

1 Quantitative Analysis of Powder Mixtures by
2 Raman Spectrometry: the influence of particle size
3 and its correction
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6 Zeng-Ping Chen^{*a}, Li-Mei Li^a, Jing-Wen Jin^a, Alison Nordon^b, David Littlejohn^b, Jing Yang^a,

7 Juan Zhang^a, Ru-Qin Yu^a
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11 *a. State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and*
12 *Chemical Engineering, Hunan University, Changsha 410082, China*

13 *b. WestCHEM, Department of Pure and Applied Chemistry and Centre for Process Analytics*
14 *and Control Technology, University of Strathclyde, Glasgow, G1 1XL, Scotland, UK*
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18 * *Corresponding author*

19 Tel.: (+86) 731 88821916; Fax: (+86) 731 88821916;

20 E-Mail Address: zpchen2002@hotmail.com (Z.P. Chen)
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22 **Abstract:** Particle size distribution and compactness have significant confounding effects on
23 Raman signals of powder mixtures, which cannot be effectively modeled or corrected by
24 traditional multivariate linear calibration methods such as partial least squares (PLS), and
25 therefore greatly deteriorate the predictive abilities of Raman calibration models for powder
26 mixtures. The ability to obtain directly quantitative information from Raman signals of
27 powder mixtures with varying particle size distribution and compactness is, therefore, of
28 considerable interest. In this study, an advanced quantitative Raman calibration model was
29 developed to explicitly account for the confounding effects of particle size distribution and
30 compactness on Raman signals of powder mixtures. Under the theoretical guidance of the
31 proposed Raman calibration model, an advanced dual calibration strategy was adopted to
32 separate the Raman contributions caused by the changes in mass fractions of the constituents
33 in powder mixtures from those induced by the variations in the physical properties of samples,
34 and hence achieve accurate quantitative determination for powder mixture samples. The
35 proposed Raman calibration model was applied to the quantitative analysis of backscatter
36 Raman measurements of a proof-of-concept model system of powder mixtures consisting of
37 barium nitrate and potassium chromate. The average relative prediction error of prediction
38 obtained by the proposed Raman calibration model was less than one-third of the
39 corresponding value of the best performing PLS model for mass fractions of barium nitrate in
40 powder mixtures with variations in particle size distribution as well as compactness.

41 **Keywords:** Quantitative Raman Spectroscopic Analysis, Particle Size Distribution,
42 Compactness, Multiplicative Confounding Effects, Powder Mixture, Dual Calibration
43 Strategy

44 **Introduction**

45 Powder blending is an important process in the manufacture of many pharmaceutical
46 products¹. Raman spectroscopy has been increasingly applied to the qualitative analysis of
47 powder mixtures²⁻⁶, because of its flexibility of sampling (solids can be analyzed with little
48 or no sample preparation), and exceptionally high chemical specificity and the use of fibre
49 optics for convenient and remote analysis, which facilitate the non-invasive in-line and real
50 time analysis of particulate systems⁷⁻¹⁷. However, some issues remain unresolved regarding
51 the quantitative in-line monitoring of particulate systems by Raman spectroscopy.

52 One of the issues is that the Raman intensities of analyte peaks depend on not only the
53 analyte concentration, but also on the intensity of the excitation source, the instrument's
54 optical configuration and the sample alignment. Therefore, to gain quantitative information
55 requires the use of internal or external standards¹⁸⁻²⁰. Band ratios between the overall Raman
56 intensities and that of an individual spectral peak arising from internal or external standards
57 are calculated and used in quantitative analysis. But the use of internal or external standards
58 can be difficult to apply accurately in many *in-situ* process analysis applications. Moreover,
59 for samples involving solids such as powder mixtures, quantitative Raman analysis becomes
60 even more difficult, because the Raman measurements from such samples depend on the
61 particle size and compactness of the mixtures, which hinders the use of an internal or external
62 standard. The application of multivariate calibration methods such as principal component
63 regression (PCR) and partial least squares (PLS) has some advantages over univariate band
64 ratio calibration models in the quantitative analysis of Raman measurements^{20,21}. However,
65 when analyzing powder mixtures using Raman spectroscopy, the variations in the physical

66 properties such as particle size and compactness of the mixtures have confounding effects on
67 the total Raman intensities. Such confounding effects cannot be effectively modeled by
68 standard multivariate calibration methods, and will significantly affect the predictive
69 accuracy of multivariate calibration models.

70 Although it has long been known that physical properties of powder samples can
71 influence the intensity of the Raman spectrum, and several studies²²⁻²⁶ have been conducted
72 on the relationship between particle size and Raman intensity, relatively little research
73 focuses on quantitative Raman spectroscopic analysis of powder mixtures. Some of the
74 present authors conducted a preliminary investigation on quantitative Raman spectroscopic
75 analysis of suspension samples²⁷. However, due to the facility limitations at that time, we
76 were unable to explicitly investigate the effects of particle size distribution and sample
77 compactness on Raman signals of powder mixtures in that work. The objectives of this study
78 are to 1) explicitly investigate the effects of particle size and compactness on Raman signals
79 of powder mixtures, 2) develop an advanced quantitative Raman calibration model for
80 powder mixtures, and 3) eventually achieve accurate quantitative analysis of powder mixtures
81 using Raman spectrometry.

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84 **Theory**

85 *Raman intensities of powder mixtures*

86 The intensity of Raman bands depends on a complex expression involving the polarisability
87 tensor of a molecule²⁸. For analytical purposes, the following less rigorous linear model

88 analogous to the Beer-Lambert law can be used.

$$I(\nu) = n \cdot r(\nu) \cdot I_o \quad (1)$$

89 Where $I(\nu)$ is the Raman intensity at Raman shift ν , I_o is the intensity of the excitation
90 radiation, n is the number of molecules of the analyte illuminated by the source and viewed
91 by the spectrometer, and $r(\nu)$ is a composite term that represents the overall spectrometer
92 response, and the self absorption and molecular scattering properties of the analyte at Raman
93 shift ν . For K powder samples comprising J constituents with amounts above their Raman
94 limits of detection, their overall Raman intensities can be expressed as the linear combination
95 of the contributions of all J constituents as well as other possible interference(s) such as
96 fluorescence.

$$I_k(\nu) = \sum_{j=1}^J [n_{k,j} \cdot r_j(\nu) \cdot I_{o,k}] + n_{k,interf} \cdot r_{interf}(\nu) \cdot I_{o,k}; \quad k = 1, 2, \dots, K \quad (2)$$

97 Where $n_{k,j}$ and $n_{k,interf}$ are the number of molecules of the j -th constituent and the
98 interference(s) in the k -th powder sample illuminated by the source and viewed by the
99 spectrometer, respectively; $r_{interf}(\nu)$ represents the molecular scattering/fluorescence
100 properties of the interference(s) at Raman shift ν .

101 Suppose m_k and V_k are the overall mass and volume of the k -th powder sample,
102 respectively. $V_{spec,k}$ denotes the volume of the k -th powder sample illuminated by the source
103 and viewed by the spectrometer. $w_{k,j}$ ($\sum_{j=1}^J w_{k,j} = 1$) signifies the mass fraction of the j -th
104 constituent in the k -th sample. M_j is the molecular weight of the j -th constituent. The
105 multiplicative parameter, p_k , is introduced to account for the effects of the particle size

106 distribution and compactness of the k -th sample on the Raman intensities^{24, 27}. Equation 2

107 then becomes:

$$I_k(\nu) = \sum_{j=1}^J [p_k \cdot \frac{m_k \cdot w_{k,j} \cdot V_{spec,k}}{M_j \cdot V_k} \cdot r_j(\nu) \cdot I_{o,k}] + n_{k,interf} \cdot r_{interf}(\nu) \cdot I_{o,k} \quad (3)$$

108 Define $q_k = p_k \cdot m_k \cdot V_{spec,k} \cdot I_{o,k} / V_k$ and $r_j^*(\nu) = r_j(\nu) / M_j$. Equation 3 can be simplified as

109 follows.

$$I_k(\nu) = \sum_{j=1}^J [q_k \cdot w_{k,j} \cdot r_j^*(\nu)] + n_{k,interf} \cdot r_{interf}(\nu) \cdot I_{o,k} \quad (4)$$

110 In equation 4, q_k is a very important model parameter. It accounts for the variations in Raman

111 intensities caused by the changes in variables other than the mass fractions of the constituents

112 in the powder mixtures, such as the intensity of the excitation source, the sample's particle

113 size distribution, sample compactness, the overall mass and volume of the powder sample as

114 well as the volume illuminated by the source and viewed by the spectrometer.

115 Suppose the j -th constituent is the target component in the powder mixtures, and the

116 Raman signals of K calibration samples have been measured over Raman shift range of $\nu_1 \sim \nu_m$.

117 As $\sum_{j=1}^J w_{k,j} = 1$, equation 4 can be rewritten as:

$$\mathbf{x}_k = q_k \cdot w_{k,1} \cdot \Delta \mathbf{r}_1^* + q_k \cdot \mathbf{r}_2^* + \sum_{j=3}^J [q_k \cdot w_{k,j} \cdot \Delta \mathbf{r}_j^*] + n_{k,interf} \cdot \mathbf{r}_{interf} \cdot I_{o,k}$$

Where, $\mathbf{x}_k = [I_k(\nu_1), I_k(\nu_2), \dots, I_k(\nu_m)]$; $\mathbf{r}_j^* = [r_j^*(\nu_1), r_j^*(\nu_2), \dots, r_j^*(\nu_m)]$, $j = 1, 2, \dots, J$ (5)

$$\Delta \mathbf{r}_j^* = \mathbf{r}_j^* - \mathbf{r}_2^*; \quad \mathbf{r}_{interf} = [r_{interf}(\nu_1), r_{interf}(\nu_2), \dots, r_{interf}(\nu_m)]$$

118 Assuming $\Delta \mathbf{r}_j^*$, \mathbf{r}_2^* , and \mathbf{r}_{interf} are linearly independent of each other, it can be seen that a

119 straightforward multivariate linear calibration model can be built only between \mathbf{x}_k and

120 $q_k \cdot w_{k,j}$ (or q_k). It is obvious that the multiplicative parameter, q_k , may be different for each

121 of the powder samples. Hence the relationship between Raman spectrum \mathbf{x}_k and the mass
 122 fraction of the j -th constituent ($w_{k,j}$) is actually nonlinear; and the multiplicative parameter, q_k ,
 123 has confounding effects on the estimation of $w_{k,j}$. In order to extract the quantitative
 124 information (mass fraction) of any constituent in powder samples from their Raman
 125 measurements, it is therefore imperative to estimate the multiplicative parameter, q_k , for each
 126 powder sample.

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128 *Dual Calibration Strategy (DCS)*^{27, 29-30}

129 For K training samples in which the mass fractions of the target constituent (say, the j -th
 130 constituent) are known, the multiplicative parameters, q_k ($k = 1, 2, \dots, K$), can be estimated by
 131 the modified Optical Path-Length Estimation and Correction method (OPLEC_m)³⁰ (the
 132 Matlab script for OPLEC_m is provided in supporting information). After the estimation of q_k
 133 ($k = 1, 2, \dots, K$), the following two calibration models can be built by multivariate linear
 134 calibration methods such as PLS.

$$diag(\mathbf{w}_j)\mathbf{q} = a_1\mathbf{1} + \mathbf{X}_{cal}\boldsymbol{\beta}_1; \quad \mathbf{q} = a_2\mathbf{1} + \mathbf{X}_{cal}\boldsymbol{\beta}_2 \tag{6}$$

$$\mathbf{X}_{cal} = [\mathbf{x}_1; \mathbf{x}_2; \dots; \mathbf{x}_K]; \quad \mathbf{w}_j = [w_{1,j}; w_{2,j}; \dots; w_{K,j}]; \quad \mathbf{q} = [q_1; q_2; \dots; q_K]$$

135 Where $diag(\mathbf{w}_j)$ denotes the diagonal matrix in which the corresponding diagonal elements are
 136 the elements of \mathbf{w}_j ; $\mathbf{1}$ is a column vector with its elements equal to unity. After the estimation
 137 of model parameters a_1 , a_2 , $\boldsymbol{\beta}_1$, and $\boldsymbol{\beta}_2$ by multivariate calibration methods such as PLS,
 138 these two calibration models could then be used to predict the mass fraction of the target
 139 constituent in any test powder sample ($w_{test,j}$) from its Raman spectrum \mathbf{x}_{test} .

$$q_{test} \cdot w_{test,j} = a_1 + \mathbf{x}_{test} \boldsymbol{\beta}_1; \quad q_{test} = a_2 + \mathbf{x}_{test} \boldsymbol{\beta}_2; \quad w_{test,j} = \frac{a_1 + \mathbf{x}_{test} \boldsymbol{\beta}_1}{a_2 + \mathbf{x}_{test} \boldsymbol{\beta}_2} \quad (7)$$

140 The mass fraction of other constituents in the test sample can be obtained in a similar way.

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143 **Experimental**

144 *Materials*

145 All chemicals were analytical grade, and were used as received without any further
 146 purification. Potassium chromate was obtained from Tianjin Windship Chemistry
 147 Technological Co., Ltd (Tianjin, China). Barium nitrate was purchased from Tianjin Kermel
 148 Chemical Reagent Co., Ltd (Tianjin, China).

149

150 *Equipment*

151 Raman spectra were acquired at room temperature on a LABRAM-0101 Laser Confocal
 152 Raman Spectrometer equipped with a 1024×256 pixels CCD detector. The microscope
 153 attachment was based on an Olympus BX41 system with a 10× objective. Radiation of
 154 632.81 nm from a 17 mW He-Ne laser was used for excitation. The widths of the entrance slit
 155 and confocal pinhole were set to 100 μm and 1000 μm, respectively. Raman spectrum
 156 between 200 and 2000 cm⁻¹ was collected with a 5 s exposure time and 3 accumulations for
 157 each spectrum.

158

159 *Raman measurements of powder mixtures*

160 The solids of both barium nitrate and potassium chromate were ground and sorted into

161 different particle sizes using standard sieves. The standard sieves were of mesh sizes 40, 60,
162 80, 100, 120, 140, 160 and 200 wires per inch. The hole sizes corresponding to the mesh sizes
163 are 425, 250, 180, 150, 125, 109, 96 and 75 μm , respectively. A total of 72 powder mixtures
164 of potassium chromate and barium nitrate powder with different weight ratios (1:0, 0.90:0.10,
165 0.75:0.25, 0.60:0.40, 0.50:0.50, 0.40:0.60, 0.25:0.75, 0.10:0.90 and 0:1) and different particle
166 sizes (425, 250, 180, 150, 125, 109, 96 and 75 μm) were prepared by mixing appropriate
167 amounts of the two constituents thoroughly (Table 1). For each of 72 powder mixtures, a
168 sample was randomly taken and loosely packed into a cylindrical sample cup with a diameter
169 of 6.9 mm and a height of 10.7 mm. The laser beam was focused at a point inside the sample
170 so as to ensure the illumination of the whole upper surface of the sample by the laser beam,
171 and then the Raman spectrum was acquired. Following this, each sample was packed more
172 firmly, and a further Raman spectrum was recorded resulting in a total of 144 spectra.
173 Seventy eight spectra (two outliers were removed) from the five mixtures with the ratios of
174 potassium chromate to barium nitrate equal to 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1
175 formed the calibration data set. The test set comprised the remaining 64 spectra from the
176 other four mixtures. Distinctive Raman peaks of potassium chromate (at around 351, 386.5,
177 396.8, 853.4, 868.4, 877.8 and 906.8 cm^{-1}) and barium nitrate (at about 1047.5 cm^{-1}) can be
178 readily observed between 292.8 and 1136.6 cm^{-1} (supporting information, Figure S-1).
179 Therefore, Raman signals in this region were selected for the subsequent data analysis.

180 Table 1: Mass ratios and particle sizes of potassium chromate and barium nitrate in powder mixtures.

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Sample No.	K ₂ CrO ₄ /Ba(NO ₃) ₂ (mass ratio)	Particle Size (μm)
1 – 8	1:0	425, 250, 180, 150, 125, 109, 96, 75
9 – 16	0.90:0.10	425, 250, 180, 150, 125, 109, 96, 75
17 – 24	0.75:0.25	425, 250, 180, 150, 125, 109, 96, 75
25 – 32	0.60:0.40	425, 250, 180, 150, 125, 109, 96, 75
33 – 40	0.50:0.50	425, 250, 180, 150, 125, 109, 96, 75
41 – 48	0.40:0.60	425, 250, 180, 150, 125, 109, 96, 75
49 – 56	0.25:0.75	425, 250, 180, 150, 125, 109, 96, 75
57 – 64	0.10:0.90	425, 250, 180, 150, 125, 109, 96, 75
65 – 72	0:1	425, 250, 180, 150, 125, 109, 96, 75

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191 *Data analysis*

192 Due to the influence of particle size and compactness on Raman intensities, it is unlikely that
193 univariate analysis will give accurate predictions of the mass fractions of barium nitrate in
194 powder mixtures. Therefore, PLS and the dual calibration strategy (DCS) were adopted for
195 the data analysis and their performance in terms of providing accurate predictions for the
196 mass fractions of barium nitrate in powder mixtures were compared. The effectiveness of
197 multiplicative signal correction (MSC)³¹, standard normal variate (SNV)³² and extended
198 inverted signal correction (EISC)³³ in correcting the confounding effects of physical
199 properties of powder samples on the Raman measurements and improving the predictive
200 abilities of PLS calibration models were also investigated. For the convenience of
201 presentation, PLS calibration models built on the mean-centred raw and preprocessed Raman
202 spectra by MSC, EISC and SNV are denoted by PLS_raw, PLS_MSC, PLS_EISC and
203 PLS_SNV, respectively. No pre-processing methods other than mean-centring were used
204 when building DCS calibration models. The optimal calibration models were selected
205 through a cross-validation procedure. During cross-validation, the Raman spectra of the
206 calibration samples with the same mass ratio of potassium chromate to barium nitrate were
207 left out in turn and the root mean square error of prediction from cross validation (RMSEP_{cv})
208 values were calculated. The calibration models with the minimal RMSEP_{cv} values were
209 taken as the optimal models, and were then used to predict the mass fractions of barium
210 nitrate in the test samples. The data analysis was performed on a Pentium class computer
211 using Matlab version 6.5 (Mathworks, Inc). All the programmes including DCS, PLS, MSC,
212 SNV, and EISC were written in house.

213

214 **Results and discussion**

215 *The sensitivity of Raman intensities to the mass fraction of barium nitrate*

216 Figure 1a shows the Raman spectra of powder mixtures samples with the same particle size
217 (250 μm) and similar compactness but different mass ratios of potassium chromate to barium
218 nitrate. The Raman peaks are relatively sensitive to the changes in the composition of the
219 powder mixtures. The Raman peak intensity at 1047.5 cm^{-1} generally increases with mass
220 fraction of barium nitrate in the powder samples. However, the relationship between Raman
221 peak intensity and mass fraction of barium nitrate deviates from a perfect linear model even
222 when samples have a similar particle size and degree of compactness (Figure 1b). Especially
223 there is a discontinuity which might be caused by the variation in excitation intensity or
224 packing density. This demonstrates the necessity to introduce the multiplicative parameter, q_k ,
225 in eq.4 to account for the variations in Raman intensities caused by the changes in variables
226 other than the mass fractions of the constituents in the powder mixtures.

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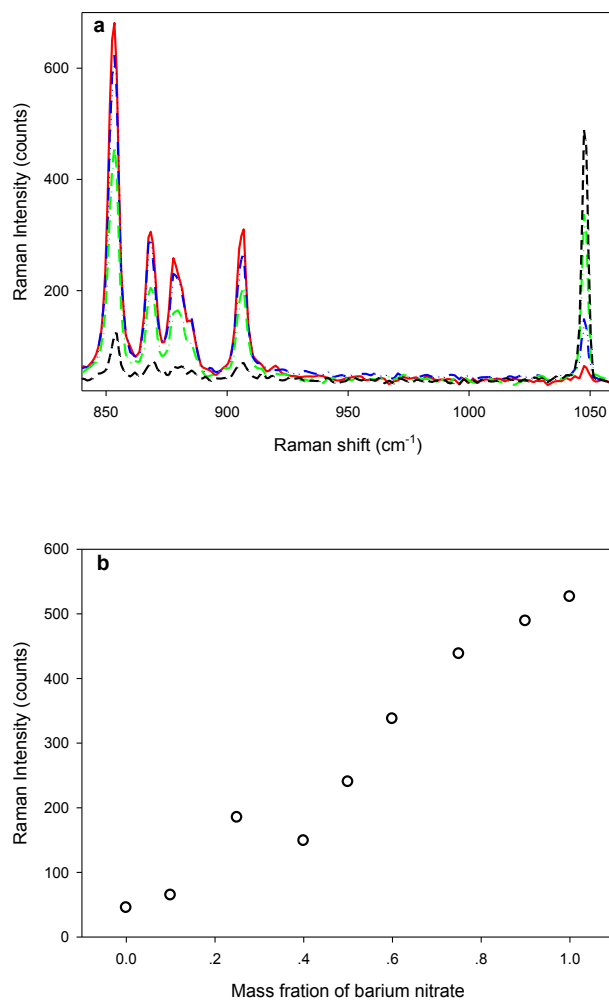


Figure 1: a) Raman spectra of loosely packed powder mixture samples (particle size: 250 μm) with different mass ratios of potassium chromate to barium nitrate (red solid line: 0.9:0.1; blue dash-dot-dot line: 0.60:0.40; green dash-dot line: 0.40:0.60; black dash line: 0.10:0.90); b) Raman peak intensity at 1047.5 cm^{-1} vs mass fraction of barium nitrate in loosely packed powder mixture samples (particle size: 250 μm).

237 *The effects of particle size and compactness on Raman intensities*

238 In addition to the mass ratio of potassium chromate to barium nitrate, the particle size and
239 compactness of the powder mixture samples also have a significant influence on the Raman
240 peak intensities. As shown in Figure 2a, a firmly packed sample has significantly more
241 intense Raman peaks than those of a loosely packed sample with the same mass ratio and
242 particle size. It has long been known that particle size differences make significant
243 contributions to the spectral variations in Raman measurements of powders²². Our
244 experimental results also show that variations in particle size of powder samples have
245 significant effects on the Raman spectra (Figure 2b). For two samples with the same particle
246 size (109 μm) but different mass ratios of potassium chromate to barium nitrate (e.g.
247 0.25:0.75 and 0.10:0.90), the difference between the peak intensities at 1047.5 cm^{-1} is 59.04.
248 While for two samples with the same mass ratio of potassium chromate to barium nitrate
249 (0.10:0.90) but different particle sizes (e.g. 109 and 75 μm), the difference between the
250 corresponding peak intensities is 113.63, which is about 1.9 times that caused by a change in
251 the mass ratio of potassium chromate to barium nitrate from 0.25:0.75 to 0.90:0.10. Moreover,
252 variation in the particle size of a sample has the same effect on all Raman peaks in the
253 spectrum. This makes it difficult to discriminate Raman intensity contributions caused by
254 changes in a sample's particle size from those due to a variation in mass fractions of the
255 chemical constituents using traditional univariate/multivariate calibration methods. If not
256 properly modelled, this difference would significantly degrade the accuracy and reliability of
257 calibration models built on Raman measurements contaminated by such confounding effects.
258 The multiplicative parameter, q_k , in eq.4 accounts for the effects of particle size and

259 compactness on the Raman intensities and so their effects can be separated from those of the
260 mass fractions of the chemical constituents in powder samples by the unique dual calibration
261 strategy.

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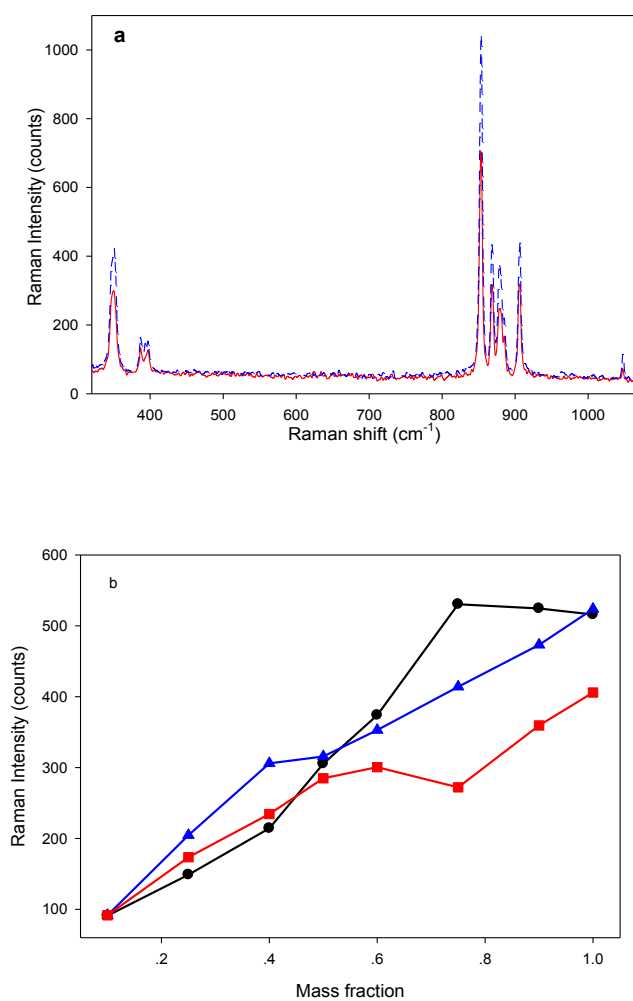


Figure 2: a) Raman spectra of a binary powder mixture sample (potassium chromate:barium nitrate: 0.90:0.10, particle size: 425 μm) with different compactness (blue dash line: firmly packed; red solid line: loosely packed); b) peak intensity at 1047.5 cm^{-1} vs mass fraction of barium nitrate with different particle sizes (black circle: 180 μm ; blue triangle: 109 μm ; red square: 75 μm).

282 *Correction of the multiplicative effects of particle size and compactness on Raman intensities*

283 As shown in the preceding section, the presence of significant multiplicative confounding
284 effects (arising from differences in particle size and compactness) caused deviations in the
285 linear relationship between the Raman intensities and the mass fraction of solid powder
286 samples. With a view to mitigate the influence of the multiplicative confounding effects
287 present in the Raman spectral data, the dual calibration strategy (DCS) was employed to
288 correct such confounding effects. For the purpose of comparison, PLS models with and
289 without the use of pre-processing methods MSC, EISC and SNV were also applied to the
290 same Raman spectral data. DCS involves the estimation of the multiplicative parameter, q_k ,
291 for each calibration sample by OPLEC_m³⁰. The implementation of OPLEC_m requires the
292 determination of the number of spectral variation sources including chemical components and
293 possible interference(s). For the powder mixture samples studied in this paper, the number of
294 spectral variation sources is two, i.e. potassium chromate and barium nitrate. The results of
295 OPLEC_m are shown in Figure 3. It is evident that different calibration samples generally have
296 different multiplicative parameter values (q_k) and the multiplicative parameter, q_k , of the
297 calibration samples varies in the range of 1 – 2.23. These results demonstrate that the
298 presence of significant multiplicative confounding effects in the Raman spectral data and the
299 introduction of the multiplicative parameter, q_k , in eq.4 is theoretically sound and also
300 practically relevant. Otherwise, the multiplicative parameter values (q_k) calculated by
301 OPLEC_m for the calibration samples would vary within a narrow range, and would also be
302 quite close to 1.

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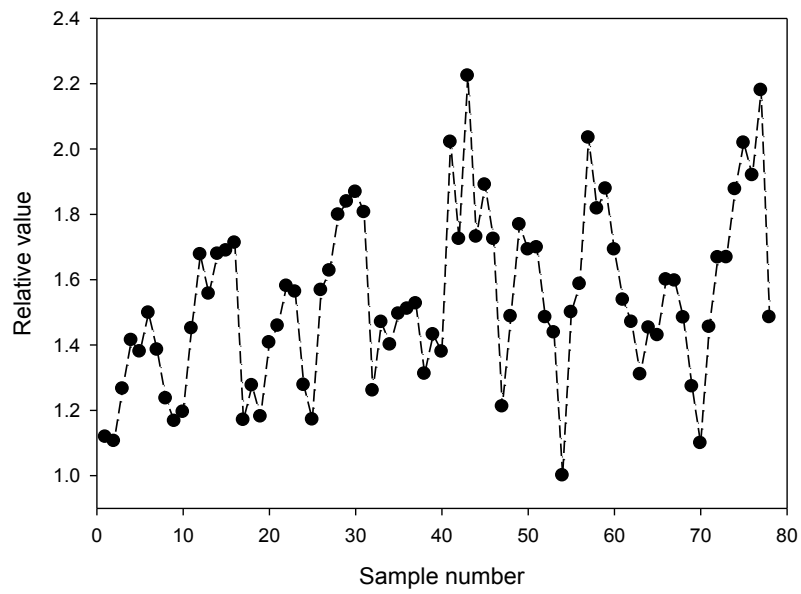


Figure 3: the multiplicative parameter q_k for the calibration samples estimated by $OPLEC_m$.

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315 After the calculation of the multiplicative parameters, q_k , for each calibration sample by
316 OPLEC_m, DCS models with different underlying components were built. Values of the root
317 mean square error of prediction from cross validation (RMSEP_{cv}) obtained by DCS and the
318 various PLS calibration models (i.e. PLS_{raw}, PLS_{MSC}, PLS_{EISC} and PLS_{SNV}) with
319 different number of latent variables are given in supporting information, Figure S-2. Both the
320 PLS_{raw} and PLS_{MSC} models attained minimum RMSEP_{cv} values of 0.08 and 0.12,
321 respectively, when two latent variables were used. For PLS_{EISC} and PLS_{SNV}, only one
322 latent variable was suggested by cross validation; however, the RMSEP_{cv} values of 0.26 and
323 0.23, respectively, were significantly larger than that for PLS_{raw}, which to some extent
324 indicates the inappropriateness of applying EISC and SNV to this particular Raman spectral
325 data set. In contrast with the above PLS calibration models, a DCS model with three latent
326 variables had a minimum RMSEP_{cv} value of 0.03, which is less than half that of the
327 corresponding value obtained with the PLS_{raw} model.

328 For a more convincing comparison, the performance of the optimal DCS and various
329 PLS calibration models for the independent test samples was investigated. As shown in
330 Figure 4 and Figure 5, the RMSEP value of the optimal PLS_{raw} model obtained for the
331 independent test samples was 0.08 (equivalent to a mean relative prediction error of 30.8%),
332 which clearly demonstrates the presence of detrimental multiplicative confounding effects
333 caused by variations in the particle size and compactness of powder samples. The application
334 of MSC, EISC and SNV resulted in a deterioration of the predictive ability of the PLS
335 calibration models. This confirms that the pre-processing methods MSC, EISC and SNV
336 cannot effectively correct the multiplicative confounding effects of particle size and

337 compactness on Raman intensities. In contrast, the optimal DCS model with 3 latent variables
338 achieved a RMSEP value of 0.04 for the independent test samples, which is equivalent to a
339 mean relative prediction error of 9.6%, less than one third of the corresponding value for the
340 optimal PLS_raw model. Even more interestingly, though the construction of the DCS model
341 requires no extra information or data compared to the PLS models, it consistently
342 outperformed the various PLS models built on the raw and pre-processed Raman spectra, no
343 matter how many latent variables were used (Figure S-3 in supporting information). The
344 significant reduction in the RMSEP value achieved with the optimal DCS model results
345 solely from the introduction of the multiplicative parameter, q_k , in eq. 4 to account for the
346 variations in Raman intensities caused by the changes in variables other than the mass
347 fractions of the chemical constituents in powder mixtures, in this case particle size and
348 compactness.

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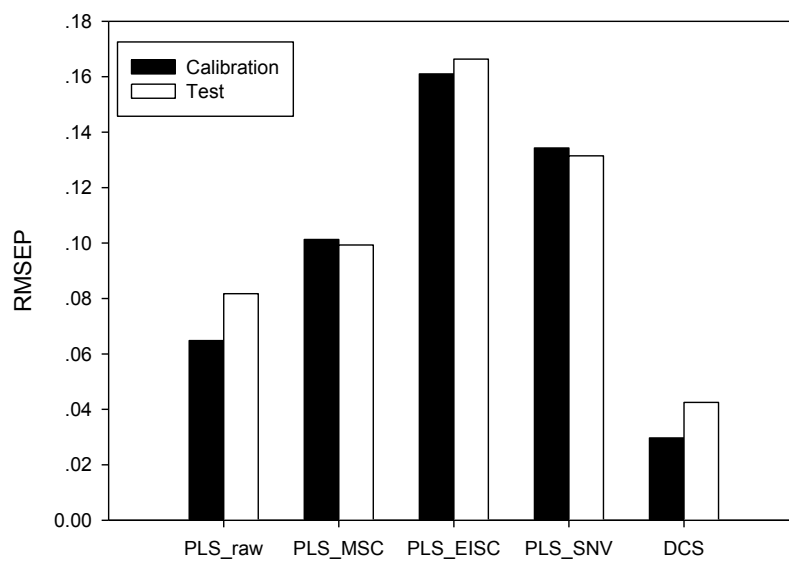


Figure 4: the RMSEP values for both the calibration and independent test samples obtained by different calibration methods.

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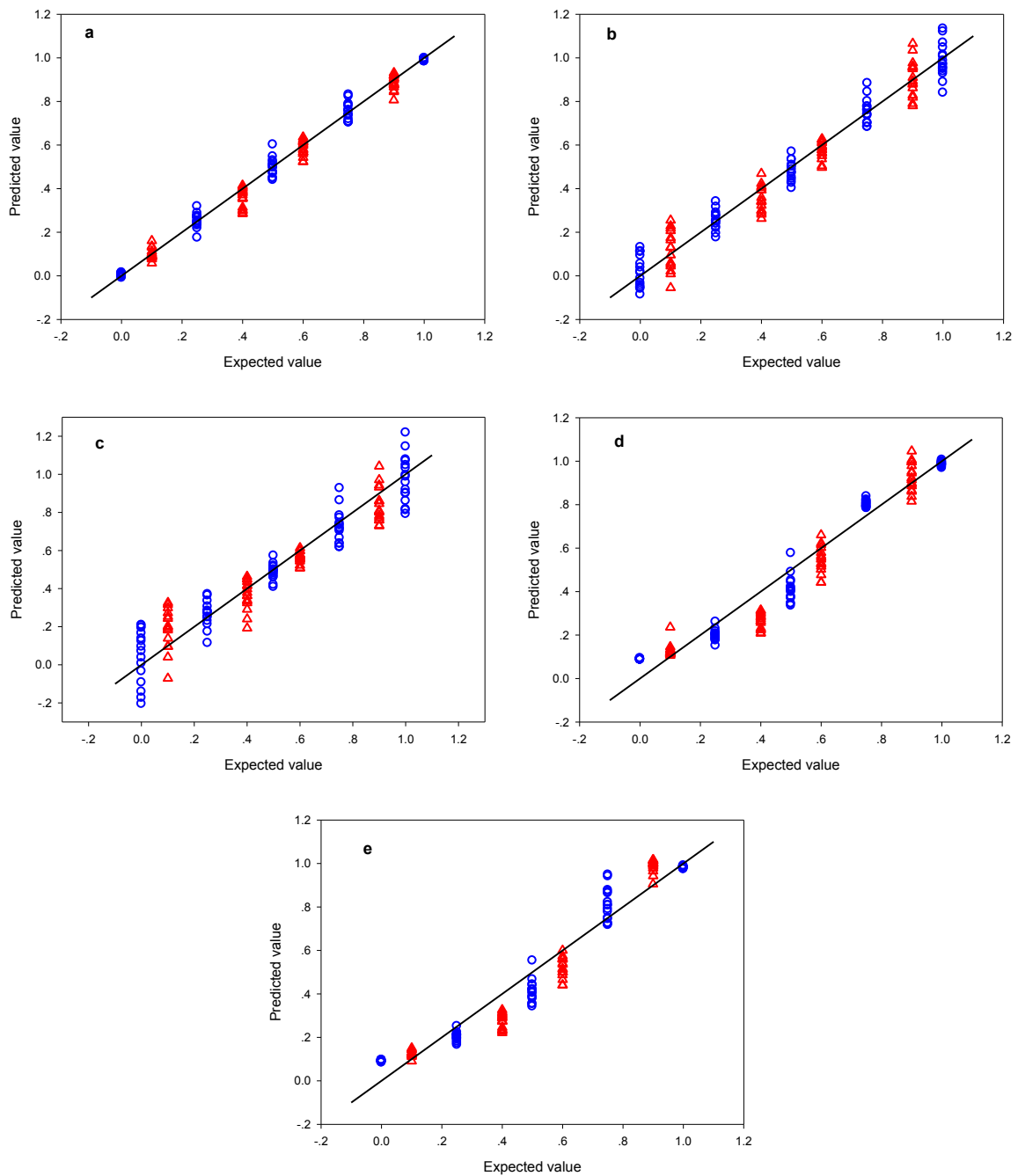


Figure 5: the mass fractions of $\text{Ba}(\text{NO}_3)_2$ in the calibration (blue circle) and independent test (red triangle) samples predicted by various calibration models (a: DCS; b: PLS_raw; c: PLS_MSC; d: PLS_SNV; e: PLS_EISC)

372 **Conclusions**

373 The Raman intensities of powder mixture samples depend on not only the mass fractions of
374 the chemical constituents but also the physical properties of samples such as particle size
375 distribution and compactness. The experimental results on a model system of powder
376 mixtures consisting of barium nitrate and potassium chromate showed that the effects of
377 particle size distribution and compactness on Raman measurements are multiplicative, which
378 cannot be effectively modelled by multivariate linear calibration methods such as PLS.
379 Pre-processing the Raman measurements with multiplicative confounding effects of particle
380 size and compactness by MSC, SNV or EISC could not improve but rather deteriorated the
381 predictive performance of Raman calibration models. In this work, we introduced a
382 multiplicative parameter in the quantitative Raman calibration model to explicitly account for
383 the confounding effects of particle size and compactness on Raman signals of powder
384 mixtures, and then eliminated the confounding effects through a unique dual calibration
385 strategy. The average relative prediction error of predictions obtained by the dual calibration
386 strategy for the independent test samples was less than one-third of the corresponding value
387 of the optimal PLS calibration models built using the raw Raman spectra and considerably
388 better than the results of PLS models based on spectra pre-processed by application of MSC,
389 EISC or SNV. These results demonstrate that the dual calibration strategy can effectively
390 mitigate the confounding effects of samples' physical properties and so improve the accuracy
391 of quantitative analysis of powders using Raman spectrometry. Hence, the dual calibration
392 strategy will be of major benefit for quantitative measurement of particulate samples such as
393 powder blends and pharmaceutical dosage forms.

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395 **Acknowledgements**

396 The authors acknowledge the financial support of the National Natural Science Foundation of
397 China (grant no. 21075034 and grant no. 21035001) and the National Instrumentation
398 Program of China (grant no. 2011YQ0301240102). AN acknowledges the award of a
399 University Research Fellowship by the Royal Society, UK.

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402 **Supporting Information Available**

403 MATLAB code for the modified OPLEC method, Raman spectra of K_2CrO_4 and $Ba(NO_3)_2$;
404 The RMSEP values from cross validation obtained by DCS and various PLS calibration
405 models with different number of latent variables built on the raw and pre-processed Raman
406 spectra by MSC, SNV, and EISC; The RMSEP values for the test powder mixtures obtained
407 by DCS and various PLS calibration models with different number of latent variables built on
408 the raw and pre-processed Raman spectra by MSC, SNV, and EISC. This material is
409 available free of charge via the Internet at <http://pubs.acs.org>.

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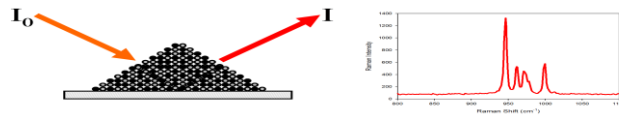
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