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Calibration sampling paradox in near infrared spectroscopy: A case study of multi-component powder blend

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ABSTRACT

The objective of this study was to illustrate the sampling paradox resulting from the different strategies of spectral acquisition while preparing and implementing the calibration models for prediction of blend components in multi-component cohesive blends. A D-optimal mixture design was used to create 24 blending runs of the formulation consisting of chlorpheniramine maleate, lactose, microcrystalline cellulose and magnesium stearate. Three strategies: (a) laboratory mixing and static spectral acquisition, (b) IBC mixing and static spectral acquisition and (c) IBC mixing and dynamic spectral acquisition were investigated for obtaining the most relevant and representative calibration samples. An optical head comprising a sapphire window mounted on the lid of the IBC was used for static and dynamic NIR spectral acquisition of the powder blends. For laboratory mixed samples, powders were blended for fixed period of 30 min and later on scanned for NIR spectra. For IBC mixed blends, the spectral acquisition was carried out in-line for 2 min and stopped for static spectral acquisition. The same cycle was repeated for the next 28 min. Partial least square (PLS) calibration models for each component were built and ranked according to their calibration statistics. Optimal calibration models were selected from each strategy for each component and used for in-line prediction of blend components of three independent test runs. Although excellent statistics were obtained for the PLS models from the three strategies, significant discrepancies were observed during prediction of the independent blends in real time. Models built using IBC mixed blends and dynamic spectral acquisition resulted in the most accurate predictions for all the blend components, whereas models prepared using static spectral acquisition (laboratory mixed and IBC) showed erroneous prediction results. The prediction performance differences between the models obtained using the different strategies could be explained in the context of relevancy and representative sample collection at the initial stage of calibration model building.

1. Introduction

While preparing near infrared (NIR) spectroscopic multivariate calibration (MVC) models, it is critical to obtain calibration samples that are both relevant and representative of the real time samples that the model will be applied to Martens and Naes (1991). The representative samples here mean that not only must the calibration sample states cover the range of samples expected during operation of the analyzer, but also the distribution of the calibration sample states should be sufficiently representative. In other words, it warrants that all potential sources of variability, such as variability in formulation aspects (e.g. concentration range of all the formulation components), variability in physical properties (e.g. particle size, shape) and other sources of variability resulting from the actual operation of the process (e.g. blending, drying, compression, etc.) should be included in the calibration samples. In the preparation of MVC models, it is often difficult to obtain calibration samples that are both highly relevant and representative. Some common strategies employed to obtain the calibration samples include: (i) laboratory synthesized calibration samples analyzed on a laboratory analyzer, (ii) laboratory synthesized calibration samples (such as simulated physical mixtures) and analyzed on an actual process analyzer, (iii) calibration samples obtained from the actual process analyzed on a laboratory analyzer and (iv) calibration samples obtained from the actual process and analyzed on an actual process analyzer (Miller, 2005). These strategies are being commonly used as routine methods for obtaining calibration samples, however, there are some paradoxical sampling issues associated with the adoption of these strategies. For example, the laboratory synthesized calibration samples are highly accurate, but they do not represent the state of samples during actual processing. The samples obtained from the process stream are representative of the actual process samples; nevertheless the analysis of these samples using laboratory techniques suffers from biases and variability due

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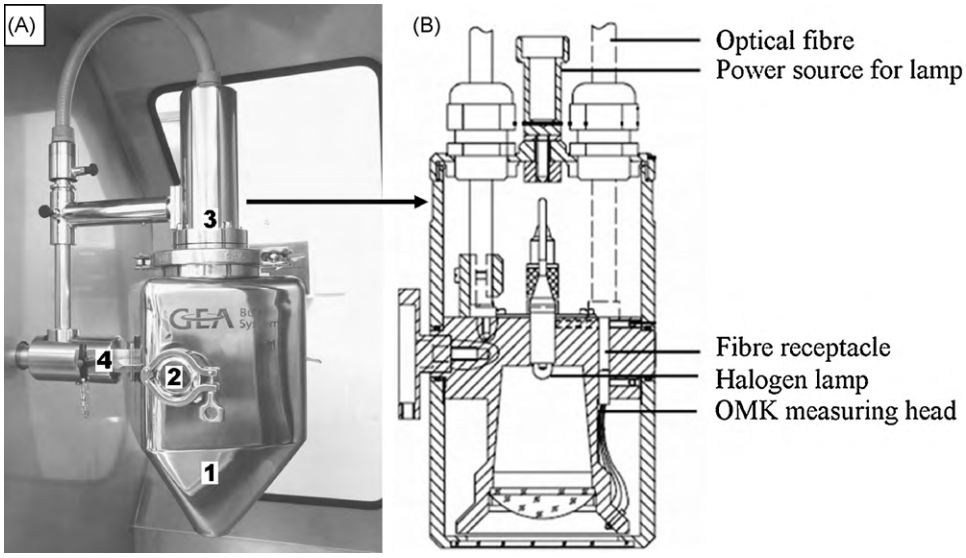

Fig. 1. (A) SP15 IBC blender (1) IBC, (2) port for prism attachment, (3) modified IBC lid with NIR sensor and (4) axis of rotation; (B) schematic of NIR sensor (optical head).

to complicated sample pre-treatments and differences in sample handling. Likewise, analysis of samples obtained from the actual process using an actual process analyzer may prove to be reason for inaccurate assignment of reference values. Design of experiment (DOE) could be used as another potential alternative to impart maximum possible relevancy and/or representative formulation and process features. Often, DOE needs a large number of runs in order to incorporate sufficient degree of variability in the calibration samples, which could incur considerable time and cost capital. In an effort to save capital, preparation of laboratory synthesized calibration samples which are usually very accurate may be employed as a method of choice. However, apart from formulation centered variability, it fails to incorporate process features which are more detrimental to successful application of MVC models for future predictions of samples from the process stream. Although a number of techniques have been adopted to obtain accurate and representative calibration samples, this sampling paradox has not been subjected to great attention. For example, in the field of dry powder blending, most of the studies carried out so far have used either laboratory synthesized calibration samples (Benedetti et al., 2007; Sulub et al., 2009; Li and Worosila, 2005; Li et al., 2006; Duong et al., 2003; Ufret and Morris, 2001; Berntsson et al., 2002) or samples obtained from the process stream and analyzed off-line to build MVC models (El-Hagrasy and Drennen III, 2006; Wu et al., 2009; Popo et al., 2002; Shi et al., 2008). These models were then used for on-line/in-line predictions or process analysis.

Recent studies carried out in this field emphasized on the prediction of blend components using NIR spectroscopy and MVC models. However, none of them have addressed the issue of sampling paradox for multi-component blends using in-process spectral acquisition of calibration samples. This work represents an effort to illustrate the issue of sampling paradox in NIR MVC models with reference to the multi-component dry powder blend. A D-optimal mixture design was used to impart the maximum possible variability in calibration samples and spectral acquisition was carried out using three different strategies: (a) simulated physical mixtures prepared in the laboratory with spectral acquisition carried out in a static manner, (b) calibration samples blended in an intermediate bulk container (IBC) blender with spectral acquisition carried out in a static manner and (c) calibration samples blended in an IBC blender with spectral acquisition carried out in a dynamic manner. These strategies were deliberately employed to illustrate the occurrence of spectral differences for the same composition powder when the spectral acquisition strategy changes.

2. Materials and methods

2.1. Materials

Materials used were chlorpheniramine maleate (CPM; Merck, Singapore, with mean particle size of 110 μm), Inhalac-230 (Meggle, Germany with mean particle size of 110 μm), lactose (Pharmatose 100 M, DMV, The Netherlands, with mean particle size of 128 μm), microcrystalline cellulose (MCC; Avicel PH102, FMC Biopolymer, USA, with mean particle size of 130 μm) and magnesium stearate (MgSt; Sigma–Aldrich, Germany). Prior to experimentation, all the materials were passed through a 355 μm aperture size sieve in order to break up any loose aggregated lumps in the bulk powders, and stored for at least 48 h at 25 °C and 50% relative humidity.

2.2. Bin blender and NIR instrumentation

An optical head comprising a sapphire window was mounted on the lid of the IBC blender (SP15, GEA Pharma Systems, UK) for NIR spectral acquisition (MCS 611 NIR 2.2 spectral sensor, Carl Zeiss, Germany) (Fig. 1) of 980–2100 nm, with 1 nm interval. Raw energy spectra were obtained from the light signals from the optical head using the spectral sensor and transferred using radio frequency to the microprocessor using the Aspect Plus (version 1.76, Carl Zeiss, Germany) and Process Explorer (version 1.1.0.6, Carl Zeiss, Germany) softwares.

2.3. Design of experiment

Preliminary experiments were carried out to examine the effect of sampling strategies on the NIR spectral features of the powder with respect to blender speed and state of the powder during spectral acquisition. In order to eliminate the spectral differences due to complexity of texture in multi-component blends, a single component powder system of inhalation grade lactose, Inhalac-230, was used. Inhalac-230 has an added advantage of narrow size distribution. The IBC was filled to approximately 60% (v/v) of its capacity and rotated at 10 and 15 rpm for 5 min. For each run, the IBC was

Table 1
D-optimal mixture design for the blend experiments.

Run	Blend components (%, w/w)			
	CPM	Lactose	MCC	MgSt
1	6	52.5	40	1.5
2	2	77.5	20	0.5
3	2	77.5	20	0.5
4	2	56.5	40	1.5
5	6	72.5	20	1.5
6	2	76.5	20	1.5
7	2	76.5	20	1.5
8	6	53.5	40	0.5
9	2	57.5	40	0.5
10	2	57.5	40	0.5
11	6	73.5	20	0.5
12	6	73.5	20	0.5
13	6	53	40	1
14	6	73	20	1
15	6	62.5	30	1.5
16	6	63.5	30	0.5
17	2	57	40	1
18	2	66.5	30	1.5
19	4	54.5	40	1.5
20	4	55.5	40	0.5
21	4	74.5	20	1.5
22	4	65	30	1
23	4	65	30	1
24	4	65	30	1

Gray shades indicates the runs used for testing the prediction performance of the different calibration models. These runs were not included in model development.

filled with fresh Inhalac-230 powder and NIR spectra were collected in real time. The static spectra of the Inhalac-230 powder were also acquired at the start and end of the blending run, and were averaged for further analysis. D-optimal mixture design (Table 1) was used to obtain the blending runs for generation of calibration models. All the blending runs were performed in the IBC blender at 10 rpm speed for 30 min.

2.4. Calibration model development

Out of 24 runs generated using D-optimal mixture design, runs 3, 10 and 23 were excluded from the calibration model development; instead, they were used as independent blend runs to test the prediction abilities of the developed calibration models. Three different strategies were used to obtain the spectral data for calibration models as described below.

2.4.1. Calibration models based on static spectral acquisition after laboratory mixing

Mixtures with respective quantities of CPM, lactose, MCC and MgSt for each calibration run were prepared in a batch size of 100 g. CPM and lactose were first premixed in a 250 mL glass bottle for 5 min using a vortex mixer (Ika, VX9/VXR, Germany). MCC and MgSt were then added to this premix and again mixed for 25 min by tumbling. These mixtures were then subdivided into 8 equivalent portions (each portion approximately 12 g) using a riffler (Retsch, Germany) and scanned by pouring onto the optical head. Each portion was scanned five times and thus, for one calibration mixture, a total of 8×5 or 40 spectra were acquired. These 40 spectra were averaged and the resultant average spectrum was used as a representative spectrum for the respective calibration mixture. Adequacy of the mixing condition was confirmed by exhibition of minimal variances of NIR spectra of the subdivided portions.

2.4.2. Static and dynamic calibration models based on spectral acquisition in the IBC

Top bottom loading pattern was used while charging the blend components into the IBC. For each run, the lactose load was divided into two equal portions. Blend components were placed in layers: lactose, drug, MCC, MgSt and lactose. The batch size of each run was 5 kg, occupying about 60% of the IBC volume. All the blending runs in the IBC were conducted for 300 rotations and NIR spectrum was captured with every rotation of the IBC.

Each blending run was divided into 15 equal segments; the blender was stopped after every 20 rotations to collect the static spectral data of the blend in the IBC. In-line spectral acquisition was carried out when the blender was in motion to obtain the dynamic spectral data of the blend in the IBC. Thus, 30 calibration models (15 from static spectral acquisition and 15 from dynamic spectral acquisition) were generated for each component per run of the D-optimal runs used for calibration model development. The reference values used for calibration runs (for both laboratory and IBC mixed blends) were calculated gravimetrically from the actual weights of the calibration blends (% ingredient $A = 100 \times$ weight of ingredient A/total weight of mixture). The calibration statistics used to decide on the performance of the models were the number of principal components (PC), X and Y explained variances, R^2 and root mean square error of prediction (RMSECV) obtained after leave one out full cross-validation.

2.5. In-line quantification of blend components

The optimal calibration models for different blend components were uploaded into the Process Explorer using the On-line Unscrambler Predictor (OLUP) software. OLUP packaged Unscrambler calibration models into a dynamic link library (DLL, 32 bit only) protocol. Through these protocols, Process Explorer was interfaced with the OLUP for obtaining in-line quantification of each of the blend components.

2.6. Chemometric data pre-processing

Chemometric data pre-processing was performed using Unscrambler 9.8 (Camo Inc., India). The spectra obtained were smoothed using moving average smoothing with segment size of three and pre-processed with standard normal variate (SNV) followed by 1st derivative employing nine smoothing points and 2nd polynomial order. Partial least square (PLS1) regression method was then used to build the calibration models for each component.

3. Results and discussion

The purpose of building MVC models is to determine the chemical concentration of the components of the formulation from corresponding NIR spectral data. This is usually achieved by obtaining calibration samples that covers the anticipated formulation variables. Such information in combination with their corresponding reference values are then employed to develop models for future predictions. In this investigation, the main thrust was to examine if sampling strategies influence the NIR spectral features and prediction performance of the MVC models. The spectra used for building the calibration models were pre-processed using SNV followed by first derivative technique. This spectral pre-processing was preferred based on the previous results obtained in our laboratory (Liew et al., 2010) to remove unwanted scattering features incorporated in NIR spectra which are often due to the differences in the size of the constituent particles and other interfering factors which do not provide any information about the chemical concentration of the analyte of interest.

3.1. Effect of sampling strategies on NIR spectra

Careful examination of the Inhalac-230 NIR spectra obtained using different strategies showed the exhibition of spectral differ-

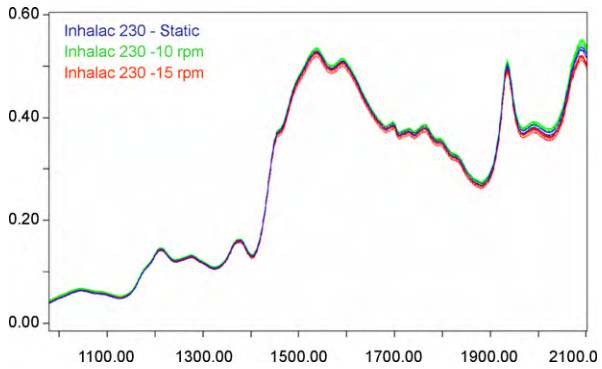

Fig. 2. Raw spectra of Inhalac-230 depicting spectral differences observed between the sampling strategies. In these plots, *X* and *Y* axes represent the wavelength (nm) and absorbance, respectively.

ences as illustrated in Fig. 2. Spectral differences were quite distinct towards the high wavelength region. Principal component analysis of the spectra obtained at three different modes was performed. Fig. 3 shows the three dimensional scores plot of the three spectral sets and reveals three distinct clusters within the scores plot. Furthermore, the spectra obtained in dynamic manner were found to be closer to one another than to the statically acquired spectra. The reason for the observed difference could be due to the difference in sample presentation for the dynamic and static samples. Considering the spectral differences between dynamic and static spectra, it was imperative to further investigate the effect of sample presentation strategies on prediction performance of the calibration models.

3.2. Effect of sampling strategies on calibration statistics

D-optimal mixture design was adopted in the present study to impart formulation variability to the calibration samples. Moreover, calibration samples were obtained from the actual process. Thus, an attempt was made to produce samples which were relevant, representative and encompassed the anticipated combination of blend components during the actual process. Considering the complexity of the formulation blend, PLS-1 regression technique was preferred over the multiple linear regression technique for building calibration models using spectra obtained by all three strategies. Raw NIR spectra of 980–2100 nm wavelength range corresponding to the calibration samples obtained were pre-processed with SNV followed by 1st derivative Savitzky–Golay using nine points. Spectral pre-processing was carried out to remove NIR spectral artifacts arising from differences in physical properties of blend components (Bellamy et al., 2008). PLS-1 calibration models were built to correlate the blend components with the NIR spectra. Leave one out full cross-validation was performed to determine the number of principal components required to minimize the standard error of prediction (SEP). Statistics obtained for calibration models with PLS-1 regression method are summarized in Tables 2–4. From the results, it can be seen that the laboratory mixed models were less accurate compared to static models in terms of their prediction performance; the number of principal components and RMSECV values obtained were much higher than those of the IBC models for all the blend components. Improved calibration statistics with the IBC models could be attributed to more efficient mixing of the cohesive blend in an IBC. The IBC used in this study has an added advantage of the prism attachment which actually increases the rate of powder turnover and occurrences of failure regions because of its divisive action which is favorable for rapid attainment of uniformity (Liew et al., 2010; Sudah et al., 2002; Castellanos et al., 1999). Between the static and dynamic IBC models, dynamic models showed further improvement in calibration statistics. These results could be explained in the context of addition of high degree of variability in the calibration models as the spectra were acquired in real time. Based on the statistics obtained with IBC based models, calibration models of each component were ranked and those with the least error of prediction and at the same time, larger R^2 values were identified as ideal models and further used for prediction of the independent runs (3, 10 and 23). The models with the bold statistics in Tables 3 and 4 were identified as ideal calibration models.

Table 2
Statistics obtained for calibration models of each blend component with PLS1 regression method.

	PC	*X*	*Y*	R^2	RMSECV
CPM	7	98.64	96.97	0.969	0.519
Lactose	3	98.31	98.28	0.983	1.580
MCC	2	95.98	96.92	0.969	1.608
MgSt	5	98.31	98.40	0.984	0.067

X and *Y* represent the *X* and *Y* explained variances (%) by the respective principal components.

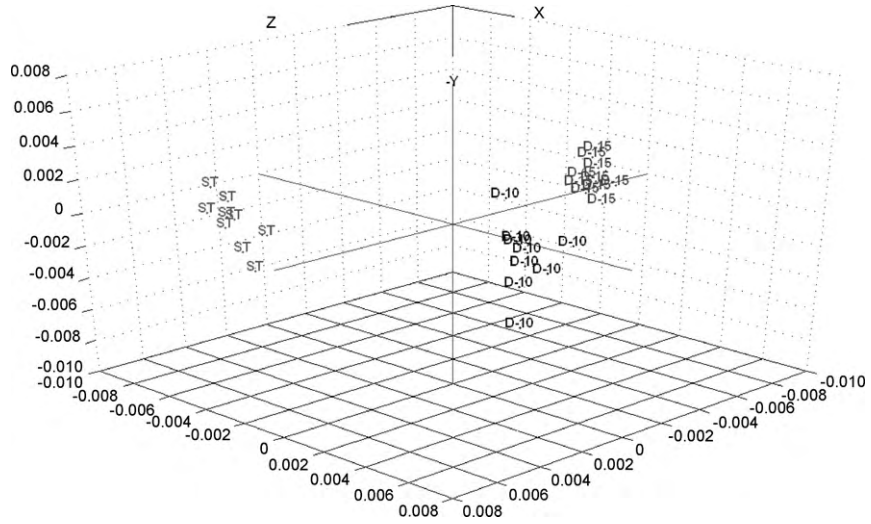

Fig. 3. 3D scores plot for the spectra obtained with different spectral acquisition modes after PCA. ST represents spectra acquired when the IBC was in static position; D-10 and -15 represent the spectra obtained when the IBC was rotated at the speed of 10 and 15 rpm, respectively.

Table 3
Statistics obtained for calibration models of CPM, lactose, MCC and MgSt based on static spectral acquisition in IBC with PLS1 regression method.

Time (min)	CPM					Lactose					MCC					MgSt				
	PC	*X*	*Y*	R^2	RMSECV	PC	*X*	*Y*	R^2	RMSECV	PC	*X*	*Y*	R^2	RMSECV	PC	*X*	*Y*	R^2	RMSECV
2	3	87.36	90.53	0.91	0.70	3	91.49	90.53	0.91	3.72	4	95.16	91.95	0.92	3.72	2	87.98	54.21	0.54	0.34
4	4	96.55	90.13	0.90	0.81	3	94.03	94.61	0.95	2.97	4	96.5	90.01	0.90	4.10	3	93.42	67.06	0.67	0.35
6	6	97.79	98.04	0.98	0.92	3	95.49	91.04	0.91	3.57	3	94.28	95.71	0.96	2.65	3	88.09	62.38	0.62	0.40
8	6	97.88	99.1	0.99	0.65	4	96.35	94.21	0.94	3.69	3	96.54	93.41	0.93	2.93	4	96.58	93.84	0.94	0.17
10	**5**	**97.59**	**99.48**	**0.99**	**0.32**	2	95.31	91.93	0.92	2.90	2	94.20	87.51	0.88	3.54	4	97.1	86.19	0.86	0.32
12	5	97.85	99.18	0.99	0.33	3	96.03	95.65	0.96	2.66	1	86.59	92.74	0.93	2.55	4	97.27	92.01	0.92	0.23
14	6	97.38	97.93	0.99	0.49	6	98.48	99.56	0.99	2.24	3	96.23	96.05	0.96	2.41	4	97.26	89.33	0.89	0.26
16	4	97.34	96.81	0.97	0.68	5	98.07	98.92	0.99	2.12	3	96.66	95.51	0.96	2.61	4	97.63	97.16	0.97	0.12
18	6	94.1	99.7	0.99	0.62	5	97.95	99.16	0.99	2.04	5	98.08	99.3	0.99	2.12	4	96.87	90.39	0.90	0.24
20	4	96.78	96.74	0.97	0.54	4	97.38	97.71	0.98	2.32	**4**	**97.5**	**99.01**	**0.99**	**1.79**	4	92.31	96.04	0.96	0.23
22	4	97.44	97.09	0.97	0.60	4	96.87	97.77	0.98	2.48	4	97.65	98.27	0.98	1.94	7	98.89	99.83	0.99	0.09
24	5	98.1	98.34	0.98	0.54	5	97.25	98.92	0.99	2.56	3	96.85	96.96	0.97	2.23	6	98.17	99.57	0.99	0.11
26	4	96.54	97.02	0.97	0.58	4	96.32	97.19	0.97	3.17	3	96.28	96.22	0.96	2.40	4	96.69	98.84	0.99	0.10
28	5	97.37	98.8	0.99	0.47	4	96.89	95.84	0.96	3.29	3	95.89	96.23	0.96	2.70	**5**	**98.41**	**99.24**	**0.99**	**0.10**
30	4	97.4	96.77	0.97	0.62	**4**	**97.68**	**98.38**	**0.98**	**1.93**	4	97.68	98.6	0.99	1.73	4	97.33	98.65	0.99	0.10

X and *Y* represent the *X* and *Y* explained variances by the respective principal components. Models with the bold statistics indicate the best models selected for testing the independent test blends.

Table 4
Statistics obtained for calibration models of CPM, lactose, MCC and MgSt based on dynamic spectral acquisition in IBC with PLS1 regression method.

Time (min)	CPM					Lactose					MCC					MgSt				
	PC	*X*	*Y*	R^2	RMSECV	PC	*X*	*Y*	R^2	RMSECV	PC	*X*	*Y*	R^2	RMSECV	PC	*X*	*Y*	R^2	RMSECV
2	4	94.68	87.83	0.88	0.97	4	93.22	92.38	0.92	4.08	3	92.72	87.42	0.87	4.06	2	89.58	80.01	0.80	0.24
4	3	95.88	93.62	0.94	0.58	3	93.17	96.33	0.96	2.37	3	93.3	96.25	0.96	2.38	3	90.85	84.54	0.85	0.22
6	3	96.44	96.72	0.97	0.41	3	95.14	98.23	0.98	1.57	4	97.49	99.15	0.99	1.15	3	92.13	79.56	0.80	0.25
8	2	95.29	96.69	0.97	0.39	4	97.71	98.54	0.99	1.55	4	97.74	98.8	0.99	1.35	5	98.15	98.64	0.99	0.09
10	3	96.02	98.42	0.98	0.31	4	97.27	98.67	0.99	1.63	4	97.33	98.83	0.99	1.47	5	98.2	98.62	0.99	0.10
12	5	98.11	99.5	0.99	0.25	4	97.31	98.02	0.98	2.09	4	97.72	99.22	0.99	1.25	5	98.01	98.79	0.99	0.09
14	5	97.97	99.6	0.99	0.25	4	97.61	99.28	0.99	1.29	4	97.34	98.25	0.98	1.95	5	98.13	99	0.99	0.09
16	**4**	**97.32**	**99.77**	**0.99**	**0.18**	4	97.49	99.06	0.99	1.38	4	97.66	99.34	0.99	1.18	5	98.1	98.96	0.99	0.09
18	4	97.00	99.53	0.99	0.21	4	97.56	99.24	0.99	1.25	**4**	**98.42**	**99.3**	**0.99**	**1.02**	5	98.12	99.18	0.99	0.09
20	3	96.05	97.76	0.98	0.37	**4**	**98.49**	**99.05**	**0.99**	**1.22**	4	96.52	99.37	0.99	1.09	5	97.99	99.03	0.99	0.09
22	4	97.19	98.81	0.99	0.34	2	94.78	98.29	0.98	1.37	5	97.9	99.62	0.99	1.01	6	98.79	99.67	0.99	0.07
24	4	96.94	98.63	0.99	0.34	4	97.03	99.18	0.99	1.26	4	97.09	99.41	0.99	1.12	6	98.86	99.76	0.99	0.06
26	3	96.19	98.28	0.98	0.30	4	96.41	99.16	0.99	1.45	5	96.97	99.69	0.99	1.09	6	98.68	99.76	0.99	0.06
28	3	95.96	98.07	0.98	0.32	4	96.8	98.9	0.99	1.50	4	97.6	98.78	0.99	1.59	**6**	**98.63**	**99.73**	**0.99**	**0.05**
30	4	97.4	96.77	0.97	0.62	4	97.68	98.38	0.98	1.93	4	97.68	98.6	0.99	1.73	4	97.33	98.65	0.99	0.10

X and *Y* represent the *X* and *Y* explained variances by the respective principal components. Models with the bold statistics indicate the best models selected for testing the independent test blends.

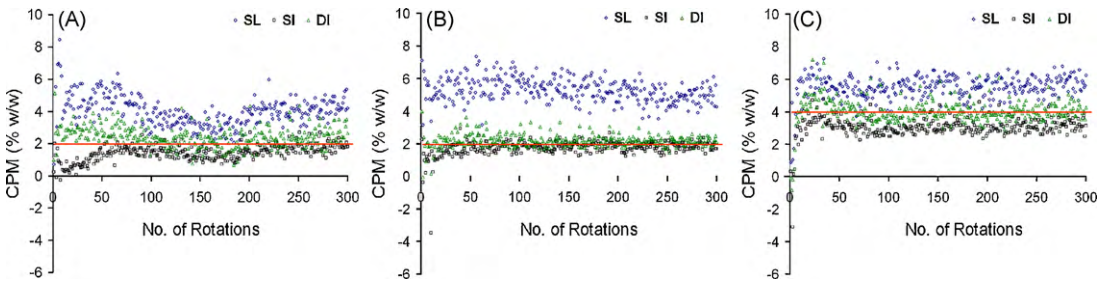

Fig. 4. Prediction results of CPM obtained using different strategies (A) run-03, (B) run-10 and (C) run-23 with laboratory mixed static (SL), IBC mixed static (SI) and IBC mixed dynamic (DI) MVC models. Red line, represents the target concentration of the respective component (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

3.3. Effect of sampling strategies on prediction performance of calibration models

The D-optimal runs, 3, 10 and 23, were used to test the prediction performance of the models built using the different strategies. The optimized PLS-1 calibration models obtained from the three different strategies were used to predict the concentration of blend components in real time during the blending process. The real time prediction of each of the blend components are depicted in Figs. 4–7. Prediction results obtained in real time showed some interesting findings.

3.3.1. Prediction results of CPM

Laboratory mixed static models resulted in predictions of CPM content that were significantly higher than the target/expected values. Calibration models prepared using blend samples within the IBC resulted in desirable prediction results. Prediction results obtained with the dynamic model exhibited low variations and were very close to the target values of the test formulations. The possible attributes behind these results could be the differences in the sample presentation during calibration model preparation and real time blending experiments. The calibration samples used for building the dynamic calibration models were exactly the same as that of real time blending experiments whereas static IBC calibration samples had only resemblance of being blended in the same blender except that the spectral acquisition was carried out when the blender was in static position. In contrast, the calibration models obtained using laboratory mixed calibration samples did not exhibit much similarity, apart from being of the same chemical composition.

3.3.2. Prediction results of lactose

Static calibration models obtained using laboratory mixed calibration samples resulted in under predictions of lactose content for all the three runs. Moreover, the results were highly variable, and the degree of variability worsened with the increase in concentration of lactose in formulation. Dynamic IBC models showed the best prediction performance followed by static IBC. Unlike CPM and MgSt, lactose prediction results were quite consistent and occurrences of aberrant prediction values were seldom observed. This could be attributed to its presence as a major component (52.5–77.5%, w/w) in the bulk powder blend at the calibration and

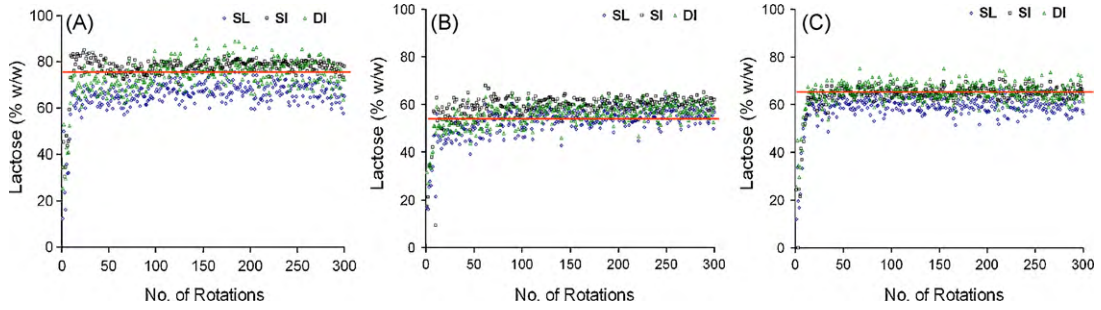

Fig. 5. Prediction results of lactose obtained using different strategies (A) run-03, (B) run-10 and (C) run-23 with laboratory mixed static (SL), IBC mixed static (SI) and IBC mixed dynamic (DI) MVC models. Red line, represents the target concentration of the respective component. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

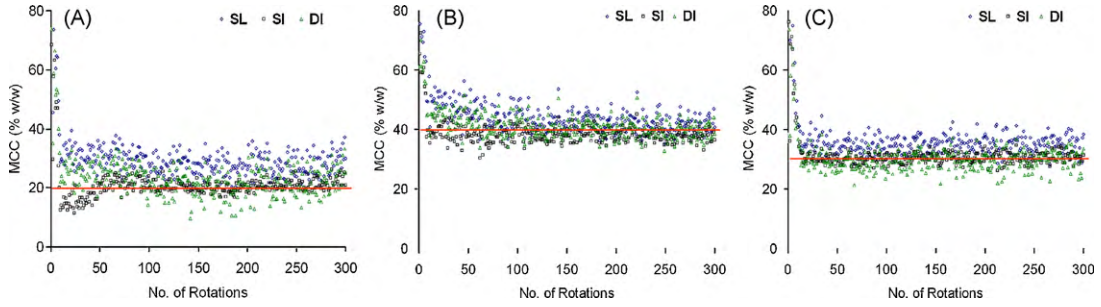

Fig. 6. Prediction results of MCC obtained using different strategies (A) run-03, (B) run-10 and (C) run-23 with laboratory mixed static (SL), IBC mixed static (SI) and IBC mixed dynamic (DI) MVC models. Red line, represents the target concentration of the respective component. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

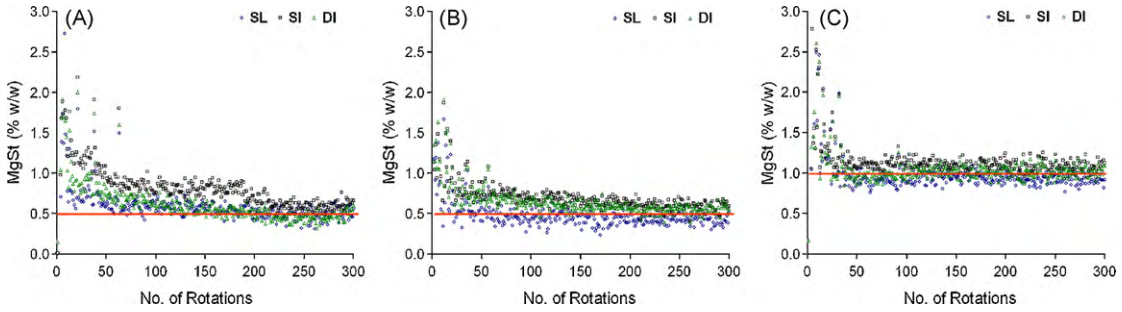

Fig. 7. Prediction results of MgSt obtained using different strategies (A) run-03, (B) run-10 and (C) run-23 with laboratory mixed static (SL), IBC mixed static (SI) and IBC mixed dynamic (DI) MVC models. Red line, represents the target concentration of the respective component. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

prediction stages which had enabled the blend to achieve uniform texture (homogeneity) rapidly.

3.3.3. Prediction results of MCC

In contrast to the CPM and lactose results, predictions results obtained with dynamic models for MCC were highly variable. The reason for these observed results could be the highly irregular morphology of the MCC particles. MCC particles are essentially highly irregular shaped fibrous particles and are known to produce high scattering effect. This could be further confirmed from the trend observed with the concentration and variability of MCC predictions in all the three runs. In addition to this, run-03 with lower concentration of MCC exhibited high variations in prediction results. These variations were attributed to the non-uniform texture of the powder blend falling on the sensor at every rotation rather than the low amount of MCC in the formulation *per se*. However, when the MCC concentration was increased to some extent, the variability decreased because of the more uniform texture of the powder blend. This improvement was quite clear with the consistent prediction results obtained using the laboratory mixed static models which could be due to static powder on the NIR sensor.

3.3.4. Prediction results of MgSt

In the case of MgSt, little difference was observed between the prediction performances of the three models. The improved performance of MgSt models could be attributed to the tendency of MgSt particles to form thin adhesive film over the coarse excipients particles, ultimately undergoing rapid ordered mixing (Liew et al., 2010). This ordered mixing actually nullified the effect of low concentration of MgSt and enabled its more uniform distribution throughout the powder bed.

4. Conclusion

This study demonstrated sampling paradox resulting from the different strategies of spectral acquisition while preparing and implementing the calibration models for prediction of blend components in a multi-component cohesive blend. Among the different strategies, dynamic sampling and analysis resulted in the best calibration and prediction results. For the static models, component concentrations of CPM and MCC were consistently predicted to be significantly higher than the actual concentrations in the target formulations. For lactose, static models resulted in under prediction of the concentration values than in the target formulations. Prediction results of MgSt were found to be the least affected irrespective of the differences in sampling strategies at calibration and real time process analyzes due to its fineness and adhesive property. In summary, prediction errors illustrate the importance of adopting appropriate and similar sampling strategies for both calibration and actual testing, i.e. real time blending process.

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