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3   ~~**liquid chromatography?**~~

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16 **Impact of particle size gradients on the apparent efficiency of chromatographic**

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18 **~~liquid chromatography?~~**

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20

21 **Abstract**

22 In this paper, the benefits of using columns packed with particles of decreasing size (particle

23 size gradient) in liquid chromatography was investigated from a theoretical point of view. It is

24 indeed well known that such columns may be useful in gradient elution, since the decrease

25 of particle size along the chromatographic column can provide extra peak focusing effect. In

26 the present contribution, several parameters (i.e., mobile phase gradient steepness, retention

27 times and operating pressures) were considered and the kinetic performance of various

28 types of columns packed with particle size gradient were evaluated.

29 In the best case, about 15-20% gain in efficiency can be expected at a given retention time

30 when utilizing a particle size gradient, compared to constant particle size. Conversely, when

31 fixing efficiency, the analysis time can be decreased by about 15% with an optimal particle

32 size gradient. However, it is also important to keep in mind that a too large a particle size

33 gradient can result in lower efficiencies than a column packed with monodisperse packing.

34 We have introduced the g_d value, which is a dimensionless measure of the particle size

35 gradient steepness that measures the relative variation of particle diameter throughout the

36 column with respect to the average. We finally observed that $g_d = 0.3 - 0.4$ provides the

37 highest gain under practically useful conditions.

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1. Introduction

In liquid chromatographic (LC) separations, the chemistry and efficiency of the stationary phase are maintained (constant) along the column, while the mobile phase composition, temperature or flow rate are often varied to adjust the selectivity or resolution [1,2]. However, an alternative approach which became more attractive in the past few years is to vary the selectivity of the stationary phase along its length [3].

A well-established method consists in serially coupling columns of different selectivity. This methodology is often referred to as stationary phase optimized selectivity liquid chromatography (SOSLC) [3,4]. This SOSLC approach has been commercialized under the name of POPLC (as phase optimized liquid chromatography, provided by Bischoff Chromatography), and several studies have reported the possible increase in selectivity resulting in improved separation quality, compared to the use of a single column [5,6,7,8]. Such approach can be considered as a discontinuous stationary phase gradient. A potential drawback of POPLC is that the connections between the coupled columns may create undesired void volume and therefore can decrease the apparent efficiency and peak symmetry [9,10]. Another limitation is that serially connected columns do not allow for cooperative interactions to take place between the various stationary phase ligands [11,12]. Continuous stationary phase gradients consisting of a gradual change in chemical functionality along the column length, can eliminate or reduce the possible difficulties of discontinuous stationary phase gradients [13]. Pucci *et al.,* first applied continuous stationary phase gradients by photografting ionizable chains onto a hydrophobic polymer monolithic in a gradient fashion for capillary electrochromatography [14]. Since then, continuous stationary phase gradients have been applied in thin layer chromatography (TLC) and also for silica monoliths used in LC [11,15,16,17]. Very recently, the principles of so-called controlled rate of infusion (CRI) were adapted to develop a new approach to fabricate continuous stationary phase gradients on packed columns [13]. Such stationary phase gradients were formed using commercial C18 columns. This new destructive method can be applied in both reversed phase (RP) and hydrophilic interaction liquid chromatography (HILIC) modes.

70   Gritti and Guiochon discussed stationary phase gradients from a different point of view [18].

71   The advantages of using columns packed with stationary phases having a composition

72   gradient, so that retention factors increase throughout the column and compounds elute with

73   isocratic mobile phase, which may provide increased peak resolution. When retention factor

74   varies linearly along the column, then band compression occurs due to a differential of

75   retention between the front and the rear parts of the peak. Their results showed that the

76   resolution has an optimum for a specific value of the retention factor gradient along the

77   column. This optimum depends on the retention factor observed at the column inlet. It is also

78   worth mentioning that this work ~~has risen some debates and discussions~~and a related

79   discussion [19] emphasized the careful treatment of band variance and the way of

80   determination of the apparent plate height along an axially heterogeneous column ~~[19]~~.

81   Besides selectivity, stationary phase gradient can also be discussed in terms of efficiency. In

82   our previous work, theory was developed for both isocratic and gradient elution modes to

83   predict separation efficiency over a coupled system which includes columns packed with

84   different particle sizes [20]. It was shown that the apparent efficiency of such column system

85   - in gradient elution mode - depends on the column order. Higher peak capacity is expected

86   when the columns are coupled in the order of increasing efficiency (decreasing particle size).

87   If the differences between the individual column efficiencies are large enough, then an

88   overall peak sharpening may occur along the column, which is never the case with a single

89   column. This approach was recently extended to combine any column formats in a serial

90   configuration [21]. For a five-column system, significant decrease of peak variance was

91   predicted when columns were placed in order of decreasing particle size (discontinuous

92   particle size gradient) versus random column order. The results suggested that a continuous

93   particle size gradient – packed in one column – may further increase the gain in efficiency.

94   As particle size gradients may also show effects from the particle size distribution (PSD), the

95   impact of PSD on column efficiency seems also interesting to study. The problem is that it

96   still remains unclear [22]. Furthermore, the literature on the topic is rather contradictory on

97   whether the width of the PSD significantly affects band broadening in LC or not [23-28]. A

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strong correlation has been observed between the width of the PSD of several commercially available HPLC particle types (both fully porous and superficially porous) and some commonly used parameters that reflect the quality of a packing, namely the minimum reduced plate height and the eddy dispersion's term [29]. In contrast, in another experimental study, different particle sizes have been mixed in various ratios, and no significant relationship was evidenced between the width of PSD and column efficiency [22]. It is now clear, that the trans-column dispersion of columns packed with superficially porous particles is significantly smaller than those of fully porous ones probably because shell particles can be packed more homogeneously across the column diameter – possibly due to their roughness. Because they are prepared through a controlled step by step growth layer process, the external surface area of these particles is not as smooth as that of fully porous particles [30]. Recently, fully porous particles were also prepared with very narrow PSD (RSD ~ 5%, while it is typically around 20%) and produced unprecedentedly low plate heights. Gritti *et al.* proposed that it is due to the small longitudinal diffusion and not due to the improved eddy dispersion [3031]. A contradictory study did not confirm the very small intra-particle diffusion of the same narrow PSD fully porous particles and highlighted that the observations may depend on the applied experimental conditions and retention of the solutes [3132]. Hence, the effect of PSD on efficiency is still a controversial question in liquid chromatography.

In this work, our aim was to study the potential benefits of columns packed with particles of continuously changing (with a linear decrease) diameter along the column, as example to demonstrate the theoretical benefits of non-uniform column format [33,34]. Various conditions (particle size gradient, mobile phase gradient steepness, operating pressure) were assumed and the expected gain in efficiency or analysis time were studied. Please note that it is a pure theoretical work, experimental verification was not feasible as such columns are not available. Moreover, to neglect possible issues related to very high pressure applications (such as radial and axial thermal effects on band broadening – caused by friction -, and

125 pressure related change of retention), relatively large particle sizes and a maximum pressure

126 drop of 400 bar were considered in this study.

127

128 **2. Theory**

129 To consider a column packed with particles possessing continuously changing diameter,

130 particle size needs to be parametrized. For a linear gradient, the following formula can be

131 written for the particle size diameter at any position (*z*) along the column:

132 $$d_p(z) = d_M \left[1 - g_d \left(\frac{2z}{L} - 1 \right) \right] \tag{1}$$

133 so that $d_p(z)$ is the particle diameter at a certain position, d_M is the average diameter, *L* is

134 the column length and g_d is a dimensionless measure of the particle size gradient steepness,

135 that measures the relative variation of particle diameter throughout the column with respect

136 to the average. As a function of the inlet and outlet particle sizes, this gives

137 $$g_d = \frac{1}{2} \left(\frac{d_p(0)}{d_p(L)} - 1 \right) \tag{2}$$

138 Positive g_d corresponds to decreasing particle sizes, which is the case in this study. As an

139 example, a column with 15 μm inlet particle diameter and 5 μm outlet particle diameter with

140 an average of $d_M = 10 \ \mu m$ would have $g_d = 0.5$. Please note, that only linear particle size

141 gradients were considered, but they should give a first reasonable approximation to the

142 effects of decreasing particle size in nonlinear gradients. Figure 1 shows a schematic view of

143 column packed with particles of continuously decreasing size.

144 Changing the particle size does not affect, up to negligible boundary effects, the available

145 cross-section of the interstitial space in the column (assuming particle shape does not

146 change as particles become smaller). This means that for a constant flow, the average speed

147 u_0 of the mobile phase in the interstitial space is also constant along the column. It will,

148 however, depend on the particle size and pressure. One can obtain the relation through the

149 Darcy's law. Written in the form of Giddings [~~32~~35], it states that the speed of flow through a

150 material made of packed particles is proportional to the pressure (*P*) differential, as

151 $$u_0 = -\frac{1}{2\phi}\frac{d_p^2(z)}{\eta}\frac{\mathrm{d}P}{\mathrm{d}z}$$ (2<s>3</s>)

152 with η the mobile phase viscosity and ϕ the column's flow resistance parameter. For fixed u_0,

153 it can be integrated along the particle size gradient to give:

154 $$P(z) = P(0) + \frac{2L\,\phi\,\eta\,u_0}{d_M^2}\frac{z/L}{1-g_d^2+2g_d(1+g_d)(1-z/L)}$$ (3<s>4</s>)

155 Evaluating this at the end of the column we can solve for the flow velocity:

156 $$u_0 = (1-g_d^2)\frac{d_M^2}{2L}\frac{\Delta P}{\phi\eta}$$ (4<s>5</s>)

157 where ΔP is the pressure differential between column inlet and outlet. Please note that for

158 fixed pressure, introducing a particle size gradient will reduce the flow velocity independently

159 on the direction of the gradient. The question is, of course, whether it pays off in terms of

160 column efficiency or not. Figure 2 shows some examples of the expected evolution of

161 pressure as function of z, for columns with particle size gradient (g_d = 0.5 and -0.5) compared

162 to a homogeneous particle size (g_d = 0). In the $g_d = +0.5$ case, particle size decreases

163 along the column. Therefore, the pressure gradient is smaller at the beginning of the column

164 than that of the constant particle size column, but increases at the column end, due to the

165 non-linear dependence of pressure drop with particle size. The overall effect is a larger

166 pressure drop than in the constant particle size case. Using an increasing particle size

167 column, with $g_d = -0.5$, the overall effect is the same however in this case, the relative

168 pressure is higher at the inlet of the column. In general, the order of different particle sizes is

169 out of concerns as pressure drops are additive (neglecting second-order effects coming from

170 higher derivatives of the particle size gradient, which should be the case in anything that

171 could be actually manufactured).

172 We remember the system of equations for peak migration and broadening from our previous

173 works [20,21]. The speed of migration u at a certain point and time in the column is given by

174 the mobile phase speed divided by the retention, which for a mobile phase gradient running

175 over a time t_G with a mobile phase variation $\Delta\phi$ is:

176 $$u(z,t) = \frac{u_0}{1 + k_0\, exp\left[-\frac{S\,\Delta\phi}{t_G}\left(t - \frac{z}{u_0}\right)\right]}$$ (~~5~~6)

177 where S and k_0 are the parameters of the Linear Solvent Strength (LSS) model (S is a

178 constant for a given compound at fixed experimental conditions, and k_0 is the extrapolated

179 value of k for $\varphi = 0$).~ This can be reparametrized with the intrinsic mobile phase gradient

180 steepness:

181 $$b = S\,\Delta\phi\frac{L}{t_G u_0}$$ (~~6~~7)

182 The intrinsic gradient steepness expresses the rate of change in log k during the gradient per

183 volume of mobile phase passing through the column.

184 Then, the system of equations determining the evolution of peak position and width is:

185 $$t'(z) = \frac{1}{u(z, t(z))}$$ (~~7~~8)

186 $$(w(z)^2)' = H(z) + 2\,\partial_z log\, u\,(z,t)|_{t=t_z} \cdot w(z)^2$$ (~~8~~9)

187 where t is the migration time to get to point z and w the spatial peak width, always assuming

188 that $w/z \ll 1$. Since particle size is not constant along the column, the local plate height

189 needs to be parametrized as a function of position, $H(z)$. The first one integrates to:

190 $$t(z) = \frac{z}{u_0} + \frac{L}{bu_0}log\left(1 + \frac{bk_0 z}{L}\right)$$ (~~9~~10)

191 The compression term is then given by:

192 $$2\,\partial_z log\, u\,(z,t)|_{t=t_z} = -\frac{2p}{L}\frac{1}{1 + p\,z/L}$$ (~~10~~11)

193 with

194 $$p = \frac{k_0}{1 + k_0}b$$ (~~11~~12)

195 A first reasonable approach for $H(z)$ (that is analytically tractable) is to parametrize it by [32]:

196 $$H(z) = \alpha\,d_p(z) + \beta\frac{d_p(z)^2 u_0}{D_m} + \gamma\frac{D_m}{u_0}$$ (~~12~~13)

197 with D_m the diffusion coefficient of the substance under migration, and α, β and γ are

198 dimensionless constants parametrizing the strength of each of the contributing effects (eddy

199 dispersion, resistance to mass transfer and longitudinal diffusion, respectively). The solution

200 for $w(z)^2$ is too large an expression (see in appendix), but importantly, it has a closed form

8

201   that we can study. Please also note that it depends on u_0 in a non-trivial way, and through it,

202   on the pressure differential of the column.

203   The observed retention time is of course the migration time to the outlet of the column:

204      $t_R = t(L)$ (~~13~~14)

205   Finally, the observed time width at the outlet, σ_t, depends on the speed of migration at that

206   point. Notice that for very steep gradients this will be close to u_0, but in general it reads as:

207      $\sigma_t = \frac{w(L)}{u(L,t_R)} = \frac{1+k_0(1+b)}{1+bk_0} \frac{w(L)}{u_0}$ (~~14~~15)

208   In these terms, the efficiency of the column can be measured by apparent plate number:

209      $N = \frac{t_R^2}{\sigma_t^2}$ (~~15~~16)

210   The key point of this analysis is that the small g_d expansion of σ_t starts with a term linear in

211   g_d as one can derive from the expression in the appendix,

212      $\frac{d\sigma_t}{dg_d} < 0$ (~~16~~17)

213   Compare this to the dependence of u_0 (and with t_R), that has a quadratic correction. Fixing

214   the pressure differential, for small enough g_d the decrease in peak width must necessarily

215   outweigh the increase in retention time caused by the particle size gradient.

216   Let us quantify this improvement. To make a meaningful comparison, one cannot just take

217   columns at fixed d_M, since changing g_d affects both efficiency and retention time. Instead, let

218   us define a fixed retention time particle size function, as:

219      $d_F(t_R, g_d)$ (~~17~~18)

220   such that

221      $t_R|_{d_M = d_F(\tau, g_d)} = \tau$ (~~18~~19)

222   that is, the particle size for which with a particle size gradient g_d we obtain precisely a

223   retention time of τ. It is easy to compute from the expression of the retention time and the

224   flow speed:

225      $d_F(t_R, g_d)^2 = \frac{1}{1-g_d^2} \frac{2L^2}{t_R} \frac{\phi\eta}{\Delta P} \frac{b + log(1+bk_0)}{b}$ (~~19~~20)

9

226   Inserting this as the value of d_M for the calculation of the efficiency, we can compare

227   columns that produce the same retention time under the same length and conditions of

228   pressure, gradient steepness, compound retention, viscosity, etc. Notice that it grows as we

229   increase the particle size gradient.

230

231   **3.   Results and Discussion**

232   **3.1. Benefits of particle size gradient**

233   As reported in our previous works, in gradient elution mode, a gain in efficiency is expected

234   when decreasing the particle size of columns along them [20,21]. Up to now, it has been

235   studied and demonstrated only for coupled columns packed with different particle sizes

236   (columns were placed in the order of decreasing particle size). Here, we focus on the

237   understanding of the impact of particle size gradient on peak width, column pressure

238   (resistance) and analysis time (retention time) when assuming a linear particle size gradient

239   along a single column.

240   To illustrate with numerical data, a compound with $log_{10} k_0 = 6$ and a diffusivity of $D_m =$

241   $10^{-5} cm^2/s$ was considered on a column's length of $L = 15$ cm. The viscosity is

242   approximately that of water at room temperature, $\eta = 10^{-3}$ Pa $\cdot$ s. Finally, the coefficients of

243   the plate height parametrization are taken from Giddings as $\alpha = \gamma = 0.1$ and $\beta = 0.5$, as well

244   as the flow resistance parameter $\phi = 300$, [~~32~~35]. Various plots have been prepared to

245   compare different particle size gradients.

246   First, the gain in apparent plate number was studied for different mobile phase compositional

247   gradients (from $b = 0$ to $b = 10$) as a function of particle size gradient (g_d). Figure 3 plots the

248   relative increase in efficiency, for fixed retention time (t_R = 10 min) and applied pressure (ΔP

249   = 100 bar), as a function of increasing particle size gradient. Notice that since we fix the

250   length of the column and the retention time, the flow velocity u_0 will only depend on the

251   retention properties of the compound. For instance, for $b = 5$ the previous parameters

252   correspond to $u_0 \simeq 6.1$ cm/min. Under isocratic conditions ($b = 0$), any modification to

253   particle size is detrimental, since the migration suffers from reduced flow rate, while having

254   no band compression mechanism to benefit from the smaller particle size at the end of the

255   column. As the mobile phase gradient increases (e.g. b = 1, 2.5, 5 or 10), so does the

256   usefulness of the particle size gradient, up to a 20% gain in efficiency was obtained in the

257   best-case scenario. However, exceeding the optimal point in particle size gradient quickly

258   results in reduced gains or even losses in efficiency. Because of this, it seems reasonable to

259   suggest a maximum value of the particle size gradient between $g_d = 0.3 - 0.4$. (e.g. a

260   column with 7 µm inlet particle diameters and 3 µm outlet diameters gives $g_d = 0.4$). Around

261   that region, the impact on compounds with low S values (resulting in low b) will be negligible,

262   while compounds with higher S values can in principle see gains in efficiency of more than

263   15%.

264   One can now ask what happens at very high mobile phase gradient steepness. As expected,

265   when the gradient is steep enough then the classical band compression already does a good

266   job even for relatively large particles, and the relative gain of using a particle size gradient is

267   diminished. This is illustrated in figures 4, 5 and 6. In figure 4, pressure was fixed at ΔP =

268   100 bar and particle size gradient at g_d = 0.3 (close to the optimal). The average particle size

269   in each column was chosen to produce a specified retention time (e.g. t_R = 10, 15, 20, 25 and

270   30 min). Increasing the mobile phase gradient improves efficiency until a certain point, after

271   which it slowly decreases. It can be seen that shorter runs benefit the most. At t_R = 10 min,

272   the efficiency increases up to b = 10, while for a t_R = 30 min separation, the relative plate

273   number increases only up to b = 3.5. Figure 5 shows the actual apparent plate numbers for

274   the same conditions as in figure 4 to have an idea about the absolute values. <u>The apparent</u>

275   <u>efficiency quickly reaches a maximum at a certain gradient steepness, after which it decays</u>

276   <u>slowly.</u>

277   A different way of comparison is illustrated on Figure 6. It shows the average particle size for

278   a g_d=0.3 particle size gradient column, which is required to produce a specified retention

279   time, at a given value of the mobile phase gradient steepness. The longer and steeper the

280   mobile phase gradient, the smaller the required average particle size is. As an example, to

11

281 perform a fast separation (t_R = 10 min) at a relatively flat mobile phase gradient (b = 2), the

282 required average particle size is d_M ~ 4.5 μm. However when performing a 30 min long

283 separation with a steeper mobile phase gradient (b = 5) then as low as d_M ~ 2 μm particle

284 size is required.

285 Figures 7, 8 and 9 show the same magnitudes for fixed particle size and mobile phase

286 gradients (g_d = 0,3 and b = 5), for varying retention time obtained at different pressures (ΔP =

287 25, 50, 100, 200 and 400 bar). Figure 7 shows the relative increase in efficiency, while

288 figures 8 and 9 show the apparent plate number itself and the required average particle size,

289 respectively. Figure 7 indicates that ~~T~~the most significant improvements can be obtained,

290 again as expected, for shorter elution times – produced by larger average particle sizes, who

291 stand to gain the most in terms of efficiency by band compression at the end of the column.

292 In other words, shorter runs and lower pressures benefit the most, since high pressures

293 already have higher base efficiency without the particle size gradients, and excessively long

294 retention times diminish the peak focusing from the mobile phase gradient. In figure 8 we

295 observe how the apparent efficiencies converge to a similar value for too large retention

296 times, with which the benefits of band compression from the gradient are less relevant.

297 Finally, figure 9 gives the necessary particle sizes to obtain these retention times under the

298 given conditions, again in the μm range.

299 Finally figure 10 shows an illustrative theoretical plot for the benefit of particle size gradient

300 (g_d = 0.3 and d_M = 3.00~~1.1~~ μm) when compared to a monodisperse packing (g_d = 0 and d_M =

301 2.68~~1.0~~ μm). Both peaks shown are simple Gaussians whose width and position follow from

302 the model proposed in the article. The particle size configurations were chosen so that the

303 apparent efficiency was identical (N = 2.594~~2~~ · 10^5) for both peaks. From figures 7 and 8 we

304 can see that low pressures and times between 20-60 minutes will see improvements while

305 keeping a good apparent efficiency. We therefore took ~~(considering~~ b = 10~~2.5~~ mobile phase

306 gradient steepness, as corresponds to large molecules with large S values, and a ΔP = ~~400~~

307 25 bar pressure drop~~)~~. A decrease (reduction) of ~ ~~44 min~~ min (14%) in the retention time

308 (analysis time) is observed thanks to the particle size gradient, as expected from the

12

309 ~ 10% – 15% improvements noticed in the previous figures. It suggests that indeed an

310 optimal particle size gradient enables to decrease the analysis time by 10 – 15 % whilst

311 maintaining the same efficiency and operating pressure in gradient elution mode.

312

313 **3.2. ~~Production of columns~~Ideas to produce columns ~~packed~~ with particle size**

314 **gradient**

315 Finally, we would like to discuss the possibility of producing/packing columns with different

316 particle sizes in a gradient arrangement. It might be feasible to inject a mixture of particles

317 that settle in a long narrow tube, so that gravity would automatically create a size gradient

318 and then force the content of this tube into a column once the particles have been "sorted".

319 However, when operating such columns at high pressure, particle rearrangement and bed

320 consolidation can be an issue in case of only gravitational particle sedimentation. Similar

321 ideas have been recently applied in production of ground silica monolith particles to improve

322 chromatographic separation efficiency [~~33~~36]. The monolithic particles after calcination were

323 separated by sedimentation into three zones using an Imhoff sedimentation cone [36]. The

324 sedimentation process was found to not only eliminate troublesome particles, but also to

325 narrow down the particle size distribution. In addition, sedimentation in a specially designed

326 centrifuge may help the procedure. Slurry packing makes sense as well, since packing

327 procedure takes typically a few minutes. A few sections (3-5) of different particle size batches

328 might be packed in a column to develop a stepwise arrangement (discontinuous particle size

329 gradient) which approaches a linear particle size gradient. Finally, we have to refer again to

330 the POPLC concept [5,6,7,8], as it offers short column segments coupled in series using an

331 optimized hardware (with minimal extra-column dispersion). However, at the time of writing,

332 only 5 and 3 µm particle size segments were available.

333

334 **4.   Conclusions**

335 The potential of columns packed with particles of decreasing size (particle size gradient) has

336 been studied. As previously seen, decreasing particle size along columns (in a coupled

337   system) can result in extra peak sharpening effect in addition to the common gradient band

338   compression [20,21]. A further increase in efficiency gain was expected with continuous

339   particle size gradient compared to discontinuous ones. Since a particle size gradient is

340   equivalent to the limit of a large number of serial columns, these efficiency gains

341   provide an upper bound on the efficiency gains of *any* number of coupled columns.

342   A measure of particle size gradient (g_d) was introduced which takes into account the

343   steepness, direction and average particle size. Then, various conditions (i.e., mobile phase

344   gradient steepness, retention times and operating pressures) were considered and columns

345   with different particle size gradients were compared to each other and to monodisperse

346   packing.

347   In isocratic elution mode, varying particle size do not present obviously any advantage.

348   However, as the mobile phase gradient increases, the benefit of the particle size gradient

349   becomes more important. In the best case, about 15-20% gain in efficiency can be expected

350   at a given retention time when utilizing a particle size gradient, compared to constant particle

351   size. Conversely, when fixing efficiency the analysis time can be decreased by about 15%

352   with an optimal particle size gradient. It also worth mentioning, that compounds with higher S

353   values resulting in higher b (large molecules such as peptides, proteins) can in principle see

354   the highest gains in efficiency.

355   It was also shown that particle size gradient should not increase without boundaries. Above a

356   given g_d value, it stops providing any benefit. Even more, too large a particle size gradient

357   can result in lower efficiencies than a column with monodisperse packing would achieve. We

358   observed that $g_d = 0.3 - 0.4$ provides the highest gain under practically useful conditions.

359

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463 **Figure captions**

464

465 Figure 1. Schematic view of column with a linear particle size gradient.

466

467 Figure 2. Pressure drop along a column with no particle size gradient, and with particle size

468 gradients $g_d = \pm 0.5$, for fixed average particle size $d_M = 0.5$ μm, mobile phase velocity

469 $u_0 = 1$ cm/s and a 15 cm long columns.

470

471 Figure 3. Relative gain in apparent efficiency between a column with no particle size

472 gradient, and column with a particle size gradient given by the value of g_d in the x-axis.

473 Average particle size in both cases is chosen so that both columns produce the same

474 retention time of 10 minutes at a pressure of 100 bar, enabling a fair comparison. Without

475 mobile phase gradient, the optimal configuration is to not have particle size gradient either

476 (since the system cannot use the late-column peak focusing effect to compensate for the

477 larger particles in the early column). Increasing the mobile phase gradient increases the

478 maximal gain available by the particle size gradient.

479

480 Figure 4. Relative gain in apparent efficiency between a column with no particle size

481 gradient, and a column with a particle size gradient of $g_d = 0.3$, as a function of mobile phase

482 gradient steepness, for several fixed retention times.

483

484 Figure 5. Actual apparent efficiency of the $g_d = 0.3$ column at each value of the mobile phase

485 gradient steepness. (Same conditions as in figure 4.)

486

487 Figure 6. The required average particle size of the $g_d = 0.3$ column to produce the indicated

488 retention time, at each value of the mobile phase gradient steepness. (Same conditions as in

489 figure 4.)

490

491   Figure 7. Relative gain in apparent efficiency between a column with no particle size

492   gradient, and a column with a particle size gradient of $g_d = 0.3$, as a function of produced

493   retention time, for several pressure drops and at a fixed mobile phase gradient steepness (*b*

494   = 5). The average particle size in each column is chosen to produce the specified retention

495   time.

496

497   Figure 8. Actual apparent efficiency of the $g_d = 0.3$ column for each produced retention time

498   when fixing mobile phase gradient steepness at *b* = 5. (Same conditions as in figure 7.)

499

500   Figure 9. The required average particle size of the $g_d = 0.3$ column to produce the indicated

501   retention time when fixing mobile phase gradient steepness at *b* = 5. (Same conditions as in

502   figure 7.)

503

504   Figure 10. Overlaid theoretical chromatograms for a compound expected with a column

505   having particle size of $d_M = \text{1.0}2.68$ μm and no particle size gradient (blue chromatogram)

506   and with a column packed with a $g_d = 0.3$ particle size gradient and average particle size of

507    $d_M = \text{1.1}3.0$ μm (red chromatogram). For both runs, $b = \text{2.5}10$ mobile phase intrinsic gradient

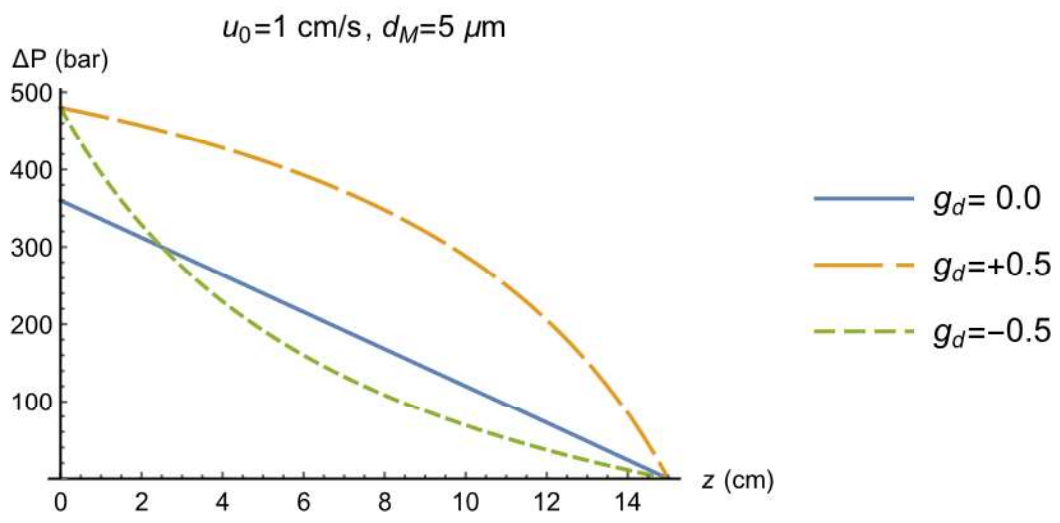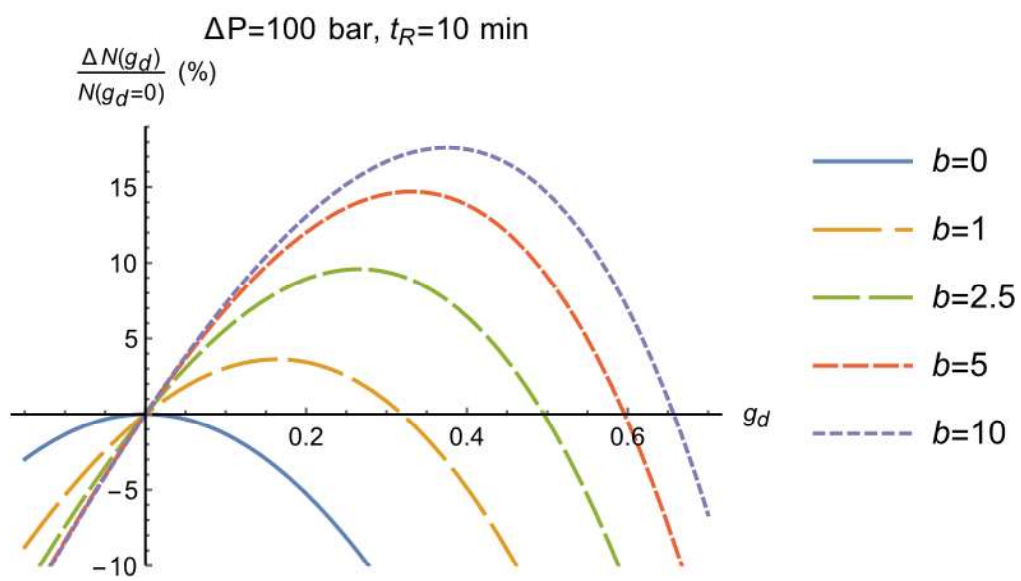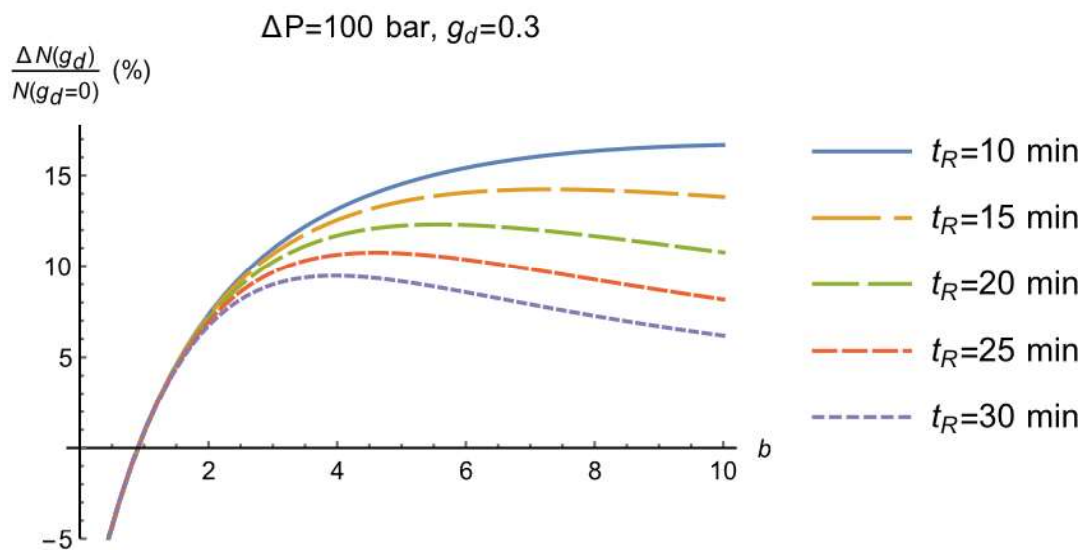508   steepness and $\Delta P = \text{400}25$ bar pressure drop were considered.
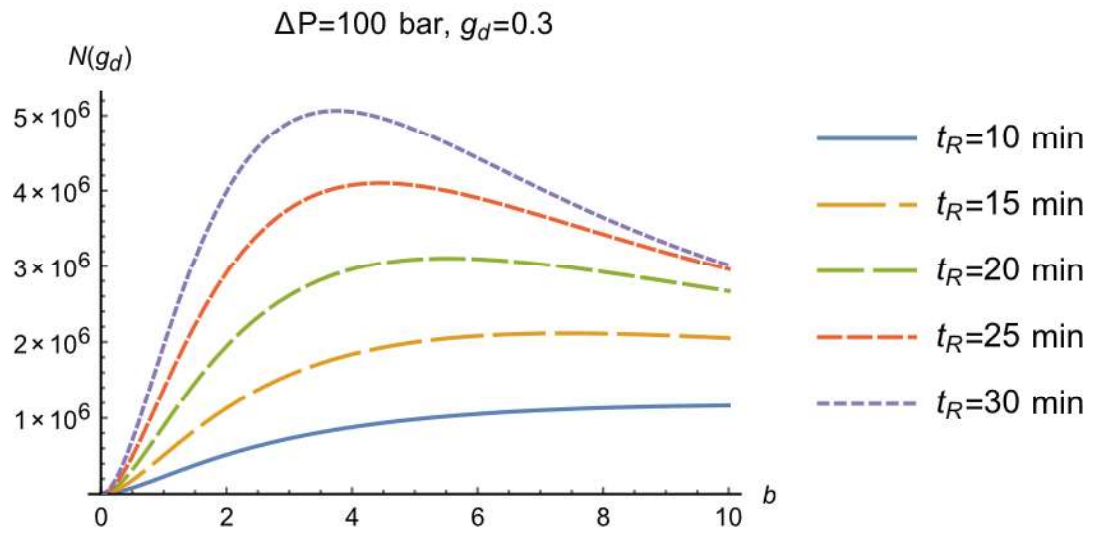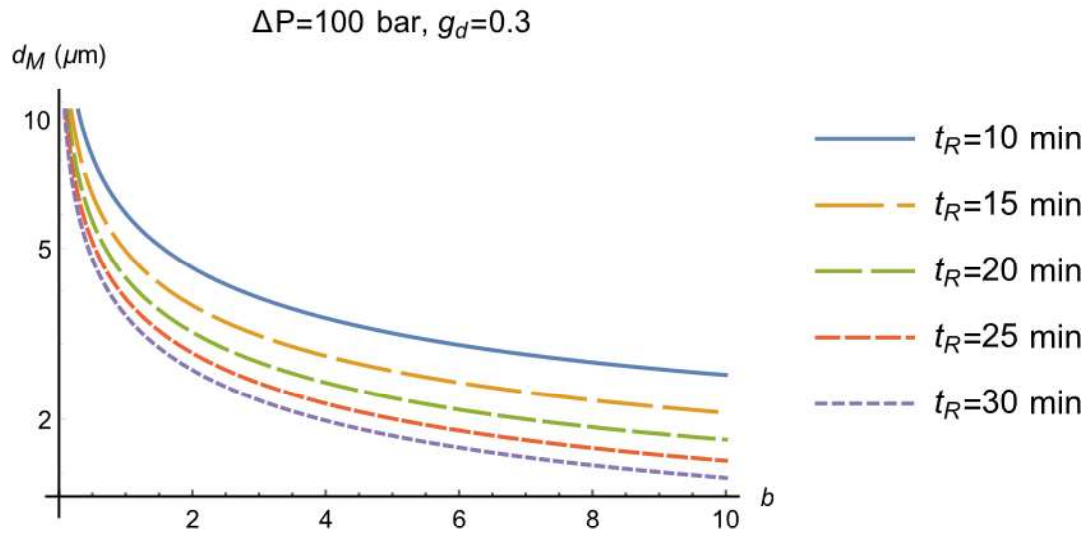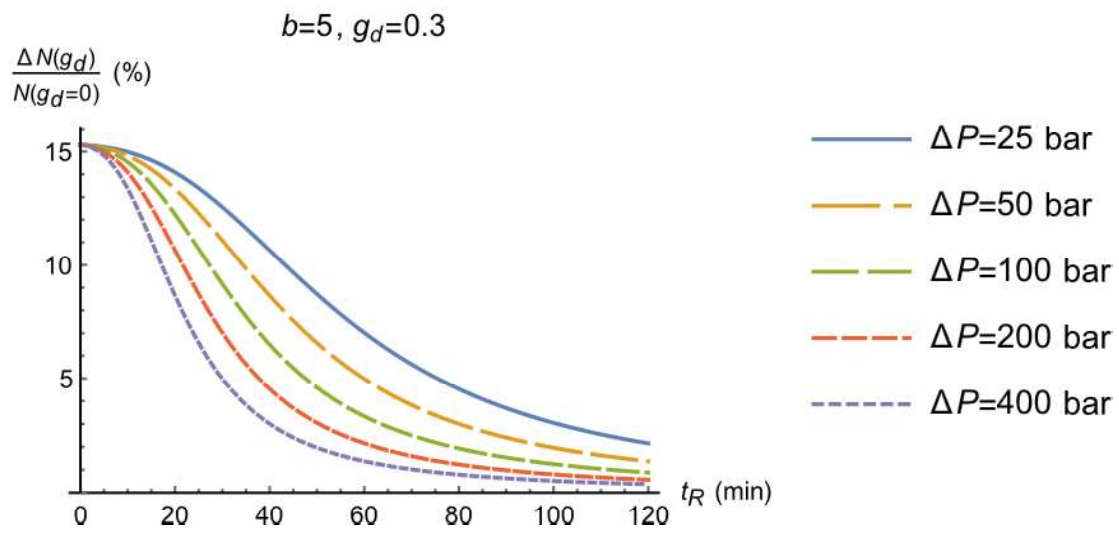
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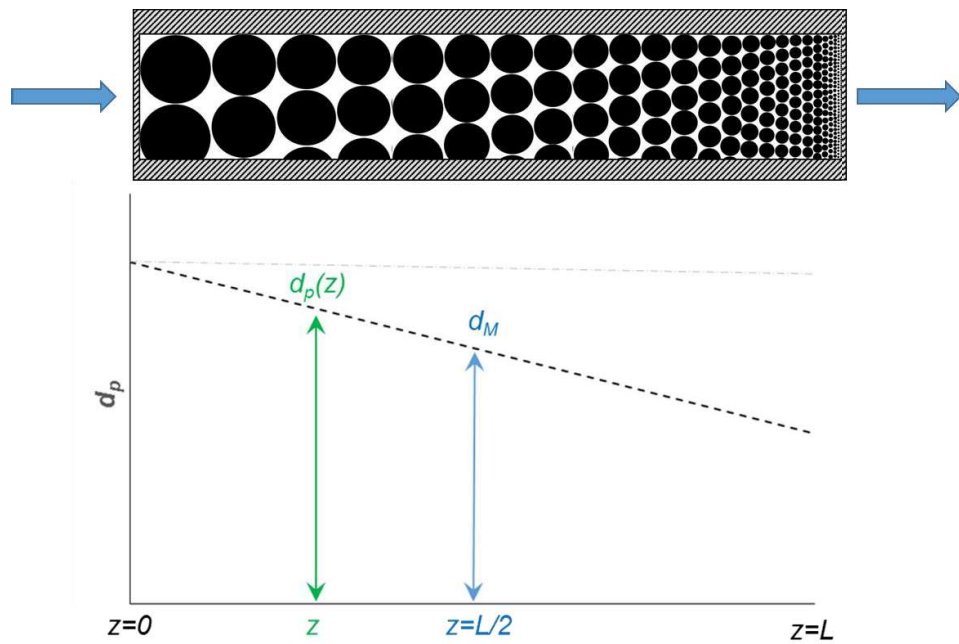
Figure 1.

Figure 2.

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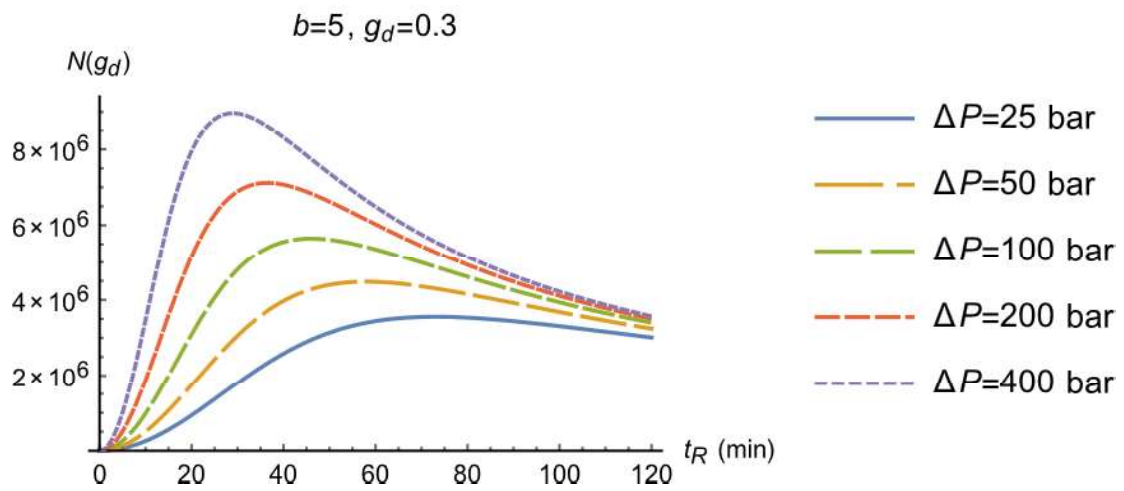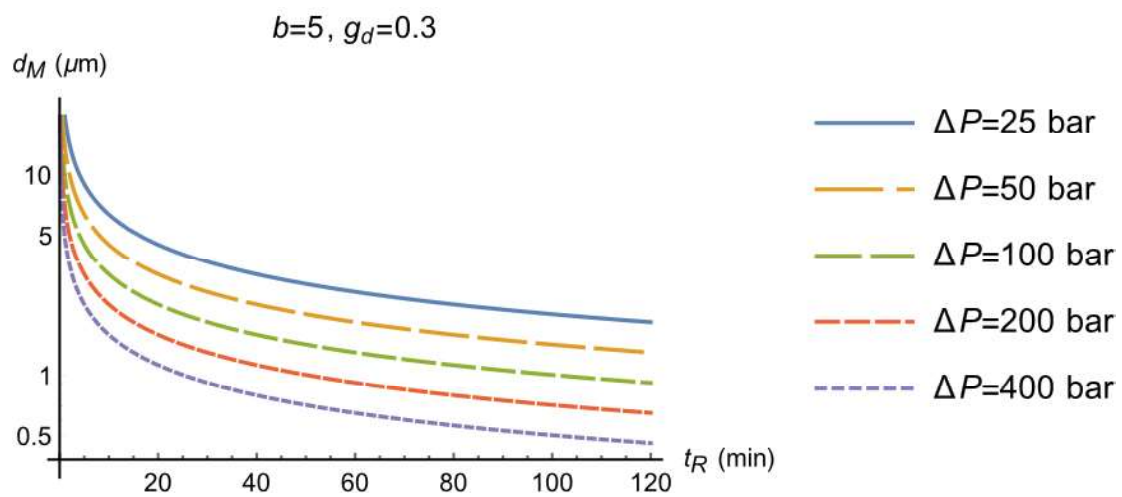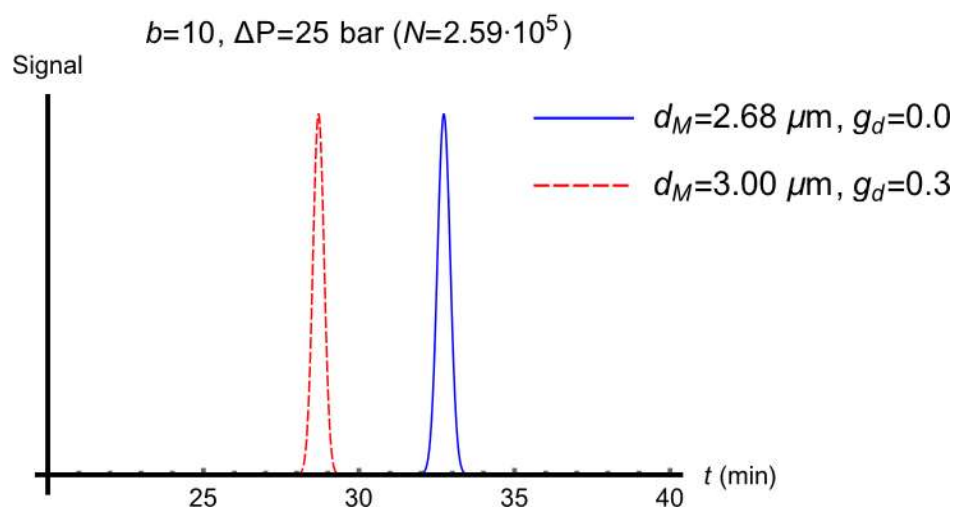
Figure 8.

Figure 9.

b=10, ΔP=25 bar (N=2.59·10^5)

Signal

d_M=2.68 μm, g_d=0.0

d_M=3.00 μm, g_d=0.3

t (min)

25   30   35   40

Figure 10.