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Automated system for release studies of salicylic acid based on a SIA method

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Abstract

The aim of this work was to describe a fully automated system for the in vitro release testing of semisolid dosage forms based on SIA technique. The system was tested for monitoring release profiles of different ointments containing 3% of salicylic acid (Belosalic, Diprosalic, Triamcinolone S). The native fluorescence of salicylic acid was used for fluorimetric detection. Phosphate buffer pH 7.4 was the receptor medium; samples were taken at 10 min intervals during 6 h of the release test; and each test was followed by calibration with five standard solutions. The linear calibration range was 0.05–10 μg ml^{-1} (r = 0.9996, six standards); the maximal SIA sample throughput for this system was 120 h^{-1}, sample volume being 50 μl and flow rate 50 μl s^{-1}. The detection limit for salicylic acid was 0.01 μg ml^{-1}.
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1. Introduction

The quality control of pharmaceutical preparations utilizes special validated methods defined by national or international rules (pharmacopoeias, statements etc.). Topical semisolid dosage forms like gels, ointments and creams are frequently used pharmaceutical preparations which are defined by tests that include identification, assay, homogeneity and in some cases viscosity, specific gravity and particle size determination. More complex information about semisolid dosage forms properties, especially to control the product after certain changes in manufacturing process or substitution of excipients is provided by a release test of the active substance (active moiety, drug). The main aim of release tests is to find the rate of release through a membrane in a specified time, a similar procedure to the well-known dissolutions test used for the solid dosage forms. Although official methods have been developed for dissolution test studies of solid dosage forms, which serve as a routine indicator of batch-to-batch uniformity or another method in the USP 25 to study active substance release from transdermal delivery patch systems, no official rule for the performance of release testing of semisolid dosage forms is given. The only existing recommendations are guidelines provided from OECD [1] and FDA [2].

Manually provided tests, where samples are taken from release medium by pipette, are time and labor consuming, and usually are followed by HPLC analysis, which in case of only one compound being present, is mostly needless. The aim of this work is to develop a simple but fully automated system for in vitro release studies based on connection of sequential injection (SIA) system and Franz diffusion cell. Sequential injection analysis is a flow method introduced by Ruzicka and Marshall in 1990 [3], derived from flow injection analysis. This system is especially suitable for long-time monitoring or fast data acquisition. The SIA has been used with dissolution tests of various pharmaceutical formulations e.g. ibuprofen

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tablets [4], analgetic tablets with aspirin, phenacetin and caffeine [5], and tablets with ergotamine tartrate [6]. Firstly, the SIA system described for monitoring of release profile was suggested by our group for tests of indomethacin [7].

The Franz diffusion cell is a device recommended both by FDA and OECD. Usually six cells are recommended, but systems with three or only one cell are mentioned as well. The Franz diffusion cell (Fig. 2) consists of two parts—donor part and acceptor part—that are separated by a membrane. The donor compartment holds the drug preparation and the acceptor compartment the receiving medium. For release experiments, normally artificial membranes are used to separate the donor and receptor compartment physically. However, the membrane should allow the active ingredient readily to diffuse receiving the medium as it is "released" from the dosage form and not be rate limiting for the diffusion. In contrast, if skin is used instead of an artificial membrane, the skin acts as a barrier and the in-vitro invasion across the skin is determined. These experiments are often called skin permeation and might be useful as a surrogate in bioavailability testing. Artificial membrane materials used are e.g. polysulphone, esters of cellulose, teflon, polycarbonate, polyvinylidene fluoride, silicone etc. [7,8].

It is desirable to have a receiving medium that is similar to the physiological condition of the skin, but the most important factor for the selection is the solubility of the active ingredient in the medium. The solubility of drug should be at least 10-times more than the highest acceptor fluid concentration achievable during the release test to guarantee that back diffusion is not significant [1]. It is imperative that the solubility in the receptor fluid is not a release rate-determining step. The receptor fluids for water-soluble compounds are usually saline solution of pH 7.4. For lipophilic substances the receptor fluid may contain beside water also organic solvents such as ethanol, polyethylene glycol (PEG) 20 oleyl ether, octoxynol-9, Poloxamer 188, PEG 400, bovine serum albumin (3% in buffer), methanol or isopropyl myristate [1,9]. The acceptor medium should be mixed to ensure the uniformity of diffusion and the concentration homogeneity. Diffusion is temperature dependent, thus the receptor fluid must be maintained at a constant temperature. For release experiments of semisolid topically applied preparations $32 \pm 1\,^{\circ}C$, which is the temperature at the skin surface, is recommended. This is secured either by water jacket or water bath. While carrying out the experiment, samples of receptor medium are drawn off through the sampling port of the Franz cell.

Salicylic acid is an *o*-hydroxy benzoic acid. It belongs to group of non-steroidal anti-inflammatory drugs. It has antipyretic, antiflogistic and analgesic effects. In higher concentrations it is used as external keratolytic therapeutic agent. For instance, it can be used to remove warts, hard corns and calluses. It also facilitates the permeation of another active substance through the skin. There are many methods for salicylic acid determination. The most common is HPLC with UV detection [10,11], electrochemical detection [12] or mass spectrometry [13]. Other techniques that can be applied for salicylic acid determination are gas chromatography–mass spectrometry [14], planar chromatography [15], spectrophotometric determination [16], surface-enhanced Raman scattering [17], fluorescence spectroscopic batch procedure [18], voltametric determination [19], flow injection analysis determination with electrochemical detection [20], flow injection with AAS [21], or sequential injection analysis coupled with monolithic chromatography column [22]. Determination of salicylic acid using fluorescence detection is based on the native fluorescence of salicylic acid. The maximum excitation wavelength is 297 nm and emission is 405 nm [23].

2. Materials and methods

2.1. Materials

The products studied were: Belosalic dermal ointment 30 g (Belupo Pharmaceuticals & Cosmetics Ltd. Koprivnica, Croatia), containing 3% salicylic acid and 0.05% betamethasone in white petrolatum with liquid paraffin; Diprosalic dermal ointment 15 g (Schering-Plough Labo N.V., Heist-opden-Berg, Belgium), containing 3% salicylic acid and 0.05% betamethasone in white petrolatum with liquid paraffin and Triamcinolon S Léčiva dermal ointment (Léčiva a.s., Prague, Czech Republic) containing 3% salicylic acid and 0.1% triamcinolone acetonide in white petrolatum, ceresin, wool fat and poloxamer.

The standards of salicylic acid were obtained from Bochemie Group, Herbacos Bofarma Ltd., Czech Republic. All solutions were prepared from a Millipore Mili-Q RG ultra pure water that served also as carrier stream. A stock solution of salicylic acid (100 $\mu g\,ml^{-1}$) was prepared by dissolving the appropriate amount of the drug in phosphate buffer pH 7.4 by sonication and was stored in amber bottles. The solution was stable at least one week at $4\,^{\circ}C$. More dilute working solutions were prepared daily by appropriate dilution of stock solution with phosphate buffer.

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 to pH 7.4 by phosphoric acid. It was degassed by means of helium for 10 min.

Membranes tested were all bought from Millipore: polycarbonate HTTP, pore size 0.45 μm; poly-carbonate GTTP, pore size 0.2 μm and mixed cellulose esters HAWP, pore size 0.45 μm. In preliminary experiments the GTTP membrane was found to be an optimal for the release test of salicylic acid. No inhibition in drug release as well as a proper separation of the donor and receptor phase was found with this type of membrane.

2.2. Apparatus

The SIA system for release tests is depicted in Fig. 1. It was built of the FIAlab 3500 (FIAlab instruments, USA)

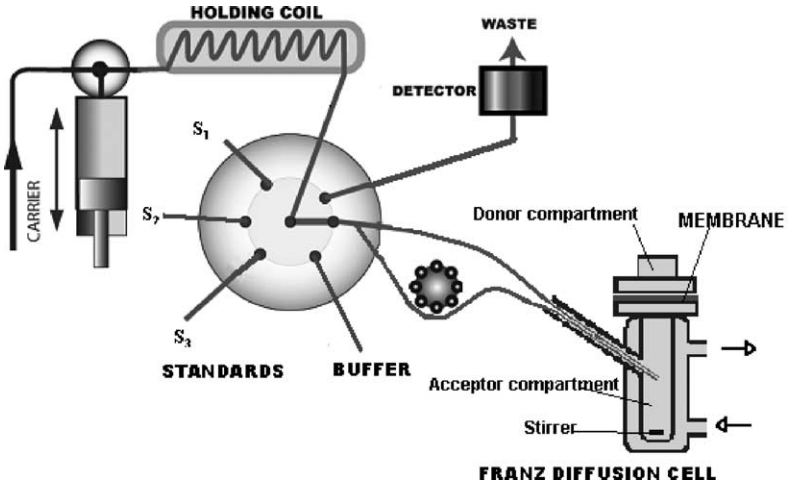

Fig. 1. Schematic view of the SIA set-up for release test.

commercial system comprising of a 2.5 ml piston pump, peristaltic pump and eight-port selection valve. Fluorimetric FIAlab detector PMT-FL with UV light source D-1000-CE was connected as a detection unit. The fluorescence signal was selected by an emission filter of 385 nm, Edmund Industrial Optics and an integration time of 200 ms was selected. The whole tubing of the SIA system was made of PTFE, i.d. 0.75 and 0.51 mm. The volume of tubing between the sampling port and the confluence connected to the two tubes linked to the acceptor compartment is approximately 5.9 μl (length 30 mm, i.d. 0.5 mm). Thus, it does not significantly influence the concentration of taken sample.

The release unit used was a Franz cell, a double-wall (thermostated) vessel with a precise inert volume of 15.00 ml, height 6.0 cm, width 3.6 cm, inner diameter 2.0 cm (acceptor compartment), cap height 2.0 cm, cap width 3.6 cm (donor compartment). The acceptor compartment of the Franz cell was filled up to the label on the side inlet. The side inlet was used for insertion of loading and filling tube (both parts of loading circuit). The solution inside was mixed (400 rpm) by an electromagnetic stirrer (IKA Labortechnik, Stauffen, Germany).

2.3. Procedure

The standards were measured by simple way of aspiration of portion of sample and its propulsion through the detection flow cell. The peak height was proportional to the sample concentration, calculated automatically by the commercially available software FIAlab.

The release tests were started with pre-treatment of the membrane by soaking for one hour in the receiving medium under constant mixing (400 rpm) and temperature setting (32 °C) as recommended by investigators [24,25]. An uniform layer of the formulation of a thickness about 1 mm was deposited on the top of the membrane (diffusion layer being about 0.1 mm) corresponding to an infinite dosing. The precise amount of applied drug is not known, but for kinetic reasons it is essential to have a reservoir of the drug always available to diffuse through.

The measurement cycle (see Table 1) consisted of following:

First sample is detected in “zero” time. The sample is aspirated from sampling circuit and subsequently propelled to the detector.

The peristaltic pump is switched on and maintained at a constant flow of receiving medium for 9 min. The pump is then switched off, 50 μl of the second sample is aspirated and detected. The acceptor compartment side of the cell is filled up by 50 μl of the release medium and the peristaltic pump is switched on. The cycle is repeated for 36-times.

At the end of the measurement, the selection valve ports are washed with calibration solutions and a calibration curve is generated.

Samples from the Franz cell were taken at 10 min intervals during 6 h of the release tests and each test was followed by calibration with five standard solutions prepared in the same medium as release. The sampling circuit with a constant flow ensured that the concentration sampled and measured is the same at the selected time as the concentration in the Franz cell. After sample aspiration, the volume of release medium was filled up by phosphate buffer. A correction to the salicylic acid concentration was taken into account by recalculation.

3. Result and discussion

3.1. Optimization and calibration

Native fluorescence of salicylic acid in the neutral medium (phosphate buffer pH 7.4) was sufficiently strong for its determination.

The sample volume was set to 50 μl, which ensured good reproducibility of sampling and did not cause big changes in

Table 1
Procedure of measuring cycle

Software report	Comments
Syringe pump valve in Syringe pump flow rate (50 μL/s) Syringe pump aspirate (1500 μL) Syringe pump delay until done Syringe pump valve out	The sample measuring in "zero" time
Valve port 7 Syringe pump delay until done Syringe pump aspirate (50 μL) Syringe pump delay until done	
Valve port 8 Syringe pump delay until done Syringe pump empty PMT start scans Syringe pump delay until done PMT stop scans	
Loop start (#) 36 Peristaltic pump clockwise (%) Peristaltic pump on Syringe pump valve in Syringe pump aspirate (1500 μL) Syringe pump delay until done Syringe pump valve out Delay (530 sec) Peristaltic pump off Syringe pump delay until done	The period of free release of drug substance (10 min)
Valve port 7 Analyte new sample Analyte name unknown Syringe pump delay until done Syringe pump aspirate (50 μL) Syringe pump delay until done	Aspiration of sample
Valve port 8 Syringe pump delay until done Syringe pump empty PMT start scans Syringe pump delay until done PMT stop scans	Measuring of sample concentration
Syringe pump valve out Valve port 6 Syringe pump delay until done Syringe pump aspirate (50 μL) Syringe pump delay until done	Steps for replenishing the acceptor compartment with the buffer
Valve port 7 Syringe pump delay until done Syringe pump empty Syringe pump delay until done	
Loop end	Afterwards the concentrations of standards for the calibration are measured

the release medium volume. The flow rate was tested in the range of 20–75 μl s^{-1}. The 50 μl s^{-1} represented the compromise between reproducibility and magnitude of the fluorescence signal.

Calibration was carried out with six solutions of following concentrations 0.05, 0.1, 0.5, 1.0, 4.0 and 10.0 μg ml^{-1}. Each standard solution was measured in triplicate. The regression equation was $y = 63503\ c + 58643$, when y is intensity of fluorescence and c is standard concentration, with a correlation coefficient 0.9996. The R.S.D. was 0.52% ($n = 10$) at concentration 1.0 μg ml^{-1}. The linear range of the calibration was 0.05–10 μg ml^{-1}. The detection limit for salicylic acid was 0.01 μg ml^{-1} (3σ) and limit of quantification was 0.03 μg ml^{-1} (10σ).

3.2. Release test

The samples taken from release medium and replenishing of medium by buffer causes decrease of the salicylic acid concentration. This is the reason for recalculation of the measured concentration to the real values of salicylic acid.

$$C_{n,\,\text{corrected}} = C_{n,\,\text{measured}} + \frac{\text{volume}_{\text{sample}}}{\text{volume}_{\text{acceptor, FDC}}} \times \sum C_{n-1,\,\text{measured}}$$

where C_n: concentratation of n-samples and FDC: Franz diffusion cell

The cumulative drug amount (Q_n) permeated at each time point related to the area of tested membrane is obtained as follows:

$$Q_n = C_{n,\,\text{corrected}} \times \frac{\text{volume}_{\text{acceptor, FDC}}}{\text{diffusion area}}$$

The in vitro drug release studies were evaluated for 6 h, with sampling each 10 min. Data were linearized using the square root of time transformation and linear plots were obtained by plotting the cumulative amounts released (μg) per square root of time (min^{-1}). (The coefficient of determination was in all cases >0.95) This was in accordance with Higuchi's model. Higuchi proposed two models, depending on if the drug is completely dissolved (I) or is suspenended (II) in the base [26].

$$\text{Model I}: Q = A \times 2c_0\sqrt{\frac{Dt}{\pi}}$$

$$\text{Model II}: Q = A\sqrt{[Dtc_s(2c_0 - c_s)]}$$

where Q = amount released at time t; A = diffusion area (cm^2); D = diffusion coefficient (cm h^{-1}); t = time (h); c_s = saturation concentration; c_0 = concentration in the semisolid preparation at $t = 0$.

Irrespective of whether the drug is suspended or solubilized, the release should be linear with the square root of time, if the release is governed via a matrix controlled diffusion process.

The slope of the regression line represents the release rate of the product. This release rate is formulation-specific and can be used to monitor product quality. An x-intercept, typically corresponding to a small fraction of an hour, is normal

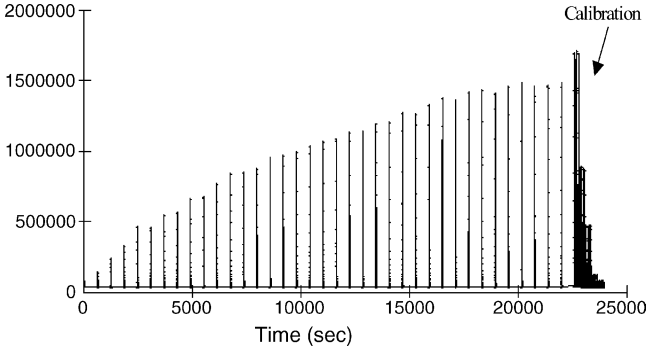

Fig. 2. Typical SIA record of release of salicylic acid with calibration (intensity of fluorescence).

characteristic of such plots and is called "lag time". The lag time corresponds to the time of delay during the first contact of the drug with the membrane surface [27] until steady state flux conditions are reached.

The drug release studies were carried out using poly-carbonate membrane with pore size 0.2 μm. This membrane was found to be optimal because of high release rate (90 $\mu g\,cm^{-2}\,h^{-1}$; poly-carbonate membrane with pore size 0.45 μm had 87 $\mu g\,cm^{-2}\,h^{-1}$ and membrane of nitro cellulose had 50 $\mu g\,cm^{-2}\,h^{-1}$), good handling characteristics and proper separation of the donor and receptor phase.

Each formulation was measured six-times for 6 h. The two evaluated preparations contained two principal substances–betamethasone and salicylic acid. The measurement of release profile of betamethasone was not provided because of its low concentration (0.05%) and none fluorescence activity. Furthermore, no influence of these substances on the fluorescence of salicylic acid could be detected.

The amount of sample taken is small and the receiving medium is immediately compensated, thus, the sampling frequency could be expanded without spoiling of the release process. Due to the high frequency of sample taking the release graph can be constructed after 3 h instead of recommended 6 h.

An example of a release profile is shown in Fig. 2. The linearized graph of six in vitro release rate measurement of Diprosalic ointment is depicted in the Fig. 3. Mean values of

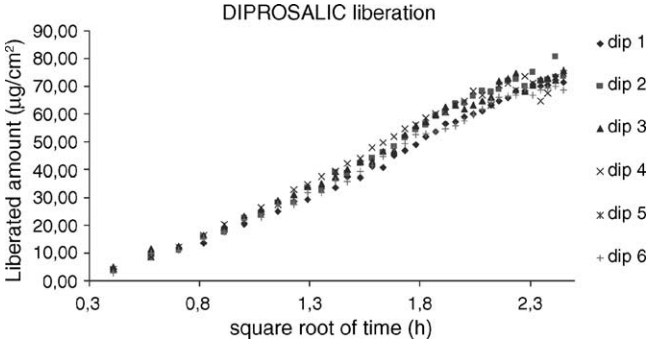

Fig. 3. Linearized release profiles of Diprosalic ointment formulation (six measurements).

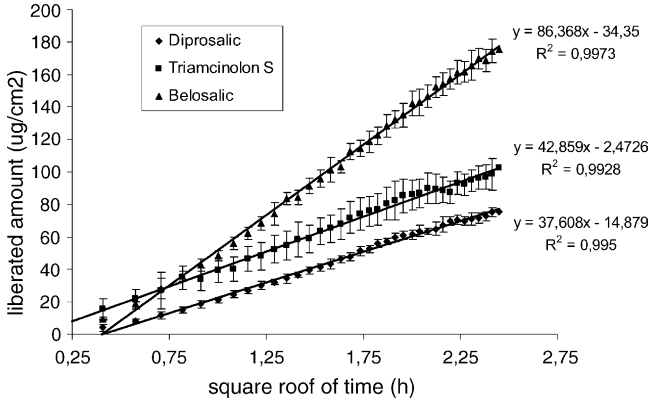

Fig. 4. Comparison of medians of release profiles of three commercial ointments (Belosalic, Diprosalic and Triamcinolone S).

release profiles of Belosalic, Diprosalic and Triamcinolone S formulations are plotted in the graph shown in Fig. 4. Mean values of release rates of these pharmaceutical preparations are adjusted in Table 2.

The statistical test used for in vitro release comparison is a non-parametric statistical method, based on a standard confidence interval (CI) procedure. This test is related to the Wilcoxon rank sum/Mann–Whitney rank test, applied to the log slopes (release rate) [2].

The in vitro release test for two preparations (A and B) is carried out and the six slopes for each paralleled preparation are calculated. A 90% CI for the ratio of the median in vitro release rate (in population) for A over the median in vitro release rate (in population) for B is computed and expressed in percentage terms. For computation of the CI form the 36 (= 6·6) individual A/B slopes ratios and order these 36 individual AB ratios from lowest to highest. In other words, arrange all possible 36 A/B ratios from lowest to highest. The 8th and 29th ordered individual ratios are the lower and upper limits, respectively, of the 90% CI for the ratio of median in vitro release rate (slopes) for A over the median in vitro release rate for B. The percentage is calculated by multiplying the ratio by 100. To accept that the release rate of two preparations is within 90% CI the values for A/B ratios should fall within the limits of 75–133.33% [2].

In our cases, the ratios 44.7–50.3% for Belosalic/Diprosalic, 51.7–56.4% for Belosalic/Triamcinolone S and 82.6–92.2% for Triamcinolone S/Diprosalic were obtained (Table 3). All preparations have hydrophobic ointment bases, but only Triamcinolone S and Diprosalic fall into this interval. The differences in the amounts released may be attributed either to the differences in the formulation and/or differences in the method of manufacture.

Table 2
Mean release rates of Belosalic, Diprosalic and Triamcinolone S ointments

Name of preparation	Median of release rates ($\mu g/cm^2/h^{1/2}$) + R.S.D. (%)
Belosalic	86.37 ± 4.17
Diprosalic	37.61 ± 3.28
Triamcinolone S	42.86 ± 3.73

Table 3
Comparison of release rates by Wilcoxon rank sum/Mann–Whitney rank test

Compared pharmaceutical preparations	Ratios of in vitro release rate in percentage
Belosalic/Diprosalic	44.7–50.3
Belosalic/Triamcinolone S	51.7–56.4
Triamcinolone S/Diprosalic	82.6–92.2

4. Conclusion

The main objective of this study was to develop an in vitro fully automated quality control procedure, analogous to the dissolution test for oral solid dosage form, and evaluate the drug release from topical semisolid formulations using Franz diffusion cell.

The fully automated system for in vitro release rate testing of salicylic acid in semisolid dosage forms based on SIA technique is presented. Release profiles of three commercial preparations were obtained and their release profiles statistically compared.

The SIA system with Franz diffusion cell is a power full tool for the automation of release studies. Automatic sampling and sample on-line analysis is advantageous because no human control is required during the experiments. Analysis is fast and economic. The analysis time could be reduced to a maximum of 3 h by using a high sampling frequency.

The flexibility of SIA computer-controlled system allows in the one-day (programming included) preparation of completely different measurements, different compounds analyses or determinations for various scientific purposes.

With the possibility of coupling six Franz cells to the system, it will be an ideal tool for on-line monitoring of release tests. Such a system could be favorably used for manufacturing process control, for monitoring of pre- and post- changes of product properties, batch uniformity monitoring, etc. SIA is a relatively simple and adapted method and could be also used as a screening device in pre-formulation and product development.

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