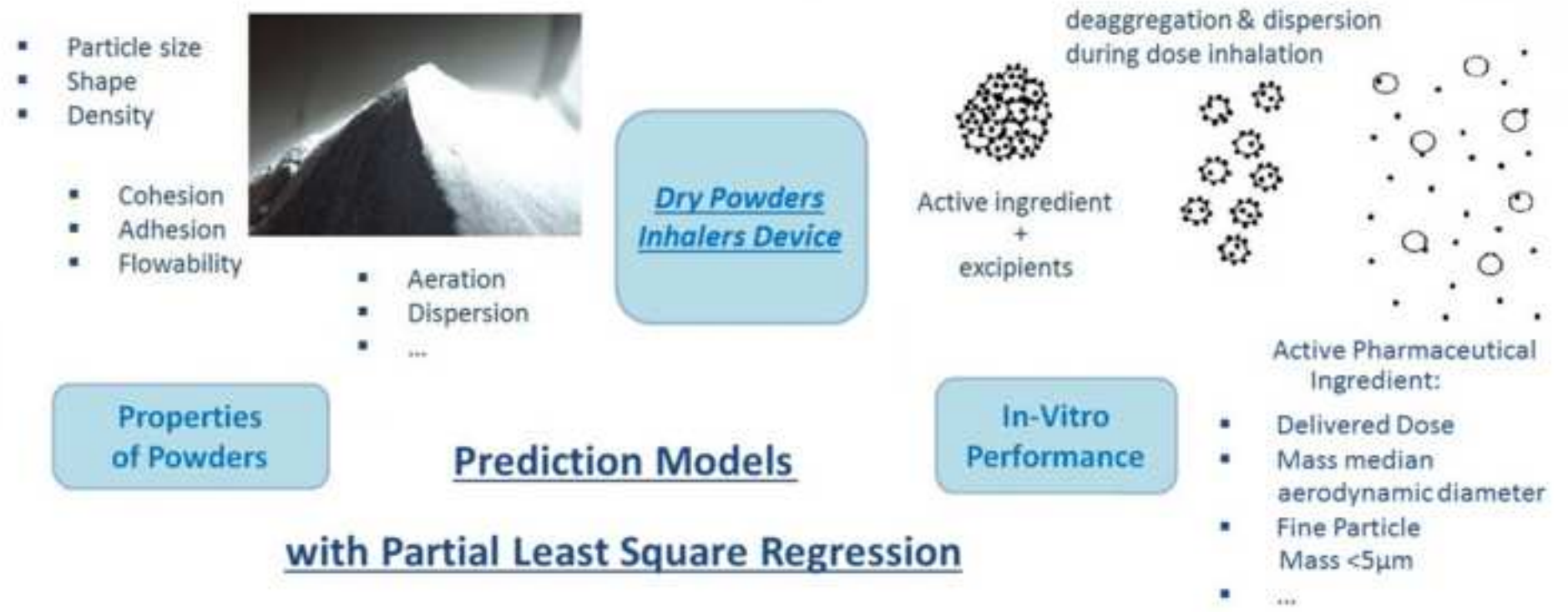
*Graphical Abstract (for review)



HIGHLIGHTS

Particle size distribution, density and flow properties have been used to characterize dry powder inhalers products

Multivariate Data Analysis provide understanding of correlation among powder properties and in-vitro performance

Predictive models of in-vitro performance allow estimation of DUSA and NGI performance

Abstract

The study aimed at investigating the correlations among the physical and bulk properties of carrier based dry powder inhaler formulations and the performance of the powder inhaler device estimated by in-vitro tests for a specific active pharmaceutical ingredient (API), and at obtaining predictive models for the in-vitro performance. Samples from scale-up process batches having different formulations, process settings and bulk size, were characterized by rheological, density and particle size tests. In vitro performance was evaluated by several parameters obtained by a dosage unit sampling apparatus (DUSA) and a next generation impactor (NGI). Correlations between powder properties and performance properties were established using partial least square regression (PLS) analysis. Variable importance in projection (VIP) was used in order to assess the most influential powder characterization variables to estimate the analytical ones. Particle size, density and rate of flowability are significant for modeling the delivered dose of the API and the total quantity of powder related to each dose. Powder characterization variables, describing the degree of cohesiveness and the flow properties of powder, are related to the total amount of the active ingredient for different formulations. DUSA test variables were satisfactory predicted on the basis of powder characterization variables, while NGI performance variables were predicted with higher error.

MULTIVARIATE DATA ANALYSIS TO ASSESS DRY POWDER INHALERS PERFORMANCE FROM POWDER PROPERTIES

Andrea Elia[a,b], Marina Cocchi[a], Ciro Cottini[b], Daniela Riolo[b], Claudio Cafiero[b], Roberto Bosi[b], Emilio Lutero[b].

[a]*Department of chemical and Geological Sciences, Via Campi 103, 41126 Modena, Italy, A.Elia@chiesi.com*

[b]*Chiesi Farmaceutici S.p.A., Dept. of Chemistry, Manufacturing & Control, Largo F. Belloli 11/A, 43122 Parma, Italy.*

Corresponding authors:

Marina Cocchi, *Department of chemical and Geological Sciences, Via Campi 103, 41126 Modena, Italy, marina.cocchi@unimore.it*

Ciro Cottini, *Chiesi Farmaceutici S.p.A., Dept. of Chemistry, Manufacturing & Control, Largo F. Belloli 11/A, 43122 Parma, Italy, C.Cottini@chiesi.com*

Abstract

The study aimed at investigating the correlations among the physical and bulk properties of carrier based dry powder inhaler formulations and the performance of the powder inhaler device estimated by in-vitro tests for a specific active pharmaceutical ingredient (API), and at obtaining predictive models for the in-vitro performance. Samples from scale-up process batches having different formulations, process settings and bulk size, were characterized by rheological, density and particle size tests. In vitro performance was evaluated by several parameters obtained by a dosage unit sampling apparatus (DUSA) and a next generation impactor (NGI). Correlations between powder properties and performance properties were established using partial least square regression (PLS) analysis. Variable importance in projection (VIP) was used in order to assess the most influential powder characterization variables to estimate the analytical ones. Particle size, density and rate of flowability are significant for modeling the delivered dose of the API and the total quantity of powder related to each dose. Powder characterization variables, describing the degree of cohesiveness and the flow properties of powder, are related to the total amount of the active ingredient for different formulations. DUSA test variables were satisfactory

predicted on the basis of powder characterization variables, while NGI performance variables were predicted with higher error.

Keywords

Dry powder inhaler; PLS regression; VIP; powder characterization tests; DUSA; NGI.

1. Introduction

Dry powder inhalers (DPI) are devices that deliver a dry powder formulation of drug to the lungs [1-3]. Their development was initially promoted as an alternative to pressurized metered dose inhaler (pMDI), following the Montreal Protocol [4] of the 1987, which provided for the non-use of ozone reducer propellants in medicinal products. In addition of being propellant free, DPIs possess higher stability with respect to the liquid state [4]. Moreover, DPI based on passive devices are directly activated by the patient's respiratory airflow, so provide a general optimization between actuation and inhalation [5-7]. DPI formulations are mainly mixtures of drug and coarse particles of lactose based excipients. α-lactose monohydrate sugar is the most used and FDA approved and it is used as the main excipient. It is able to fluidize and disperse the drug whose particles are in the breathable size range, while it is not delivered to the lungs. Other components, such as fine particle lactose and magnesium stearate, can be used in the formulation in order to optimize the de-agglomeration of drug particles from the large carrier particles. The active ingredient can be designed as a target for a generic or specific respiratory disease, such as asthma and COPD (chronic-obstructive-pulmonary-disease). It has to adhere to the carrier's surface during the manufacturing process and keep this status during the shelf-life of the product, but it has to de-aggregate during the delivery phase, to follow the inspiratory flow and to reach the deposition site. As powder is transported from the device to the lungs of the patient as an aerosol, it becomes important to understand how it flows under gravity when consolidated, unconsolidated, aerated or even fluidized, and how readily it will entrain air and release it again. Important powder properties include particle size, shape, density, cohesion, aeration, dynamic flow and shear properties [8]. Several studies have shown that physico-chemical carrier properties and cohesive-adhesive force balances between drug and carrier have an influence on the in-vitro aerosol deposition [9-10]. In particular, performance was optimized when the drug-carrier cohesion–adhesion balance ratio was slightly cohesive. Moreover, aerosol performance resulted dependent also on the device's

design and patient's inspiratory force. Devices with greater aerosol resistance resulted in greater FPF values. In this sense, tuning of the resistance to airflow in the design of a dry powder inhaler may improve the drug deposition in the respiratory tract, as in the case of passive devices [11]. Understanding powder flowability, fluidization, de-agglomeration and in general all physico-chemical powder properties, leads to a better knowledge of the overall delivery system [12].

It is also important to understand how these properties may be affected by process parameters, especially during the scale-up of a product [13-14]. Mixing, sieving, filling, granulation and in general all process operations can modify powder characteristics, and their effects should be taken into account in order to obtain a reliable process and a product with the required characteristics [15-18]. In this sense, measuring powder properties within or at the end of the manufacturing process, can give information on the quality of the product, before executing in-vitro analytical tests of performance. These tests are essential and mandatory before proceeding to the subsequent phases of the drug development, but are very time-consuming. NGI (next generation impactor) and DUSA (dosage unit sampling apparatus) analysis can take few days of execution and, even more important, can be preceded by a quarantine period of samples storage. Consequently, other scale-up batches may have been already manufactured during this period, without taking advantage of the suggestions that the previous results can give.

Powder characterization tests [19] require a shorter time of execution. Some tests, such as aeration, density and flowability tests require less than 15-20 minutes of execution, while others, such as shear-cell test, usually last less than one hour. All tests employed in this work can be performed in a half-day work per sample, ideally at the end of the production of powder bulk. Gathering information in a shorter time can bring benefits in scale-up activities, which often require shortcuts.

Most of the research undertaken in this area has emphasized the influence of some chemical-physical properties on the DPI performance, mainly by studying one property at time, i.e. using a univariate approach [20-21]. However, studying the properties altogether can lead to a better understanding of the performance in term of the overall behavior of the powder as determined by the inter-play of several physico-chemical properties. It is well recognized that a multivariate approach improves data analysis efficacy and process understanding [22-25]. Here, we apply multivariate data analysis to the data arising from several powder characterization tests and in-vitro performance tests. In particular, Principal

Component Analysis (PCA) is used as an explorative data analysis tool in order to extract salient traits of the studied batches and the correlation structure of measured variables, and Partial Least Square Regression (PLS) is used in order to establish correlations among one or more performance variables and the powder characterization variables with the aim of obtaining predictive models. In order, to assess which are the most relevant features to estimate the performance variables and to improve the overall interpretability of the models, the Variance Importance in Projection (VIP) parameter has been used [26-27]. To the best of our knowledge this is the first study attempting to predict DPI performance from powder characterization tests in scale-up phase.

2. Materials and Methods

2.1 Samples

The study is undertaken at the scale-up step of drug development, i.e. at the phase where, after laboratory scale optimization the product is tested at pilot plant level before passing to plant manufacturing. A total of 27 samples were taken at the end of pilot manufacturing process of batches of powder. Formulations consisted of excipient and one active ingredient. The excipient used was alpha-lactose monohydrate based. The active pharmaceutical ingredient (API) was a target molecule designed for a generic or specific respiratory disease, such as asthma and COPD (chronic-obstructive-pulmonary-disease). Five different formulations were used, respectively named A, B, C, D and E, increasing the amount of active ingredient from A to E. The batches differed for bulk size, process parameters and starting materials. The manufacturing process consisted in the excipient mixing, the addition of the active principle, mixing and sieving step. Process parameters were set according to the batch size and the information derived from the scale-up process. The formulation, as well as the process, is under development, so that any additional detail cannot be provided at the moment. Despite of this, the manufacturing procedure was optimized for each formulation, and all the results in terms of blend uniformity analysis were satisfactory. In particular, the expected quantity of active ingredient (as mean of 40 samples of 20 mg each taken from the bulk) was inside the acceptance criteria (90.0-110.0 percent), with a relative standard deviation of no more than 5.0 percent.

The resulting powder was placed into a generic dry powder inhaler either manually (22 samples) or using an instrumental filling procedure (5 samples).

Each sample consisted of 500 grams of powder and two devices taken at the end of the manufacturing process. The 500 grams were characterized by powder characterization tests within one-two days from production, while the two devices were analyzed for the in-vitro performance by DUSA and NGI tests in a period that ranges from fourteen to twenty days, this is due to the higher time required for the in vitro tests and the internal organization of the laboratory at the company. All test were performed in controlled conditions of humidity and temperature. The different time periods between characterization and in-vitro tests support the aim of this study, which is to obtain predictive information on DPI performance as soon as possible during the scale-up activities. As mentioned before, this work is about the investigation of all possible correlations between the properties of the powder and its inhalation performance. To this aim, having collected batches that well span the manufacturing variability is an advantage in order to obtain a general correlation model.

The evaluation of the effects of the parameters and materials on performance is not reported here, and it will be dealt in a coming study.

2.2 Powder characterization tests

Density

Poured Density and Tapped Density of powder samples were measured using the jolting Stampf Volumeter STAV 2003 (from Engelsmann, Germany). A measured amount of powder was introduced into a cylinder of 250ml. Poured density refers to the initial mass/volume ratio. Tapped density was measured by mechanically and vertically tapping the cylinder under its own weight and considering the final volume obtained.

Particle Size Distribution

Particle Size determination of powder samples was performed with the Vibratory Sieve Shaker AS 200 Control (from Retsch, Germany) on the powder samples. The powder was fractionated according to the different sieves: 425µm, 355µm, 300µm, 250µm, 212µm, 200µm and 180µm. The achievement of full sieving was assured with a sieving time of 25 minutes, as tested in our laboratory. The corresponding variables are the percentage of powder with particle size under these sieve size values.

Stability & Variable Flow Rate

FT4 Powder Rheometer (from Freeman Technology, UK) was used in order to measure dynamic flow and shear properties of powder.

Stability & Variable Flow Rate properties were determined by combining seven conditioning and test cycles (for the Stability Test: test1- test7) and four conditioning and test cycles (for the Variable Flow Rate: test8-test12). Measurements were performed in triplicates. The used vessel size was 25mm. Blade tip speed was 100mm/s for the Stability test cycles, while 100, 70, 40 and 10mm/s for the variable flow rate test cycles. The measured parameters were the Basic Flowability Energy BFE (mJ), the Stability Index SI, the Flow Rate Index FRI, the Specific Energy SE (mJ/g) and the Conditioned Bulk Density CBD (g/ml). BFE is the energy required to move a conditioned and stabilised powder at a given speed of rotation of the blade. SI is a factor describing how the flow energy changes during repeated testing (SI=test7/test1). FRI is a dimensionless parameter that describes how the energy changes when the flow rate is reduced by a factor of ten (FRI=test11/test8). SE represents the energy needed to displace 1 g of conditioned powder using a lifting and shearing movement. CBD is the bulk density of the conditioned powder.

Aeration

Aeration test is aimed at determining how powder flow properties change as a result of the aeration. It consists of a combination of six conditioning and test cycles. All test cycles were at 100mm/s of blade tip speed. The air supply was off during the first test, then was introduced and increased in velocity for each subsequent test. The used vessel size was 25mm. The measured parameters were the Aeration Energy AE (mJ) and the Aeration Ratio (AR_n). AE is the flowability energy at 42 mms^{-1} air velocity, while AR is the factor by which the BFE is reduced by aeration ($AR_n=AE_0/AE_n$). AR values of about 1, between 1 and 20 and more than 20 respectively indicate very cohesive powders not sensitive to aeration, powders with average sensitivity to aeration, and powders very sensitive to aeration that tend to be fluidised. Measurements were performed in triplicates.

Compressibility

Compressibility test is aimed at determining how density changes as a function of applied normal stress. The results depend on several properties, such as cohesivity, particle size and shape. Powder was placed into a 50mm vessel, and subjected to three initial steps of conditioning. Then 8 compression tests (starting from 0.5kPa to 18kPa) were made, each of

them consisting of a period of 60 seconds. Results were in terms of bulk density after the compression test.

Permeability

Permeability test gives information on how easily a powder can transmit the air. As for compressibility, powder was placed into a 50mm vessel, and subjected to three initial steps of conditioning. Then 8 compression tests (starting from 1kPa to 15kPa) were made, at an air velocity of 2mm/s. Results were in terms of bulk density after the permeability test.

Shear Cell

Shear properties were measured with the Shear Cell Test. During the test, both vertical and rotational stresses were applied to the powder through a shear head. The shear head moved downwards inducing a normal stress until the required normal stress, σ, was reached. Then shear head begun a slow rotation inducing a shear stress, τ. Shear stress increased until the powder bed failed or sheared, maintaining constant the normal stress. The maximum shear stress was the Yield Point, or the Point of Incipient Failure. The measured parameters were Cohesion, Unconfined Yield Strength (UYS), Major Principal Stress (MPS), Angle of Internal Friction (AIF), Flowability (FF_c) and Bulk Density (BD). Cohesion is the shear strength at zero normal stress. UYS is the compressive strength. MPS is the major consolidation stress given by Mohr stress circle of steady state flow. AIF is the angle between the axis of normal stress (abscissa) and the straight line given by the values of shear stress as a function of the values of normal stress. FF_c is defined as MPS/Cohesion. Higher values of Cohesion and UYS, with consequently low values of FF_c describe generally cohesive powders.

More detailed information about the Shear Cell Test, as well as all the other tests, can be found in the manuals of Freeman Technology (http://www.freemantech.co.uk/).

Names, acronyms, related test and instrument for each characterization variable used in this work are reported in the following table.

Table 1 to be inserted here

2.3 In vitro performance tests

Dosage Sampling Unit Apparatus (DUSA) and Next Generation Impactor (NGI) were used in order to assess the in vitro performance of devices, after having verified that the active

pharmaceutical ingredient was well distributed within the entire bulk of powder. This was done by High Pressure Liquid Chromatography (HPLC) analysis of samples taken from the bulk. DUSA and NGI instruments were provided by Copley Scientific, UK. HPLC Waters Agilent 1100, 1200, 1290 and Alliance 2695 with PDA2998 detector were used for chromatographic analysis.

DUSA test allows the quantification of the Delivered Dose (DD), which is the total amount of drug emitted from the device and hence available to the patient. The powder weight of each dose is measured by an analytical balance as weight difference (Shot Weight, SW). After capturing the dose, the active drug is dissolved in solvent and an aliquot of the solution is then analysed by using High Pressure Liquid Chromatography. All measurements were made at the same conditions. 10 shots were collected for each device, arranged so as to cover the full range of inhalations (3 shots at the beginning, 4 at middle and 3 at the end). Shot Weight and Delivered Dose of each device were taken as the average based on the 10 shots.

NGI test is a high performance particle classifying cascade impactor. It has seven stages plus a micro-orifice collector (MOC). The air flux transports the powder within the various stages of the impactor through a series of nozzles having a gradually reduced diameter. The most important parameters taken into account by analysing the amount of drug deposited on the various stages are the total or calculated delivered dose (CDD), the fine particle mass (FPM) and the mass median aerodynamic diameter (MMAD). FPM represents the amount of drug which particle size is under 5μm. As well as for DUSA test, analysis is made on different shots taken during the whole range of inhalation.

At least two devices for each sample were analyzed, for both DUSA and NGI tests.

2.4 Data Analysis

Principal component analysis (PCA) has been used as an explorative multivariate data analysis tool [28] to assess batches differences in terms of powder properties and in vitro performance, as well as to get a first insight of their correlation structure.

Partial Least Squares (PLS) Regression [29] has been used to derive predictive models of in vitro performance considering as dependent variables, **Y** (PLS-2), all variables arising from DUSA and NGI tests. In order, to establish the most significant explanatory variables that affect the response variables [30] the Variable Importance in Projection (VIP)

parameter has been used [26]. VIP values represent the influence of each variable, x_j of the data matrix **X**, on the model of the responses matrix **Y** and are computed by using the PLS weight, w_j, weighted by how much of **Y** is explained in each model dimension, according to the following equation:

$$v_j = \sqrt{p \sum_{a=1}^{A} \frac{\left[SSY_a \left(w_{aj}/\|w_a\|\right)^2\right]}{\sum_{a=1}^{A}(SSY_a)}} \qquad \text{(eq. 1)}$$

Where SSY_a is the sum of squares of estimated **Y** by the a^{th} PLS component and w_{aj} is the PLS weight for the variable x_j in the a^{th} component. This formula may be referred to a single y_m variable (considering $SSY_{m,a}$) or all **Y** variables altogether [24]. It is generally assumed that a significant variable can be selected when $v_j > 1$, since the sum of squared VIP values close to the number of **X**-variables in the data set.

Data preprocessing, PCA and PLS analyses were performed in R environment [31] by using the chemometric packgages developed by Varmuza and Filzmoser [32]. VIP calculation was performed using in-house routine implemented in R.

Data sets and preprocessing

Different data sets were considered to derive PCA and PLS-2 models, because some tests were not performed on all samples. The PCA data set includes all variables, i.e. thirty (the 25 reported on Table 2 and the five in vitro performance variables described in *2.3 section*), for twenty-two samples, corresponding to batches obtained by five different formulations.

PLS-2 regression models were calculated both considering all responses, and separately for DUSA variables (SW and DD) and NGI variables. Global model includes twenty-eight variables (23 explanatory variables and 5 response variables) for twenty-two samples. PLS-2 models for DUSA and NGI include respectively twenty-five variables (23 explanatory variables and 2 response variables) for twenty-four samples and twenty-seven variables (23 explanatory variables and 3 response variables) for twenty-seven samples. CBD_Comp and CBD_Perm were not used as explanatory variables in the PLS-2 models in order to not reduce the dimension of the data sets in terms of samples. Moreover these variables resulted directly related to the other density variables, so their exclusion in the regression models should not constitute a limiting factor in terms of prediction.

All data sets were pre-processed with autoscaling.

Validation of PLS Models

Both PLS-1 (only one response variable) and PLS-2 models were evaluated. When the responses variables to be modelled are not correlated an overall PLS-2 model can be less effective than the single response respective PLS-1 models. Therefore, a global PLS-2 model was first considered, and then PLS-2 models were built taking into account only the groups of related response variables.

Considering the very limited number of samples for proper validation, all PLS-Models were obtained according to the following schema:

Assessment of model dimensionality: for each data set about 100 different splits in training and test (four samples) sets were randomly generated from the initial data table, but constraining the samples with the most extreme values of Y to be included in the training set, in order not to reduce the range of the Y values in calibration step. Root Mean Square Error in Cross Validation (RMSECV), using Leave-One-Out procedure, was assessed for each model, corresponding to a given split, as function of the number of PLS components. The average RMSECV value and its standard deviation were then used in order to select the most appropriate model dimensionality.

The PLS model with a number of components corresponding to the minimum of the average RMSECV values was then used to estimate the test set samples for each split. The average RMSEP and its standard deviation were obtained and used to assess the predictive performance.

Analysis of the residuals and leverage was used for outliers identification and removal.

Estimation of significant explanatory X variables: VIP values where calculated for each permutation model.

After identifying the most significant explanatory variables, new PLS models were generated in order to predict the analytical variables using only the powder characterization tests containing the significant variables. The results were compared.

PLS models were obtained including as samples all available batches also when having different formulation. Performance variables such as DD, FPM and CDD were converted

into percentages calculated on the respective target value related to each formulation, according to the following equation (example for DD):

$$DD = \frac{DD_{original\ value\ relative\ to\ formulation\ i}}{DD_{teoric\ value\ relative\ to\ formulation\ i}} * 100\ \% \quad \text{(eq. 2)}$$

This choice was made in order to reduce the prediction error of the models, which was found to be initially high (respectively 53, 42 and 50%).

3. Results and Discussions

3.1 Exploratory data analysis

An overview of samples trend and variables correlation structure can be gathered by PCA scores and loadings plots (Figures 1a and 1b). The first two principal components, which explained the 57% of the data variance, depicted the most structured information. Samples were partially grouped according to their formulation, as shown in the Scores Plot (Figure 1a). Samples that differ for increasing amounts of active ingredient had consequently higher values of DD, CDD and FPM (Delivered Dose of DUSA and NGI Test, Fine Particle Mass). These variables are placed at positive values of PC1 in the Loadings Plot (Figure 1b). When the quantity of active ingredient increases, going from formulation A to E, and thus increasing the number of micronized particles, there is a general increase in the cohesiveness of the powder, and in flow properties (in particular BFE, SE, FRI). In fact, BFE, FRI and SE are directly correlated with the amount of active ingredient. Low BFE values are generally associated with powders that have good flow properties.

Samples related to formulation A and B have BFE values of 130-300 mJ while formulations C, D and E have values of 300-650 mJ. FRI describes the flow rate sensitivity of powders. FRI varied approximately between 0.73 and 1.13 when increasing the amount of the active ingredient for the samples taken into account, describing powders with low flow rate sensitivity (characteristics observed for values of about 1 or <1.0). Powders with these characteristics can be processed with low shear mixing operations, minimising the possibilities of particle attrition and increase of electrostatic charge while still ensuring homogeneity.

SW and all density variables (green coloured) are directly correlated. This is a consequence of the drug delivery system: the quantity of powder per single delivered dose is equal to the

quantity of powder that is placed gravimetrically within a volume defined by a bulk reservoir.

The increase of density seems associated with powders having a higher percentage of fine particles in the region under 355µm, whose corresponding variables have positive PC1 values in the Loadings Plot, and thus a lower percentage of particles of particle size between 425µm and 355µm (coarse particles). Particle size lower than 212µm is very close to density variables, hence directly correlated, this is confirmed by the Pearson correlation coefficient of 0.73 between particle size <212µm and Tapped Density.

Flowability and aeration properties (AIF, FF and AE_42) are placed opposite to SW and densities (Figure 1b). Aeration Ratio (AR) varied approximately between 9 and 60, describing powders with average and high sensitivity to aeration. Lower values of energy of aeration describe powder that are easily subjected to flow; samples with this behaviour are also those to which correspond an higher value of shot weight, that is the quantity of powder related to the delivery system.

Figure 1 to be inserted about here

3.2 Regression analysis

A global PLS-2 regression model was obtained considering all the five analytical variables (DUSA and NGI tests), the predictive capability of the model is reported in Table 2. The PLS **X**-weights and **Y**-Loadings plots are reported in Figure 2 (respectively 2a and 2b), for the PLS-2 model corresponding to one of the split. For this model, percentage values of DD, CDD and FPM were used, as reported in the 2.4.2 section. This explain why they show a slightly different correlation pattern in the space of the first two PLS components with respect to the results of the PCA model, in terms of a decrease in the degree of correlation between cohesiveness and performance variables.

Table 2 to be inserted here

Figure 2 to be inserted about here

The other variables confirm the relationships previously observed in PCA space.

However PLS results, in term of prediction, improved when PLS-2 models were obtained considering as **Y**-block groups of analytical variables correlated. Thus, distinct PLS-2

models were developed considering as Y-block Shot Weight (SW), Delivered Dose (DD) of DUSA test, and FPM, CDD, and MMAD of NGI test, respectively.

3.2.1 DUSA variables

A PLS-2 model was developed for each of the splits in training and test sets (obtaining 99 models), as described in the 2.4.2 section. The resulting average Root Mean Square Error in Cross Validation (RMSECV) is reported for each response variable versus the number of components (Figure 3). The minimum RMSECV value corresponds to three components, after which it increases, along with its uncertainty.

Figure 3 to be inserted about here

Figure 4 to be inserted about here

Inspection of the PLS inner relationships plots supported the choice of three PLS components as optimal model dimensionality (Figure 4, for one of the split). The PLS regression coefficients, for SW and DD of one of the split model, are reported in Figure 5 and show quite similar trends: density, flowability (FF) and particle size (<212µm and <355µm) present the most positive values of regression coefficients (direct correlation), while Energy of Aeration (AE), Cohesivity (MPS) presented the most negative ones (inverse correlation).

Figure 5 to be inserted about here

Figure 6 to be inserted about here

As mentioned in Methods section, VIP values were calculated, separately for SW and DD (Figure 6), in order to assess significant explanatory variables. The density variables (CBD, PDensity, TDensity and BD), the energy of aeration (AE), the particle size (in particular the 212µm) and MPS are significant for both response variables.

These variables, considered for each formulation, can depict the effect of process parameters such as mixing and sieving and of starting materials that confer to the powders different profiles of particle size, different density and aeration energy. The cohesiveness variables, correlated with the amount of active ingredient are, at variance, not so useful for prediction of SW and DD.

Figure 7 to be inserted about here

Figure 7 shows the predicted versus measured values (for each sample there are several repetitions that correspond to estimated response by each of the 99 PLS models); black numbers indicate the samples when included in the training set (fit) and red numbers indicate the samples when used as a test (predicted). The average Root mean squares error for test samples (RMSEP), i.e. red points, was 2.57% and 2.02% for SW and DD respectively. This performance was quite satisfactory considering the uncertainty of the reference method, which is respectively 4.95% and 5.60%.

The results of the PLS-2 model obtained reducing the number of descriptor variables by considering only the most significant variables according to their VIP values, namely Stability and Aeration variables, are summarized in Table 3. The model was computed considering 3 PLS components.

Table 3 to be inserted here

The performance of the models with reduced variables was similar meaning that SW and DD can be estimated with the two mentioned tests, thus reducing the time and analysis costs.

3.2.2 NGI variables

A PLS-2 model with one latent variable, according to minimum average RMSECV, was developed for each of the 100 splits of training and test sets.

Percentage values for CDD and FPM were used. This PLS model however, was not as performing as the models for SW and DD. RMSEC and RMSEP values are shown in Table 4.

Table 4 to be inserted here

PLS regression coefficients are reported in Figure 8 (for one of the split), while Figure 9 reports the plot of VIP for each response variable (average and standard deviation based on all splits).

Figure 8 to be inserted here

Figure 9 to be inserted here

It can be seen that SI variable from Stability test is significant for all considered responses, together with PDensity, TDensity, BD, AIF and particle size variables. SI, Density and particle size under 355µm had negative values of regression coefficient for CDD and FPM, while positive for MMAD. FF and particle size under 425µm had positive values of regression coefficient for CDD and FPM, while negative for MMAD. The observed different influence of particle size on MMAD depending on the fine fraction considered seems in agreement with the complex relation, as observed by Podczeck et al. [10] among fine carrier particles, the micronized drug particles and the surface roughness of carrier.

SI is a parameter that describes the change in energy between test 1 and test 7 of the Stability test. Several factors can be responsible for instability of powder, resulting in SI values higher or lower than 1. Some of these factors are de-aeration, agglomeration, segregation, electrostatic charging, which led to SI values higher than 1, and attrition, de-agglomeration, blending of a flow additive, which led to SI values lower than 1. SI values range from 0.9 and 1.1. This variable is directly correlated to MMAD for each formulation, meaning that attrition phenomena, changing the physical size and shape of particles through mechanical stress can result in different values of mass median aerodynamic diameter, and thus in an increase or decrease of performance in terms of FPM and CDD. Density, flowability (AIF, FF) and particle size variables have an influence on performance and the same considerations given in the Explanatory data analysis section hold.

These results are consistent with previous studies [10,17, 33-37], which highlighted how shape, size and powder flow properties can influence the mixing and DPI performance. In terms of DPI performance, Kaialy *et al.* [33] supported that the shape of carrier particles in terms of elongation ratio (ER) directly influence the amounts of active delivered to lower airway regions indicating enhanced DPI performance. In addition, Jones & Price [36] argued that the addition of fine particles of lactose or one of many other excipients to a formulation increases formulation performance. In terms of mixing performance, Muzzio *et al.* [17, 37] investigated the effect of flow properties and shear environment in continuous mixing, observing that bulk density showed a significant effect in terms of significant resident time and that impeller speed and cohesion of powder showed a significant interacting effect on the axial dispersion coefficient.

4. Conclusions

Multivariate data analysis of powder characterization tests and in-vitro performance tests allowed capturing the salient aspects that influence the performance of DPI devices. Powders resulted to be more cohesive when increasing the quantity of the active ingredient. The increase of density was associated with powders having a lower percentage of particles having particle size between 425µm and 355µm (coarse particles). The delivery system was mainly affected by properties such as density, particle size, flowability and aeration properties.

Moreover, predictive models were derived to estimate in-vitro performance from powder characterization tests. Notwithstanding the entity of the errors these model can always be used to assess with less efforts batches quality and thus to aid tuning of process settings in the scale up phase.

Among all variables, SW and DD (DUSA tests) were the ones better predicted. In particular using only variables measured by Stability and Aeration tests, which took a few hours of executions, the error provided by these models in predicting unknown samples was respectively of 2.3% and 1.8% for SW and DD. This result was due to the good correlation of SW and density variables with the energy of aeration (powder property of being aerated), which in turn affects the total delivered dose, as the result of the reservoir-based device. Performance response variables (NGI test) were predicted with a greater error, going from 4.3% up to 11.4%. The differences in performance relative to each formulation were mainly due to the particle size and shape of the powder, which in turn affects density and stability of powder during the execution of the Stabilty Test. These considerations are in agreement with results reported by several studies [10,17, 33-37].

In order to meet the needs of the scale-up process, in which the number of tested batches was kept to the minimum necessary, PLS predictive models were obtained for samples having different formulations. In NGI prediction, probably a PLS model for each single formulation could provide better results.

Finally, as far as the multivariate data analysis approach is concerned recent studies [38-39] have shown as a quality by design approach allowed developing predictive models for powder flowability as a function of particle size and shape distribution [38] as well as to obtain optimal flow properties for four-components powder mixtures [39]. These

approaches are extremely useful to develop dry powder inhaler formulation and for process optimization.

The predictive models developed in the present work address the stage of scale-up where for the limited number of batches allowed not always a proper experimental design approach is feasible/accepted; in this context models for faster estimation of in vitro performance may aid finding best process setting.

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Caption of Figures and Tables.

Figure 1: (a) PC1 vs PC2 Scores plot. Batches are coloured by formulation; (b) PC1 vs PC2 Loadings plot. Variables are coloured according to type of test: green – Density variables; grey – Particle size; blue – Aeration test; red – Stability test; violet – Shear Cell test; light blue – Ability to Settle; black – Dusa variables; orange – NGI variables. Names of variables are reported in Table 1.

Figure 2: PC1 vs PC2 of PLS X-weights (a) and Y-Loadings (b). X Variables are coloured according to type of test: green – Density variables; grey – Particle size; blue – Aeration test; red – Stability test; violet – Shear Cell test; light blue – Ability to Settle. Names of variables are reported in Table 1.

Figure 3: RMSECV versus the number of PCs. (a) SW and (b) DD response variables (DUSA Test).

Figure 4: PLS Inner Relationships of one of the 99 PLS-2 models (the same trends are observed for all the other models), for the first three principal components.

Figure 5: PLS regression coefficients for SW (a) and DD (b) for one of the split model.

Figure 6: VIP values for SW (a) and DD (b) responses. Error bar corresponds to uncertainty estimated by considering the 99 permutation models.

Figure 7: Predicted vs measured values for SW (a) and DD (b) responses. In black training samples, in red test samples.

Figure 8: PLS regression coefficients for CDD (a), FPM (b) and MMAD (c) for one of the split model.

Figure 9: VIP values for CDD (a), FPM (b) and MMAD (c) responses. Error bar corresponds to uncertainty estimated by considering the 100 permutation models.

Table 1: Description of powder properties variables.

Table 2: PLS-2 model performance for DUSA and NGI responses. The average error and the corresponding standard deviation, in fit and prediction, over the 93 permutation models are reported.

Table 3: PLS-2 model performance for DUSA responses. The average error and the corresponding standard deviation, in fit and prediction, over the 99 permutation models are reported.

Table 4: PLS-2 model performance for NGI responses. The average error and the corresponding standard deviation, in fit and prediction, over the 100 permutation models are reported.

Figure 1

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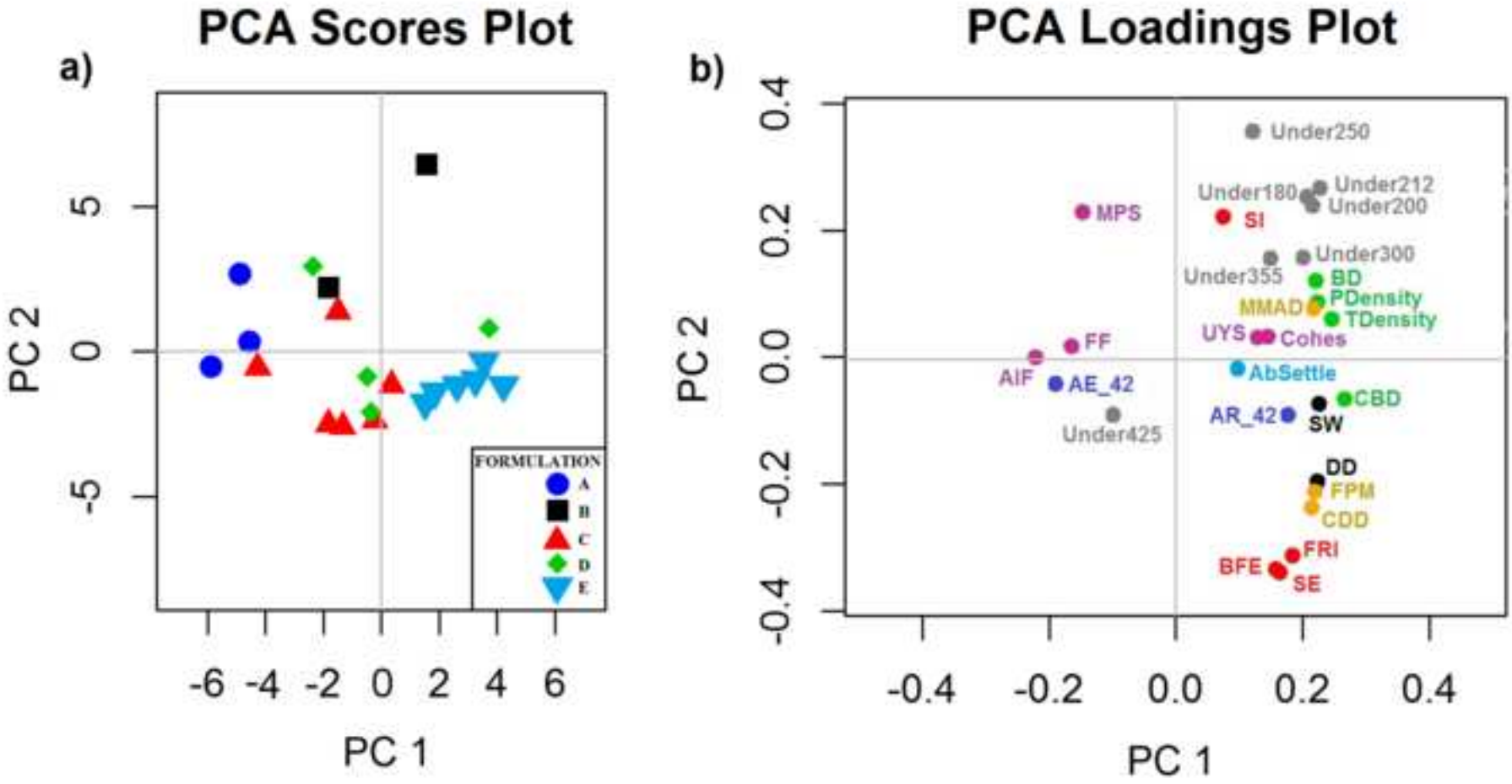

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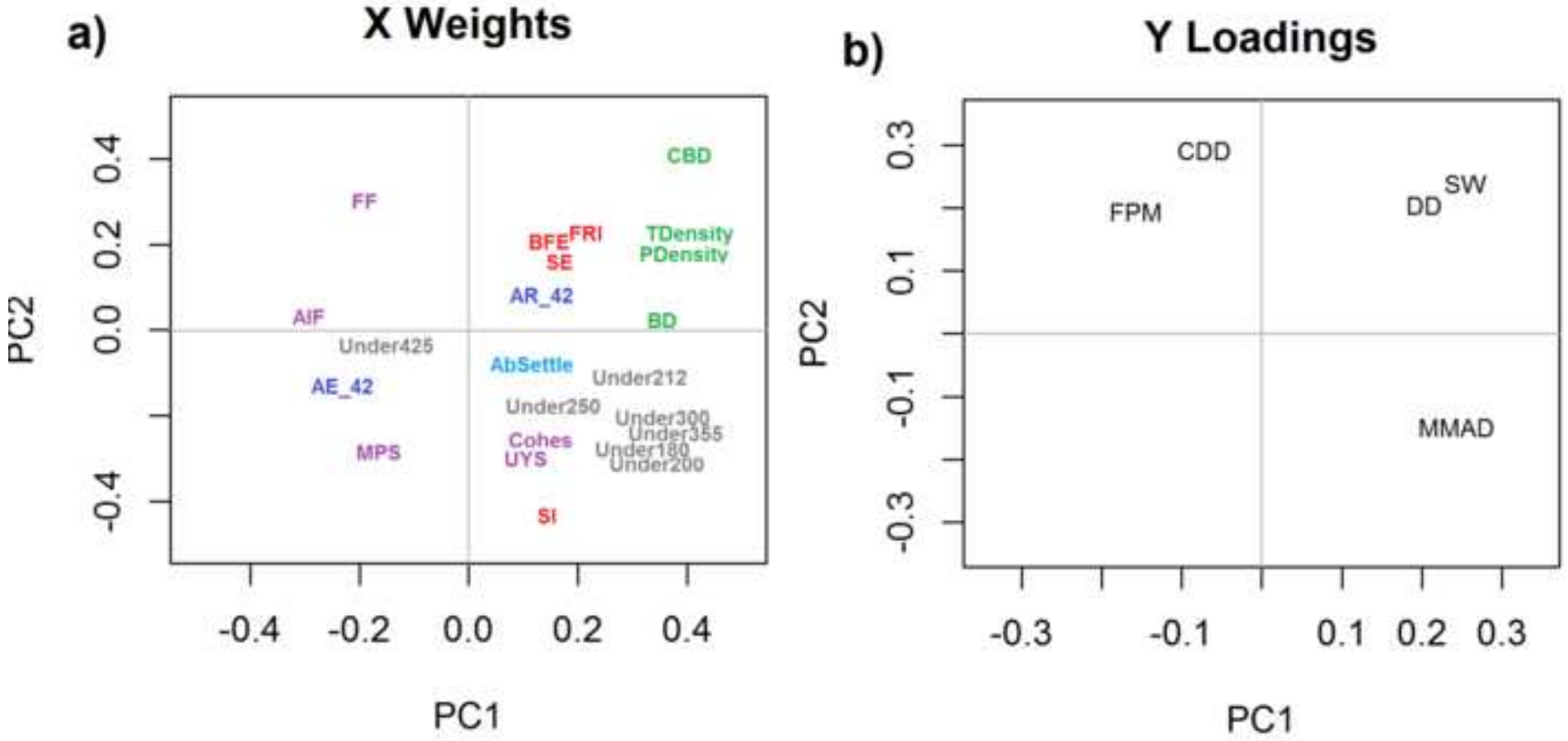

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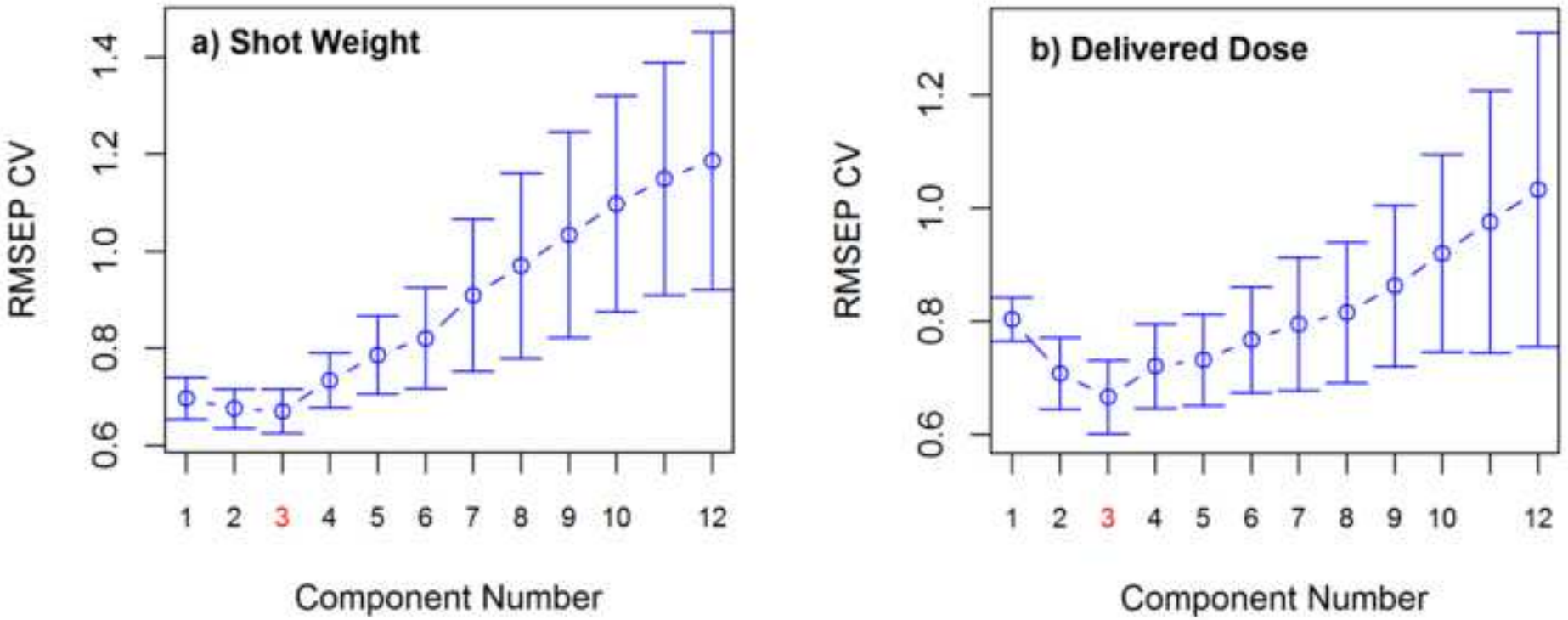

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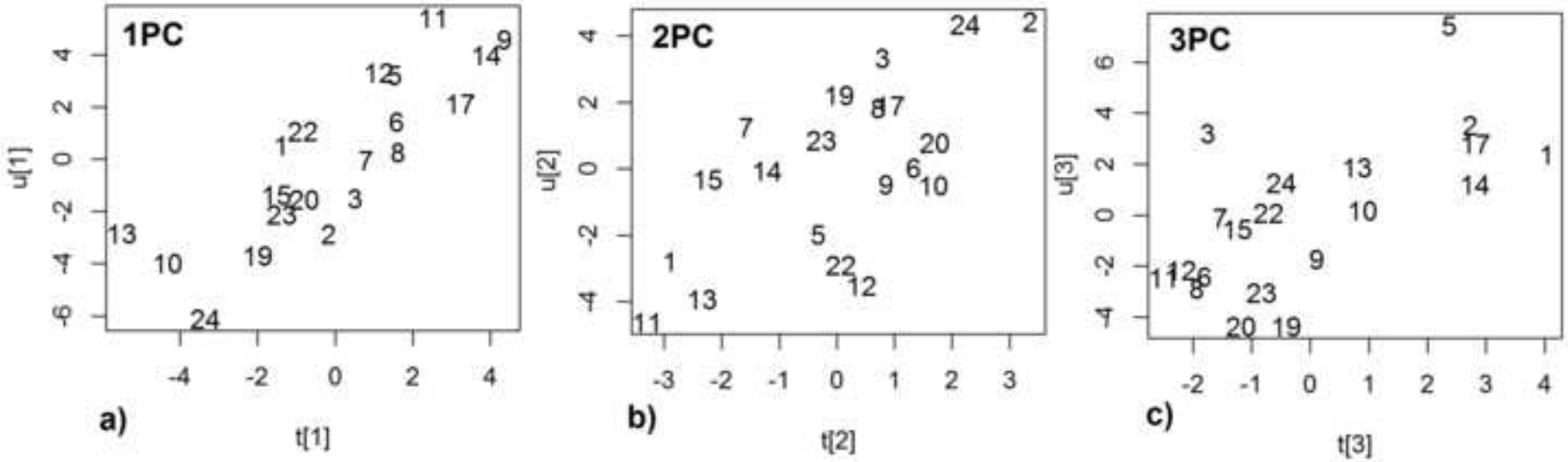

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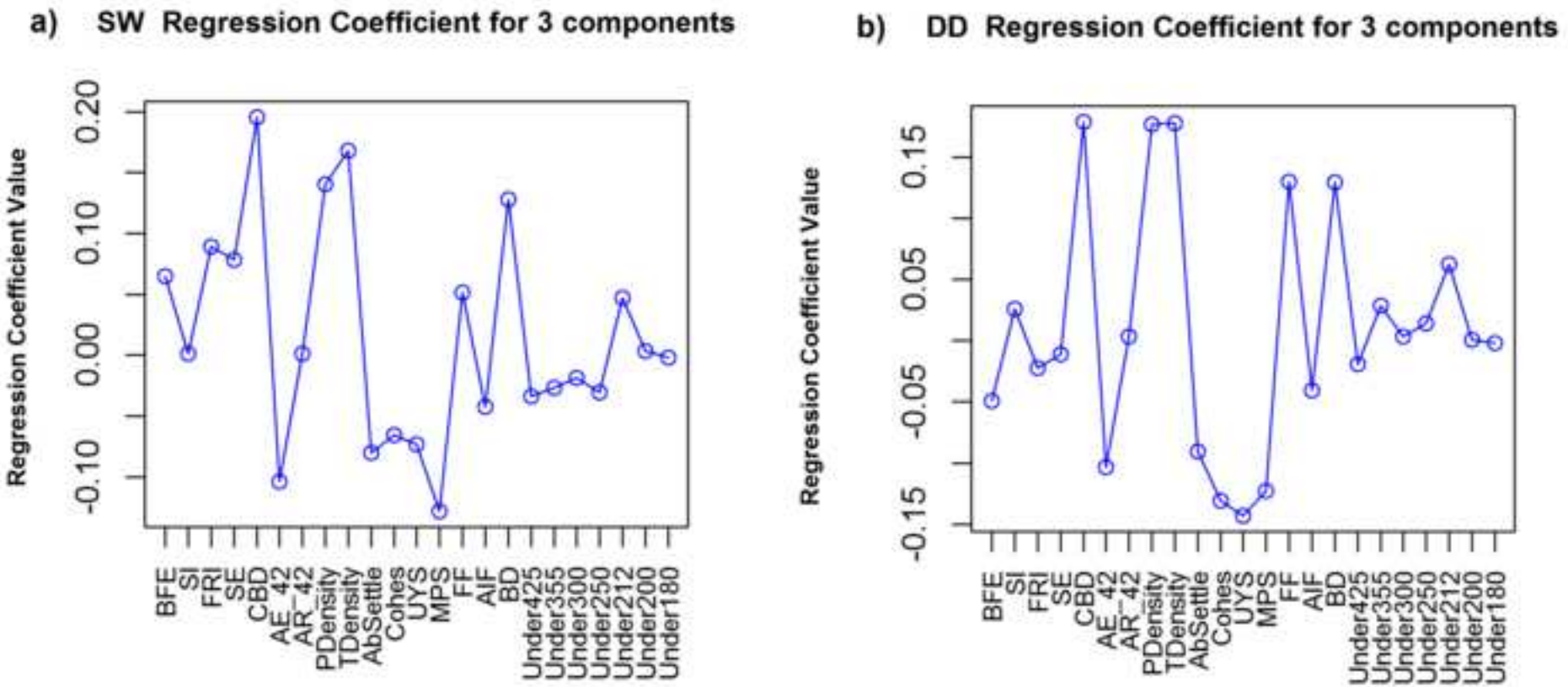

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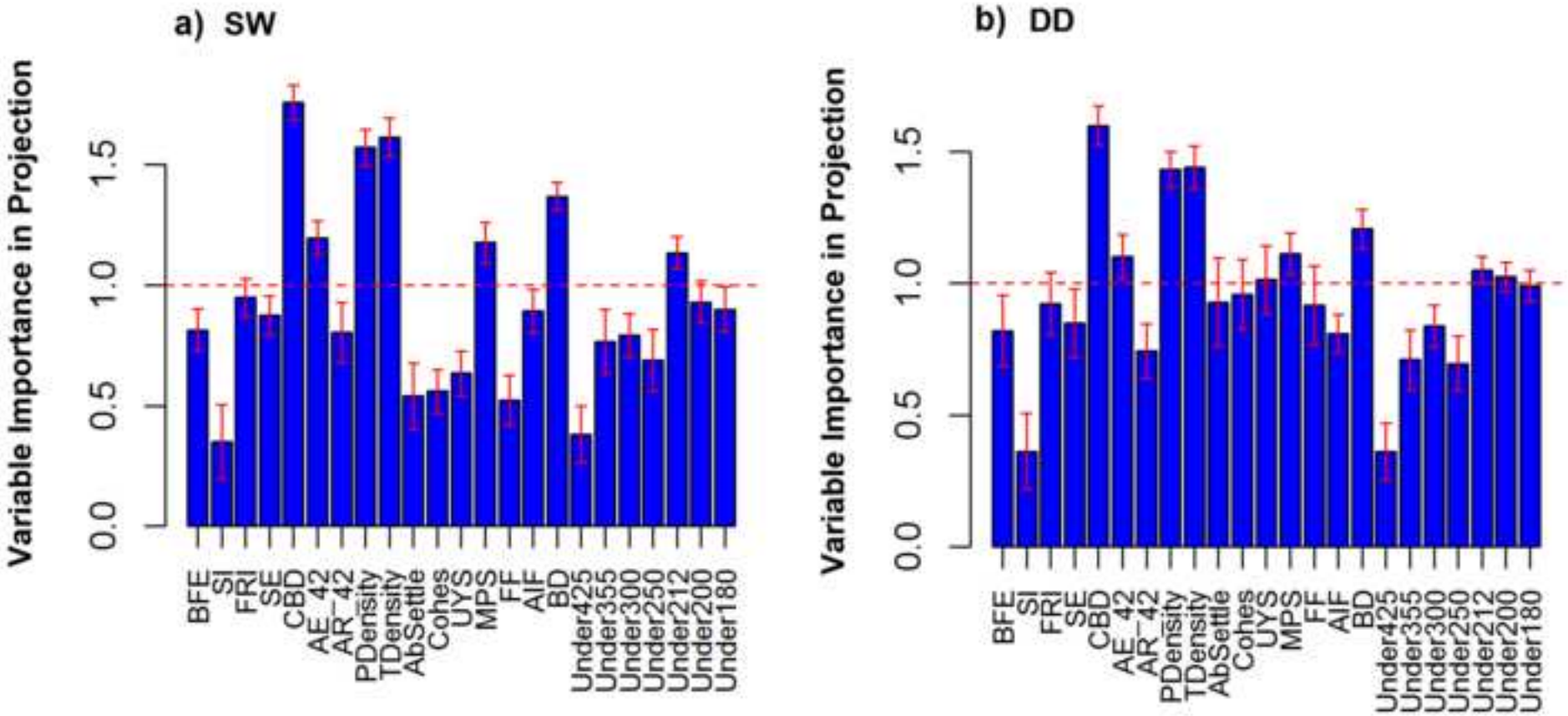

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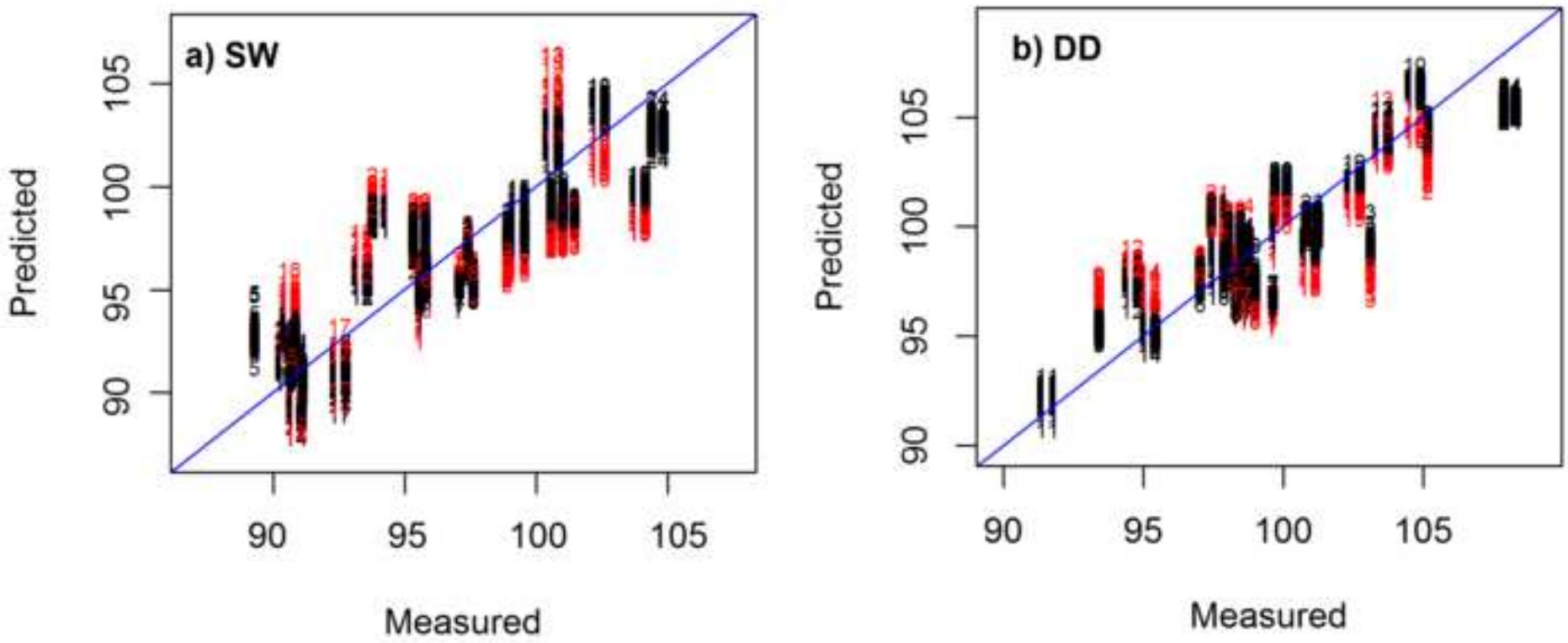

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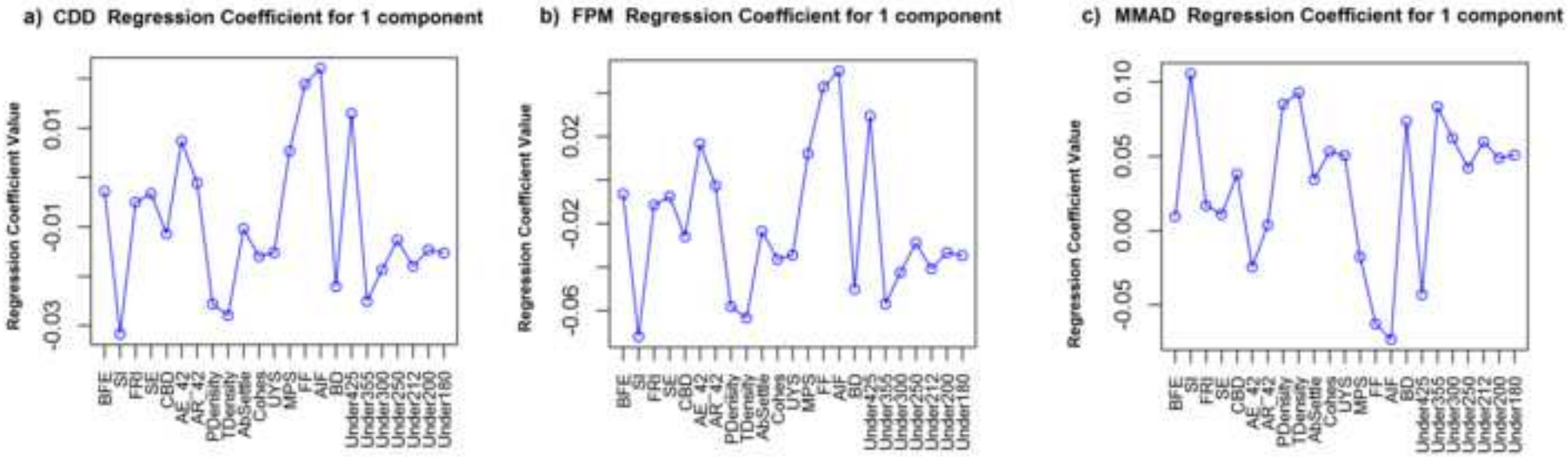

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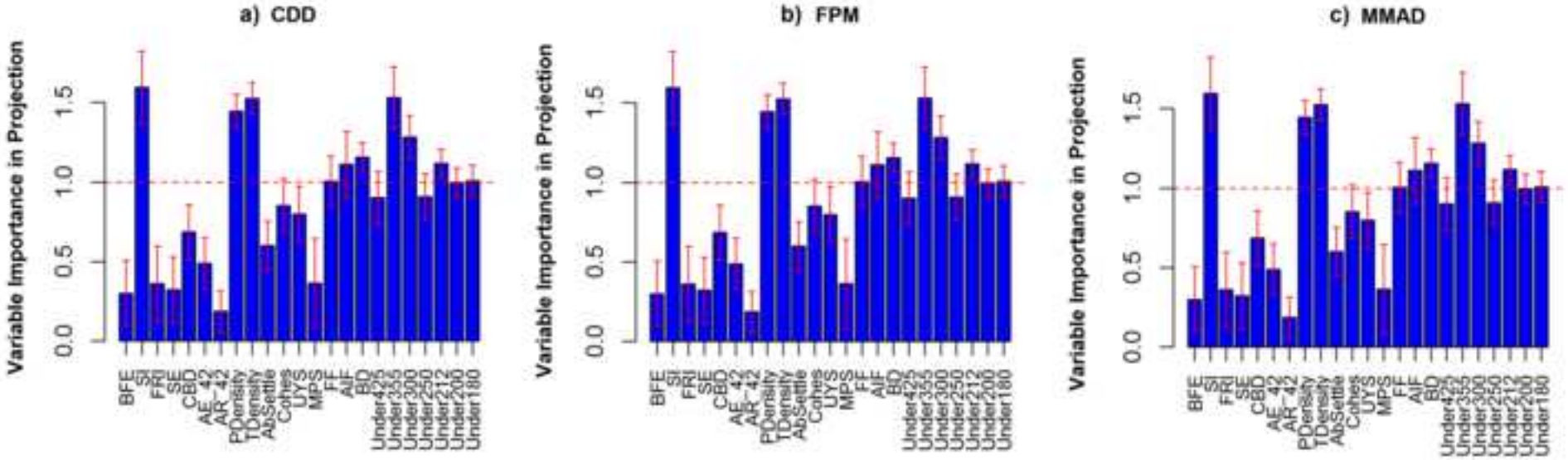

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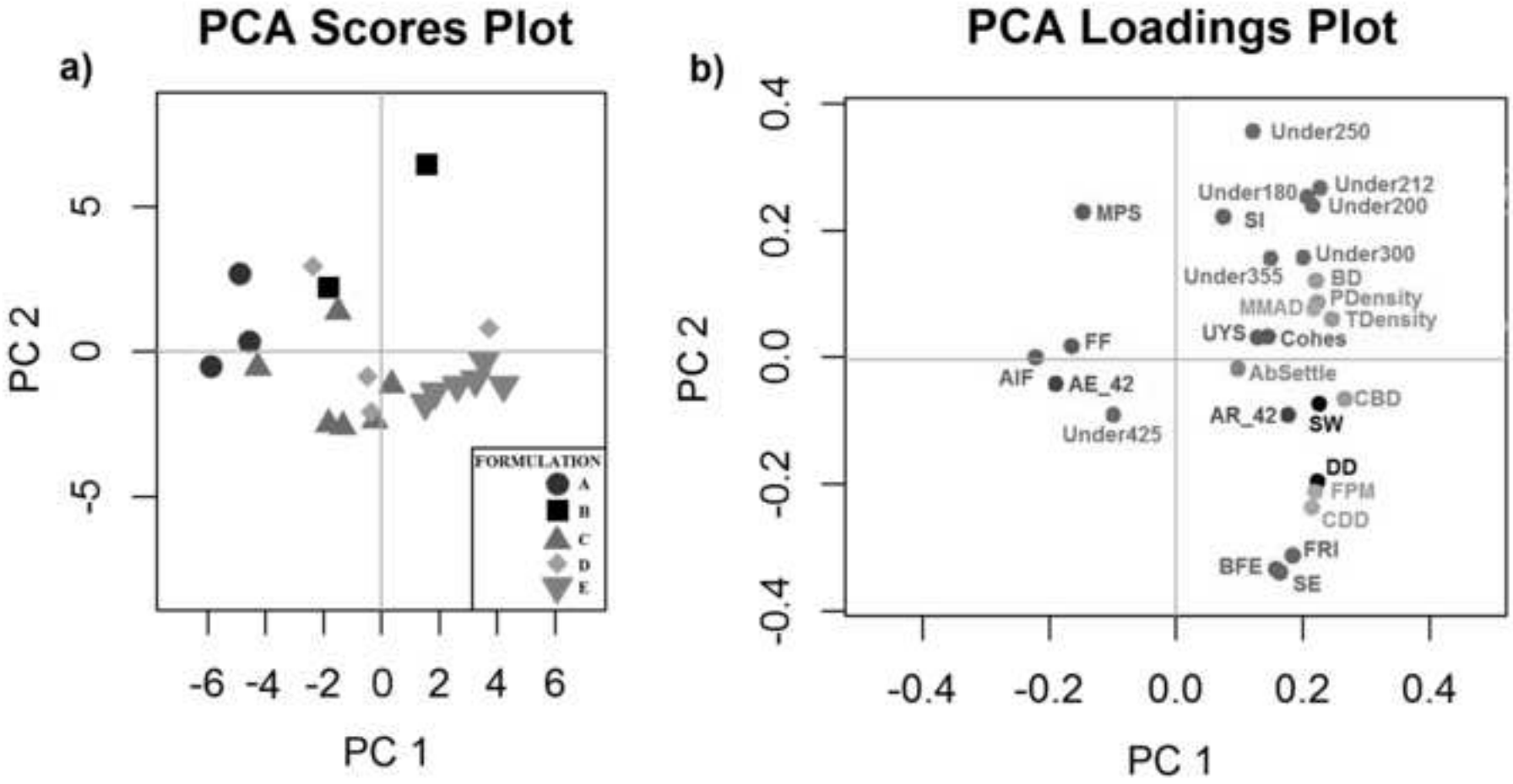

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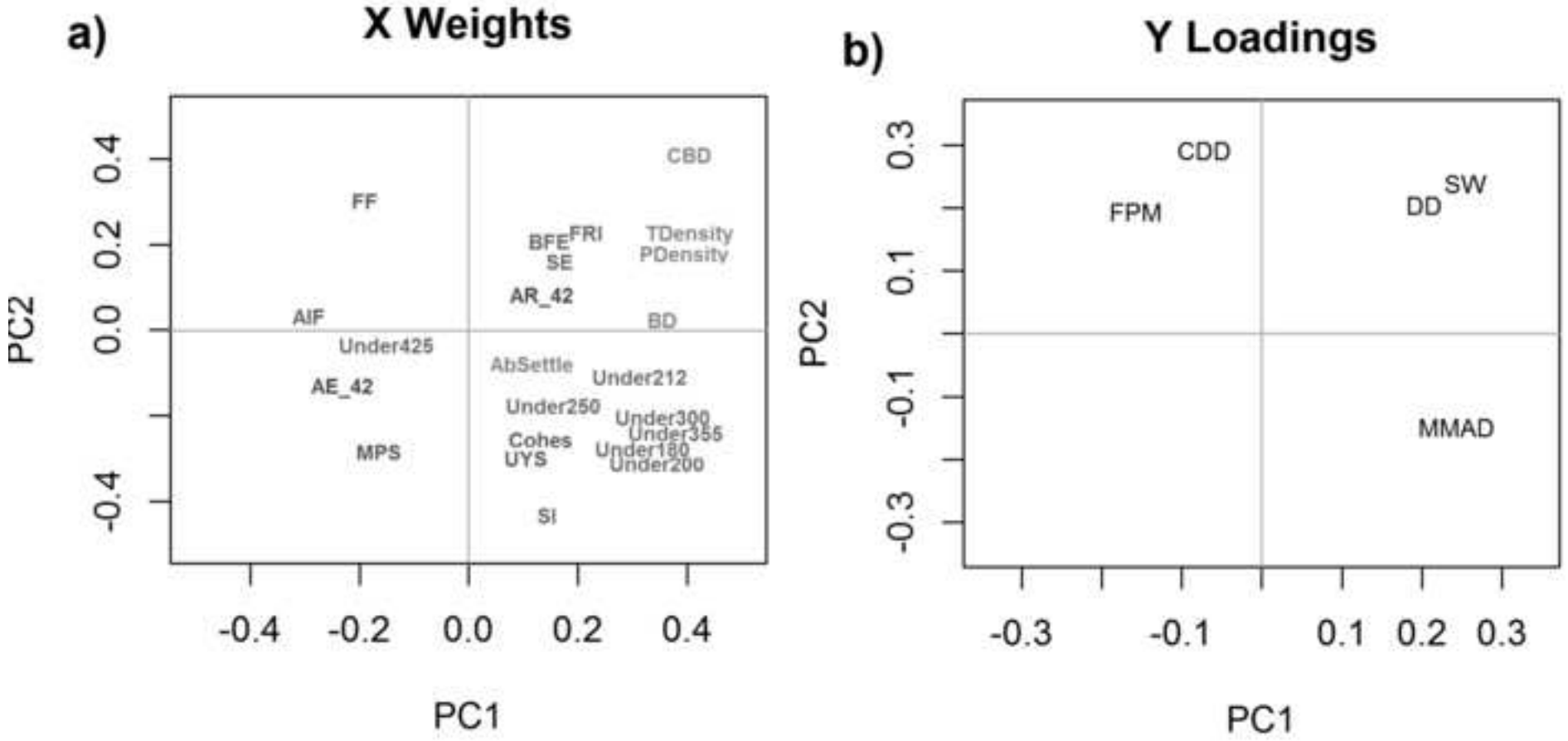

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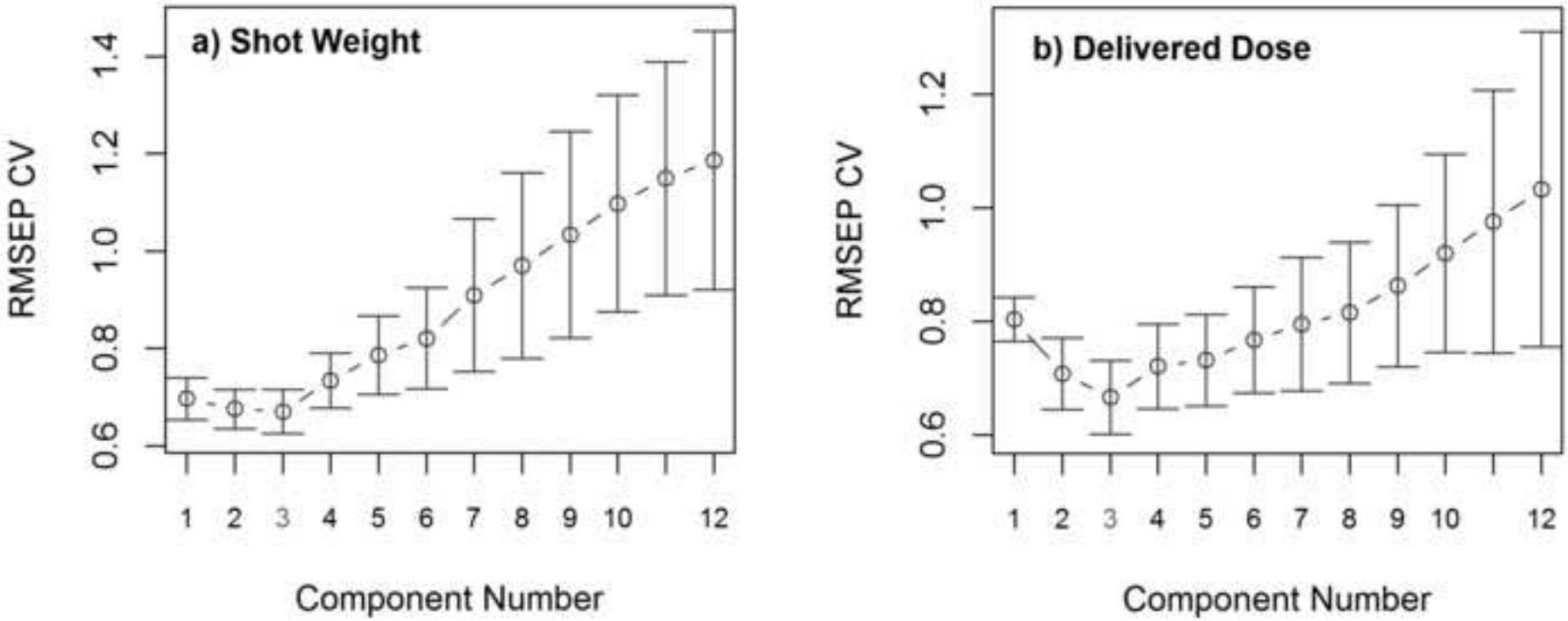

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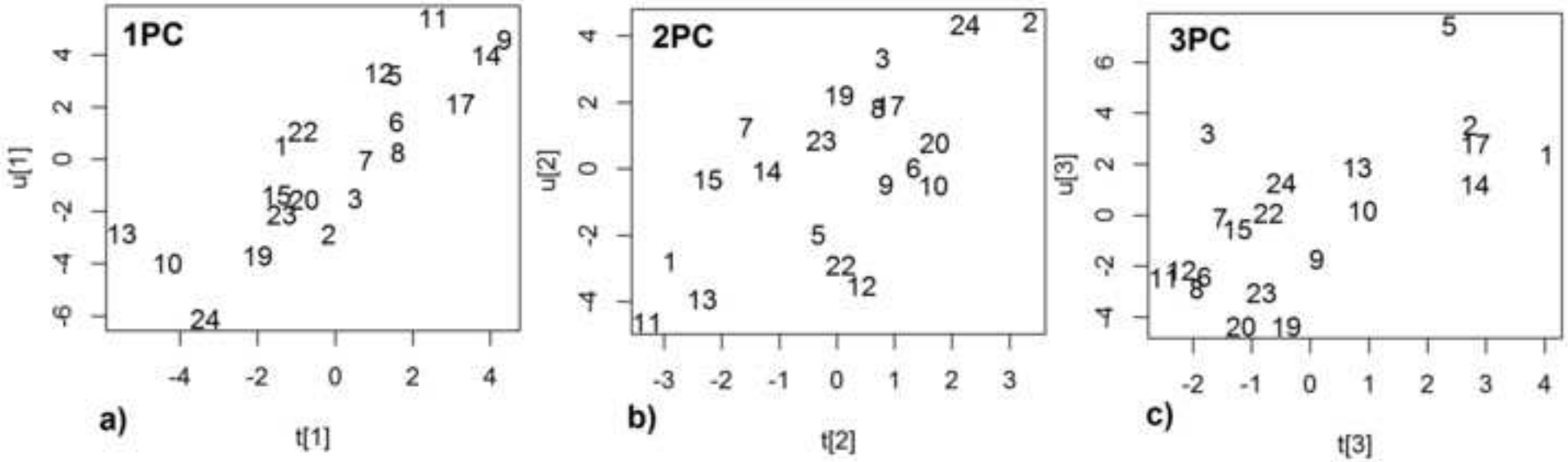

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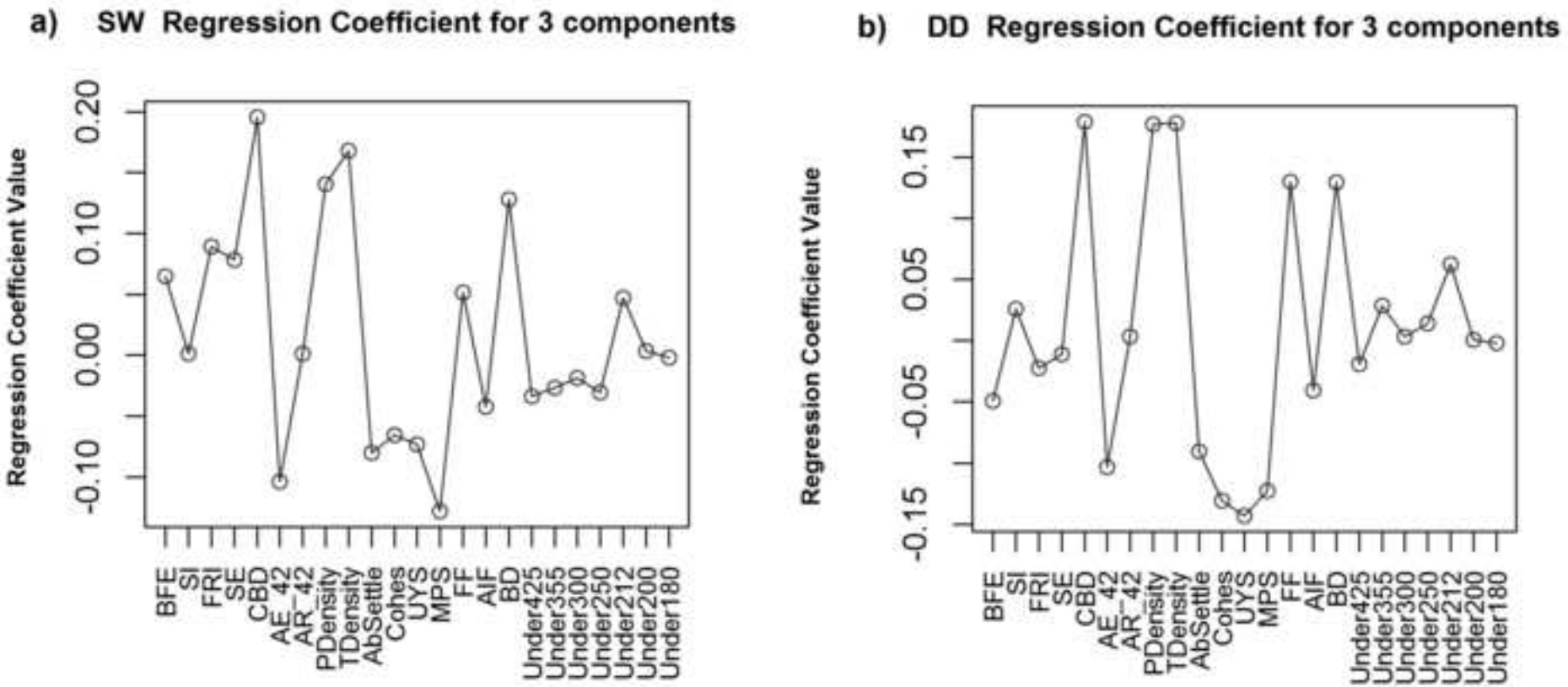

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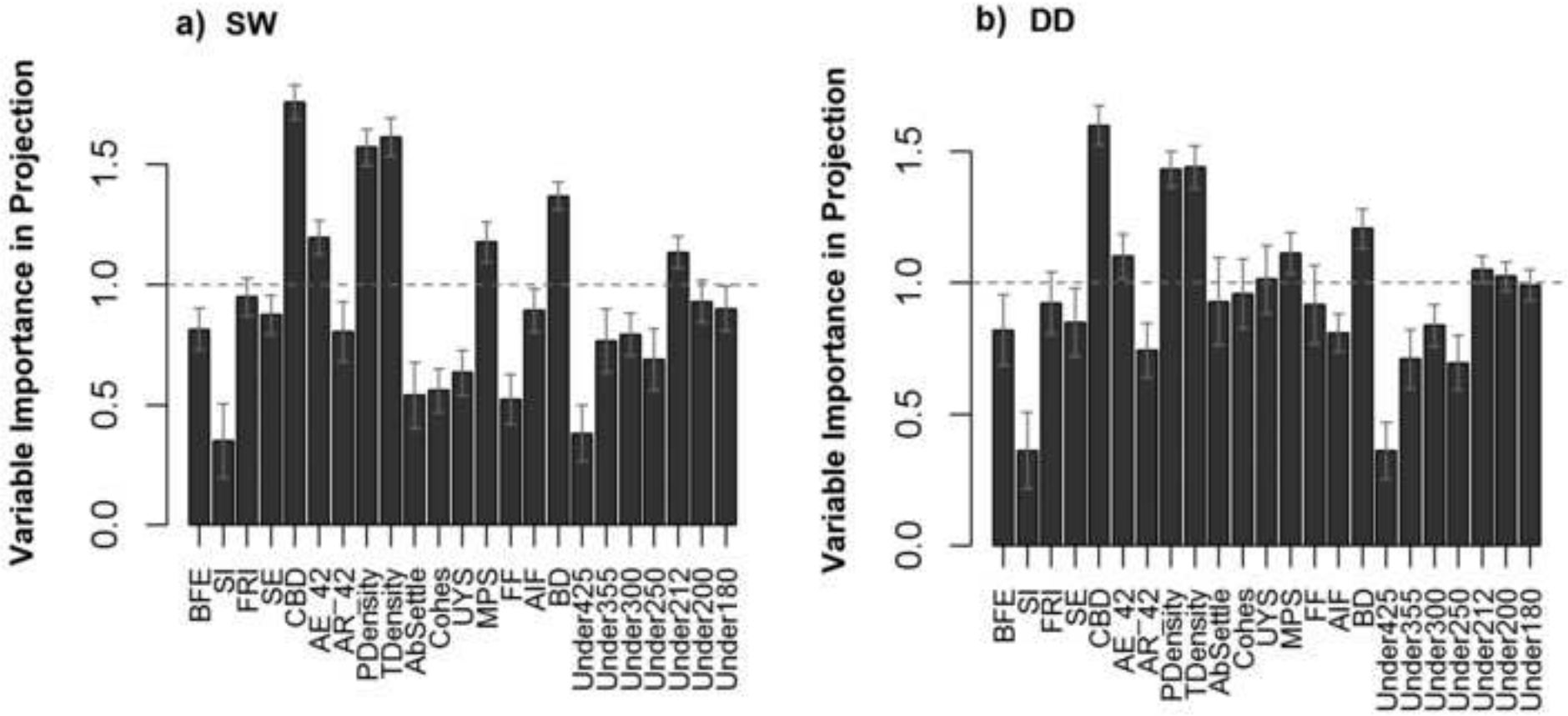

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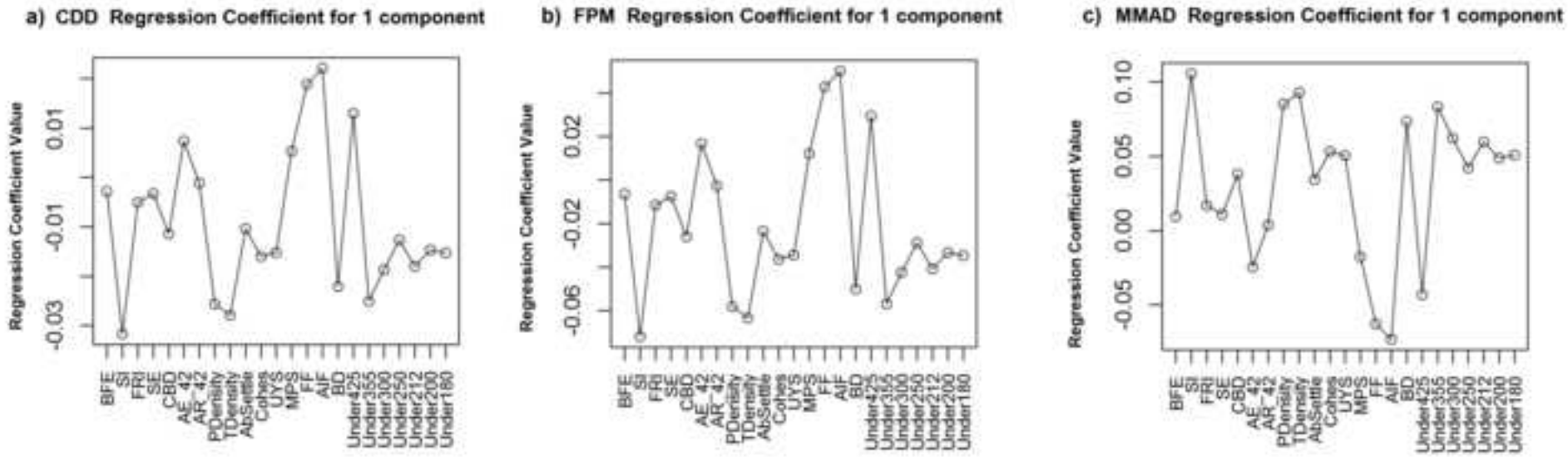

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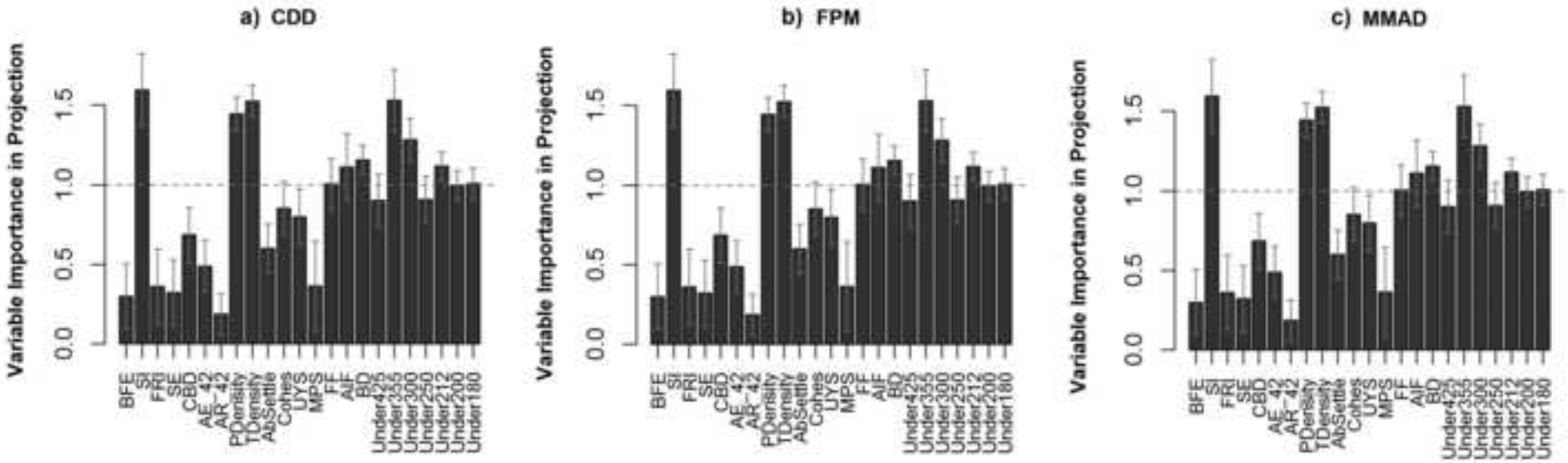

Table 1: Description of powder properties variables.

Acronym	Variable Name	u.m.a	Test	Instrument
PDensity	Poured Density	g/ml	Density	Tapped Density Tester IG4
TDensity	Tapped Density	g/ml		
AbSettle	Ability to Settle	ml		
Under X	% of powder with particle size under X, where X is: 425µm, 355µm, 300µm, 250µm, 212µm, 200µm and 180µm	%	Particle Size	Vibratory Sieve Shaker AS 200 Control
AE	Aeration Energy	mJ	Aeration	FT4 Rheometer
AR	Aeration Ratio	Dimensionless		
BFE	Basic Flowability Energy	mJ	Stability	
SI	Stability Index	Dimensionless		
FRI	Flow Rate Index	Dimensionless		
SE	Specific Energy	mJ/g		
CBD	Conditioned Bulk Density	g/ml		
Cohes	Cohesion	kPa	Shear Cell	
UYS	Unconfined Yield Strength (UYS)	kPa		
MPS	Major Principal Stress (MPS)	kPa		
AIF	Angle of Internal Friction (AIF)	°		
FF	Flowability (FFc)	dimensionless		
BD	Bulk Density (BD)	g/ml		
CBD_Comp	Conditioned Bulk Density of Compressibility	g/ml	Compressibility	
CBD_Perm	Conditioned Bulk Density of Permeability	g/ml	Permeability	

Table 2: PLS-2 models performance for DUSA and NGI responses. The average error and the corresponding standard deviation, in fit and prediction, over the 93 permutation models are reported.

Y response variable	Average RMSEC / std dev.	Average RMSEP / std. dev.
SW	2.12%+/-1.37%	3.26%+/-1.66%
DD	1.87% +/- 1.30%	2.68% +/- 2.14%
CDD	3.50% +/- 3.24%	5.56% +/- 4.67%
FPM	9.65% +/- 7.58%	11.61% +/- 7.85%
MMAD	9.27% +/- 5.91%	15.02% +/- 9.43%

Table 3: PLS-2 models performance for DUSA responses. The average error and the corresponding standard deviation, in fit and prediction, over the 99 permutation models are reported.

Model	**X-variables**	**y**	**Average RMSEC / std dev.**	**Average RMSEP / std. dev.**	**y**	**Average RMSEC / std dev.**	**Average RMSEP / std. dev.**
I	All	SW	1.83 +/- 1.30%	2.57 +/- 1.77%	DD	1.49 +/- 1.06%	2.02 +/-1.36%
II	Only variables obtained by Stability and Aeration Tests	SW	2.09 +/- 1.28%	2.33 +/- 1.54%	DD	1.47 +/- 1.11%	1.82 +/- 1.26%

Table 4: PLS-2 models performance for NGI responses. The average error and the corresponding standard deviation, in fit and prediction, over the 100 permutation models are reported.

Y response variable	Average RMSEC / std dev.	Average RMSEP / std. dev.
CDD	4.65% +/- 3.88%	4.34% +/- 2.97%
FPM	9.95% +/- 7.44%	10.67% +/- 8.07%
MMAD	9.27% +/- 5.42%	11.42% +/- 7.66%