Graphical Abstract

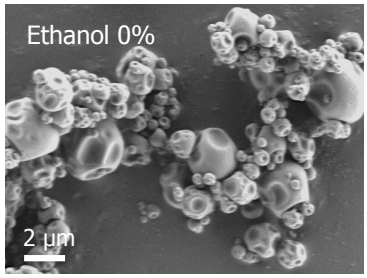
Amikacin dry powders for inhalation

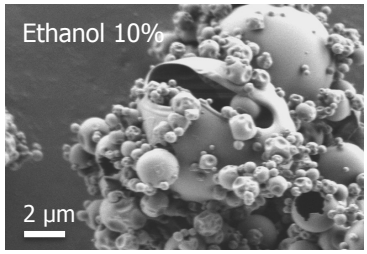
Central Composite Design

↓

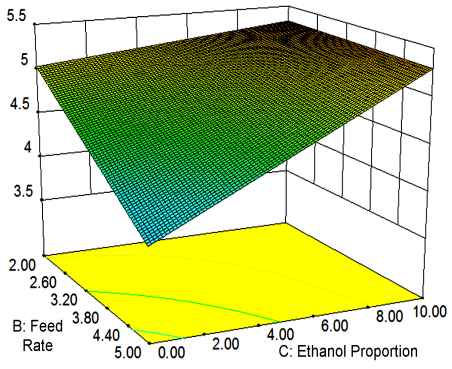
Effect of ethanol on the formation of microparticles

Peclet number and drug solubility





Fine Particle Dose



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Spray-dried amikacin sulphate powder for inhalation in cystic fibrosis patients: the role of ethanol in particle formation.

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KEYWORDS
amikacin sulphate; dry powder inhaler; Peclet number; microparticles; cystic fibrosis

Abbreviation section

CCD	Central Composite Design
CF	Cystic Fibrosis
CQAs	Critical Quality Attributes
CPPs	Critical Process Parameters
DoE	Design of Experiments
ED	Emitted Dose
FPD	Fine Particle Dose

Chemical compound studied in this article:

Amikacin sulphate (PubChem CID: 45357036)

Abstract

A Central Composite Design (CCD) was applied in order to identify positive combinations of the production parameters of amikacin sulphate spray-dried powders for inhalation, with the intent to expand the experimental space defined in a previous half-fractional factorial design. Three factors, namely drying temperature, feed rate and ethanol proportion, have been selected out of the initial five. In addition, the levels of these factors were increased from two to three and their effect on amikacin respirability was evaluated. In particular, focus was given on the role of ethanol presence on the formation of the microparticles for inhalation.

The overall outcome of the CCD was that amikacin respirability was not substantially improved, as the optimum region coincided with areas already explored with the fractional factorial design. However, expanding the design space towards smaller ethanol levels, including its complete absence, revealed the crucial role of this solvent on the morphology of the produced particles. Peclet number and drug solubility in the spraying solution helped to understand the formation mechanism of these amikacin sulphate spray-dried particles.

1. Introduction

Lung infections in Cystic Fibrosis (CF) patients caused by *Pseudomonas aeruginosa* are efficiently managed with antibacterial drugs. These treatments require high doses of antibiotics. However, using the pulmonary route, the inhaled drug is directly deposited on the site of infection providing higher local concentrations with lower doses compared to systemic administration. Dry powder inhalers are able to deliver high payloads of drug in a shorter time, offering a convenient alternative to solutions for nebulization [1]. However, high doses of powders can raise adverse effects during the administration, such as cough and choking. Consequently, there are two approved administration strategies for delivering high doses of powdered drugs to the lung of the patients [2]. The first used a single pre-metered capsule reservoir containing the whole dose to be extracted by successive inhalation acts, such as with the Colobreathe product [3]. The second strategy consisted in splitting the dose in multiple capsule reservoirs. In Tobi Podhaler, the dry powder of tobramycin formulation (112 mg dispersed in approximately 200 mg of powder) is administered by the consecutive inhalation of four capsules content. An evolution of these delivery systems is the use of new disposable devices, capable to gradually release the dose loaded in the device reservoir in alternative to hard capsules [4,5].

The performance of a dry powder inhaler is governed by formulation characteristics. Particle engineering strategies have been adopted to optimize size, morphology and structure of microparticles, in order to maximize the respirable fraction of the drug, without compromising the powder flow properties [6,7]. Since the antibiotics are administered at high doses (up to 100-150 mg), formulation techniques should avoid the use of carrier excipients, to limit the mass of the powder to be inhaled [8].

Spray drying is a suitable technology towards this direction, as it is capable of providing respirable microparticles for lung administration with acceptable flow properties [9]. The

method has been used for the preparation of antibiotic [10-12], anti-inflammatory compounds [13,14] and insulin dry powder [15,16]. The shape and density of the spray-dried particles can be modified by controlling the parameters affecting the evaporation process of the sprayed droplets [17 - 19].

In a previous study [20], a half-fractional factorial experimental design was applied as a statistical tool for the construction of amikacin sulphate spray-dried pulmonary powders. The mathematical relationships between six Critical Quality Attributes (CQAs) of the finished product and five Critical Process Parameters (CPPs) were established. Drying temperature, feed rate, ethanol:water ratio, concentration of amikacin sulphate in spraying solution and presence of PEG-32 stearate, as respirability adjuvant, were investigated. The results obtained showed that the proposed adjuvant did not benefit the quality of the spray-dried powders and the best factor combination led to an amikacin sulphate powder with an Emitted Dose of 85% and a respirable fraction reaching 58% of the loaded dose.

In the present study, a Central Composite Design has been applied, aiming to expand the experimental space previously defined in the hypothesis to discover further positive combinations of the manufacturing parameters. Therefore, among the previous CPPs, the three most important were amplified at three levels including unexplored regions assumed favourable for increasing amikacin powder respirability. In detail, ethanol proportion, drying temperature and feed rate were evaluated at three levels, including new settings for the first two factors. Special attention has been given to the role of ethanol as solvent in the sprayed solution, with respect to the effect of its absence/presence on final product structure and inhalation performance.

2. Materials and methods

2.1 Materials

Amikacin sulphate was obtained by ACS DOBFAR S.p.a. (Milan, I). All solvents used were of analytical grade. Water was purified by reverse osmosis (MilliQ, Millipore, Guyancourt, France). Hydroxypropylmethylcellulose (HPMC) capsules (size 3) were received from Capsugel (Colmar, France). RS01 Dry Powder Inhaler device flow rate 60 L/min (gift of Plastiape S.p.a. (Osnago, LC, I).

Amikacin sulphate solubility was measured in purified water, in ethanol 95.6° and water ethanol mixtures, using the amikacin assay method of Ph.Eur. 8.

2.2 Design of Experiments (DoE)

A face centred-CCD with three factors at three levels was employed, and the experimental matrix is presented in Table 1. The design was constructed and analysed using Design-Expert® Software, Version 9.0.1 (Stat-Ease, Inc., Minneapolis, USA).

2.3 Preparation of spray-dried powders

2.5 g of amikacin sulphate were dissolved in water at room temperature. Ethanol was added under stirring to obtain the proportions reported in Table 1, while drug concentration was kept 2% w/v. The solutions prepared were spray-dried using a Büchi Mini Spray Dryer B-290 (Büchi Labortechnik, Flawil, Switzerland) coupled to a B-296 de-humidifier, adopting the process parameters reported in Table 1. Aspirator rate was kept constant at 90%, while atomizing air velocity and nozzle cleaning interval were adjusted at 600 L/h and level 5 respectively.

The spray-dried powder was quantitatively recovered from the product collection vessel and weighed on an analytical balance (E50S, Gibertini, Italy). The yield was expressed as

percentage of the solid dissolved in the sprayed solution. The dry product was then stored at room temperature in a 25 ml cylindrical glass vial, sealed with a rubber stopper and aluminium cap. Part of the product was agglomerated into microparticle clusters by sieving as described in a previous publication [20].

2.4 Powder and agglomerate characterization

The morphology of the spray-dried powders was assessed by Scanning Electron Microscopy (SEM) (Sigma HD, Carl Zeiss, Germany), at extra high tension of 1.00 kV. Microparticle samples were placed on a double-sided adhesive tape pre-mounted on an aluminium stub and analysed after a 30 min depressurization.

Particle size distribution of spray-dried powders was measured by laser light scattering (SprayTec, Malvern, UK). Approximately 10 mg of sample were dispersed in 20 ml of cyclohexane containing 0.1% w/v of sorbitan monooleate (Span 80) and sonicated for 5 min. The results were expressed in terms of median volume diameter $D_{(v,90)}$, percentiles $D_{(v,10)}$, $D_{(v,50)}$ and Span.

The residual water content (%) of the spray-dried powders was measured by Karl Fischer volumetric titration using TitroMatic Karl Fischer (Crison Instruments, S.A., Barcelona, Spain).

The bulk density was determined as the ratio of the sample mass and its unsettled apparent bulk volume. The latter was directly measured in a 25 ml cylindrical glass vial.

The true density was measured using a helium pycnometer (APS AccuPyc 1330 Gas Pycnometer, Micromeritics, Norcross, GA, USA).

The agglomerates were observed by optical microscopy (magnification 3x), and the diameter of the projected area assumed as spherical, was measured using Image J software (U. S. National Institutes of Health, Bethesda, Maryland, USA).

153 The aerodynamic assessment of the spray-dried powders was carried out using the Fast
154 Screening Impactor (FSI) (Copley Scientific, UK). The FSI divides the aerosol particles
155 emitted from the inhaler into two parts, i.e. the coarse and the fine fractions, the latter
156 corresponding to sizes lower than 5 μm considered as respirable fraction. The Coarse Fraction
157 Collector (CFC) is equipped with an insert that enables the 5 μ m cut-off at 60 L/min. The
158 particles not captured in the CFC follow the airstream and deposit in the fine fraction
159 collector (FFC) where they are captured by a filter (A/E glass filter, 76 mm, Pall Corporation,
160 USA).
161 In detail, an accurately weighed amount of powder equal to 10 ± 0.2 mg, was manually
162 introduced into a size 3 hard HPMC capsule. The capsule was then inserted into the holder
163 chamber of the RS01 device and pierced. The latter was connected to the FSI and flushed by
164 the air stream for 4 s at 60 L/min. The FFC filter was weighed before and after the air
165 actuation, in order to determine the amount of powder deposited, termed as Fine Particle Dose
166 (FPD). Each powder was tested in triplicate before and after the agglomeration process.
167

168 ## 2.5 Determination of evaporation rate of spray-dried solutions

169 The evaporation rates of the spray-dried solutions were measured by thermogravimetric
170 analysis (TGA, Mettler Toledo, Columbus, OH, USA). An accurate amount of solution was
171 introduced in an aluminium-crucible 40 μl pan (Me-26763 without pin, Mettler Toledo). The
172 sample was heated into the apparatus furnace at constant temperature of 85 °C, corresponding
173 to the outlet temperature of spray drying, while purging nitrogen at a flow rate of 20 ml/min.
174 The weight loss was recorded as a function of time [19].
175

3. Results and discussion

In all experiments the yields of amikacin spray-dried powders exceeded 80%. The residual water content was lower than that of amikacin sulphate active substance, which was 10.7% (Table 2). The lowest residual water content value (4.92%) was obtained for the combination of the high drying temperature (180 °C), low feed rate (2 ml/min) and absence of ethanol in the feed solution (experiment # 6). In contrast, the maximum water content was measured when the low drying temperature (150 °C) was combined with the high level of feed rate (5 ml/min) and ethanol proportion (10%) (experiment # 3).

The ANOVA analysis of residual water content (numerical data not shown) and the corresponding contour plot (Figure 1) indicated feed rate (B) and ethanol proportion (C) as the most influential factors. The contour plot illustrates that at the drying temperature of 165 °C, the highest residual water values are in the red zone, where high percentages of ethanol and feed solution rates are used. Although the effect of increasing the feed rate on residual water is practically self-explanatory, attributing higher water content of dry particles to the increase of ethanol in feed solution required further consideration. This result could be attributed to the different vapour tension of the two miscible liquids. During the drying process, different compositions between the solution to evaporate and the condensed vapour, richer in ethanol, were obtained. Since the evaporation time of the droplets and the drying temperature were constant, the more volatile ethanol, when present, subtracted part of available heat energy to water evaporation, so leaving more residual water in the solid.

3.1 Morphological analysis

The SEM images of the powders produced at different ethanol concentrations (experiments # 13-15) reveal peculiar morphological differences between the microparticles (Figure 2). Almost all particles produced without ethanol (experiment # 14) are shrunk. In contrast, in the

201 powders prepared from ethanol solutions (experiments # 13 and 15), together with shrivelled
202 particles, numerous large spherical particles have been observed, captured as either swollen
203 by an inner pressure or 'exploded'. This condition was more evident at high level of ethanol
204 in the feed solution The blown up or ruptured particles, compared to the shrivelled (collapsed)
205 ones in the absence of ethanol, indicated that water/ethanol evaporation rate during the drying
206 process was the determinant of particle morphology.
207
208 3.2 Particle size distribution and density of powders and agglomerates
209 All spray-dried powders showed a median diameter, $D_{(v,50)}$, between 2.49 and 4.36 μm,
210 suitable for pulmonary administration (Table 2). Confirming the size observed in the SEM
211 pictures, the presence of ethanol and the increase of feed rate resulted in particles with larger
212 volume diameter and span.
213 With respect to true density, no significant differences were measured, as values ranged
214 between 1.5 and 1.6 g/cm^3 (data not shown). However, bulk density was strongly affected
215 when ethanol was present in the spraying solution of amikacin sulphate. As shown in Figure
216 3, the powders with the highest bulk density values (experiments # 5 to 8, and 14) were
217 obtained from feed solutions without ethanol. Connecting this result with the SEM picture
218 observation, the ethanol-generated large exploded microparticles are the responsible of the
219 powder volume increase and thus, of the bulk density reduction.
220
221 In general, the spray-dried amikacin sulphate powders poorly flowed, since they appeared as
222 lumps of particles having different sizes when collected from the spray drier cyclone. This
223 behaviour made the powder non homogenous, anticipating negative expectations about the
224 operation of loading the device reservoir for drug product preparation (dry powder inhaler).
225 As a consequence, the powders were agglomerated to form soft pellets, in order to
226 homogenize the lumps and improve their flowability and packing characteristics.

227 Agglomeration made the powders free-flowing and increased the bulk density with few
228 exceptions (see Figure 3).
229 During the agglomeration process it was also observed that the spray-dried powders gave rise
230 to two distinct size groups of soft pellets, one group with a projection diameter smaller than
231 0.5 mm (0.16 to 0.47 mm), (powders # 5 to 8 and 14), and the second group with a diameter
232 larger than 0.5 mm (0.58 to 0.85 mm). Agglomerates obtained from powders prepared with
233 feed solutions without ethanol belonged to the first group, whereas the powders prepared with
234 5 or 10% of ethanol entered the second group (Figure 4).
235
236 Having seen that ethanol in the spray-dried amikacin sulphate solution caused different
237 particle morphologies, it can be reasonably assumed that bulk density, water content and size
238 of agglomerates changed in dependence on the ethanol presence in the feed solution. In
239 summary, the agglomeration process, performed by means of a short process of sieve
240 vibration of the microparticles, produced free flowing powders, which facilitated the reservoir
241 dosing of the inhalers.
242

243 ## 3.3 Aerodynamic performance

244 The aerodynamic performance of the powders before and after agglomeration was tested *in*
245 *vitro* using the Fast Screening Impactor. The values of Emitted Dose (ED) and Fine Particle
246 Dose (FPD) obtained are shown in Table 3. The Emitted Doses of the amikacin sulphate
247 powders and agglomerates studied in this work exceeded 72% in many cases, with few
248 significant differences before and after agglomeration. However, it was noticed that the
249 lowest ED values, for both powders and agglomerates, were found when powders were
250 produced without ethanol in the feed solution. FPD values before and after agglomeration
251 ranged between 3.45 - 5.59 mg and 2.86 - 5.30 mg, respectively. The highest FPD values
252 were obtained for powders produced using a feed solution containing 10% of ethanol, which

253 was also the optimum region identified for this factor in the previous fractional factorial
254 design studying the process.
255
256 The graphs in Figure 5 illustrate the values of ED and FPD before and after agglomeration for
257 each powder produced. The graphs did not allow clearly differentiating groups of powders
258 and agglomerates having similar aerodynamic behaviour in dependence on the CPPs.
259 Nevertheless, statistical analysis confirmed that ethanol proportion in the feed solution was
260 the major parameter influencing the powder aerodynamic behaviour, in consequence of the
261 variations in particle structure determined by the solvent presence or absence. This is depicted
262 in the perturbation plots and tridimensional graphs on ED and FPD obtained from the design
263 analysis (Figures 6 and 7).
264
265 While ED is clearly affected only by ethanol presence, FPD is influenced by all three studied
266 factors. In other words, it is evident that ethanol proportions govern ED values, irrespectively
267 of the other two CPPs. Furthermore, a curvature occurs at high ethanol proportions towards
268 the maximum of 10%. This was the optimum ethanol concentration also identified previously
269 [20], in which its levels ranged between 10 and 20%, thus validating the former fractional
270 factorial design.
271 At the same time, ethanol proportion in the same region promotes FPD, while the contribution
272 of Feed Rate and Drying Temperature for this CQA is also significant.
273 As a result of the above, robust regions for the CPPs were identified for optimizing both
274 CQAs simultaneously. For instance, settings assuring high ED are located at 10% ethanol
275 levels, where FPD also maximizes with appropriate adjustment of the other two CPPs, which
276 in turn do not deteriorate ED, as the latter is practically unaffected from their changes.
277

278 3.4 Mechanism of particle formation

279 The amikacin sulphate particle formation mechanism can be identified by determining the
280 Peclet number (P_e) applied to the evaporation of the sprayed droplets of drug solution. P_e
281 depends on the drying rate (k) of the droplet and the diffusion coefficient (D) of drug in the
282 droplet solution, according to the following equation:

283 $$P_e = \frac{k}{8D} \qquad \text{(equation 1)}$$

284 where k is the evaporation rate constant in cm^2s^{-1} and D is the diffusion coefficient of
285 dissolved substance in the solution. When $P_e \leq 1$, the diffusion velocity of drug molecules in
286 the droplet is faster or of the same order of magnitude of the drying rate. In this case, if the
287 solute has a high solubility in the solvent, during the evaporation process drug precipitation is
288 delayed, leading to dense particles. When $P_e > 1$, drying rate is faster than diffusion rate of
289 solute molecules which accumulate and precipitate at the droplet surface, leading to empty
290 shell particles [9].

291 In this work, evaporation rates of amikacin sulphate in the different feed solutions have been
292 determined by TGA. Solutions containing amikacin sulphate showed a slower evaporation
293 rate compared to the solvent mixtures. The profiles of mass fraction evaporated versus time
294 were linear and the slope was measured as s^{-1}. Since for P_e calculation the evaporation rate
295 constant is measured in surface over time units (cm^2/s), the slope of the evaporation curves
296 (1/s) was multiplied by the evaporating area exposed in the TGA pan (0.26 cm^2), which
297 remained constant during the analysis. The values obtained are shown in Table 4.

298 The amikacin coefficient of diffusion was calculated at 298 K using the following equation
299 [11]:

300 $$\text{Log } D = -4.113 - 0.4609 \log Mw \qquad \text{(equation 2)}$$

301 D value of amikacin sulphate in water was determined as 3.58 10^{-6} cm^2 s^{-1}. Then, assuming
302 that the temperature of evaporating solution equals the outlet temperature during spray drying

303 (85 ° C, i.e., 358 K) and applying the Stokes-Einstein equation [12], D value at 85 °C was
304 determined. Disregarding the presence of ethanol, D approximated equal to 1.30 10^{-5} cm^2 s^{-1}.
305 The P_e values obtained in this study were higher than 1.0 (Table 4) and not significantly
306 modified by the ethanol presence. This indicates that molecules did not diffuse to the inner
307 part of the droplet because the evaporation rate was faster than diffusion. Thus, amikacin
308 sulphate particle formation was described as a fast recession of the droplet surface with the
309 precipitation of solute at the surface, resulting in formation of a shell void particle. SEM
310 pictures confirmed this predicted formation of void particles. In the particle pictures, the shell
311 of some broken particles and the differences in size depending on the feed solution
312 composition are clearly visible. In fact, numerous swollen and often exploded amikacin
313 sulphate particles have been obtained from the feed solutions containing ethanol. On the
314 contrary, the particles obtained from the feed solution without ethanol were smaller,
315 shrivelled and evidently empty.
316 The formation of these different particle populations has to be attributed to the different
317 amikacin sulphate solubility. The measured solubility of amikacin sulphate in the solvents and
318 their mixtures used is shown in Table 4. Amikacin sulphate is freely soluble in water but
319 practically insoluble in ethanol. The presence of several large particles in the powders made
320 from ethanol:water solutions was attributed to the fact that amikacin sulphate dissolved in
321 water droplets precipitated at the surface later than in the droplets containing ethanol. This
322 was due to the higher solubility of amikacin sulphate in water than in the mixtures with
323 ethanol. Thus, ethanol, decreasing the amikacin sulphate solubility, anticipated its surface
324 precipitation and promoted the formation of swollen, void, often exploded microparticles due
325 to the vapour tension of the ethanol entrapped inside the particle. Amikacin sulphate particles
326 obtained from the feed solution without ethanol were also void, but remain smaller.
327

4. Conclusions

Using a Central Composite Design including new combinations of the three selected spray drying process and formulation parameters, no further aerodynamic improvement of powders and agglomerates was observed, compared to the previous half-fractional factorial experimental design.

In this study, the role of ethanol in the solution to be sprayed was identified as crucial on the formation of amikacin sulphate microparticles and the properties of corresponding powders. Large microparticles with low aerodynamic diameter, high density powders, agglomeration easiness contributed to enhance the respirability of powders obtained in presence of ethanol in sprayed solution, in particular close to 10%. The solubility of amikacin sulphate in water/ethanol mixtures and the evaporation rate (Peclet number) of the sprayed solutions helped to understand the formation mechanism of the deriving spray-dried particles. The effect of ethanol in the sprayed solution was revealed by the appearance in the obtained powder of swollen from inside, empty, often exploded large amikacin sulphate microparticles. The precipitation of amikacin sulphate in the drying droplet of the lower solvent water/ethanol and pressure of ethanol entrapped into the shell particle have been the determinants of the structure of these highly respirable amikacin sulphate microparticles. Other drugs could benefit of this mechanism provided that the solubility and solution composition activate a microparticle formation similar to amikacin sulphate.

Acknowledgments

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Figure Legends

Figure 1. Contour plot of water content as function of feed solution rate and ethanol proportion at the drying temperature of 165 °C (red: high water content; blue low water content).

Figure 2. SEM pictures of three spray-dried powders at two magnifications (in brackets combinations of factor levels are presented): powder #14 (0% EtOH – 3.5 ml/min – 165 °C); powder #15 (5% EtOH – 3.5 ml/min – 165 °C) and powder #13 (10% EtOH – 3.5 ml/min – 165 °C).

Figure 3. Bulk density of the spray-dried powders. In the square, the amikacin powders made in absence of ethanol.

Figure 4. Optical microscope pictures of agglomerated powders: batch #14 and batch #15 bis.

Figure 5. Emitted Dose (left) and Fine Particle Dose (right) values of spray-dried powders (open circle: before agglomeration; black circle: after agglomeration).

Figure 6. Perturbation plots for Emitted Dose (ED) and Fine Particle Dose (FPD). A: drying temperature; B: feed rate; C: Ethanol proportion.

Figure 7. 3D plots for Emitted Dose (left) and Fine Particle Dose (right) as a function of feed rate and ethanol proportion at the medium level of drying temperature (165 °C).

Tables

Table 1. Matrix of the face centered-CCD showing the studied parameters, their levels and the experiment number (#) including the replicated center points (#15).

Exp. #	A. Drying Temp (°C)	B. Feed Rate (ml/min)	C. Ethanol (%w/w)
1	150	2.0	10
2	180	2.0	10
3	150	5.0	10
4	180	5.0	10
5	150	2.0	0
6	180	2.0	0
7	150	5.0	0
8	180	5.0	0
9	150	3.5	5
10	180	3.5	5
11	165	2.0	5
12	165	5.0	5
13	165	3.5	10
14	165	3.5	0
15	165	3.5	5
15 bis	165	3.5	5
15 ter	165	3.5	5

Table 2. Residual water content and particle size distribution (volume diameter and span) of amikacin spray dried powders (n=3).

#	Residual water	Volume Diameter (μm)			
	(%)	$D_{(v,10)}$	$D_{(v,50)}$	$D_{(v,90)}$	Span
1	6.98 ± 0.35	1.23 ± 0.07	2.80 ± 0.25	7.41 ± 0.58	2.19 ± 0.04
2	7.99 ± 0.41	1.19 ± 0.03	2.72 ± 0.08	7.21 ± 0.25	2.22 ± 0.02
3	9.02 ± 0.27	1.32 ± 0.06	3.23 ± 0.33	8.71 ± 0.83	2.29 ± 0.01
4	7.70 ± 0.42	1.39 ± 0.02	3.25 ± 0.12	8.64 ± 0.68	2.40 ± 0.09
5	6.53 ± 0.51	1.45 ± 0.01	2.53 ± 0.07	4.48 ± 0.39	1.20 ± 0.12
6	4.92 ± 0.28	1.35 ± 0.01	2.37 ± 0.01	4.08 ± 0.03	1.16 ± 0.01
7	7.45 ± 0.41	1.44 ± 0.07	2.32 ± 0.09	4.69 ± 0.32	1.40 ± 0.08
8	6.57 ± 0.51	1.36 ± 0.04	2.49 ± 0.07	4.52 ± 0.05	1.29 ± 0.04
9	8.19 ± 0.23	1.41 ± 0.03	2.60 ± 0.04	4.71 ± 0.00	1.27 ± 0.03
10	8.29 ± 0.40	1.29 ± 0.01	3.21 ± 0.12	8.88 ± 0.42	2.37 ± 0.04
11	7.70 ± 0.19	1.21 ± 0.02	2.64 ± 0.09	6.89 ± 0.37	2.19 ± 0.06
12	8.84 ± 0.39	1.40 ± 0.04	3.68 ± 0.13	9.68 ± 0.22	2.25 ± 0.03
13	8.81 ± 0.35	1.18 ± 0.10	2.73 ± 0.12	8.30 ± 0.08	2.37 ± 0.08
14	5.50 ± 0.10	1.44 ± 0.01	2.60 ± 0.08	4.59 ± 0.18	1.21 ± 0.03
15	8.30 ± 0.11	1.32 ± 0.02	3.33 ± 0.07	8.29 ± 0.30	2.26 ± 0.00
15 bis	8.07 ± 0.22	1.36 ± 0.01	3.22 ± 0.11	8.26 ± 0.79	2.14 ± 0.18
15 ter	7.60 ± 0.06	1.37 ± 0.03	3.54 ± 0.01	9.54 ± 0.20	2.31 ± 0.04

Table 3. Aerodynamic assessment of the spray dried powders. Emitted Dose (ED) and Fine Particle Dose (FPD), (n=3)

#	Before agglomeration		After agglomeration	
	ED (mg)	**FPD <5µm (mg)**	**ED (mg)**	**FPD <5µm (mg)**
1	8.80 ± 0.20	5.54 ± 0.59	8.47 ± 0.93	5.30 ± 1.00
2	8.60 ± 0.30	5.59 ± 0.17	7.53 ± 1.04	4.48 ± 0.91
3	8.93 ± 0.23	5.02 ± 0.88	7.83 ± 0.67	5.09 ± 0.92
4	8.77 ± 0.25	5.41 ± 0.46	9.27 ± 0.81	5.28 ± 0.42
5	7.27 ± 0.76	4.70 ± 0.44	6.77 ± 0.55	3.72 ± 0.14
6	7.77 ± 0.47	5.65 ± 0.21	7.70 ± 1.00	3.70 ± 1.10
7	7.00 ± 0.69	3.45 ± 1.14	8.43 ± 1.40	4.24 ± 0.17
8	7.23 ± 0.51	3.87 ± 0.79	7.53 ± 0.49	3.94 ± 0.80
9	8.63 ± 0.23	4.67 ± 0.83	7.93 ± 0.67	3.39 ± 0.27
10	8.93 ± 0.46	5.47 ± 0.53	8.17 ± 0.23	3.56 ± 0.34
11	8.10 ± 0.85	4.19 ± 0.20	7.87 ± 0.92	3.64 ± 0.43
12	8.83 ± 0.98	4.84 ± 0.79	8.53 ± 0.64	3.71 ± 0.50
13	7.80 ± 0.30	5.30 ± 0.39	7.87 ± 0.06	5.18± 0.51
14	7.87 ± 0.38	4.81 ± 0.48	6.80 ± 0.10	2.86 ± 0.35
15	8.47 ± 0.63	4.72 ± 0.62	8.67 ± 0.45	4.10 ± 0.65
15 bis	8.97 ± 0.60	4.98 ± 0.53	8.50 ± 0.17	3.97 ± 0.14
15 ter	8.43 ± 0.55	4.58 ± 0.53	9.10 ± 0.52	4.63 ± 0.47

Table 4. Ethanol:water ratio, mean slope of the TGA straight lines, evaporation rate constants (k), Peclet numbers and solubility of amikacin sulphate in the feed solution solvents

EtOH: Water	Slope (s^{-1})	k (cm^2/s)	Peclet Number	Amikacin Sulphate solubility (mg/ml)
0 : 100	$1.27\ 10^{-3}$	$3.30\ 10^{-4}$	3.17	309 ± 2
5 : 95	$1.28\ 10^{-3}$	$3.33\ 10^{-4}$	3.20	298 ± 3
10 : 90	$1.32\ 10^{-3}$	$3.43\ 10^{-4}$	3.29	104 ± 3
100 : 0	-	-	-	$3.6\ 10^{-3} \pm 0.4\ 10^{-3}$

Figure1

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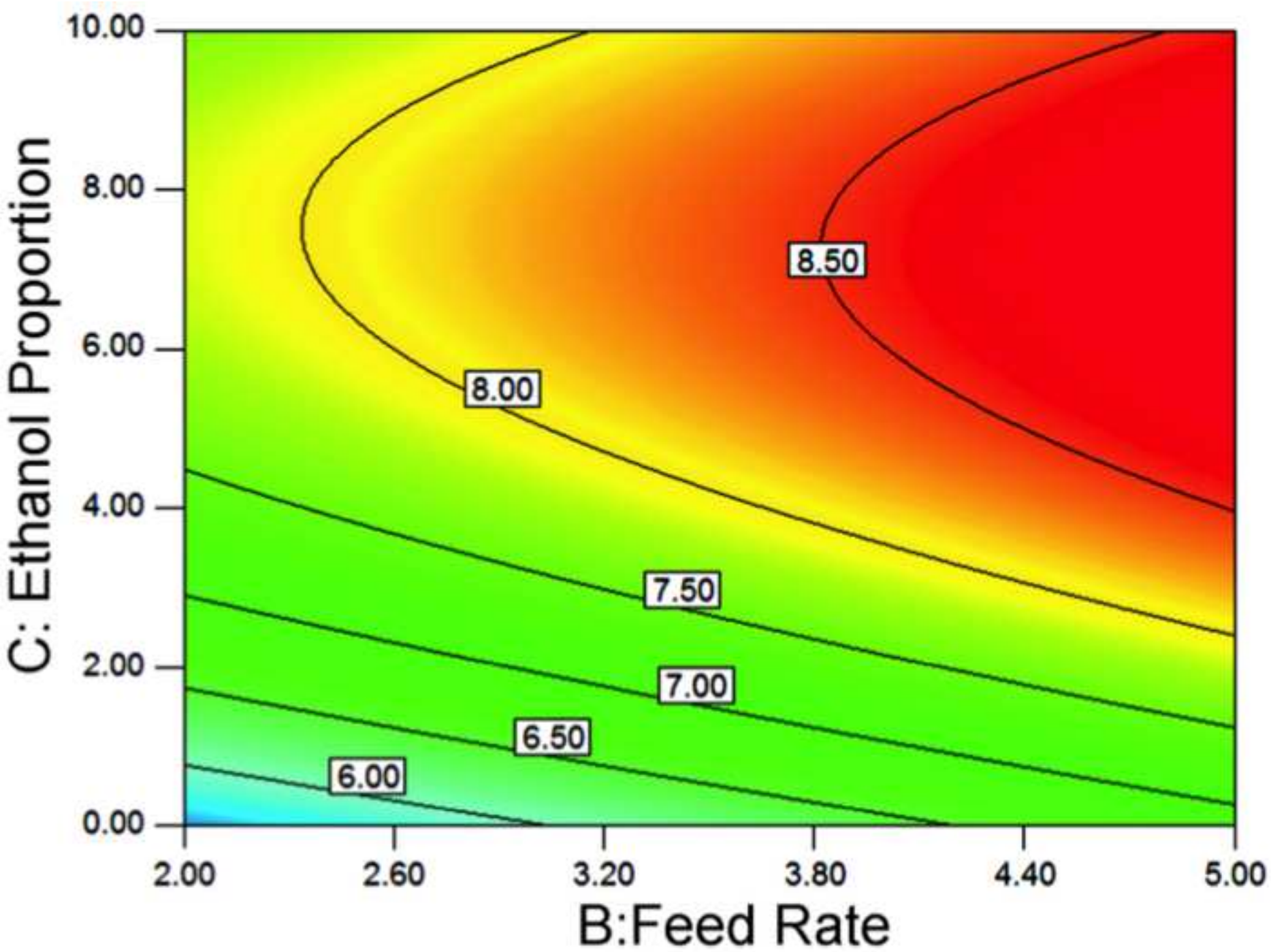

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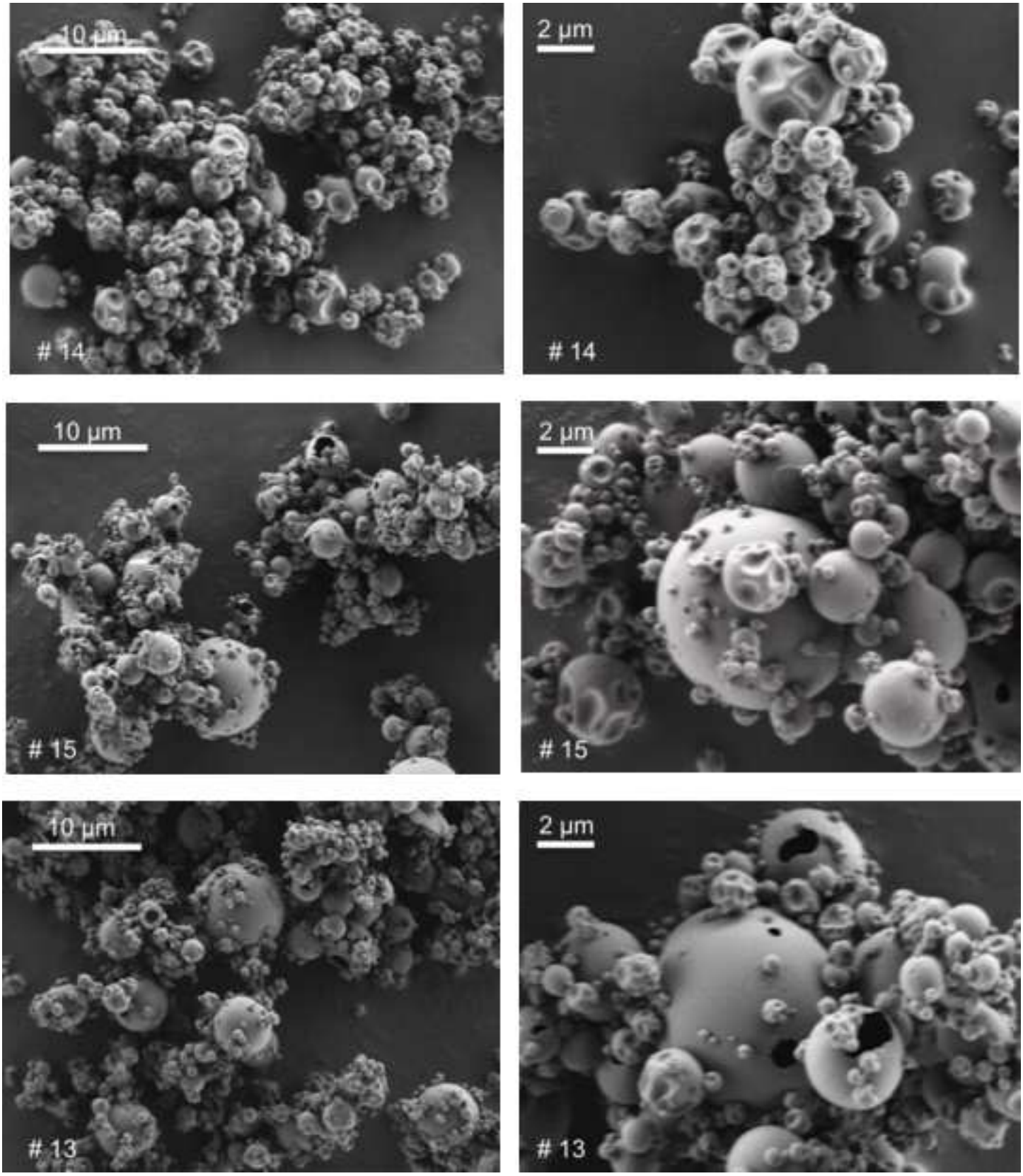

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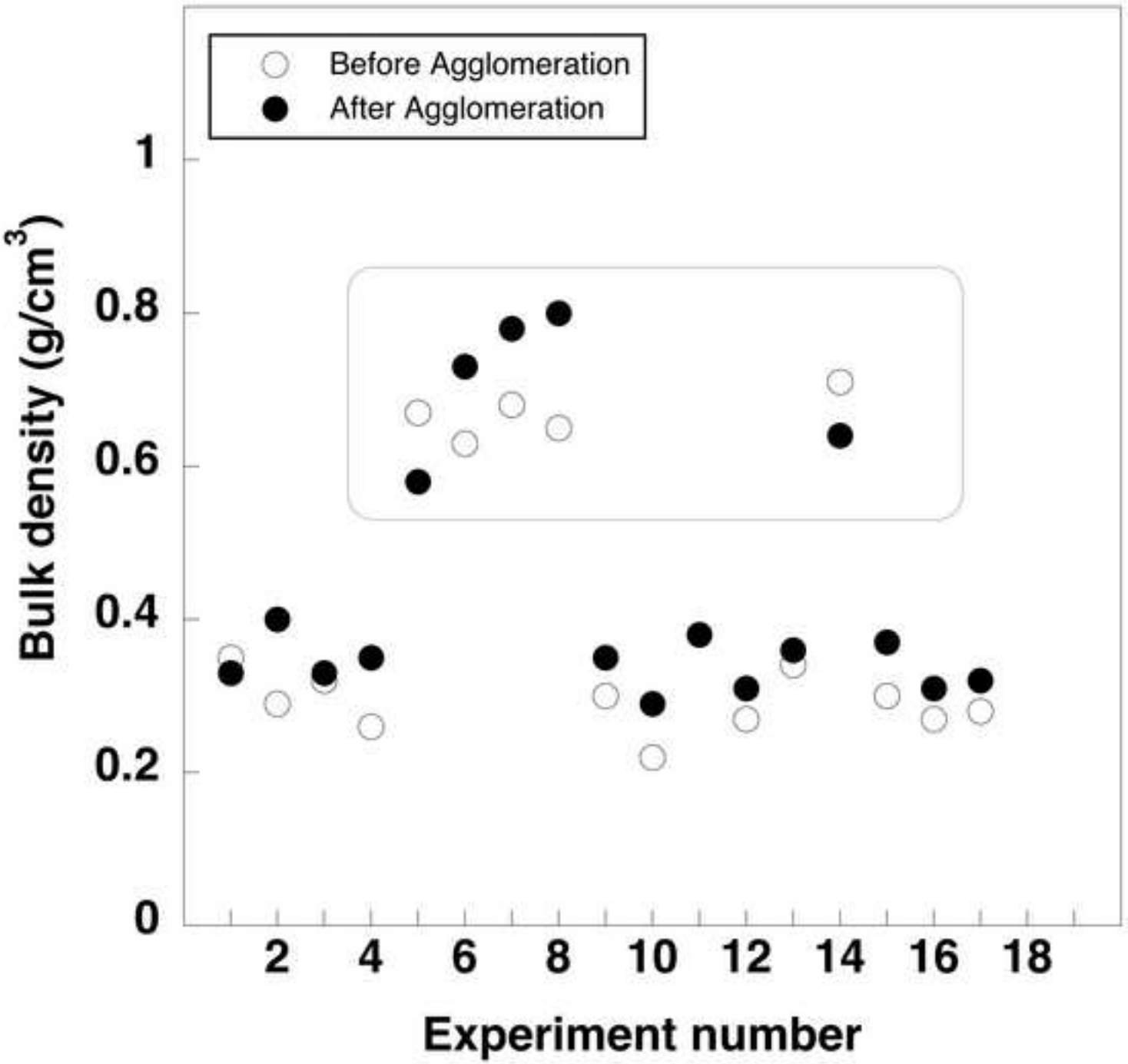

Figure 4a

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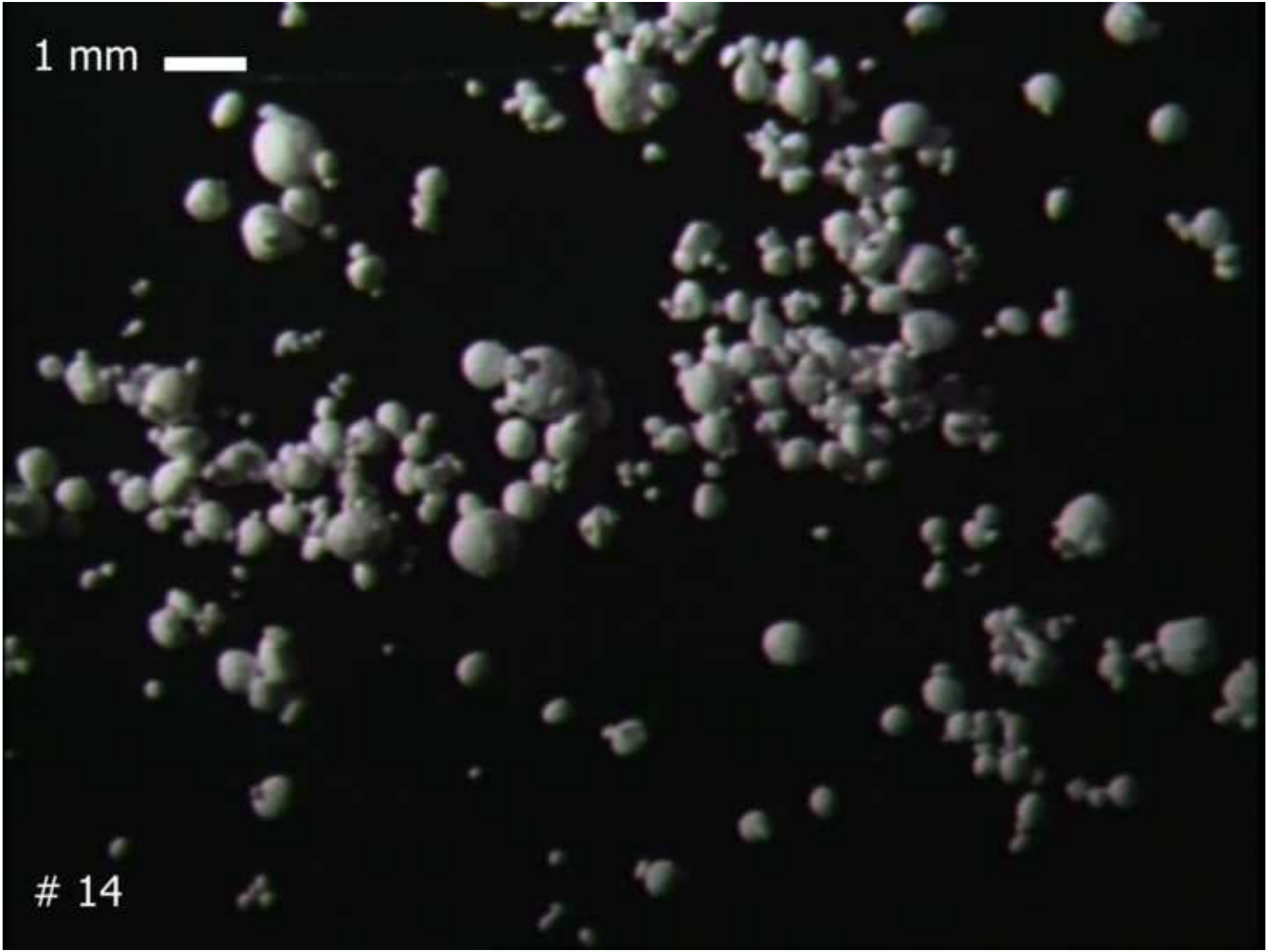

Figure 4b

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Figure 5a
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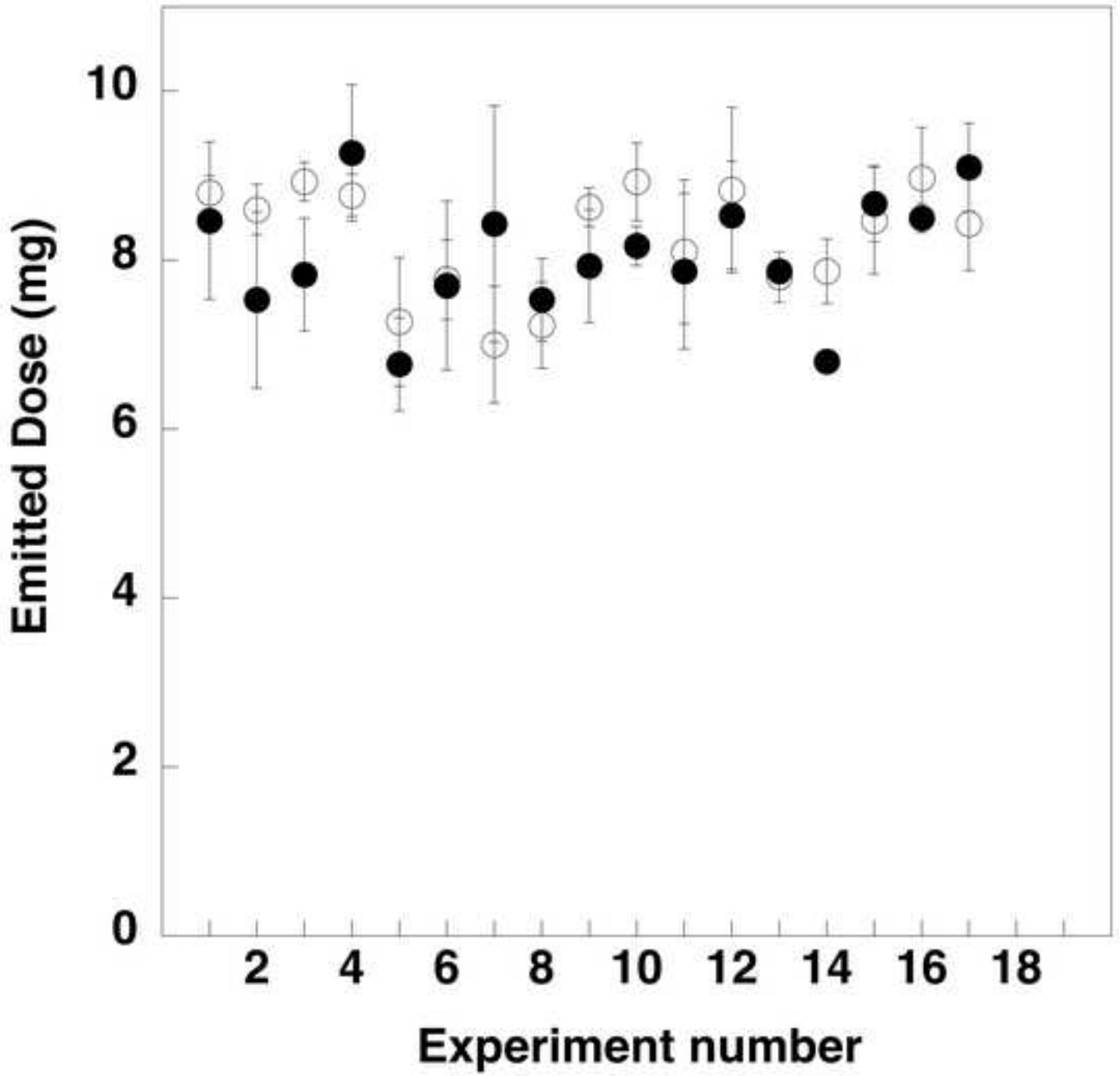

Figure 5b

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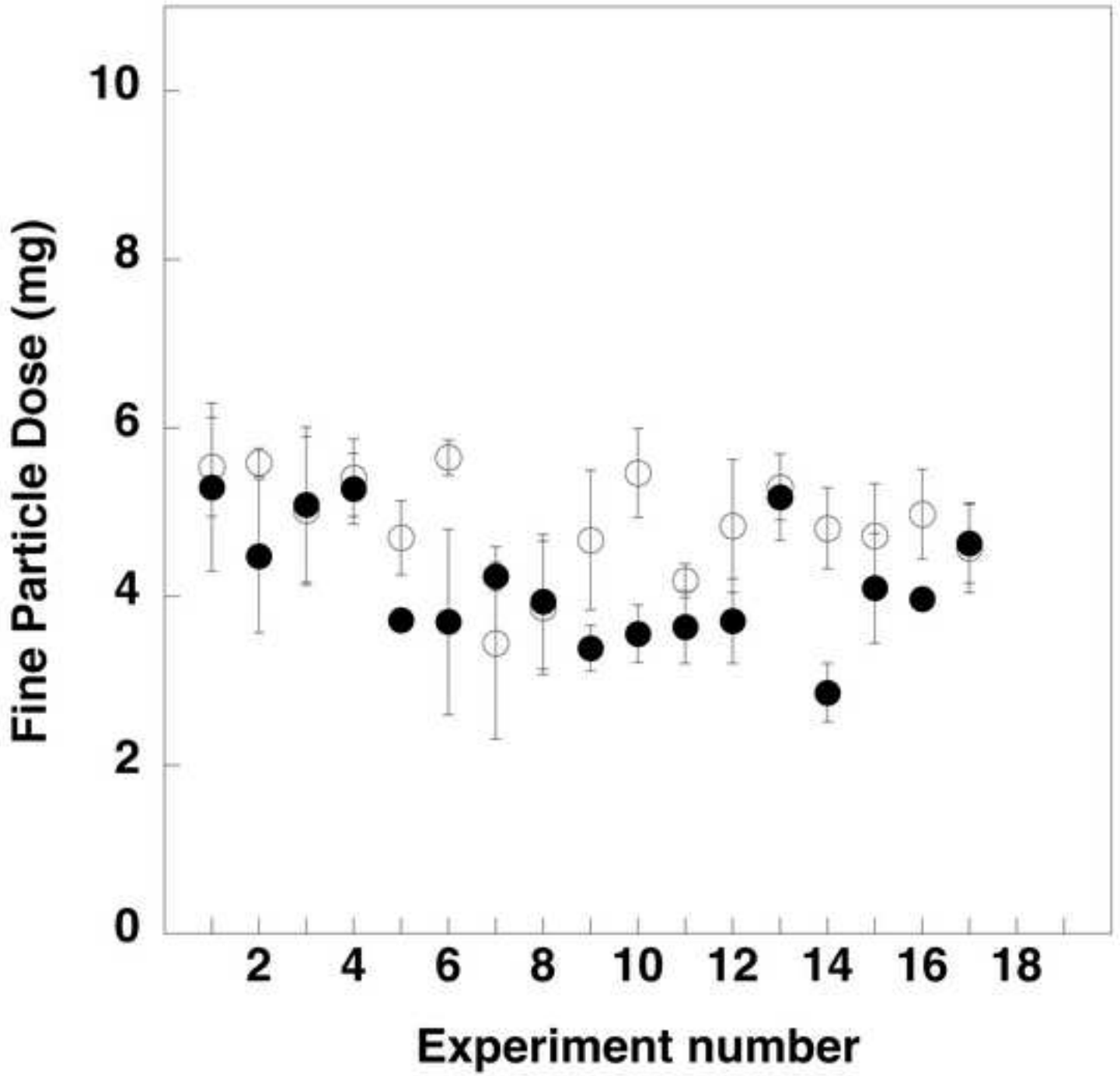

Figure 6

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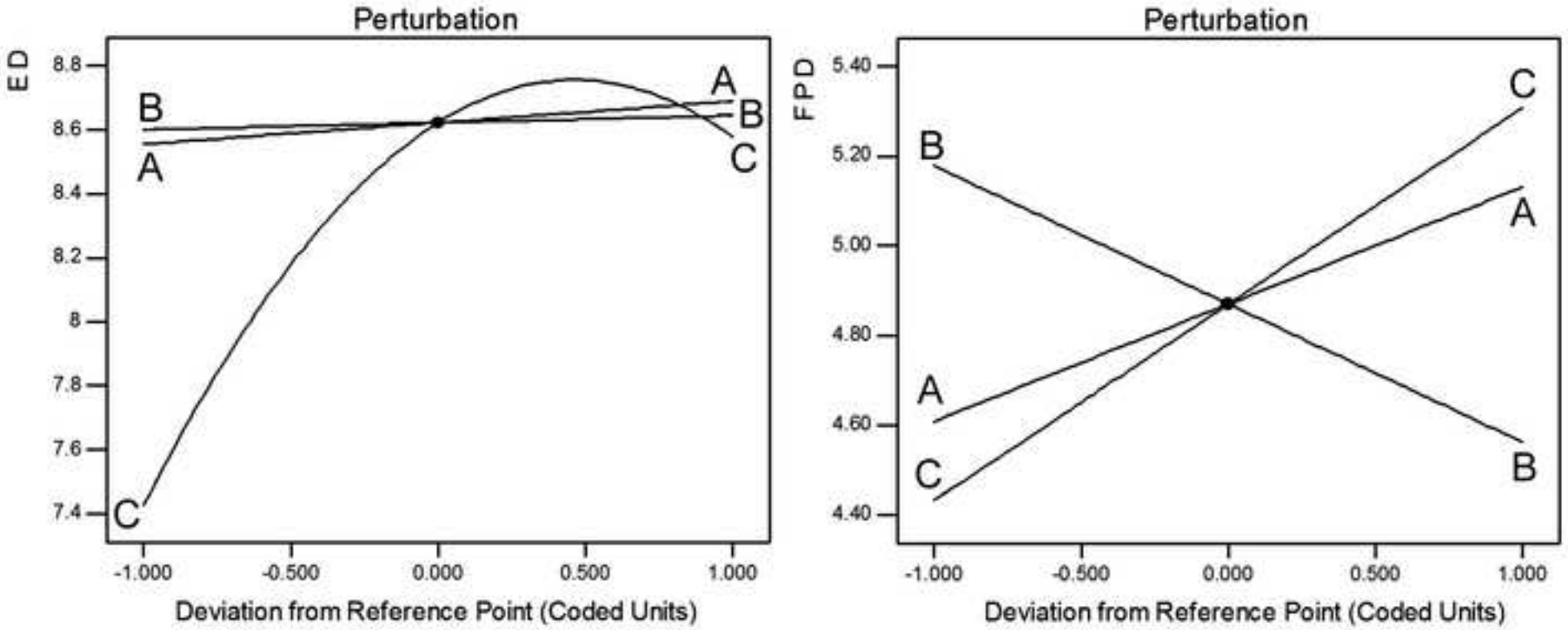

Figure 7a

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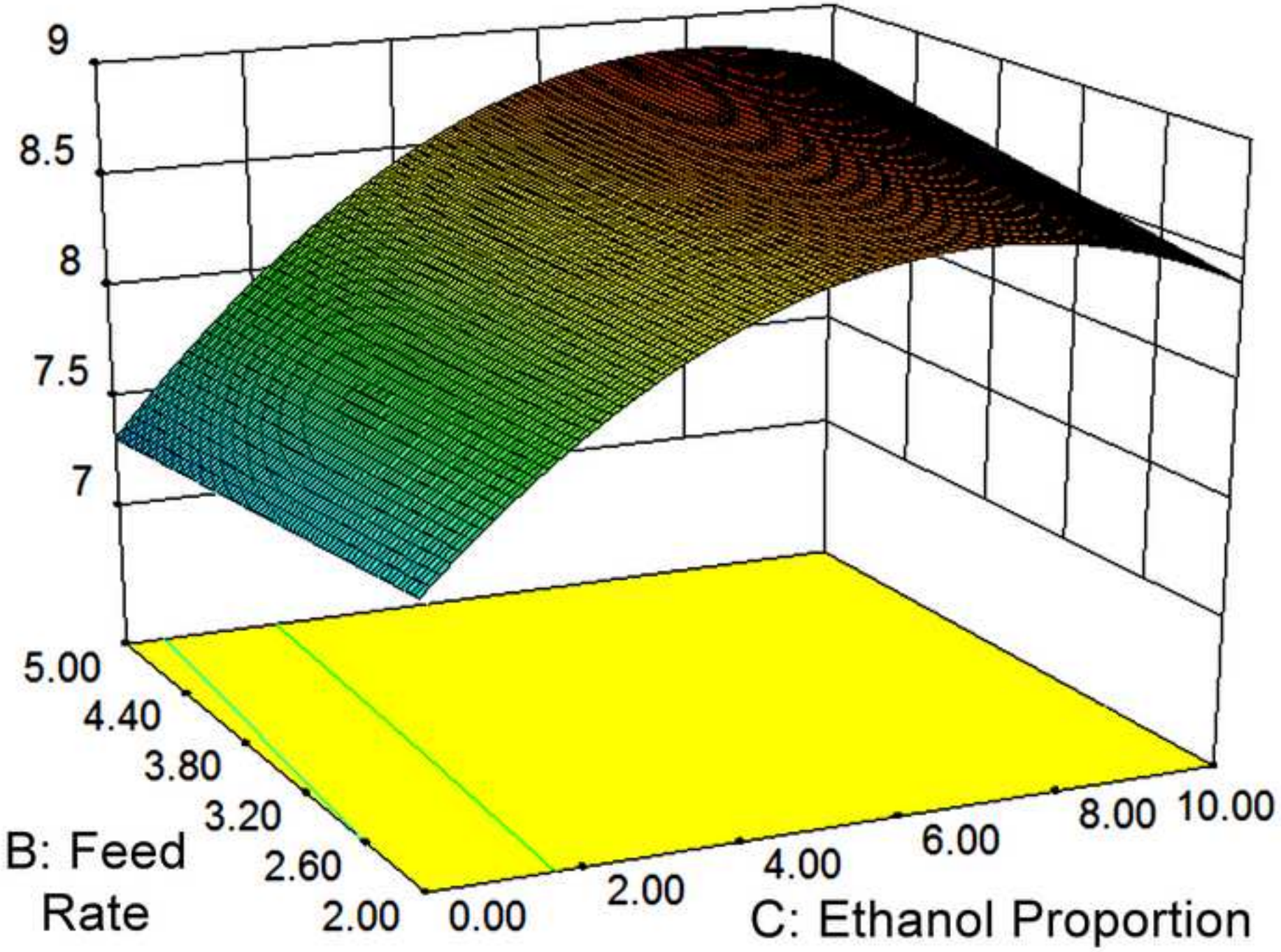
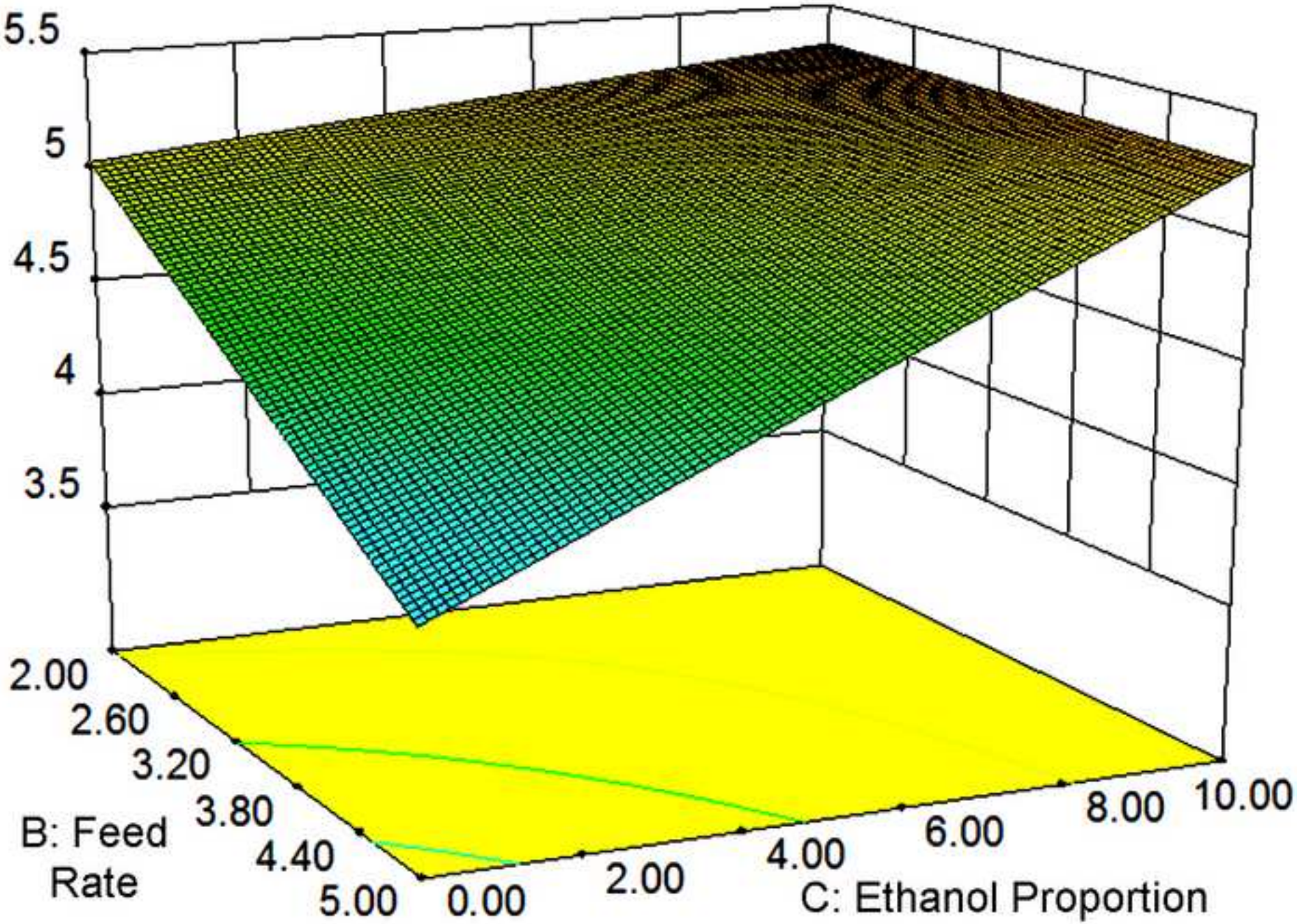
Figure 7b
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