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Research paper

Near InfraRed Spectroscopy homogeneity evaluation of complex powder blends in a small-scale pharmaceutical preformulation process, a real-life application

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ABSTRACT

Near InfraRed Spectroscopy (NIRS) is a potentially powerful tool for assessing the homogeneity of industrial powder blends. In the particular context of hospital manufacturing, we considered the introduction of the technique at a small pharmaceutical process scale, with the objective of following blend homogeneity in mixtures of seven components. This article investigates the performance of various NIRS-based methodologies to assess powder blending.

The formulation studied is prescribed in haematology unit, as part of the treatment for digestive decontamination in children receiving stem-cell transplantation. It is composed of the active pharmaceutical ingredients (APIs) colimycin and tobramycin and five excipients. We evaluated 39 different blends composing 14 different formulations, with uncorrelated proportions of constituents between these 14 formulations. The reference methods used to establish the NIRS models were gravimetry and a High Performance Liquid Chromatography method coupled to an Evaporative Light Scattering Detection.

Unsupervised and supervised qualitative and quantitative chemometric methods were performed to assess powder blend homogeneity using a bench top instrument equipped with an optical fibre. For qualitative evaluations, unsupervised Moving Block Standard Deviation, autocorrelation functions and Partial Least Square Discriminant Analysis (PLS-DA) were used. For quantitative evaluations, Partial Least Square Cross-Validated models were chosen. Results are expressed as API, and major excipient percentages of theoretical values as a function of blending time. The 14 different formulations were only satisfactorily discriminated by supervised algorithms, such as an optimised PLS-DA model. The homogeneity state was demonstrated after 16 min of blending, quantifying three components with a precision between 1.2% and 1.4% w/w.

This study demonstrates, for the first time, the effective implementation of NIRS for blend homogeneity evaluation, as early as the preformulation step in a small hospital manufacturing unit. It shows how NIRS involving sampling with an optic fibre can be useful to characterise, optimise and control a small-scale mixing processes on the basis of the distribution of APIs and excipients during blending.

1. Introduction

1.1. Clinical and pharmaceutical context

In the haematology unit of the Robert Debré Children's Hospital, children receiving bone marrow transplantation are concomitantly treated for digestive decontamination to prevent bacterial spread. The current formulation is poorly palatable, leading to non-compliance by most of our paediatric patients. This jeopardised patient outcome and consequently, the AGEPS (the Department for Drug Development of Paris Hospitals) has formulated oral powders for paediatric digestive decontamination. These preparations were composed of two APIs *i.e.* colimycin (C, 0.1 w/w) and tobramycin (T, 0.2 w/w), with five excipients: sucrose, citric acid, colloidal silica, magnesium stearate, and flavourings.

The role of the AGEPS is to develop and manufacture pharmaceutical forms intended for distribution to the hospital pharmacies. When the needs do not justify industrial manufacture by the AGEPS, individual accredited hospital pharmacies have themselves to

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manufacture the pharmaceutical products. Accredited hospital pharmacy compounding units, such as that at Robert Debré Hospital, can be considered to be very small-scale pharmaceutical industries, and have to apply pharmacopoeia regulations, although producing very small batches. In this context, a first project was successfully developed in the Robert Debré Hospital Pharmacy Department to monitor the powder blend and the uniformity of the content of the busulfan-containing capsule production process [1]. This demonstrated the feasibility of the implementation of Process Analytical Technologies (PAT) at the hospital scale and the substantial benefits of using NIRS to monitor this cytotoxic API with a narrow therapeutic index.

In this study, we considered the implementation of NIRS to set-up and validate our manufacturing process as early as the preformulation step and the blending operation.

1.2. Brief review about NIRS and blend homogeneity monitoring

The overall performance of a blending operation depends on many variables, including the physical properties of the mixing materials, type and geometry of the blender, and operating conditions, which influence the degree of mixing. Conventional methods for determining uniformity are all sample destructive, time consuming and costly. Indeed, the most time consuming part of a blending process is not the blending operation itself but the analytical controls to confirm the endpoint of mixing [2]. Therefore, the development of a fast and non-destructive method to determine blend homogeneity would be valuable and may provide feedback to assist in process development.

Various methodologies have already been described for investigating the homogeneity of a blend by NIRS; they include off-line, at-line, on-line, and in-line approaches. The main differences between them are the objectives (qualitative or quantitative homogeneity determinations), the process scale (and the adapted apparatus) and the chemometric methods used. For qualitative assessment of homogeneity, algorithms such as Moving Block Standard Deviation (MBSD) calculated between NIRS spectra [2–5] are used with in-line spectrometers providing a substantial volume of spectral data. High sampling frequency is certainly required with such techniques to obtain sufficient variability in the calibration set. Some authors have evaluated qualitative pattern recognition algorithms to assess their utility in controlling powder blending [2,6,7]; they include Soft Independent Modelling of Class Analogy (SIMCA [2,6]), the Principal Component Modified Bootstrap Error Adjusted Single Sample Technique (PC-MBEST [6,7]) and Guided Principal Component Analysis (G-PCA [2]). G-PCA requires the inclusion of pure component spectra in the dataset. The limitations of these techniques for low blend concentrations may be circumvented by the global approach proposed by El-Hagrasy which involves introducing different processing conditions [6]. Some authors [2,8] highlight the difficulty of evaluating the significance of the observed differences between spectra, and suggest that such qualitative techniques may underestimate blending end points.

To overcome these limitations, Berntsson proposed quantitative homogeneity evaluation based on mean spectrum methodology and non-linear partial least squares calibration using reflectance probes [9,10] during blending (which require a large representative calibration set). The authors state that using an average spectrum from a sufficient number of sub-fractions of the same sample aliquot may improve the representation of the "true" value of the content in the entire aliquot. They explain that the heterogeneity of powder mixtures does not allow true reference concentration values to be assigned to NIRS spectra collected from bulk powders by a fibre-optic probe. Bodson referred to a conformity test, checking at each wavelength whether or not the NIRS spectra are included in an accepted range of specifications, and tested the reliability of their quantitative model by tracing the relative bias ß expectation tolerance limits [11]. Most often, NIRS measurements are recorded from the same site in the mixer over time. The problem is the spectra lack of spatial information about overall homogeneity and that, for example, "dead spots" cannot be detected. Consequently, various alternatives have been described such as an experimental setting including six optical windows on the surface of the blender from where the monitoring is performed [12]. However, with this approach, information is only gathered from the surface regions of the blender. In similar conditions, El-Hagrasy et al. [13] collected their NIRS spectra from 12 sapphire windows and also by using a modified multi-compartment thief probe to record more information. The authors compared different pretreatments and models to characterise inter shell vs intra shell powder mixing kinetics. Popo sampled powder blends in motion and preferred sampling at small time intervals rather than repeatedly collecting samples at the same preselected sites [5]. They present stream sampling as the best compromise for identifying segregation problems related to the emptying of the blender. In the same way, Li compared the adequacy of on-line sensors [14], at-line probes and off-line bench top instrument [15] measurements to determine blending profiles. They explain how NIRS fibre-optic probes can be used not only to provide complete blending profiles for all components, but also to detect clumps and aggregates in the blending mixture. They highlight the potential use of NIRS for comparing blending dynamics related to physical and surface properties [15]. They also reveal the inadequacy of on-line NIRS sensors which may underestimate the variances of the blends because of the "averaging" effect caused by large beam size [14] which could be circumvented with adaptable beam size. In view of the difficulty of defining the most appropriate sampling protocols, it may be useful to transpose Muzzio's recommendations to NIRS analyses [16]. To avoid any such "averaging" effect, they propose limiting the size of samples used for characterization of content uniformity to, at most, three times the unit dose.

This brief review reveals the growing interest in NIRS for monitoring blending and highlights its advantages and limitations in several blending contexts. Nevertheless, there have been no published studies of NIRS methodology for assessing the homogeneity of seven-component blends, such as our formulations. So, the overall aim of this work was to compare different chemometric approaches using NIRS for assessing blend uniformity in the context of the specific constraints of complex seven-component blends and small batches. We considered the use of an optical probe and a bench top instrument, as the most appropriate NIRS apparatus for our purpose, *i.e.* at a preformulation step with small-scale blends, and blender unsuitable for on-line NIRS sensors.

2. Materials and methods

2.1. Materials and equipment

The studied formulations contained tobramycin base (Unipex®, Rueil Malmaison, France), colimycin sulfate (Inresa®, Bartenheim, France), sucrose (Tereos®, Lille, France), citric acid (Cooper®, Melun, France), colloidal silica (Brenntag spécialités®, Sartrouville, France), magnesium stearate (Univar®, Fontenay sous Bois, France), and flavouring agents (Givaudan® SA, Vernier, Switzerland). All the ingredients complied with their corresponding European Pharmacopeia monographs, except the flavouring agents which were GRAS labelled (Generally Recognised As Safe by the FDA). We decided to retain three different flavouring agents – strawberry, banana and caramel – in our target formulation because the flavouring agent had not yet been chosen at this preformulation stage.

The powders were mixed in a Turbula® mixer (Willy, A Bachofen AG Maschinenfabrik, Basel, Switzerland), a three-dimensional blender combining rotation, translation and inversion movements which was stopped to allow NIRS spectra acquisitions at various time points.

A NIRFlex N-400 FT-NIR® spectrometer (Büchi, Flawil, Switzerland) was used to obtain spectra for all samples described in this article. This spectrometer is equipped with a reflectance fibre optical probe module, a 10 W light source and a PbS detector maintained at 30 °C. A total of 16 scans were co-added to produce final spectra covering the wavelength range 4000–10,000 cm^{-1} at 12 cm^{-1} intervals. NIRCal® 4.21 Chemometric Software (Büchi, Flawil, Switzerland), and SIMCA-P® 11 (Umetrics, Umeå, Sweden) were used for data acquisition and treatment.

Reference data were measured for purposes of API quantification with a validated High Performance Liquid Chromatography method coupled to an Evaporative Light Scattering Detection system (HPLC–ELSD). Trifluoroacetic acid (TFA) was purchased from Acros Organics (Geel, Belgium) and was flushed with nitrogen after each use. Ultrapure water was obtained with a Milli-Q system from Millipore (Bedford, MA, USA). Acetonitrile (HPLC grade) was obtained from Carlo Erba (Milan, Italy).

2.2. Methods

2.2.1. Raw material identification

A qualitative spectral analysis was first performed to check the potential discrimination of the raw materials by a Nircal® Cluster analysis before monitoring any blending operation.

2.2.2. Experimental design

Fourteen different formulations, corresponding to a volume of 200 capsules per batch, were manufactured. This sample size was chosen so that the sample thickness stayed within the effective range for NIRS reflectance mode, and to allow estimation of the future manufacturing process. Three of the formulations (differing in flavour) may be used in clinical practice (see Table 1). The other 11 formulations (from 0 to 10, detailed in Table 2) were manufactured and analysed to construct a sufficiently extended calibration range, with uncorrelated constituent proportions to avoid co-linearities. The appropriate distribution was established using the "rand()" function in Excel® software (Microsoft®), checking that the correlation between two constituent proportions did not exceed 0.5 for all formulations. Calibration ranges were established for each constituent according to its proportion in the target formulation. We adapted the ranges according to the concentrations of the various constituents because NIRS is not a sensitive method. Thus, for sucrose, which was the most concentrated constituent, we chose a range of ±10%, whereas for each colloidal silica and magnesium stearate (the least concentrated) the range was ±80%. We studied three series for each working formulation batch, and two for each target formulation batch; a total of 39 blends were manufactured.

2.2.3. Mixtures

Powders were placed in a 250 ml bowl according to the following procedure: 1/3 of the sieved sucrose, sieved APIs and citric acid, the second 1/3 of the sieved sucrose, sieved colloidal silica, magnesium stearate and flavourings, and the last 1/3 of the sieved sucrose. The powders were mixed in a Turbula® mixer at 67 rpm for 19 min.

2.2.4. NIRS and chemometric optimisation

Spectra were collected with a fibre-optic probe introduced into the powder blends. To increase the repeatability of the measurements, a pierced lid was adapted for the bowl to present the optical fibre into the powder in a repeatable way (see Fig. 1). The mixer was stopped at various times *i.e.* 0, 4, 7, 10, 13, 16 and 19 min, and NIRS acquisitions were recorded. The reflectance fibre-optic probe was manually inserted as gently as possible into the powder bed for acquisitions, so as to minimise perturbations during blending operations. Two sampling locations were chosen for each acquisition time to obtain representative samples throughout the blender as recommended by Popo et al. [5].

Qualitative and semi quantitative models have been widely reported in the literature for chemometric optimisation [2–8]. All these publications concerned binary to quaternary mixtures,

Table 1
Constituent proportions in target formulations.

Target formulations	Total weights (g)	API		Sucrose % w/w	Citric acid % w/w	Colloidal silica % w/w	Magnesium stearate % w/w	Strawberry flavour % w/w	Banana flavour % w/w	Caramel flavour % w/w
		T % w/w	*C* % w/w							
S-target	52.3	19.1	10.1	62.7	5.7	0.9	0.9	0.6	0.0	0.0
B-target	52.3	19.1	10.1	62.7	5.7	0.9	0.9	0.0	0.6	0.0
C-target	52.5	19.1	10.0	62.5	5.7	0.9	0.9	0.0	0.0	0.9

S-target, strawberry target formulation ($n = 2$); B-target, banana target formulation ($n = 2$); C-target, caramel target formulation ($n = 2$).

Table 2
Uncorrelated constituent proportions of the 11 working formulations.

Formulations	Number of batches	Tobramycin (mg)	Colimycin (mg)	Sucrose (mg)	Citric acid (mg)	Colloidal silica (mg)	Magnesium stearate (mg)	Strawberry flavour (mg)	Banana flavour (mg)	Caramel flavour (mg)
0	3	8000	3158	31,810	4440	243	94	0	495	0
1	3	6010	6843	27,880	2640	93	168	0	165	0
2	3	13,010	7896	34,760	3000	542	244	0	495	0
3	3	15,000	5790	32,810	1510	767	318	495	0	0
4	3	9020	4738	29,840	1200	617	393	165	0	0
5	3	14,010	3685	33,790	2290	393	468	0	0	250
6	3	5000	7369	35,750	1930	319	543	0	0	750
7	3	11,010	2633	36,730	4800	168	618	0	165	0
8	3	12,010	4211	30,830	3720	693	592	0	0	250
9	3	10,010	6317	28,860	4080	842	767	165	0	0
10	3	7010	5270	37,730	3370	468	842	0	0	750

Tobramycin gradient from 9.7 to 26.4 % w/w = formulations 6, 10, 1, 0, 4–9–7, 2, 8, 5, 3. Colimycin gradient from 4.7 to 15.6 % w/w = formulations 7, 0, 5, 8, 10, 3, 4, 9, 2, 6, 1.

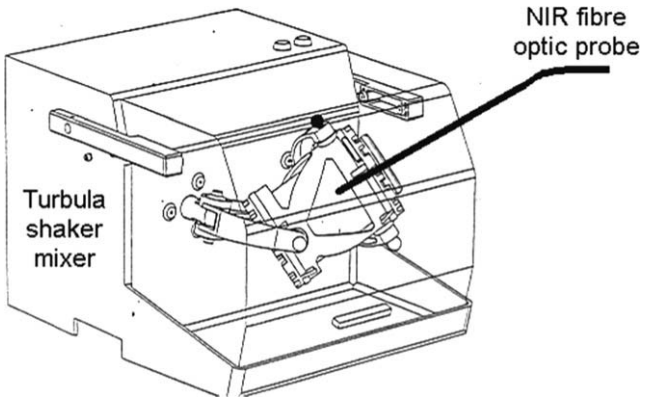

Fig. 1. Turbula® shaker-mixer equipment with NIRS reflectance probe.

mostly assessed by on-line measurements. When the only cause of variance arises from quantitative homogeneity, then the spectral homogeneity may be established by qualitative unsupervised techniques. If there are other causes of variance in the data (for example, different powder granulometries, packing, water content), more sophisticated qualitative or quantitative models have to be developed. The complexity of the models does not depend only on the objectives, but also on the data and experimental system (including sample composition, chosen apparatus, laboratory conditions, repeatability of manipulations).

First, we compared several pretreatments to reduce the spectral variability associated with the physical characteristics of powders, and the instrumental variability. Standard Normal Variate (SNV), Multiplicative Scatter Correction (MSC), Second Derivative, Orthogonal Signal Correction (OSC) and Wavelet Denoise effects were compared on the basis of the discriminating capacity of qualitative and quantitative models. SNV, applied spectrum by spectrum, consists in centring and reduction of each intensity value for all the different variables included in the spectra [17]: this transformation reduces the multiplicative interferences of scatter and particle size. With MSC, each individual spectrum is transformed by least squares to fit as closely as possible the averaged calibration spectrum [17]: this compensates for different scatter and particle sizes. A five-point Second Derivative was also tested to minimise spectral variations due to positioning and/or processing conditions [8,18]. Then, an OSC was performed from one projection. OSC is expected to improve the calibration model when the signal contains large systematic variation in X (spectral information) not related to Y (quantitative information), such as baseline shifts [19]. Lastly, wavelet denoise using the Daubechies function of the 4th order was selected and optimised according to Chalus et al. [20] and Trygg et al. [21].

Two qualitative chemometric evaluations were conducted employing unsupervised and supervised algorithms for our time series data.

2.2.5. Qualitative unsupervised evaluation methodology

In a first attempt to evaluate the homogeneity of powder blends, we searched for the simplest and most appropriate methodology. For example, we tried direct spectral comparison using Moving Block Standard Deviation (MBSD) method as a means of assessing homogeneity. MBSD calculates the standard deviations of the spectral intensities at each wavelength measured across a block of different spectra, grouping by time of analysis, and then summed over all wavelengths. This algorithm was also tested from score data after decomposition by Principal Component Analysis (PCA). For the unsupervised qualitative analysis, we tested an autocorrelation function follow-up, consisting of the calculation of the correlation between consecutive spectra as a function of time shifts.

2.2.6. Qualitative supervised evaluation methodology

A supervised qualitative discrimination of the different formulations was carried out. The Partial Least Squares Discriminant Analysis (PLS-DA) algorithm was assessed for predicting the homogeneity of the different formulations. PLS-DA consists of a classical PLS regression where the response variable is a categorical one (replaced by the set of dummy variables describing the categories) expressing the class membership of the statistical units. Such methods require building a calibration set that includes spectra representative of homogeneous blends. However, representative samples are difficult to select. If there is no doubt about the non-homogeneity of a powder blend at the beginning of the mixing, the dynamic procedure to reach homogeneity presumably depends on many factors and may vary from one formulation to another. A prolonged mixing time is not a guarantee of homogeneity, since demixing is possible when blending is continued for an excessive period. From the analytical point of view, this is a paradoxical situation in which the information needed by the analyst is that that they are trying to predict. The determination of powder blend homogeneity by a reference method typically involves the withdrawal of analytical samples from defined mixer locations, and subsequent quantification of target constituents. Generally, quantification is restricted to the API(s) and the distribution of individual constituents is considered to be homogeneous when the active ingredient is uniformly distributed. Such invasive analytical reference methods imply notions that cannot be extrapolated to NIRS analyses: the sample volume explored with conventional methods is much larger than that scanned with a small-beam-size optical probe, and the homogeneity monitored with NIRS determinations depends on chemical and physical distributions of every component. Therefore, we decided first to select for the calibration the spectra from the end of mixing (t16, t19 min), as they were likely to reflect homogeneous blends. Then, the PLS-DA method was applied to attempt to create groups of homogeneous spectra (one class per formulation). The composition of the calibration set was refined by excluding spectra whose scores were not consistent with the other members of the corresponding formulation class. The calibration set was then enriched with spectra corresponding to shorter mixing times that fit into the corresponding formulations classes. Thus, spectra for t4–t19 min were used for calibration. The relevance of the approach was then tested by the evaluation of the predictive capacity of external validation spectra for the homogeneity determination. In practice, 25 blends were independently manufactured to be used for calibration purposes (two batches per working formulation and three of the target ones). The 350 corresponding calibration spectra were recorded and tested with PLS-DA algorithm (14 time series, two spectra at each time point). An external validation set was composed from 196 spectra from 14 additional blends (one batch per working formulation and three target formulations), recorded at the same times between t0 and t19 min. Of the 196 external validation spectra, 42 were for the three independent target formulations.

2.2.7. Quantitative evaluation methodology

Quantitative approaches were tested to approximate the homogeneity of the different target blends included in the independent validation set. The mixing characteristics were explored by NIRS quantifying the two APIs. In addition, a quantitative evaluation of the excipients was attempted on the basis of the mass balance results of excipients introduced into each batch. Colloidal silica was not quantified because of the absence of specific absorption bands in its NIRS spectrum (Fig. 2a, spectrum 7). The flavourings were also not quantified as they differed between formulations. Inde-

pendent PLS Cross-Validated (PLS-CV) models were optimised to quantify tobramycin, colimycin and excipient contents. The choice of adequate model for comparing different pretreatments and spectral ranges was difficult. Indeed, the criteria retained were the correlation coefficient, the overfitting tendency, the precision parameters, and the robustness. The model used had the lowest number of components. Other options have also been reported in the literature, and include the use of the Residual Predictive Deviation (RPD) [22,23], bias corrected Standard Error of Prediction (SEP_c) [23], and accuracy profile [11]. There is currently no evidence that any one of these evaluation tools is superior to the others.

Note that the goal of this study was to assess methods for monitoring powder blending at the preformulation step. Indeed, calibration involved several candidate formulations, the target formulation being at the centre of the plan. Individual models were optimised to predict amounts of APIs and excipients, and then tested with 42 target blend spectra included in the independent validation set. Quantitative predicted determinations were only considered by PLS when the corresponding probability of membership of a model exceeded 5%.

2.2.8. Reference assays

Two reference methods were used: gravimetry and HPLC-ELSD.

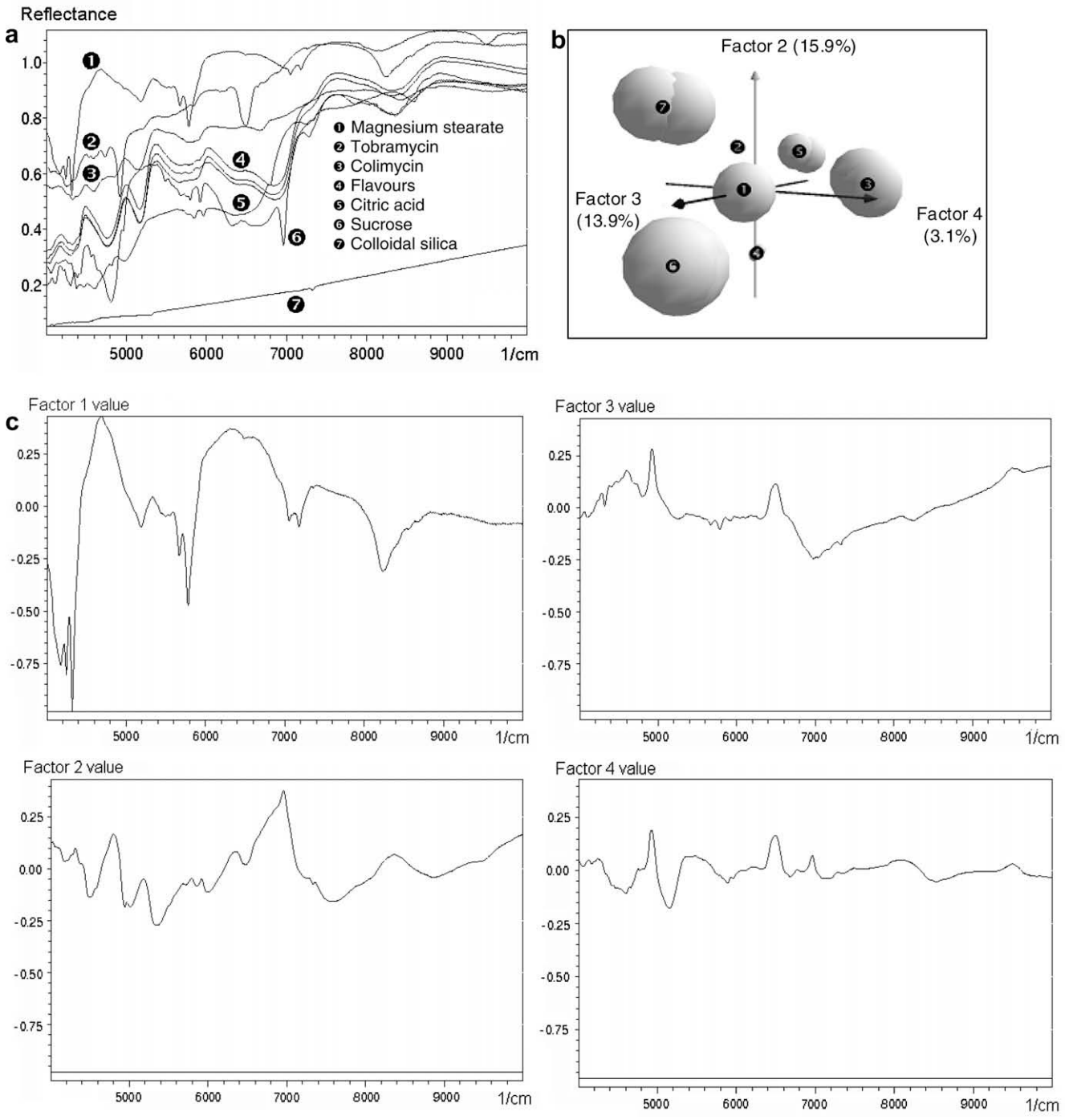

Fig. 2. Raw material spectra (a) and corresponding cluster plot (b) and loading plot (c).

Gravimetric data were used to test our NIRS models for excipient quantification. Each ingredient was weighed in Class I balances (e = 1 mg, d = 0.01 mg) (Metler Toledo, Viroflay, France).

An HPLC-ELSD method was optimised and validated to quantify the two APIs simultaneously. The HPLC system consisted of a TSP model P1000XR pump (Thermoseparation Products®, Fremont, CA, USA), and a TSP model AS1000XR autosampler (Thermoseparation Products®, Fremont, CA, USA) set to inject 20 μL. The ELSD was a Sedex 75 model from SEDERE® (Alfortville, France) equipped with a normal flow nebulisation head (SEDERE®, Alfortville, France) with a gain set at 7, an air pressure of 3.0 bars and an evaporation temperature of 60 °C. The analytical column was a Zorbax SB C18 (Agilent Technologies Inc.®, Santa Clara, CA, USA) 150 mm × 4.6 mm i.d., 3.5 μm reversed phase column. The column temperature was controlled at 30 °C with a Croco-Cil external oven (Thermoseparation Products®, Fremont, CA, USA). The mobile phase consisted in a binary mixture of TFA 37.5 mM (phase A) and acetonitrile containing TFA 37.5 mM (phase B) in a gradient elution mode (98/2 for 5 min, 98/2–70/30 in 1 min, 70/30 for 7 min, 70/30–98/2 in 1 min and 98/2 for 6 min) at a flow rate of 1.0 mL/min. Fresh standard solutions were prepared daily by dissolving the appropriate amount of tobramycin and colimycin sulphate standards in 37.5 mM TFA. For pharmaceutical samples, the corresponding solution was ultrasonicated for 1 min and was filtered through a regenerated cellulose Chromafil filter from Macherey-Nagel® (Düren, Germany) before injection. The HPLC-ELSD reference method was validated for specificity, precision (repeatability RSD < 3.0%, intermediate precision RSD < 3.4%), linearity, accuracy and stability [24], according to the International Conference of Harmonisation (ICH) guidelines [25].

3. Results and discussion

3.1. Raw material identification

NIRS evaluation of blend homogeneity must ensure that it generates spectral differences that can be exploited in discriminant analyses. We obtained spectra for the raw materials (Fig. 2a), and applied four-factor cluster analysis; their projection on the cluster plot (Fig. 2b) confirmed appropriate discrimination of the different constituents. Tobramycin (spectrum 2) presented two bands that were very narrow and intense compared to those obtained with colimycin (spectrum 3). The flavourings were all included in the same cluster; this was probably because they were all adsorbed on the same maltodextrin, resulting in similar spectra. This was not a problem for the approach, as the final formulation will only contain one flavouring agent. The loading plot of factor 1, depicted in Fig. 2c, mainly referred to information concerning magnesium stearate (Fig. 2a, spectrum 1) and explained 63.1% of the spectral variation, creating two groups in a score plot of factors one and two (data not shown): magnesium stearate, and the six other ingredients. Therefore, the score plot presented in Fig. 2b illustrates factors 2, 3 and 4: their loading plot profiles (Fig. 2c) suggested combined information from the six other ingredients. This model (involving factors 1–4) explained 96.0% of the spectral information. Hence, as depicted in Fig. 2, APIs and excipients present exploitable spectral differences that allow their discrimination. These results support the view that NIRS can be used to monitor the blend homogeneity of tobramycin/colimycin formulations.

3.2. NIRS homogeneity determinations

3.2.1. Unsupervised time series qualitative analyses

We first tested a MBSD method for following the mixing operation by direct spectral comparisons. In this method, data were arranged into a time by wavelengths matrix, with one matrix for each blend. Standard deviations (SD) of intensities were calculated over all wavelengths for the different time blocks (from 0 to 19 min of blending, with two spectra per time of acquisition). Then, root sum square differences (RSSD) of intensities were calculated for two consecutive spectra (at the same time of analysis or consecutive lag times). Fig. 3 shows this type of representation of the variations during mixing for a unique target blend (14 spectra, 7 times of analysis). The RSSD plotted as a function of time is expected to decrease and to reach a baseline state when blend homogeneity is achieved [2,3,5]. The oscillating plot presented in Fig. 3 indicates that experimental data were somewhat more complex to interpret. Indeed, no baseline state was observable in our data, suggesting that the blending process did not achieve a stable state after 19 min blending. This, however, is very unlikely and there are several other possible explanations, including significant instrumental variability, or several simultaneous causes of deviations in spectra. In an attempt to describe the causes of variance in the spectral data better, the MBSD was calculated on score data after computing a PCA. The basic principle of the PCA is to describe data variability as a small number of uncorrelated variables, accounting for the largest possible variance in the dataset; redundant information, random variation and spectral noise should be eliminated from the data. The same 14 spectra originating from one target blend analysed at several times during mixing were compressed into a 2 PC space. The loading plot (Fig. 4) indicates two causes of variance mainly depending on the two major components (sucrose and tobramycin). The PC1 loading plot, explaining 85% of the X variance, included information concerning tobramycin and sucrose, whereas PC2, accounting for the remaining 15% of the X variance, was composed of information concerning magnesium stearate and sucrose. As nearly 100% of the spectral information was modelled by the two PCs, the MBSD calculated with the scores from the two PCs resulted in a plot nearly identical to that in Fig. 3. This reduction of variables did not lead to a more interpretable representation of the spectral evolution during blending, and demonstrated the need for further mathematical treatment of the spectra collected. The variation associated with PC1 loading (Fig. 4) could be attributed to baseline shifts. Indeed, pretreatment, such as a first derivative, enhanced the information extracted by PC1, with a distribution accounting for 47% on PC1 and 34% on PC2. However, MBSD profiles established with these 81% X transformed data were again comparable to those in Fig. 3. Similarly, MBSD qualitative methods did not lead to interpretable results. The difficulty may have been due to the use of an optical probe together with the complexity of our mixtures (number of constituents, different particle sizes). In similar context, El-Hagrasy reported a variation of the outlier percentage depending on the thief probe location in the powder bed [8]. Moreover, this tech-

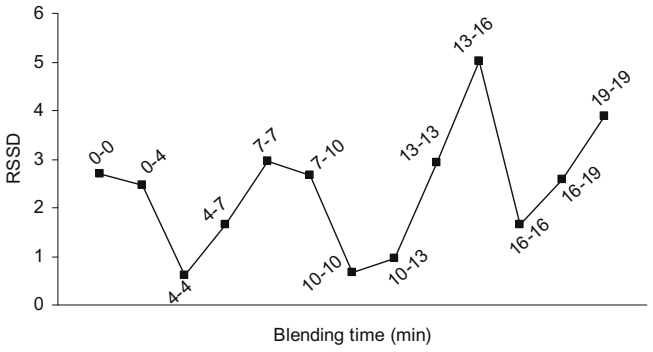

Fig. 3. Root sum squares differences of intensity values (RSSD) of all spectra ($n_t - n_{t+1}$ min) for a single blend.

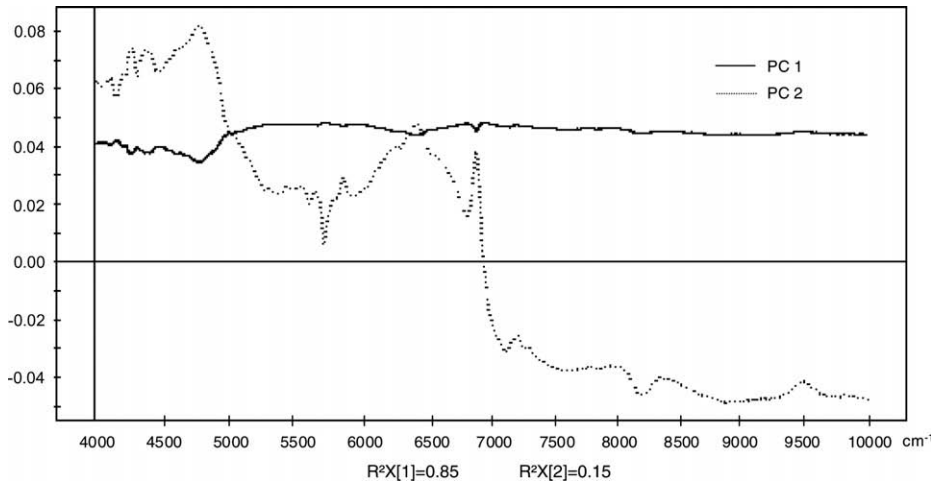

Fig. 4. Loading plot corresponding to a two PCs model optimised from 14 spectra of a single blend.

nique seems more appropriate for following the blending profiles of simple binary [5] or ternary [3,4] mixtures. The only published studies describing such qualitative approaches involving slightly more complex mixtures (four components) were reported by Sekulic et al. [2,26], using an on-line automated system. Such an unsupervised algorithm seems to be simple and straightforward, but was not adapted to our experiments, probably because we did not have enough data to increase the size of the blocks. Using a larger block size, like Lyon with NIRS imaging data [4], could have been useful to smooth the curves but was not possible with our technical set-up. Therefore, it appeared that qualitative techniques tend to require a large number of spectra, from many different points in the blender, and are therefore more appropriate for on-line spectra acquisitions. Indeed, Li proposed the following equation to calculate the number of samples required to assess blend uniformity correctly: $N = L^{c-1}$, where N is this number of samples, L the number of levels for each component and c the number of components [27]. A direct calculation of this equation for our mixtures (seven components) indicates that 12^6 spectra would have been necessary to apply NIRS successfully to study blend uniformity.

Another approach, widely used in time series analyses, is the exploitation of the autocorrelation function [28]. The autocorrelation function shows how a signal correlates with itself as a function of time shift. This function should decrease as the process becomes stationary. We plotted the autocorrelation function (Fig. 5) using the same data as for the MBSD study. At the beginning of blending, the probe is likely to be exposed to only one of the blend components, so substantial variability between spectra is expected. The autocorrelation function profile that is depicted in Fig. 5, calculated along the different principal components, decreased rapidly after 10 min of blending, and reached a steady state after 16 min. It was, however, difficult to establish a decision rule about the acceptation of homogeneity from one profile to another, as this requires a decision on standard deviation tolerance and on the length of the baseline state.

3.2.2. Supervised qualitative approaches

The qualitative discrimination between formulations was achieved by projecting calibration spectra on a PLS-DA score plot after pretreatment involving application of MSC to each class of formulation. Indeed, MSC was calculated classwise, each spectrum of a particular class of formulation being corrected with reference to the average spectrum of this class. Among the 350 calibration spectra, 162 were considered to represent homogeneous blends and were retained for calibration. Fig. 6 shows the PLS-DA score plot of the 12 decorrelated formulations: the discrimination between formulations was established. In this model, 12 principal components were required, but 96% of the X variance was supported by the first two latent variables. However, the predictive ability of the model appeared to be poor when confronted with the external validation set. This qualitative model was inappropriate for mixing endpoint determination, but was, however, effective for establishing a first qualitative discrimination and a representative calibration set of spectra for the following quantitative analyses.

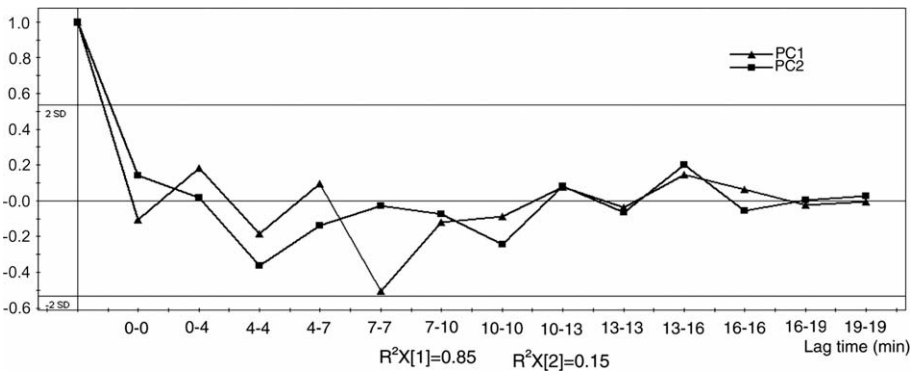

Fig. 5. Autocorrelation function profile for a single blend as a function of time.

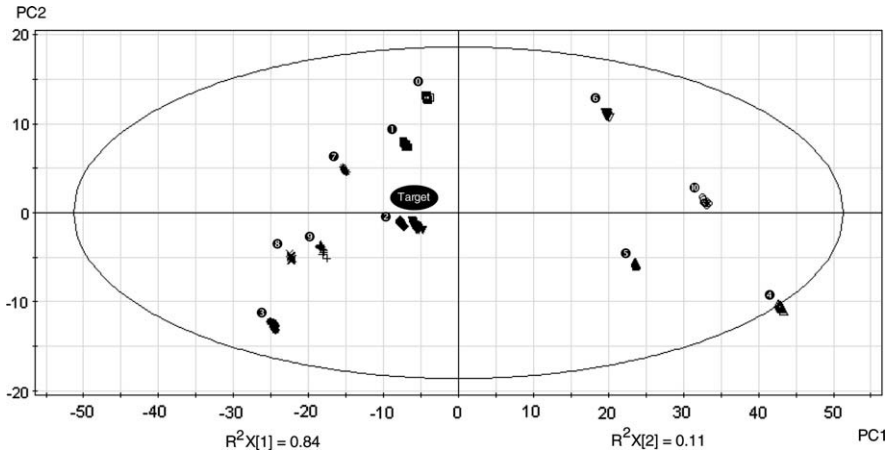

Fig. 6. PLS-DA Score plot after MSC class pretreatment.

3.2.3. Supervised quantitative approaches

We next considered quantitative determinations to assess the homogeneity of these blends. Quantitative NIRS development was justified in this context by the complexity of these analyses of two APIs, and the improvement in understanding the process using a non-destructive technique. We applied an MSC per class and then PLS-CV, using the same 162 spectra qualified by PLS-DA as reflecting homogeneous blends; this approach led to the optimised results presented in Table 3. PLS-CV regressions were optimised between NIRS-predicted and HPLC-ELSD tobramycin and colimycin contents. Three other models were optimised from the three-excipient mass balance data, using the conditions detailed in Table 3. Cumulative fractions of total variation extracted by the components ($Q2_{cum}$) were between 0.81 and 0.93. The numbers of PLS factors were six for tobramycin, seven for colimycin, six for sucrose, the major constituent in the formulation, nine for citric acid, and 13 for magnesium stearate, the minor constituent. The NIRS precision, expressed as RMSEP, was between 0.2% and 1.4% w/w (see Table 3). Also, the RMSEE was of the same order of magnitude as RMSEP, which indicates the absence of any overfitting tendency. It is clear that magnesium stearate and citric acid are not followed quantitatively because of the number of factors included in their corresponding models and their poor $Q2_{cum}$. Bias between the two quantitative methods (HPLC-ELSD and NIRS) was evaluated from all the t_{19} spectra of the external validation set for which the model probability membership exceeded 5%. The *t* test, performed at a 5% risk, demonstrated the absence of bias (no significant difference with 0) in the models for the two APIs. Close agreement between HPLC-ELSD and NIRS was thus demonstrated. We expect that the corresponding calibrations for quantification of APIs and sucrose will be robust enough to predict their amount in the powder blends before they reach homogeneity.

According the acceptance criteria set by the FDA [29], a blend is considered homogeneous if the percentage potency is between 90% and 110% of the label claim and the RSD is less than 5%. For pharmaceutical dose forms, the EMEA recommends a target potency between 95% and 105% of the stated amount for the APIs, and between 90% and 110% for the excipients [30].

The 42 target formulation external validation spectra were used to establish homogeneity profiles for individual blends calculated so as to evaluate both the predictive ability of the models and their applicability to monitor blending. All the spectra scanned before the start of the mixing operation (t_0) were qualitatively considered as corresponding to non-homogeneous blends, because they were rejected from the model prediction (membership probability <5%). In contrast, most of the spectra recorded after the start of mixing were considered to be sufficiently similar to the calibration spectra to be predicted. For each blending profile shown in Fig. 7, an average of 10 of the 12 spectra was predicted. Fig. 7 illustrates the predicted amounts of active ingredients (Fig. 7a for tobramycin and Fig. 7b for colimycin) and excipients (Fig. 7c–e) expressed as percentages of the target values together with the corresponding absolute differences (AD) of the two measurements. The degree of mixing was thus individually assessed by inspection of the distributions of the APIs and excipients, and of the AD for the successive determinations. The interpretation of the different blending profiles of the APIs suggests that a state of homogeneity was reached at 16 min. The trend was similar for sucrose, which shows that an approximate calibration established using the major constituent can also provide some valuable information. Considering the two other excipients, the picture is less clear, but certainly indicates that the calibration based on the PLS vs mass balance data was not satisfactory for predicting the amounts of minor components. All the three external validation blends can thus be considered to be homogeneous after 16 min of blending according to the FDA criteria. This analysis indicates that the quantitative model can now be used to predict the uniformity of our various formulated blends.

Table 3
NIRS calibration and prediction results for five ingredients after independent PLS-CV optimisation.

PLS-CV models	Target strength (w/w)	Strengths range (w/w)	PLS factors	RMSEE (w/w)	RMSEP (w/w)	$Q2_{cum}$
Tobramycin (vs HPLC-ELSD)	0.191	0.097–0.264	6	0.011	0.013	0.93
Colimycin (vs HPLC-ELSD)	0.100	0.047–0.156	7	0.010	0.012	0.87
Sucrose (vs mass balance results)	0.625	0.565–0.692	6	0.015	0.014	0.84
Citric acid (vs mass balance results)	0.057	0.026–0.092	9	0.007	0.010	0.81
Mg stearate (vs mass balance results)	0.009	0.002–0.015	13	0.001	0.002	0.85

RMSEE, Root Mean Square Error of Estimate calculated from the external calibration set; RMSEP, Root Mean Square Error of Prediction calculated from the external validation set; $Q2_{cum}$, Cumulative fraction of total variation extracted by the components.

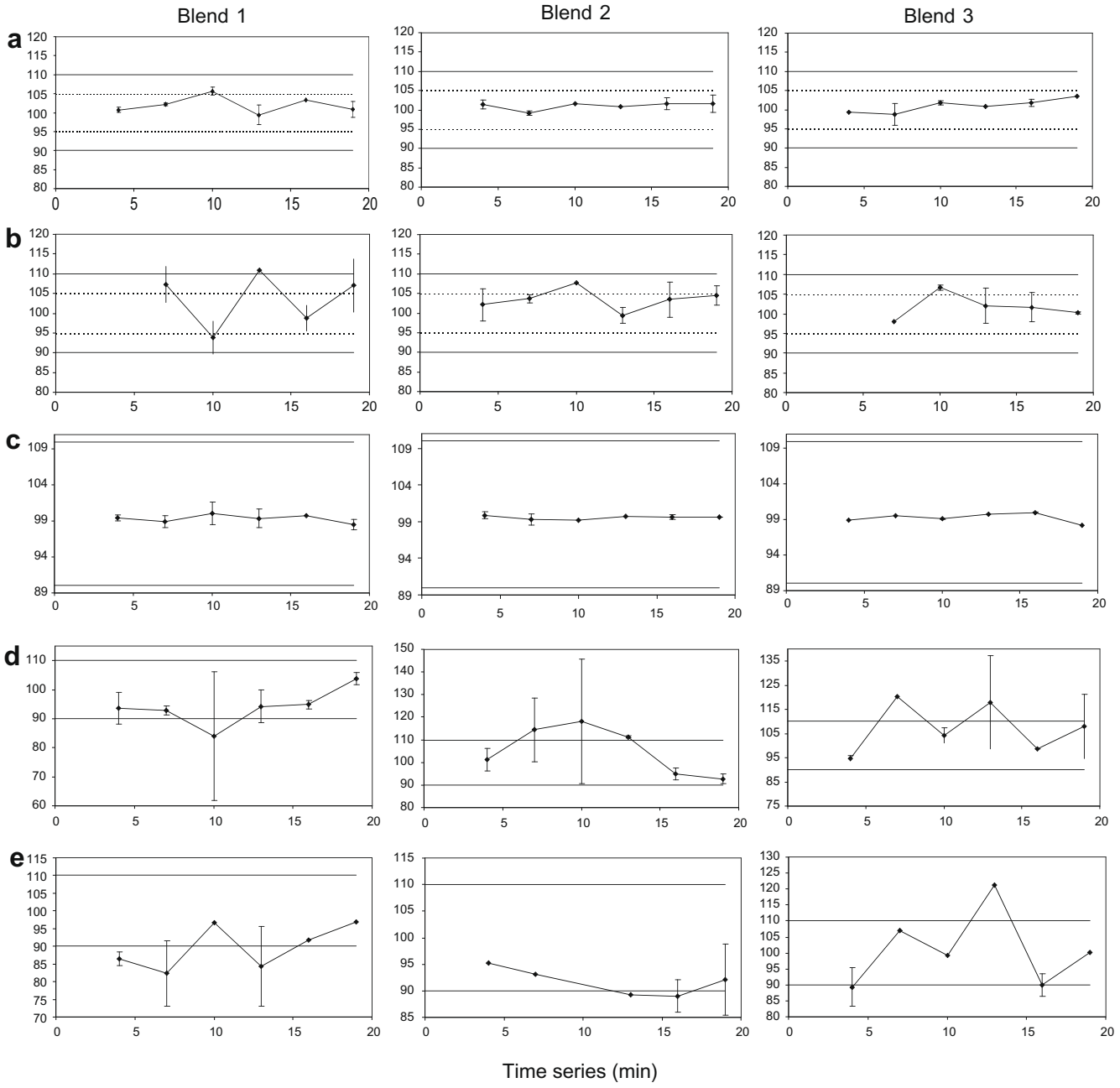

Fig. 7. Percentages of predicted amounts compared to target predicted values for tobramycin (a), colimycin (b), sucrose (c), citric acid (d) and magnesium stearate (e) for target formulations, as a function of the time of blending (from 4 to 19 min).

4. Conclusion

This study demonstrates, for the first time, the effective implementation of NIRS, as early as the preformulation step in a small manufacturing unit in a hospital. It shows how NIRS can be used to characterise, optimise and control a small-scale mixing process on the basis of the distribution of APIs and excipients during blending. The technique involved sampling with a fibre-optic probe and using models optimised from a large range of preformulations of APIs and excipients.

At the hospital, this is a real advance because magistrals are generally prepared manually by technicians and only final controls are performed. Our approach will be used to standardise daily practice and improve quality in our compounding unit towards the standards in industrial production. For clinical use, this is a significant breakthrough as it will allow the hospital pharmacist to secure extemporaneous dispensing medicines efficiently and should lead to improved paediatric patient outcome.

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