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Application of automated flow injection analysis to drug liberations studies with the Franz diffusion cell

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The flow-injection method with sensitive fluorimetric detection is used to monitor the liberation profiles of a topical dermatological formulation containing the model compound salicylic acid. The connection of a standard Franz diffusion cell with the automated flow-injection system enables an acquisition of multi-point liberation data in a form of series of fluorescence peaks in a short time. Examples of liberation profiles for a topical dermatological formulation containing salicylic acid are shown.

1. Introduction

Monitoring of semisolid pharmaceuticals has grown over the past decade [1]. One of the most widely accepted standard methods is to determine the rate of release of active pharmaceutical ingredients with respect to the time. Recently, with an increasing emphasis and recommendation of FDA [2], the use of the Franz diffusion cell system for liberation of topical preparations is recommended. The studies should provide the multipoint-profile testing (optimally 20–30 measurements per hour), which is difficult to achieve with HPLC methods (usually with relatively less sensitive UV detection). HPLC is also time-consuming, with a low sampling frequency and is relatively expensive.

The Franz diffusion cell system is a well established system for the determination of *in vitro* release of topical dosage forms. Normally, the cell is fitted with a synthetic membrane. The test product is placed on the upper side of the membrane in the open donor chamber of the diffusion cell and a sampling liquid is placed on the other side of the membrane in a receptor cell. Diffusion of drug from the topical product to and across the membrane is monitored by assay of the sequentially collected samples of the receptor fluid. Sample collection can be automated, but usually manual collection of samples followed by HPLC analysis is provided. A plot of amount of drug released per unit area against the square root of time yields a straight line, the slope of which represents the release rate. This release rate measure is formulation-specific and can be used to monitor product quality.

Many different methods have been described for the determination of salicylic acid in pharmaceuticals with the flow-injection technique [3–5]. There is only one flow method that uses fluorescence detection for the determination of salicylic acid, based on the formation of ion-pairs [6]. No other method that uses native fluorescence of salicylic acid in the neutral medium has yet been described.

The interfacing of a computer-aided flow injection analyser (FIA) with a fluorescence detector and Franz diffusion cell leads to a fully automated monitoring system capable of providing real time analysis and a multipoint liberation profile. An automated flow-injection apparatus, controlled by home-made software FIA-MOD 2.2 [7], was used for the optimisation of the analytical method and of the liberation test conditions. The programme enables to display real-time signal peaks (FI-gram). The analytical signal (as both peak height and peak area) is automatically calculated, displayed and stored.

The aim of this work was to develop a rapid, sensitive and fully automated method for the determination of salicylic acid based on the fact that the compound exhibits an intensive fluorescence in neutral pH. After optimising the FI system, the method has been used for determination of liberation rate of the salicylic acid from an ointment by using Franz diffusion cell.

2. Investigations, results and discussion

2.1. Optimisation of the FI system

The similar manifold was used to investigate optimisation of variables, for the analytical application and release tests. After optimisation of excitation wavelength, the flow-injection variables (volume of injected sample, flow rate, length of the coils) were also optimised (changing one variable in every turn and keeping the others at their optimum values). All experiments were performed at constant laboratory temperature. The effect of the various experimental variables was studied with respect to the sensitivity, precision and sampling rate. A fixed sample loop of 75 μl was finally used for the routine measurements. To maintain sink conditions in the basolateral receptor phase, Soerensen phosphate buffer solution pH 7.4 was used. Additionally, phosphate buffer solution of pH 7.4 was found best in maintaining the baseline stability and the reaction sensitivity. Flow rate was set at $1.0\ ml \cdot min^{-1}$. The optimal excitation wavelength was found to be 292 nm (maximum), with an emission filter >370 nm.

2.2. Analytical application

Using the optimal experimental parameters, a calibration curve was constructed for the determination of salicylic acid. The peak height, calculated and stored automatically by the computer was chosen for the calculation of analytical parameters. The calibration curve was linear over the range 0.01–0.4 $\mu g \cdot ml^{-1}$ ($n = 8$) with a correlation coefficient of 0.9996 and a relative standard deviation (RSD) of 0.68% ($n = 3$; $c = 0.1\ \mu g \cdot ml^{-1}$). The linear regression equation was: intensity of fluorescence $I_F = 2106.7.\ c + 1.593$. The detection limit ($3 \times SD$ of the most dilute standard) was $0.002\ \mu g \cdot ml^{-1}$, while the quantification limit ($10 \times SD$) was $0.005\ \mu g \cdot ml^{-1}$. The typical measurement throughput was about $60\ h^{-1}$.

2.3. Determination of liberation profile

The Franz diffusion cell (Fig. 1) was filled with 15.00 ml of the acceptor medium (phosphate buffer pH 7.4) and the temperature of the water bath was set up on 32 °C. After

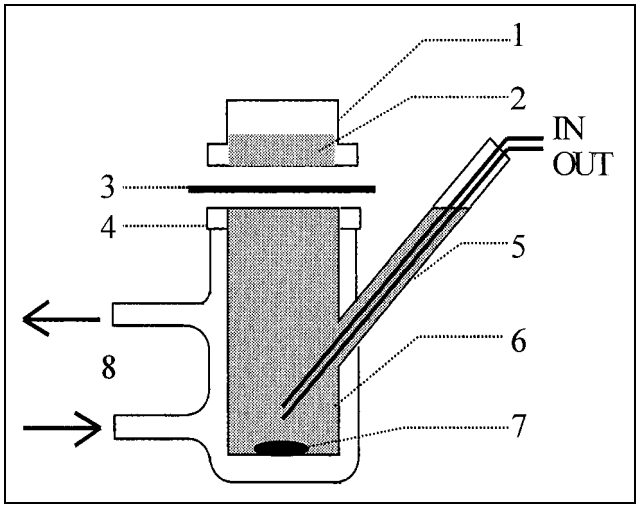

Fig. 1: Schematic diagram of the Franz cell
1 – cap – 20 mm I.D.; 2 – ointment tested – 0,9% salicyclic acid; 3 – membrane – neutral cellulose; 4 – glass cell – double-walled; 5 – side-arm – sampling – connected to FIA; 6 – inner part of cell – volume 15.0 ml; 7 – stirring bar – 400 rpm; 8 – outer part of cell – thermostated for 32 °C

15 min, about 200 mg of the ointment was placed onto the membrane. For liberation testing, ointment containing 0.9% salicylic acid in wool alcohols ointment according to DAB 2000 [8] was used. Standard solution was injected before and after the measurement.

A series of six repeated tests was measured. The release rate is represented by a slope, given by the plot of amount of drug released per unit area against the square root of time. As expected, a straight line is formed Fig. 2. The recommended time for the analysis of the liberation profile is 6 h, with sampling done at 0.5, 1, 2, 4 and 6 h after beginning the procedure. We have found that with more frequent sampling (which can be done by using an automated FI system) a straight line can be constructed (release rate) after only about 3 h, instead of the usual 6 h. It can be concluded, that by a FI procedure with the possibility of analysing many samples per hour, the release rate can be calculated in about half the time of usual measurements. This is one of the major advantages of the described method.

The use of a fluorimetric detector increases sensitivity in comparison with the conventional UV detection normally used in HPLC. Moreover, the flow-injection system can be fully automated and is also very flexible and versatile. The implementation of another channel to the FIA system can accommodate more complicated chemistry used for the detection of the analysed compound. This example shows the attractivity and potentiality of FIA for automated monitoring of liberation of active drug substances from topical applied dermatological formulations.

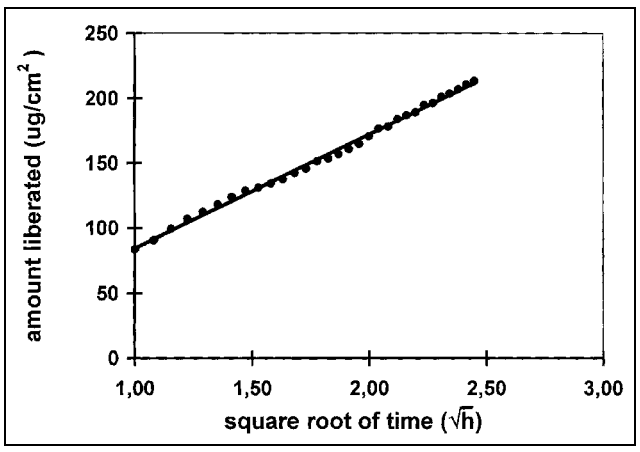

Fig. 2: Linearized liberation profile of formulation containing salicylic acid – equation $F = 87{,}44.\ \sqrt{t} + 2{,}839$ – correlation coefficient $R = 0{,}9992$

3. Experimental

3.1. Chemicals

Salicylic acid (precipitated) was obtained from Merck (Darmstadt, Germany). A stock solution of salicylic acid (100 $\mu g \cdot ml^{-1}$) was prepared by dissolving the appropriate amount of the drug in phosphate buffer by sonication and was stored in amber bottles. The solutions remained stable for at least 1 week at 4 °C. More dilute working solutions were prepared daily by the appropriate dilution of stock solutions with the same solvent.

The phosphate buffer was prepared by weighing 3.67 g of sodium hydrogen phosphate and 1.00 g of potassium dihydrogen phosphate and diluted to the volume of 500 ml in water. The solution was adjusted, if necessary to pH 7.4 by phosphoric acid. The phosphate buffer solution pH 7.4 was found to be an optimal carrier solution.

All solutions were filtered through a membrane filter (Millipore, Austria – pore size 0.85 μm) and degassed under reduced pressure prior to use. All reagents used were of analytical-reagent grade and distilled water was used throughout.

3.2. Flow-injection system

The two-channel FI manifold developed and optimised for the determination of salicylic acid is shown in Fig. 3. It consists of a peristaltic pump (Gilson-Minipuls 3, type M3, Gilson Medical Electronics, USA), a sample-loop injector (4-way) controlled by an electric actuator (Model 99T, Watrex, Prague, Czech Republic) and a built-in Rheodyne valve (Model 5020, Cotati, CA, USA); a fluorimeter (Spectra-Physics, type FS 970, USA) equipped with a flow cell (void volume of 8 μl) and a cut-off emission filter; an IBM compatible personal computer (486-DX66, 8 MB RAM) to which the analogue output of the fluorimeter was fed using a home-made interface circuit based on a 10-bit analogue-to-digital converter (ADC 1005 CCJ, National Semiconductor, USA). The pump and the injection valve were controlled by the computer with a home-made software FIA-MOD 2.2., which enables the control of the multichannel peristaltic pump, injection valve status and signal processing from the detector. A detailed description of a previous version of this program has already been presented [7]. All connections and mixing coil were made from 0.5 mm i.d. PTFE tubing.

3.3. Franz diffusion cell

The Franz diffusion cell consisted of two glass parts. An upper open cap, ground glass surface with 15 mm diameter orifice and total diameter of 25 mm, in which topical preparation of ointment was layed out onto the membrane. The membrane used was high permeability cellulose membrane, m.w.c. 10 000 (Dianorm No. 10.16, Muenchen, Germany), and was placed in an acceptor medium 2 h before use. The lower double-walled

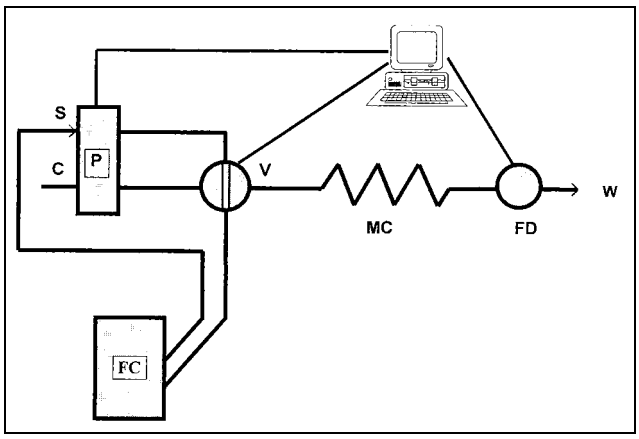

Fig. 3: Flow injection manifold for the liberation studies
P – pump 1.0 ml · min; S – sample liberated 0.9% salicylic acid; V – valve 75 μl; C – carrier phosphate buffer pH 7.4; MC-mixing coil 0.5 m; FD – detector $\lambda_{ex} = 292$ nm; $\lambda_{em} \geq 370$; W – waste; FC – Franz cell 32 °C, 400 rpm, volume 15.0 ml

glass vessels contained the receptor medium thermostated to 32 °C, stirred with a small magnetic stirrer at constant speed 400 rpm. Receptor medium was phosphate buffer, pH 7.4, of volume 15.00 ml. The vessel contained a sampling side arm, from which the solution was continuously pumped through an injection valve and after a suitable period an aliquot of 75 µl was injected and transferred to the detection system. Multiple replicates (six samples are recommended) were analysed for 6 h taking the samples every 10 min. If necessary, samples can be taken every 1–2 min.

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