Research Article

Comparison of Univariate and Multivariate Models of ^{13}C SSNMR and XRPD Techniques for Quantification of Nimodipine Polymorphs

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Abstract. The focus of the present investigation was to explore the use of solid-state nuclear magnetic resonance (^{13}C ssNMR) and X-ray powder diffraction (XRPD) for quantification of nimodipine polymorphs (form I and form II) crystallized in a cosolvent formulation. The cosolvent formulation composed of polyethylene glycol 400, glycerin, water, and 2.5% drug, and was stored at 5°C for the drug crystallization. The ^{13}C ssNMR and XRPD data of the sample matrices containing varying percentages of nimodipine form I and form II were collected. Univariate and multivariate models were developed using the data. Least square method was used for the univariate model generation. Partial least square and principle component regressions were used for the multivariate models development. The univariate models of the ^{13}C ssNMR were better than the XRPD as indicated by statistical parameters such as correlation coefficient, R^2 , root mean square error, and standard error. On the other hand, the XRPD multivariate models were better than the ^{13}C ssNMR as indicated by precision and accuracy parameters. Similar values were predicted by the univariate and multivariate models for independent samples. In conclusion, the univariate and multivariate models of ^{13}C ssNMR and XRPD can be used to quantitate nimodipine polymorphs.

KEY WORDS: nimodipine polymorphs; X-ray powder diffraction; solid-state nuclear magnetic resonance; univariate; multivariate.

INTRODUCTION

Solid-state characterization of drug is very important, especially for polymorphs. The polymorphs differ in their physicochemical properties which may impact drug product performance. Commonly used techniques for the polymorphs characterization are near and mid/far infrared, Raman, differential scanning calorimetry (DSC), hot stage microscopy, powder X-ray diffraction (XRPD), and solid-state nuclear magnetic resonance (^{13}C ssNMR) (1–3). However, in order to understand the impact of the polymorphs present in the drug substance or formulations, it is paramount to quantify them. Often times, the polymorphs may change in a bulk drug substance or formulation due to processing or storage conditions. Generally, the DSC and XRPD are commonly used techniques for quantitative analysis of the polymorphs. However, the ^{13}C ssNMR can also be used for quantitative

analysis of the polymorphs. The polymorphs have different configuration and hence chemical environment. The ^{13}C ssNMR spectrum represents the finger-print of a material. It is likely that two polymorphs may have different spectra due to differences in chemical environment (4–6). Similar to the XRPD, the ^{13}C ssNMR has advantage of being non-destructive. However, it takes longer time to collect data; instrument is expensive and requires expertise to collect and analyze the data (7).

Nimodipine belongs to a calcium channel blockers pharmacological class. Originally, it was developed for lowering blood pressure but now a days used primarily for the treatment of ischemic neurological deficits caused by spasms of cerebral vessels after subarachnoid hemorrhage (8). However, its oral bioavailability is dependent upon aqueous solubility and dissolution because it belongs to biopharmaceutical class II (9). Bioavailability is further compounded by existence of two polymorphic forms, namely, metastable form I (modification 1) and stable form II (modification 2, conglomerate) (10,11). Both forms differ in their physicochemical properties. The aqueous solubility of form I is twice that of form II and can transform to form II at room temperature (10). Because of these reason, it is commercially available in a solubilized dosage form (cosolvent) as a soft gelatin capsule (8). However, there is always a possibility of drug recrystallization in a cosolvent formulation if not properly formulated, change in manufacturing process, or low temperature storage during use or transport. Class I recall was issued to

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Fig. 1. a ^{13}C NMR spectra and b XRPD diffractograms of nimodipine form I and form II

nimodipine generic product due to the drug crystallization in the cosolvent formulation. The recrystallized drug could be form I or form II. The drug may not be bioavailable, and hence, effectiveness and safety may be compromised (12). Our research group published on the root cause of nimodipine recrystallization in the formulation. Findings indicated that poorly formulated and low storage conditions promote drug crystallization (13,14). We also quantitated the polymorphs by spectroscopy (infrared, near infrared, and Raman) and thermal methods (15,16). However, no one reported the use of the ^{13}C ssNMR and XRPD univariate and multivariate models in the quantification of nimodipine polymorphs to the best of our knowledge. The objective of the present investigation was to compare the ^{13}C ssNMR- and XRPD-based univariate and multivariate models in quantification of the polymorphs and validate the models by the known sample and drug recrystallized from the cosolvent formulation.

MATERIALS AND METHODS

Materials

Nimodipine was obtained from LEAPChem (Hangzhou, China), and Super Refined® polyethylene glycol (PEG) 400 NF was purchased from CRODA (Edison, NJ). Ethanol and glycerol were purchased from Fisher Scientific Co. (Norcross GA). All other chemicals and solvents used were of analytical grade.

Methods

Preparation of Cosolvent Formulation

Nimodipine (2.5%) was dissolved in a mixture of PEG 400 (40% w/w) and glycerol (30% w/w) by sonication for 15 min. Water (30% w/w) was added to resultant solution. Final formulation was kept at 5°C for 4 weeks to allow dissolved drug to

Fig. 2. a ^{13}C NMR spectra and b XRPD diffractograms of sample matrices

Fig. 3. Univariate models calibration plots of **a** ^{13}C ssNMR and **b** XRPD data of sample matrices

crystallize. The recrystallized drug was obtained by centrifugation at 4500 rpm for 30 min, filtered, and washed with water. It was air-dried for 24 h followed by vacuum drying at 30°C for another 24 h. The polymorph quantification in the recrystallized sample was done using the univariate and multivariate models.

Preparation of Nimodipine Polymorphs

The purchased nimodipine was form I, and form II was prepared according to the method reported in a literature (17). Nimodipine form I was dissolved in ethanol and the solvent was allowed to evaporate to dryness. Form I and form II were confirmed by FTIR, Raman, DSC, XRPD, and ^{13}C ssNMR before using them in sample matrix preparation. The polymorphs were mixed in various ratios to prepare the sample matrices containing 0–100% of form I or form II.

The samples matrices were mixed by manual shaking 50 times in transverse and longitudinal directions.

Solid-State Nuclear Magnetic Resonance

The ^{13}C ssNMR experiments were performed on a Varian VNMR 400 spectrometer (Agilent, Santa Clara, CA) at 75 MHz using a Varian T3 narrow-bore double resonance probe fitted with a 4-mm PENCILTM module. Samples (approximately 45 mg) were packed into zirconia rotors and sealed with Teflon end caps. Spectra were acquired using cross-polarization (CP) (18,19) magic-angle spinning (MAS) (20) at a rate of 7 kHz. 3-Methylglutaric acid was used to optimize the spectrometer settings and set the reference frequency (21). The ^{13}C ssNMR experiments were carried with a 90° proton pulse, 12.5-s recycle delay (spin-lattice relaxation, ^1H T_1 of form I and form II were 2.5 s and 2.2 s,

Table I. Univariate and Multivariate Models Statistical Parameters of XRPD and ^{13}C ssNMR Data

Data	Model method	Component	Model	Sample no.	Slope	Offset	Correlation	R^2	RMSE	SE	Bias
XRPD	PLSR	Form I	Calibration	36	0.999	0.042	0.9996	0.9992	0.920	0.933	-3.709e-07
			Validation	30	1.007	-0.005	0.9994	0.9989	1.264	1.254	0.278
		Form II	Calibration	36	0.999	0.042	0.9996	0.9992	0.920	0.933	5.828e-07
			Validation	30	1.007	-0.740	0.9994	0.9989	1.264	1.254	-0.278
	PCR	Form I	Calibration	36	0.999	0.055	0.9995	0.9989	1.051	1.066	8.477e-07
			Validation	30	1.009	-0.108	0.9996	0.9991	1.102	1.088	0.266
		Form II	Calibration	36	0.999	0.055	0.9995	0.9989	1.051	1.066	-4.768e-07
			Validation	30	1.009	-0.877	0.9996	0.9991	1.102	1.088	-0.266
	Univariate	Form I	Calibration	10	0.009	0.069	0.9857	0.9716	14.378	15.156	-1.243e-15
		Form II	Calibration	10	0.009	-0.013	0.9857	0.9716	14.378	15.156	2.842e-15
^{13}C ssNMR	PLSR	Form I	Calibration	6	1.000	0.014	0.9998	0.9997	0.597	0.654	1.907e-06
			Validation	4	0.977	0.116	0.9962	0.9911	2.935	3.147	-1.088
		Form II	Calibration	6	1.000	0.016	0.9998	0.9997	0.597	0.654	-1.907e-06
			Validation	4	0.977	2.23	0.9962	0.9911	2.935	3.147	1.088
	PCR	Form I	Calibration	6	0.999	0.026	0.9997	0.9994	0.811	0.888	1.272e-06
			Validation	4	0.978	0.006	0.9961	0.9909	2.967	3.179	-1.106
		Form II	Calibration	6	0.999	0.029	0.9997	0.9994	0.811	0.888	-6.358e-07
			Validation	4	0.978	2.164	0.9961	0.9909	2.967	3.179	1.106
	Univariate	Form I	Calibration	8	0.010	-0.024	0.9995	0.9991	2.123	2.269	-8.882e-16
		Form II	Calibration	8	0.010	0.003	0.9995	0.9991	2.123	2.269	-3.775e-15

Fig. 4. Residual plots of **a** ^{13}C ssNMR and **b** XRPD univariate models

respectively, and recycle delay was five times the ^1H T_1 of form I), 4-ms contact time, and 5000 scans (total acquisition time 17.5 h). Single spectra of all the samples were recorded at an ambient temperature.

X-ray Powder Diffraction

The XRPD patterns of individual polymorph, sample matrices, and recrystallized sample were collected using a Bruker D8 Advance DaVinci diffractometer (Bruker AXS, Madison, WI) equipped with the LYNXEYE scintillation detector and Cu K α radiation ($\lambda=1.5405\text{\AA}$) at a voltage of 40 kV and a current of 40 mA. Corundum was used as an external standard to calibrate the XRPD instrument. Powder sample equivalent to 100 mg was used for data collection, and six replicates were obtained for each sample matrices. Weighed powder

was placed in a sample holder, pressed using zero diffraction plate, and scanned over 2θ range of $4\text{--}40^\circ$ with a step size of 0.0103° at 1 s per step (3497 total steps). Samples were rotated at 15 rpm during measurements to get average diffractogram of the samples. The XRPD data collection was achieved through Diffract.Suite V2.2 (Bruker AXS, Madison, WI).

Statistical Analysis

Diffract.Evaluation V2.0 (Bruker AXS, Madison, WI) was used to analyze and calculate net area of the peaks. In the case of ^{13}C ssNMR, line fitting and analysis of the spectra were performed using MNovo version 8.1.4 (Mestrelab Research, Escondido, CA). UnscramblerX software (version 10.1, Camo Process, Oslo, Norway) was used for the multivariate data analysis. Numbers of latent variables (LV) or factors or principle component (PC) are

Fig. 5. Score plots of **a** PLSR and **b** PCR models of ^{13}C ssNMR, and **c** PLSR and **d** PCR models of XRPD data

very critical for PLSR (partial least squares) and PCR (principle component) regression model development. Selecting too many or few LV results in over or under fitting of the model, and hence impacts performance of the model. The optimum number of LV was determined by the lowest value of RMSECV (root mean squared error of cross validation) using “leave one batch out” method. Correlation coefficient, R^2 , RMSEC, RMSEP (root mean square error of calibration and prediction), and SEC and SEP (standard error of calibration and prediction) were used to evaluate performances of various models.

RESULTS AND DISCUSSION

Polymorphs Characterization

Solid-State Nuclear Magnetic Resonance

FTIR, Raman spectra, and DSC thermograms of form I and form II concurred with previously reported data (not shown)

(13). Because of different arrangement of atoms in a molecule due to polymorphism or, alternatively, different electronic milieu around a nucleus, the resonant frequency or chemical shift of the nuclei varies. The ^{13}C ssNMR spectra of form I and form II are shown in Fig. 1a which concurred with literature (11). The spectra of form I and form II were referenced to 40.81 ppm (peak due to quaternary carbon of the nitrogen ring, b). Spectra of both forms were similar; however, there were some clear differences in peak positions or chemical shifts, especially, in aliphatic and aromatic regions of the spectra. For example, the peaks for the several methyl groups (a) in the form I were very distinct and present between 17.02 and 22.27 ppm. On the other hand, form II showed overlapped peaks of methyl groups and was present between 19.33 and 20.94 ppm. Other noticeable differences were observed for methoxy, methylene, quaternary carbon, and ester groups. The methoxy ($-\text{OCH}_3$, c), methylene (d), and quaternary carbon (e) groups were observed at 57.42, 61.68, and 71.90 ppm and 58.96, 64.00, and 69.40 ppm for form I and form II, respectively. Four quaternary carbon of nitrogen (f)

Fig. 6. Loading plots of PLSR models of **a** ^{13}C ssNMR and **b** XRPD data

showed two peaks at 101.31 and 103.49 ppm, and 101.49 and 104.59 ppm for form I and form II, respectively. Single peak for ester group (h) was showed at 166.14 ppm for form I. On the other hand, form II showed two distinct peaks at 166.07 and 167.45 ppm for the ester groups. However, both forms showed peaks for benzene ring carbons (g) between 122 and 152.33 ppm. As expected, the sample matrices showed additive spectra of both the polymorphs (Fig. 2a).

X-ray Powder Diffraction

Diffraction patterns of nimodipine polymorphs are shown in Fig. 1b. Similar to the ^{13}C ssNMR, the XRPD of the form I and form II showed differences. Unlike the ^{13}C ssNMR, most of the peaks of the form I and form II were overlapping with the adjacent peaks. Nonetheless, there were some unique peaks present in both the polymorphs that can be used to distinguish between them. The unique peaks of the form I and form II were observed at 6.55° , 12.89° , and 17.37° and 10.32° , 11.96° , 15.23° , 15.90° , 20.76° , and 26.94° , respectively (Fig. 1b). These observations were in concurrence with reported values (17,22–24). Furthermore, the sample matrices that contained varying percentage of form I and form II showed the peaks of both the polymorphs (Fig. 2b).

Quantification Models

Univariate Models

Recycle delay of five times of ^1H T_1 was selected for collecting the ^{13}C ssNMR spectra of sample matrices. Two non-overlapping peaks for form I at 62.58 ppm and form II at 64.09 ppm, respectively, were selected for quantification model development as these peaks were distinct. The peak areas were determined by deconvolution method using MNova software. Area ratio for form I (62.58 ppm) and form II (64.09 ppm) were determined (area ratio=area of 62.58 ppm (form I) or 64.09 ppm

(form II) peak/total area of both peaks). Similar to the ^{13}C ssNMR data, the XRPD peaks for form I and form II were selected at 2θ 17.37° and 15.90° , respectively, for the univariate model development. Unlike the ^{13}C ssNMR, the peaks were overlapping with the adjacent peaks of form I or form II in the sample matrices of the XRPD data; however, overlap was minimal in the selected peaks compared to other peaks in the diffractogram. The net area of the selected peaks was calculated, and net area ratio was determined (net area ratio=net area of 17.37° (form I) or 15.90° (form II) peak/total net area of both peaks). The univariate models for ^{13}C ssNMR and XRPD were generated using least squares regression method between percentage of form I or form II and area or net area ratio. The results of univariate models are shown in Fig. 3 and Table I. The models showed linear relationship between form I or form II area ratio and their actual percentage for the ^{13}C ssNMR data as indicated by correlation coefficient of 0.9995 (Fig. 3a). However, the relationship was curvilinear, and correlation was not strong in the case of the XRPD models as suggested by correlation coefficient of 0.9857 (Fig. 3b). Coefficient of determination, R^2 , determines how well data fit into the statistical model and total variation explained by the model. The ^{13}C ssNMR models were better than the XRPD as indicated by R^2 . The variation explained by the models was 99.91% for the ^{13}C ssNMR data compared to 97.16% for the XRPD data. The predicted values of form I and form II were not close to actual values as indicated by residual plots and residual value as high as 9% were observed for the XRPD models (Fig. 4). The residual figure patterns for form I and form II were concave and convex, respectively, as these models were for binary mixture and two components of the models were interrelated. Root mean square (RMSE) and standard error (SE) values were low for the ^{13}C ssNMR compared to the XRPD models. This suggested that the ^{13}C ssNMR models have low error compared to the XRPD models. One of probable reason for poor fitting of the XRPD data was due to overlapping peaks, and the software that we used does not have capability to deconvolute the true area of the selected peaks.

Fig. 7. PLSR models calibration and prediction plots of **a** form I and **b** Form II of ^{13}C ssNMR, and **c** form I and **d** form II of XRPD data

Fig. 8. Residual plots of **a** ^{13}C ssNMR and **b** XRPD PLSR and PCR multivariate models

Multivariate Models

Data showed an increase/decrease in peaks intensity with an increase/decrease in percentage of form I/form II in the sample matrices (Fig. 2). The ^{13}C ssNMR and XRPD data from 15 to 168 ppm and 15.6 to 31.7° were used, respectively, for the multivariate models development due to presence of major peaks of the polymorphs in those regions. After qualitative data analysis, the PCR and PLSR multivariate models were built for both the ^{13}C ssNMR and XRPD data. Test method was used in which the data were divided into two sets. The first data set was used to build calibration model, and the other data set was used to validate the model (prediction model). Two LVs (PC or PLS) were selected based on lowest value of RMSECV and found to explain $\geq 98\%$ and $\geq 99\%$ variation in X and Y block of all the data, respectively, for the PLSR and PCR models. First, LV was found to explain maximum variation in the data, e.g., PC1 or PLS1 explained 94 and $>99\%$ variation in X and Y block, respectively, of the ^{13}C ssNMR and XRPD. Additionally, each PC or PLS is decomposed into score and loading plots. These plots work in harmony to identify the trend in the data and variable impacting them. Score plots can also be used for detecting outlier in the data. Each sample was distinctly located in the

score plots, and furthermore, replicate samples were grouped together which indicated absence of outlier (Fig. 5). The PLSR models score of ^{13}C ssNMR data showed an increase in PLS1 score number with an increase in form I percentage in the sample matrices (Fig. 5a). On the other hand, PC1 of the PCR models showed reverse trend as that of the PLSR models (Fig. 5b). Moreover, similar trend was observed for the PLSR and PCR models score plots of the XRPD data (Fig. 5c, d). This observation indicated that PLS1 or PC1 might be related to form I or form II percentage in the sample matrices. This can be confirmed by comparing PLS or PC loading plots with form I and form II spectra. PLS1 loading plot of the ^{13}C ssNMR PLSR models showed upward and downward peaks (Fig. 6a). The upward peak matched with form I while downward peaks matched with form II. On the other hand, PLS2 resembles with form II (Fig. 6a). Thus, PLS1 was related to form I and form II while PLS2 was related only to form II. Loading plots of the PCR models showed the same trend as that of the PLSR (figure not shown). Furthermore, loading plots of the PLSR and PCR models of the XRPD data showed the same pattern as that of the ^{13}C ssNMR models (Fig. 6b). The PLSR and PCR calibration models showed linear relationship between actual and predicted polymorphs percentage as indicated by correlation coefficient >0.996 for

Table II. Univariate and Multivariate Models Predicted Nimodipine Polymorphs Amount

Data	Model	Actual polymorphs (%)		Predicted polymorphs (%)	
		Form I	Form II	Form I	Form II
XRPD	PLSR	70%	30%	67.49±0.67	32.51±0.67
	PCR	70%	30%	67.46±0.59	32.54±0.59
	PLSR	Formulation		49.63±3.03	50.37±3.03
	PCR	Formulation		49.44±2.73	50.56±2.73
	Univariate	70%	30%	71.35±0.08	28.65±0.08
	Univariate	Formulation		53.93±0.22	46.07±0.22
^{13}C ssNMR	PLSR	70%	30%	67.49±1.97	32.51±1.97
	PCR	70%	30%	67.45±1.98	32.55±1.98
	PLSR	Formulation		43.41±3.74	56.59±3.74
	PCR	Formulation		43.01±3.77	56.99±3.77
	Univariate	70%	30%	70.07	29.93
	Univariate	Formulation		41.43	58.57

all the models (Fig. 7 and Table I). Additionally, residual values (the differences between actual and model predicted values) were small and showed random distribution which suggested good fit of the data (Fig. 8). However, residual values were higher in the models of ¹³C ssNMR data. The calibration models were evaluated by intercept, slope, RMSEC, and SEC. For ideal models, slope should be close to one and intercept close to zero. Slopes were close to one, and intercept, RMSEC, and SEC values were low for all the models. However, the values of these parameters were better for the XRPD models in comparison to the ¹³C ssNMR models. The calibration models were validated by data of samples matrices (predicted model) not used in the calibration models. It was assessed by slope, intercept, correlation coefficient, R^2 , RMSEP, and SEP. The predicted values of these parameters should be close to the calibration models for a good model. The slope, intercept, correlation coefficient, and R^2 of predicted models were close to calibration models. RMSEP and SEP measure the precision and accuracy of a calibration model. Values were close to the calibration models for the XRPD data. However, values of these parameters were higher for the ¹³C ssNMR models. This indicated a higher precision and accuracy for the XRPD models compared to the ¹³C ssNMR models. This was probably due to smaller number of samples used in the ¹³C ssNMR multivariate models and higher noise in the ¹³C ssNMR data compared to the XRPD.

Model Prediction

The performance of the univariate and multivariate models of the XRPD and ¹³C ssNMR was evaluated by plugging data of known and unknown samples. The known sample contained 70% form I and 30% form II, and unknown sample was crystallized drug from the prepared formulation kept at 5°C for 4 weeks. The model predicted values of form I and form II are shown in Table II. The univariate models of ¹³C ssNMR and XRPD predicted similar values for the known sample. Additionally, the univariate model predicted values for the known sample were very close to the actual value. In the case of multivariate models, the predicted values differed by 2.5% from the actual value. However, higher errors were observed in the ¹³C ssNMR multivariate model predicted values compared to the XRPD multivariate models as indicated by standard deviation. High noise in the ¹³C ssNMR data contributed to high error in the ¹³C ssNMR multivariate models. However, the univariate models showed different trend for the unknown sample. The ¹³C ssNMR and XRPD univariate model predicted values for the unknown sample differed by about 12.5%. The reasons for dissimilar values might be due to overlapping peaks observed in the XRPD diffractogram. On the other hand, the difference between the ¹³C ssNMR and XRPD multivariate models predicted values for the unknown sample was about 6%. Furthermore, the PLSR and PCR models predicted similar results for the known and unknown samples. However, greater differences were observed for the XRPD multivariate and univariate model predicted values for the known and unknown sample when compared to the ¹³C ssNMR models.

CONCLUSION

It is important to have a good quantitative analytical method to estimate the fractions of the polymorphs in a formulation to assess impact of the formulation, manufacturing, or storage conditions on the product performance. The developed univariate and multivariate models can quantitate the polymorphs in the mixture. The univariate and multivariate models of the ¹³C ssNMR data are similar as indicated by statistical parameter such as correlation coefficient and R^2 , and smaller values of RMSE and SE. On the other hand, multivariate models of XRPD are better than its univariate. Furthermore, the univariate models and multivariate models produced similar values for independent sample not used in the model building. We conclude that the ¹³C ssNMR univariate models perform equally well as the XRPD multivariate models. However, there are limitations for the ¹³C ssNMR, and the obvious one is longer time to collect data when compared to the XRPD which many investigators may find it difficult to use for routine analysis for quantitative purpose.

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