Introduction

The use of inkjet printing (IJP) technology is gaining considerable interest for additive manufacturing of pharmaceutical drug delivery systems (1, 2). Printing offers multiple advantages for manufacturing of drug products such as precise dosing, production of multi-dose regimens and ultimately personalized medicine, optimized for the treatment of the specific patient (3). When conducting IJP of pharmaceuticals, the active pharmaceutical ingredient (API) is either dissolved or suspended in an ink base and then precisely deposited on the substrate, i.e., a carrier, from a printer's nozzle in a digitally controlled way (4).In spite of the evident benefits of IJP, production challenges exist in regard to using inkjet printing for manufacturing of personalized medicine. Inkjet printing of pharmaceuticals is currently a low-output method, meaning that the number of dosage forms produced in IJP is limited. While it is easy to conduct API content analysis using well-established methods such as UV spectroscopy and HPLC analysis, these methods require the use of destructive sample preparation that is costly. Therefore, the API content analysis of the printed dosage forms should ideally be conducted using non-destructive, robust and fast methods (5). This is especially important in the future to realize on-demand and near the end-users pharmacoprinting of personalized medicine (6) or to integrate it in a continuous manufacturing setup (7, 8) according to the emerging regulatory framework (9). Recently, multiple methods have been described for non-destructive API content analysis in printed dosage forms. Vakili et al. used near-infrared chemical imaging for content analysis of IJP theophylline using copy paper as the model substrate (10). Researchers from the same group have used a colorimetric technique for content determination of IJP propranolol with colorant added, using edible rice paper and edible icing sheets as the substrates (11) and was also used for quantitative determination of IJP vitamin B2 doses on edible rice paper and copy paper (12). More recently, a handheld near-infrared spectrometer has been reported for API quantification on the IJP dosage forms, containing levothyroxine sodium and prednisolone. Edible icing sheets and solvent-casted films, containing cellulose derivatives, were used as the substrates (13). ATR-FTIR was used for quantifying loperamide and caffeine printed on poly-tetrafluoroethylene films (14). Recently, our group used Raman spectroscopy for the quantification of IJP haloperidol on inorganic compacts and commercial paracetamol tablets (15). Raman spectroscopy has also been used for the assessment of polymorphism of prednisolone IJP onto polytetrafluoroethylene (PTFE) fiber glass films (16). In addition, confocal Raman mapping was conducted to describe drug distribution in the multi-layered films with three jet-dispensed model drugs (17). While the analytical techniques mentioned here have the potential to be used in a manufacturing-on-demand setting (such as in a community pharmacy), they also have drawbacks. For example, the colorimetric technique determines the content of the printed API indirectly by quantifying the coloring agent, added to the ink along the API.

This could potentially lead to errors in estimating the actual API content due to, for example, degradation of the API, discoloration and/or migration of colorants. Measurements with spectroscopic techniques, such as near-infrared (NIR) and Raman spectroscopy, put certain demands on the morphology of the substrates, for instance, near-infrared chemical imaging requires the substrate to be completely flat in order to achieve accurate focusing of the diffusely reflected light (18). The handheld NIR method can in turn be prone to localized variation of the obtained spectra within the printed dosage form, e.g., if the substrate is porous and there is a variation in the absorption of the ink, or if the API crystallizes on the surface of the substrate, and therefore, different regions of the same printed area may give different results leading to errors in the correct determination of the drug content in the dosage form. Raman spectroscopy is also prone to variations in the material density of the substrate, e.g., when a highly porous vacuum-oven dried hypromellose was used as substrate, it was unsuitable for quantifying the drug content using spectroscopic methods (15). Besides that, measurements with NIR would be affected by residual solvents, in particular moisture from vapor sorption. It is evident from these examples that there is a need for gaining a better understanding about the analytical technique(s) in terms of its sensitivity and suitability to determine the drug content in a porous substrate.

In this study we describe inkjet printing of three APIs on novel porous substrates prepared from hypromellose and the investigation of two spectroscopic techniques for the non-destructive and accurate determination of the inkjet printed drugs. Three model APIs, namely montelukast, haloperidol and propranolol in various doses were printed on the custom-developed substrates. The printed doses were analyzed using NIR spectroscopy and Raman spectroscopy and the resulting spectra were correlated to the API content measured by HPLC using Partial Least Squares (PLS) regression. The resulting models were compared in regards to their accuracy and prediction power.

Materials and Methods

Three model active pharmaceutical ingredients (APIs) were used in this study: haloperidol and propranolol hydrochloride were purchased from Sigma Aldrich (St Louis, MI, USA), and montelukast sodium was obtained from Matrix Laboratories (Hyderabad, India). Propylene glycol (PG) and lactic acid (LA) were obtained from Sigma Aldrich, ethanol (96 %) was supplied by Altia OY (Helsinki, Finland). Hypromellose (hydroxypropyl methylcellulose), HPMC, Metolose® 60SH-4000, was purchased from Shin-Etsu Chemical Co. (Tokyo, Japan), macrogol 4000, polyethylene glycol 4000, PEG4000, and polysorbate 20, Tween® 20, were purchased from Fluka Analytical (Seelze, Germany). Erythrosine (E127) was supplied by Merck

(Darmstadt, Germany), blue food coloring liquid, containing brilliant blue (E133), was supplied by Dr. Oetker (Bielefeld, Germany).

Preparation of substrates

A single substrate (sample name S3) was produced as described in a recent paper (19). It was prepared by mixing 5 g HPMC with 0.5 g macrogol 4000, glycerol and polysorbate 20 and 2.1 g poloxamer 188. This mixture was added to purified water at 70°C under stirring. The total mass of the mixture was 100 g. After cooling the mixture to room temperature, it was cast into several silicone molds (10×28 cm^2). Casting was done so that the approximate height of the liquid in the mold was 5 mm. The formulation in the casting molds was cooled to 5 ± 3 °C for 24h in order to allow complete hydration of the polymer. The samples were then freeze-dried using an Epsilon 1-4 LSCplus Pilot Scale Freeze Dryer (Martin Christ, Osterode am Harz, Germany). The samples were cooled to -30°C over 3 h, then held isothermally for 3 h after which the pressure was reduced and kept at 0.12 mbar while the temperature was increased to 0°C over 16.5 h during primary drying.

Ink formulations

Propranolol hydrochloride ink (50 mg/ml) was prepared by mixing PG:water in a ratio of 3:7. 10 drops of blue liquid fruit coloring were added through a cellulose pore filter (0.45 µm, Phenomenex, Torrance, CA, USA) to 100 ml of this mixture. 2.5 g propranolol hydrochloride was dissolved in this mixture in a 50.0 ml volumetric flask. The mixture was allowed to stand in an ultrasound bath for 30 min in order to ensure that API was dissolved.

Montelukast sodium ink (200 mg/ml) was prepared by mixing PG:ethanol in a 3:7 ratio in a 100 ml volumetric flask. Two grains (approx. 1 mg) of erythrosine were added to the mixture. 10.0 g montelukast sodium was added to the mixture in a 50.0 ml volumetric flask. The mixture was put in an ultrasound bath for 2 h in order to ensure that montelukast sodium is dissolved completely.

The haloperidol ink (160 mg/ml) was prepared by mixing LA:ethanol in a 16:84 ratio in a 50.0 ml volumetric flask. 1 drop of blue fruit coloring liquid and 1 grain of erythrosine was added resulting in slightly purple color. 4.0 g haloperidol was combined with the solvent mixture in a 25.0 ml volumetric flask. The mixture was put in the ultrasound bath for 30 min to dissolve haloperidol.

Inkjet printing

Inkjet printing was done on a PiXDRO LP50 piezoelectric inkjet printer (Roth & Rau, Eindhoven, Netherlands) mounted with a Spectra SL-128 AA print head with 128 nozzles (Fujifilm, Tokyo, Japan). The ink for printing was loaded into the ink container through a syringe equipped with a cellulose pore filter (0.45 µm, Phenomenex®). Propranolol ink was printed using a voltage of 90 V and a pulse ratio of 90 % with an ink pressure of -21.0 mbar. Montelukast ink was printed using a voltage of 120 V and a pulse ratio of 85% with an ink pressure of -22 mbar. The haloperidol ink was printed with a voltage of 120 V and a pulse ratio of 90 % with a pressure of -23.9 mbar. All the inks were printed in 1 × 1 cm^2 squares and 6-8 samples were printed for each dosing step. The number of layers printed and the dosage regimen for each API is described in Table I. Drop shape and size analysis on all the inks were done using Advanced Drop Calculation software (Meyer Burger Technologies, Eindhoven, Netherlands).

Table I. Overview of printed samples on the different substrates.

API	Number of layers printed	API content per step, mg	Dose regimen, mg (calculated)
Propranolol hydrochloride	5-35 in steps of 5	0.6	0.6; 1.2; 1.8; 2.4; 3.0; 3.5; 4.1
Montelukast sodium	5-30 in steps of 5	2.1	2.1; 4.2; 6.3; 8.4; 10.5; 12.6
Haloperidol	2-14 in steps of 2	0.6	0.6; 1.2; 1.8; 2.4; 3.0; 3.6; 4.2

Dynamic viscosity and surface tension

The viscosity of the API-containing inks was measured on an AR-G2 rheometer (TA Instruments, New Castle, DE, USA), equipped with a cone-plate geometry (Ø = 60 mm). The cone angle was 0.9811°. 1.05 ml of each ink solution was applied on the plate, thermostated to 25 °C, and subjected to a stationary shear stress ramp from 10 to 1000 s^{-1}. The average of three measurements of the viscosity at 10, 100 and 1000 s^{-1} was calculated and used to obtain the viscosity value of the ink by using Rheology Advantage software v5.7.2 (TA Instruments).

The surface tension of the inks was measured with a DSA100 Drop Shape Analyzer (KRÜSS GmbH, Hamburg, Germany). The data were analyzed using Drop Shape Analysis 1.90.0.22 software (KRÜSS).

Microscopy

Visible light microscopy was conducted on a DM LM microscopy (Leica Microsystems GmbH, Wetzlar, Germany) equipped with an Evolution MP Camera (Media Cybernetics, Rockville, MD, USA) controlled by Image-Pro Insight software v 8.0 (Media Cybernetics). The microscope was operated in both reflected-light mode and polarized-light mode using a 10X objective. The surface and cross-sections of the printed samples were imaged in reflected-light mode, taking multiple exposures, each focusing on a different part of the sample and the resulting images were stacked in Adobe Photoshop CC 2018 v 19.0.1 (Adobe Systems Inc, San Jose, CA, USA), using the Auto-Blend Layers Function.

High-performance liquid chromatography (HPLC)

API quantification was performed on an Infinity 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA) using a C18 column (5 µm, 150 mm × 4.6 mm). The HPLC system was controlled by Infinity 1260 software (Agilent Technologies). The mobile phase for analysis of propranolol consisted of 0.067 mM phosphate buffer (pH 3) and acetonitrile in a 60:40 ratio. For montelukast, the mobile phase was 1 mM acetate buffer (pH 5.9):acetonitrile in a 10:90 ratio . Haloperidol mobile phase consisted of 50 mM phosphate buffer (pH 2.5):acetonitrile in a 75:25 ratio. Standard curves were done for all APIs and linearity was observed (R^2 = 0.998-0.999).

Porosity and internal structure of the samples

The porosity of the substrates was assessed using a custom-developed oil absorption method (20) and X-ray computed microtomography (µCT). Samples of approximately 8 by 10 mm were cut out using a scalpel. The length, width and height of the samples were measured using a caliper (Mitutoyo Corporation, Kawasaki, Japan) and the mass was measured. The samples were then placed in paraffin oil (ρ = 0.862) in a desiccator at a pressure of 40 mPa for 24 hours. Then the samples were removed and excess oil was wiped off using a tissue. The samples were then weighed and the porosity, Φ, was calculated according to Eq. 1.

$$\Phi = \frac{(M_o - M_s)/\rho_o}{V_s} \quad \text{Eq. 1}$$

V_s is the volume of the sample, M_o is the sample weight after oil absorption, M_s is the sample weight before oil absorption, ρ_o is the density of the oil.

X-ray computed microtomography (µCT) analysis

Samples of approximately 5 by 5 mm were cut and analyzed using a SkyScan 1172 µCT scanner (Bruker Corporation, Antwerp, Belgium). The samples were imaged at an isotropic voxel resolution of *5* µm. the 3D imaging was done by rotating the object through 180° in steps of 0.4°, recording the projection images

using a cone beam configuration. 10 images were averaged for each position. A total of 1034 cross-section images per sample were generated with each sample requiring an acquisition time of about 1.2 h.

Spectroscopic analysis of samples

NIR spectroscopy of the surface of the dosage forms was conducted on a BOMEM MB-160 spectrometer (ABB, Zürich, Switzerland), controlled by Horizon MB software version 3.2.5.2 (ABB). 32 scans were obtained for each spectrum covering the range from 4000 to 12000 cm^{-1} and using a resolution of 8 cm^{-1}. A spectralon reference standard (LabSphere Inc, North Sutton, NH, USA) was used to obtain a reference measurement before analyzing the samples. The samples were placed with each printed square centered on top of the analysis window.

Near-infrared transmission (tNIR) spectroscopy was conducted on the BOMEM MB-160 equipped with a tablet sampler (Tablet Sampl*ir*, ABB). The sampler has 4 signal enhancement levels, depending on the opaqueness of the samples. The level was kept at 1 (lowest) throughout the measurements. 64 scans were obtained for each spectrum covering 5800 to 12000 cm^{-1} at a resolution of 8 cm^{-1}. A transmission spectralon was used to obtain the reference measurement. The spectralon was kept in place while measuring the samples in order to decrease signal intensity and avoid oversaturating the detector.

Raman spectroscopy in a backscattering setup was done on a Kaiser RXN1 Microprobe (Kaiser Optical Systems, Ann Arbor, MI, USA) with a PhaT-probe (Kaiser Optical Systems), controlled by HoloGRAMS software v 4.1. The laser wavelength used was 785 nm and the Raman shift from 150 to 1900 cm^{-1} was measured, each spectrum comprising 5801 data points. The laser spot size was 6 mm on the center of the printed samples and 6 exposures of 10 s each were averaged for each sample, giving a total exposure time of 60 s. A transmission setup was used as described earlier (21). The excitation fiber was placed directly beneath the sample and the Raman scattered light was collected by the PhaT-probe. The power of the laser was 200 mW at the output of the fiber. 6 exposures of 5 s each were averaged, giving a total exposure time of 30 s.

Multivariate data analysis

Spectral data (NIR and Raman) were analyzed using MatLab R2015a (Mathworks, Natick, MA, USA) and PLS Toolbox 8.0.1 (Eigenvector Research Inc, Manson, WA, USA). All the data were subjected to preprocessing in the form of Standard Normal Variate (SNV) transformation followed by Savitzky-Golay smoothing. Different window sizes were used, but all data were fitted to a 2^{nd} order polynomial of which the 2^{nd} derivative was taken. The regions in the spectra with the most contribution from the API were selected.

After preprocessing and spectral selection, the data were modeled using Partial Least Squares (PLS) regression. The samples were randomly split into a calibration and validation set, the calibration set containing 2/3 of the samples and the validation set 1/3. Cross-validation was done using venetian blinds with 6 splits and 1 sample per blind.

Results and Discussion

Ink formulation and substrate preparation

In this study, three different APIs, propranolol hydrochloride (propranolol), montelukast sodium (montelukast) and haloperidol were inkjet-printed on the porous sponge-like HPMC substrate. The substrate was developed as previously reported by our group to possess good absorption properties for the ink and good mechanical properties, i.e., to be flexible but at the same time, mechanically strong (22). The substrate was prepared from high-molecular weight hypromellose, containing various excipients: glycerol and macrogol 4000 were added as plasticizers, whereas polysorbate 20 was used as a surfactant, foaming agent and plasticizer. The resulting substrate was porous and the morphology of the surface pores reflected the shape of sublimated ice crystals. It is well-known that the shape and size of the formed ice crystals within the sample during freeze drying cycle would affect the microstructure and surface topography of the dried sample (23).

The formulation of haloperidol- (15) and propranolol-containing (22) inks were done according to previously published work. The montelukast ink was formulated based on the solubility of the API in ethanol, and then modified so that the rheological and surface tension characteristics of the ink would be suitable for printing. In order to maximize the possible printed dose, the printable solvent system with a high concentration of the drug in it was selected, i.e., 200 mg/ml of montelukast in 3:7 PG:Ethanol ratio. The viscosity and surface tension of the drug-containing inks were all within the printable range (Table II).

Table II. Surface tension and dynamic viscosity of the printed API-containing inks.

Ink	Surface Tension, mN/m	Viscosity, mPa·s
Propranolol	47.95 ± 0.12	3.11 ± 0.02
Montelukast	27.81 ± 0.15	10.95 ± 0.87
Haloperidol	28.34 ± 0.01	3.87 ± 0.05

The use of porous substrates made of water-soluble polymer (HPMC) enables printing of multiple layers of the ink on the dosage forms without the risk of smearing of the ink during subsequent handling and thereby uncontrolled loss of the API. Indeed, the ink could absorb into the pores of the substrate. The choice of HPMC as the polymer was based on it being water soluble, pharmaceutically approved and being compatible with and APIs and the most common ink solvents, used for dissolving/suspending the APIs. It is known that HPMC is slightly soluble by ethanol. However, it was expected that the ethanol-based ink, jetted in picoliters during IJP, would rather absorb into the porous substrate than dissolve it. In contrast, it was expected that printing a water-based ink would dissolve the surface of the substrate. The substrates could easily be handled after printing and there was visually observed no smearing of the ink for any of the APIs (Fig. 1).

The behavior of each ink in contact with the substrate was dependent on whether the ink was ethanol- or water-based (Fig. 2). The ethanol-based ink containing montelukast dissolved the surface of the substrate slightly when a high number of layers were printed, due to HPMC being soluble in ethanol to some extent (Fig. 2B). The haloperidol ink contained ethanol and also lactic acid, which has been shown to dissolve HPMC (24). However, the ink was only printed in a maximum of 14 layers which did not appear to change the surface significantly.A slight dissolution of the surface was observed when analyzed by microscopy (Fig 2C). The water-based ink containing propranolol dissolved the surface of the substrate (HPMC dissolves/hydrates in water,) and a continuous polymer film (skin) was formed on the surface of the substrate, which was particularly evident when a higher number of layers was printed. The porous nature of the substrates enabled them to quickly absorb the ethanol-based inks during printing without signification dissolution of the surface of the substrate, highlighting the suitability of HPMC-based porous substrates for IJP of ethanol-based inks.

Despite a slight surface dissolution of the substrate by montelukast inks with 30 layers printed (Fig 3B), the ethanol-based inks penetrated into the porous substrate as expected (Fig 3B and 3C). In general, the penetration depth of the ink was dependent on the localized variation of the density of the polymer within the substrate, i.e., the ink penetrated deeper into areas with larger pores compared to areas with smaller pores as shown in Fig 3B. The penetration depth, evaluated by optical microscopy varied between 500-600 µm for the montelukast ink, but it was difficult to assess the penetration depth for the haloperidol ink due to its weak color and the low amount of layers printed. The water-based propranolol ink concentrated at the surface of the substrate without penetration into pores of the carrier and propranolol was crystallized at the surface (Fig. 3D).

Porosity and microstructure

The new substrate was designed to be porous to facilitate the absorption of ink, and therefore the porosity of the carrier is a crucial parameter. Two different methods were investigated, a custom-developed oil absorption method and μCT for assessing the porosity of the substrate. While the oil absorption method only gives a single value of the porosity for a given type of substrate, μ-CT is able to visualize the internal structure of the samples, e.g., the pore size, their distribution and potential anisotropy of the material (25). The porosity of the freeze-dried substrate as measured by the oil absorption method was 0.92 ± 0.02 (n = 3), showing that the freeze dried foams were highly porous as expected. The apparent porosity of the samples was lower for the μCT measurements, which gave values between 0.7-0.8 (results not shown). This is likely due to the different nature of the techniques with μCT being non-destructive and non-invasive compared to oil absorption, where interactions between the oil and the substrate take place. Despite the differences, both methods indicate that highly porous substrates were prepared using the freeze-drying method. The internal structure of the samples as measured by μCT revealed pores of varying size, presumably due to the variation in the size of the ice crystals during the freeze drying process. Two similarly prepared substrates had different apparent pore sizes (Fig. 4). This difference could be due to internal variation within the freeze drier during the freeze drying process, e.g., in one part of the freeze drier the formation of ice crystals is faster, resulting in small ice crystals and thereby in small pores, while in another part the nucleation is slower resulting in larger crystals and thereby larger pores. It could also be due to the preparation method: variation in the distribution of the components within the formulation could affect the morphology of the product after freeze-drying. Better control of the freeze drying process parameters to affect the formation of ice crystals might alleviate this (23). Despite the observed differences in the porosity level and pore size, the samples appeared relatively homogenous.

Drug content of the printed dosage forms

In this study, the doses selected for printing of montelukast and haloperidol reflect clinical doses, i.e., haloperidol has a recommended daily dose between 0.5 and 4 mg for treatment of first-episode psychosis (26) and between 1 and 4 mg for treatment of schizophrenia (27). For montelukast the range of clinically available doses is 4- 10 mg (28). For propranolol, the doses correspond to the daily uptake for treatment of infantile haemangioma (29), but higher doses are required in other indications (30). Despite not achieving the doses for adult treatment, they were printed in order to assess the effects of a water-based ink on the new substrates and the suitability for spectroscopic analysis.

The amount of the API deposited per layer is dependent on the concentration of API in the ink formulation, the droplet size and the area printed. For all three APIs, the content measured by HPLC correlated linearly with the amount of layers printed, indicating that the printing parameters did not vary significantly during

the process (Figure 5). There was a slight deviation from the calculated content, but it was deemed acceptable.

Spectral analysis

Development of models

The Raman and NIR spectra contain both physical and chemical information and must therefore be treated using preprocessing algorithms prior to modeling. In this study the focus was on the quantitative analysis of the API, therefore spectral variation from physical effects, such as density variation and pore size of the substrate, had to be addressed. This was done by systematic pretreatment of all the data. The optimal window size, polynomial fitting and derivative applied is highly dependent on the nature of each analysis (31), therefore various approaches for the optimization of the Savitzky-Golay preprocessing were attempted and combinations of different preprocessing parameters were used as described in Table III.

The substrate had a weak Raman scattering signal, likely a combination of two factors: (i) the primary ingredient of the substrate being HPMC which itself shows weak Raman scattering, and (ii) the low density of the substrate due to the sponge-like structure weakens the Raman signal. This makes the developed HPMC-based highly porous wafers good candidates for non-destructive analysis of printing APIs by Raman spectroscopy. Montelukast proved to have a strong Raman scattering signal in both transmission and backscatter modes. Haloperidol had a weak signal in backscatter mode, but little to no signal in transmission mode. Analysis of the propranolol-containing samples was complicated by the presence of brilliant blue in the ink, which induced fluorescence, making Raman spectroscopy unsuitable for analysis of the propranolol containing samples. The montelukast samples, both measured in transmission and backscatter modes, contained a strong Raman contribution from montelukast in the region from 700-1700 cm^{-1} . For haloperidol, the signal contribution was very low in transmission mode, however the signal was strong enough in backscatter mode to achieve a good signal useful for modeling.

Raman spectroscopy has limitations in regard to quantitative analysis of inkjet printed pharmaceuticals. For instance, the presence of fluorescence is a disturbing factor, which can arise from the substrate, the API or the ink constituents. In addition, different compounds can possess varying Raman scatterering abilities that have to be taken into account when selection the ink-substrate combination. For example, if the substrate has a strong Raman contribution while the API has a weak contribution, determining the printed API content from the Raman spectrum can be challenging. Furthermore, when using a backscatter Raman setup, the penetration depth of the laser is of paramount importance; If the printed ink has penetrated deeper than the Raman laser, an incorrect API content may be predicted by the analysis.

All the samples were analyzed by transmission NIR (tNIR). While all the APIs have strong and characteristic spectra when measured as pure powders, very little signal could be obtained when printed on the substrate. The obtained PLS models contained a large number of LVs and had poor prediction power. This technique was therefore discarded for quantitative analysis of the printed dosage forms. There can be multiple reasons for the failure of tNIR in analyzing the printed dosage forms. First of all, the used tNIR setup was optimized for tablet samples. Secondly, the used substrates are porous and require the reduction of the signal strength in order to not oversaturate the detector. This may cause the reduction in the spectral contribution from the APIs. Due to the failure of tNIR, where the entire bulk of the printed dosage form was analyzed, focus was then switched to surface NIR. Here, only the surface of the printed dosage forms was analyzed. All the APIs had strong signal contributions from the drug-printed samples compared to the blank substrates. Therefore, the resulting spectra contained contributions from both the substrate and the studied API (supplementary material). The PLS models for surface NIR showed excellent predictive performance for both montelukast and propranolol, while haloperidol showed worse predictive performance.

The quality of the PLS models for the prediction of the API content was assessed by evaluating the number of latent variables (LVs), the relative contribution of each LV, the root-mean square error of cross-validation/prediction (RMSECV and RMSEP, respectively) and the R^2-value of cross-validation and prediction. The number of latent variables gives information on the complexity of the model and it should be evaluated against the RMSECV and RMSEP values that should be as small as possible. The optimal selection of LVs gives usually a relatively low RMSECV and RMSEP and using a higher number of LVs does not improve predictive power of the model. The resulting selection of LVs should also ideally give a high R^2 value for both cross-validation and prediction, indicating that the predicted content gives a value close to the content measured by HPLC. The optimal selected models for all APIs and techniques are gathered in Table III.

Table III. Summary of working PLS models for the different methods and APIs.

API/substrate	Method	Variable selection, cm^{-1}	Preprocessing, (window size, polynomial, derivative)	LVs	RMSECV/RMSEP, mg	R^2 CV/ R^2 P
Montelukast	Transmission Raman	150-1900	SNV, SG (81, 2, 2)	3	0.39/0.42	0.99/0.99

	Surface Raman	150-1900	SNV, SG (81,2,2)	4	0.81/0.86	0.96/0.97
	Surface NIR	3800-6400	SNV, SG (15, 2, 2)	4	0.34/0.39	0.99/0.99
Haloperidol	Surface Raman	150-1900	SG (51, 2, 2)	2	0.15/0.15	0.98/0.99
	Surface NIR	4100-6600	SNV, SG 31,2,2	5	0.24/0.20	0.96/0.97
Propranolol	Surface NIR	4100-6300	SNV, SG 31 2 2	5	0.21/0.23	0.98/0.99

316 RMSECV: root mean square error of cross-validation, RMSEP: root mean square error of prediction, R^2 CV:
317 correlation coefficient of cross-validation sets, R^2 P: correlation coefficient of validation set

318 Table IV. Summary of the applicability of the spectroscopic methods for the non-destructive quantification
319 of different APIs printed on porous substrates.

	Montelukast	Haloperidol	Propranolol
Transmission Raman	Yes	No	No
Surface Raman	Yes	Yes	No
Transmission NIR	No	No	No
Surface NIR	Yes	Yes	Yes

320

321 Comparing the different spectroscopic techniques and models studied does not yield an ideal method for
322 all cases (Table IV). The appropriate analytical and modeling methods are API-dependent, and are affected
323 by the resulting ink formulation and the substrate. Montelukast, being a strong Raman scatterer, gave good
324 results when using the transmission setup, however poor fitting was achieved for the surface Raman setup.
325 The good fitting for the transmission model can be ascribed to the transmission setup measuring the entire
326 bulk of the dosage form, independent of penetration depth of the ink or variation in the density of the
327 porous substrate. Surface Raman spectroscopy had a poorer performance, which can be due to the Raman
328 laser not penetrating deep enough to get a linear correlation between the number of layers printed and the
329 resulting spectra. Furthermore, both methods required a combination of SNV and Savitzky-Golay

smoothing algorithm for preprocessing of the raw spectra. SNV, originally developed for standardization of NIR spectra, first transforms each spectrum to mean zero and unit standard deviation after which the Savitsky-Golay preprocessing smoothens the spectra and enhances shoulders and subtle differences in the spectra. While surface Raman yielded a poor model for montelukast, the surface NIR method yielded a good fitting, indicating that the penetration depth for surface NIR spectroscopy being high enough to measure the entire amount of the printed API. The best fitting for the predictive quantitative analysis of haloperidol was shown by the surface Raman method, compared to the NIR method. Interestingly, the best fit was achieved when using only Savitzky-Golay preprocessing without SNV transformation. The reason for this can be found in the raw spectra, which contained weak contributions from haloperidol and the substrate, plus a strong background contribution inherent to the instrument. Applying SNV means that the background contribution would be enhanced in the SG processed spectra, thereby weakening the prediction ability of the model (supplementary material). For propranolol, due to the addition of brilliant blue to the ink, it induced fluorescence with Raman spectroscopy, only NIR was usable, where a good predictability of the API content was achieved.

Conclusion

The use of spectroscopic techniques made it possible to fast and accurately determine the API content in dosage forms prepared by inkjet printing on porous substrates suitable for the printing process. Selecting a single optimal method for non-destructive determination of the API content in any printed sample is close to impossible as each technique has its advantages and drawbacks. That said, Raman in both transmission and reflectance mode and NIR spectroscopy in surface mode could be used in combination, complementing each other. However, in specific cases when one method fails for a given API (e.g. Raman spectroscopy due to fluorescence), another technique (NIR spectroscopy) could be used alone for assessing the API content.

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