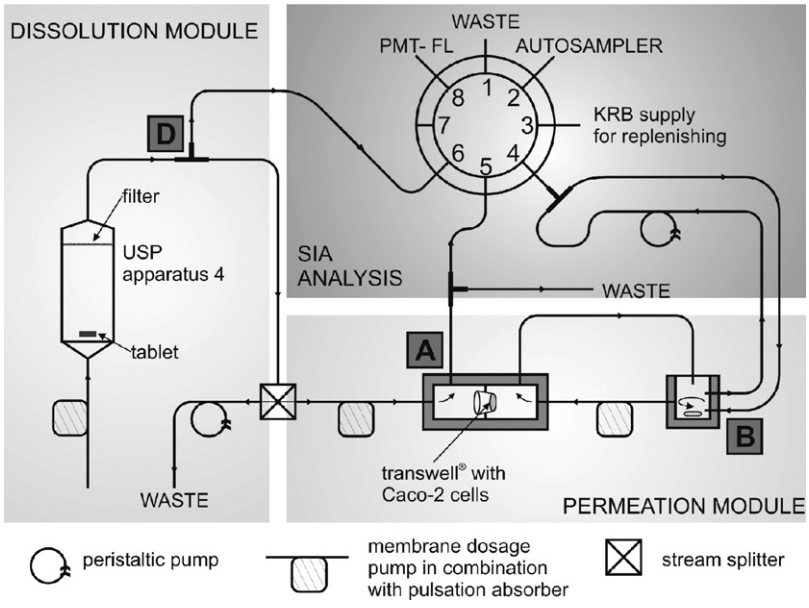

Fig. 1. Schematic depiction of the automated apparatus for simultaneous measurement of dissolution and permeation. Sampling ports are indicated with capital letters, D for dissolution, A for apical and B for basolateral. The multipositional valve and its port assignment was as follows. Port 1 was connected with the waste, port 2 with the autosampler for aspirating the standard solutions, port 3 was connected to the KRB supply for replenishing the volumes taken from the basolateral compartment. Port 8 was connected to the fluorescence detector PMT-FL and ports 4, 5 and 6 are assigned to sampling ports B, A and D, respectively.

the fluorimetric analysis, and afterwards 75 μL of KRB were dispensed back into the measurement circuit.

All tubing of the apparatus for combined measurement of dissolution and permeation and the SIA system were made of Teflon® to minimize adsorption; inner diameter of tubing in the dissolution module was 1.55 and 1.0 mm in the permeation module (Upchurch, WA, USA). Connection tubing to the multipositional valve had an inner diameter of 0.50 mm. The flow through dissolution cell was equipped with filters (type GF/D, Whatman International Ltd., Maidstone, UK) preventing undissolved drug and excipients from leaving the dissolution module. The flow through permeation cell and the basolateral reservoir vessel were made of polyetheretherketone (PEEK). The basolateral vessel was magnetically stirred (MINI 07, H+P, Oberschleißheim, Germany). Prior to each experiment, all media were properly degassed by stirring for 10 min under reduced air pressure. Before inserting in the apparatus, Caco-2 cells were washed twice with KRB and were allowed to equilibrate for at least 30 min in KRB at 37 °C. Then, TEER was measured and the Transwell® with a cell monolayer exceeding 350 Ω cm^2 was inserted into the flow through permeation cell. Subsequently, the flow was adjusted to 1.0 mL min^{-1} and cells were equilibrated for another 10 min before the experiment was started.

Cumulative amount (A_c) appeared at sampling ports D and A have been calculated from the measured concentrations using Eq. (1), which is derived from the trapezoidal rule:

$$A_c = \sum_{i=1}^{n-1} \left[\frac{c(t_{i+1}) + c(t_i)}{2} (t_{i+1} - t_i) \right] \cdot \text{FLOW} \quad (1)$$

whereas $c(t)$ is the concentration (μg mL^{-1}) measured at time t (min) and $c(t_{i+1})$ is the concentration measured at time t_{i+1}. FLOW designates the flow rate (mL min^{-1}) in the respective module.

Mean dissolution and mean permeation time was calculated according to Eqs. (2)–(4):

$$\text{mean time at D}: \quad \text{MT}_\text{D} = \frac{(t \times m_\text{D}) - \text{AUC}_\text{D}}{m_\text{D}} \quad (2)$$

$$\text{mean time at A}: \quad \text{MT}_\text{A} = \frac{(t \times m_\text{A}) - \text{AUC}_\text{A}}{m_\text{A}} \quad (3)$$

$$\text{mean time at B}: \quad \text{MT}_\text{B} = \frac{(t \times m_\text{B}) - \text{AUC}_\text{B}}{m_\text{B}} \quad (4)$$

whereas t is the time duration over which dissolution or permeation was measured (typically 120 min), m_D and m_A are the masses which cumulatively appeared at the respective sampling ports after time t, m_B is the mass which permeated after time t. AUC_D, AUC_A and AUC_B are the areas under the dissolution or permeation curve, respectively (both were calculated using trapezoidal rule).

As the apparent permeability (P_app) is generally defined as flux over donor concentration, apparent permeability was calculated from the concentrations assessed at sampling ports A and B. Flux at the respective measurement point (flux_n) was estimated by interpolation using the following equation:

$$\text{flux}_n = \frac{\text{mass}_{n+1} - \text{mass}_{n-1}}{t_{n+1} - t_{n-1}} \quad (5)$$

Mass_{n+1} is the mass permeated at measurement point $n+1$ and t_{n+1} is the time of the measurement point $n+1$. Mass_{n-1} is the mass of the measurement before n, t is the time of the measurement point before n. The apical concentration at the respective time points were calculated using linear regression between two measurement points at A.

3. Results and discussion

3.1. Calibration

Signal of native fluorescence of propranolol HCl in aqueous media [22] was sufficiently strong for direct detection in the matrix KRB. Experiments with placebo tablets (the API propranolol HCl was replaced by lactose) led to signals comparable to those obtained from blank KRB, thus, indicating that no interference of excipients with the analysis occurred. Standards were provided by aspiration from the autosampler, and peak height was correlated with standard concentrations. Flow rate of the analyte through in the PMT-FL was found to be ideal in terms of reproducibility and signal height at 50 μL s^{-1}. Since concentrations at sampling ports D, A and B differ strongly, different aspiration volumes were found to be optimal. Sampling port D and A exhibiting sampling ports with the highest concentration had an aspiration volume of 25 μL; however, sample aspiration volume at port B, where only low concentration are expected, was increased to 50 μL to assure sufficient sensitivity at low concentrations. The lower aspiration volume was shown to yield lower fluorescent signals combined with an increased linear part of the regression line. Consequently, higher aspiration volumes caused higher fluorescent signals and led to earlier nonlinear behavior. Nevertheless, it was not feasible by decreasing the aspiration volume to 25 μL to fit the calibration curve to a straight line as it can be seen in Fig. 2, and thus calibration for sampling port D and A was performed with an first order poly-

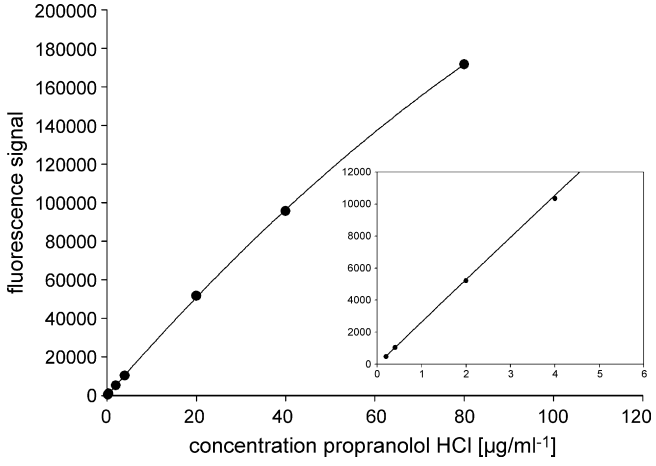

Fig. 2. Calibration plot for propranolol HCl in KRB (injection volume 25 μL). The inserted plot shows an extract of the regression curve for the lower standards.

nomial equation. For 50 μL injection, calibration was performed with five standard solutions and for 25 μL injection with seven standards. Standard concentration for 50 μL injection were 0, 0.05, 0.2, 0.4, 2.0, 4.0 μg mL^{-1} of propranolol HCl. Standards concentrations for 25 μL injection were 0, 0.2, 0.4, 2.0, 4.0, 20, 40, 80 μg mL^{-1} of propranolol HCl. All standards were measured in sextuple. Prior to regression, blank values obtained injecting KRB were subtracted. Relative standard deviation for the 50 μL injection at the lowest standard was 2.35% ($n = 6$) and at the highest standard 1.63% ($n = 6$). Linear regression led to the following equation: $y = 4246.8c - 44.9$ (y = fluorescence signal, c = concentration (μg mL^{-1}), $R^2 > 0.999$). Limit of quantification was calculated as 0.04 μg mL^{-1} (10σ); limit of detection 0.02 μg mL^{-1} (3σ). Relative standard deviation at the lowest standard concentration for 25 μL injection was 2.82% ($n = 6$) and for the highest standard concentration 3.09% ($n = 6$). Regression led to a first order polynomial equation (see Fig. 2): $y = -6.55c^2 + 2669.1c - 18.7$ (y = fluorescence signal, c = concentration (μg mL^{-1}), $R^2 > 0.9999$). The values for quantification and detection limit are consistent with values found for comparable substances in literature [14,15]. In conclusion, it can be pointed out that an accurate and sensitive quantification method for propranolol HCl was found and that no interference with excipients occurred.

3.2. Automated measurement of dissolution and permeation

Sampling and analysis were performed alternating at sampling ports D and A, intermitted by a measurement at sampling port B after every third loop of measurement at D and A. Sampling together with quantification took 0.7 min at sampling port D, 0.9 min at A and 1.3 min at B. Time for sampling at port B lasted longer because of the time needed for replenishing KRB. Sampling frequency of approximately 60 h^{-1} was found to be sufficiently high, although more measurement points at early time points of the experiments might be desirable. Fig. 3 plots the concentration measured at sampling port D and sampling

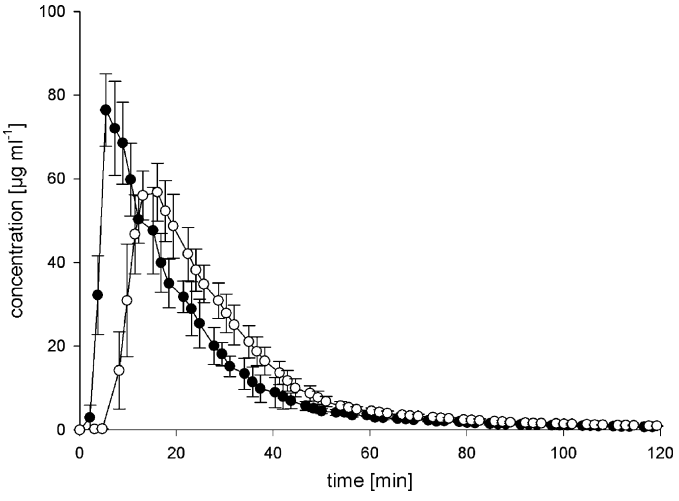

Fig. 3. Closed circles (●) represent the propranolol HCl concentrations measured at sampling port D and open circles (○) the concentration assessed at A for a 10 mg immediate release tablet ($n = 7$, all values as mean ± S.D.).

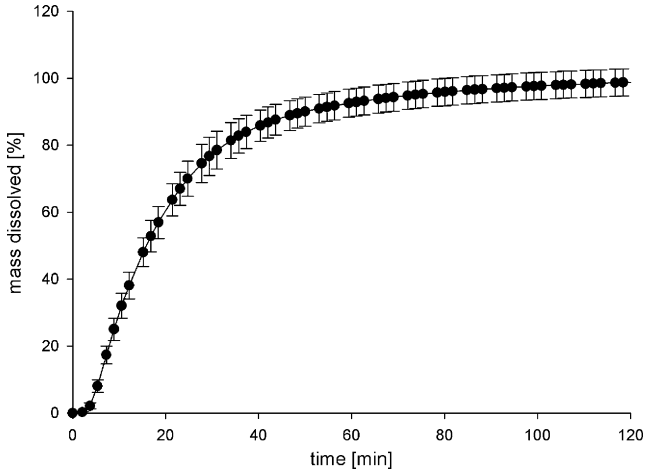

Fig. 4. Cumulative mass of propranolol HCl calculated according to Eq. (1) from the concentration obtained at sampling port D is plotted vs. time. After 120 min tablets released 98.7 ± 4.0% mg of the labeled amount ($n = 7$, all values as mean ± S.D.).

port A against time. Concentration assessed at sampling port D yielded higher concentrations than at sampling port A since convectional mixing and dilution throughout the passage of the apparatus broadened the peak and decreased peak height. In addition, a time shift between the peak maxima at D and at A of approximately 7.5 min was detected. Fig. 4 plots the percentage of released propranolol HCl calculated according to Eq. (1) against time. An almost complete release of 98.7 ± 4.0% of the labeled amount of propranolol HCl could be detected strongly suggesting correct calibration. Cumulative amount appeared at A yielded a mass of 1.48 mg at 120 min (curve not shown). Since the stream splitter is dividing the incoming 6.5 mL min^{-1} stream from the dissolution device into an 1.0 mL min^{-1} for the apical compartment and a waste stream, the mass appeared at A should be 1/6.5 = 15.4% of the amount appeared at D. The observed value of 15.5% matched sufficiently precise the targeted value, indicating that flow rates were adjusted correctly and that the stream splitter worked properly. Fig. 5 shows on the primary ordinate the concentration measured at sampling port A versus time, hence, the concentration which was led over the Caco-2 monolayer. The amount of propranolol HCl measured in the basolateral compartment against time is plotted on the secondary axis. It is evident that the greatest increase of propranolol HCl in the basolateral compartment is accompanied with the maximum peak concentrations at sampling port A. After 120 min, a total amount of propranolol of 4.07 ± 0.37 μg ($n = 7$) was detected in the basolateral compartment. When forming the ratio between the amount which permeated and the cumulative amount appeared at A, a value of 0.275% is found. This value is comparable with values found in literature for high permeability compounds [2]. According to Eq. (5), apparent permeability (P_{app}) was calculated from seven time points as indicated with asterisks in Fig. 5. P_{app} was estimated as $(2.61 \pm 0.63) \times 10^{-5}$ cm s^{-1} (mean of seven independent calculations). Vogelpoel et al. [20] stated that P_{app} values for propranolol HCl through Caco-2 cell monolayers are reported in literature to be in a range of $(1.12 \pm 0.05) \times 10^{-5}$

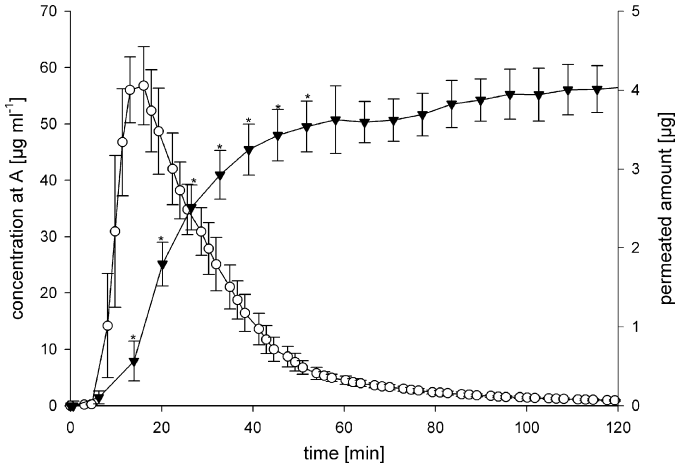

Fig. 5. Open circles (◯) represent the concentration measured at A (primary ordinate), triangles down (▼) represent the permeated amount of propranolol HCl derived from concentration measured in the basolateral compartment (secondary ordinate). Asterisks indicate the values from the P_{app} value was calculated ($n = 7$, all values as mean ± S.D.).

to $(4.30 \pm 0.36) \times 10^{-5}$ cm s^{-1}. Therefore, it can be pointed out that the value found with the here described apparatus is consistent, both with previous experiments of the authors and with literature. Caco-2 monolayers retained their integrity throughout the experiments indicated by a TEER before and after each experiment being above 350 Ω cm^2 with blank filter subtracted.

3.3. Comparison between manual sampling and automated sampling

Synopses of data obtained from manual sampling with subsequent quantification by HPLC as described in [7] and data obtained from the above described SIA method are graphically juxtaposed in Fig. 6. The same propranolol HCl 10 mg immediate release tablets of the same batch were used for both sets of experiments. Before interpreting the data, it is worth to mention that aside from automation the apparatus for combined measurement of dissolution and permeation underwent some further changes compared to the one previously described. The inner diameter of the tubing and the length of the tubing was decreased wherever feasible since lag time and time shift were found to be improvable. In addition, the volume of the basolateral compartment was decreased in order to be able to measure higher concentrations. As it can be seen in Fig. 6, concentration trends at sampling ports A and B obtained from either manual or automated sample processing appear to be comparable. The optimized and automated setup yielded at sampling port D higher peak maximum concentrations of approximately 75 µg mL^{-1} versus 65 µg mL^{-1} of the previously described apparatus. This may be contributed to the shorter and thinner tubing and to higher resolution due to more sampling points. Maximum concentrations derived from sampling port A behave similar (57 µg mL^{-1} versus 50 µg mL^{-1}). Standard deviation of the automated apparatus seemed to increase slightly. This phenomenon might be due to the higher sampling volumes of the not automated apparatus where 500 µL of sample were withdrawn and analyzed

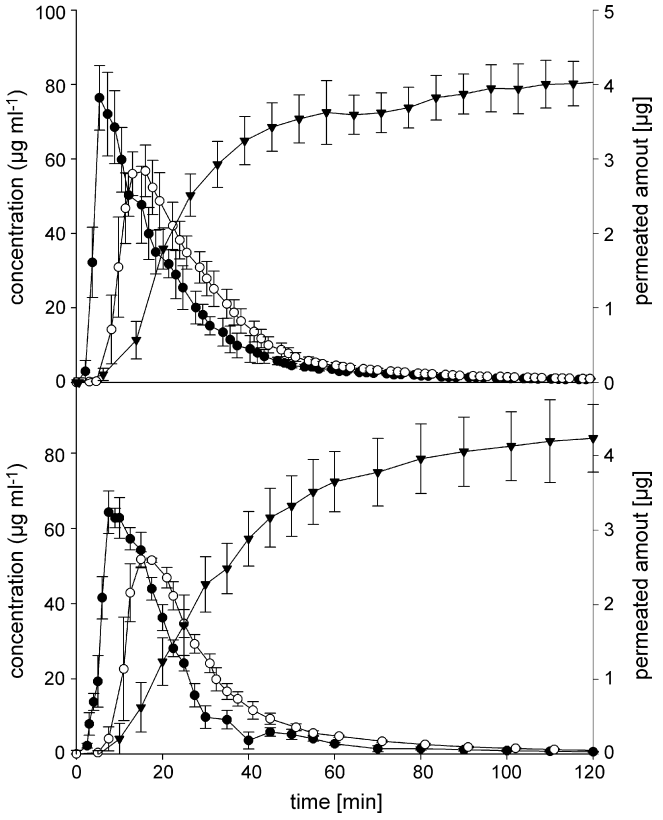

Fig. 6. Comparison between manual sampling and analysis via HPLC (lower diagram) ($n = 5$) and automated sampling and fluorescence detection using SIA (upper diagram) ($n = 7$). Closed circles (●) represent the concentration at D, open circles (◯) the concentration measured at A (both primary ordinate) and triangles down (▼) represent the permeated amount of propranolol HCl (secondary ordinate).

(compared to 25 µL at the SIA). Sampling higher volumes will average the concentration variation within this volume and thus apparently decrease variation of concentrations. Aside from that, peak shape was not significantly affected. By means of the automation, sampling frequency was increased nearly by the factor two compared to manual sampling and fairly increased temporal resolution compared to the previous setup. At the peak concentrations, however, higher sampling frequency for the SIA system would be appreciated. This might be subject for further optimization. The concentration trend measured in the basolateral compartment changed only slightly. It appears that for the newer setup a plateau is reached earlier (Fig. 6). The reason for that might be – additionally to shorter tubing – the decreased volumes in the basolateral compartment (5.25 mL compared to 10.5 mL). However, after 120 min, a comparable amount of propranolol HCl in the basolateral compartment was detected (4.07 ± 0.37 µg for SIA versus 4.23 ± 0.45 µg for manually and HPLC treated samples, respectively). Additionally, decreased standard deviations of the concentrations for the automated setup were found for concentrations measured at B; however, sample number is too small to generalize this finding. Table 1 shows in summary the mean times at the different sampling ports D, A and B calculated according to Eqs. (2)–(4). Generally speaking, a trend to shorter mean times at the different sampling ports can

Table 1
Mean times calculated according to Eqs. (2)–(3) and Eq. (4) in comparison ($n = 5$ for manual sampling according to data in [7] and $n = 7$ for SIA analysis)

	Manual sampling followed by HPLC analysis (min)	Automated sampling and analysis by SIA (min)
MT_D	23.45 ± 0.96	22.32 ± 2.32
MT_A	31.37 ± 1.12	30.35 ± 1.56
MT_B	34.98 ± 2.27	30.02 ± 2.56

be seen for the automated and optimized apparatus. Since sampling port D is located very early within the apparatus, only a minor decrease of the MT_D is be detected. A similar behavior can be observed for sampling port A, where the MD_A time was slightly decreased in the automated setup. A more pronounced effect could be detected for the MT_B, which has been calculated from the mass increase in the basolateral compartment. Here, the MT_B shifted distinctively for approximately 5 min. Since the mean times for sampling ports D and A did not change significantly, the change of the MT_B can exclusively be assigned to changed setup. This entails that a small change in tubing and dead volumes can greatly effect the concentration trends and intends for future studies that comparing results from different apparatus for combined measurement of dissolution and permeation is difficult if even impossible.

4. Conclusion

Two putative limitations of SIA are set concerning sampling frequency and selectivity. Analysis, filling and washing requires time and restricts sampling frequency to maximum 60–80 measurements per hour. In addition, not every API is accessible selectively and sensitively via fluorescence, hence forcing the researcher to search for alternative solutions for detection. Nevertheless, automation of the apparatus by means of SIA facilitated flexible and sufficient rapid sampling from different sampling ports with varying washing steps, varying aspiration volumes and in case of sampling from sampling port B automated replenishing of the withdrawn buffer. Detection with fluorescence permitted sensitive and selective online determination and quantification of propranolol HCl in KRB at a higher temporal resolution compared to the previous setup. In addition, online measurement could reduce issues when working with unstable compounds. Furthermore, automation decreased labor intensive manual sampling, considerably dropped the costs for HPLC accessories, and dramatically increased the throughput. In summary, an automated and rather unique tool for assessing the permeability of solid dosage forms through Caco-2 monolayer has been successfully developed.

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