



HAL
open science

Cross-frontal mode: An alternative methodology for Taylor dispersion analysis of monomodal sample

Jérémie Gouyon, Ariane Boudier, Arnaud Pallotta, Célia Boukoufi, Igor Clarot

► **To cite this version:**

Jérémie Gouyon, Ariane Boudier, Arnaud Pallotta, Célia Boukoufi, Igor Clarot. Cross-frontal mode: An alternative methodology for Taylor dispersion analysis of monomodal sample. *Journal of Chromatography A*, 2023, 1694, pp.463913. 10.1016/j.chroma.2023.463913 . hal-04026838

HAL Id: hal-04026838

<https://hal.univ-lorraine.fr/hal-04026838v1>

Submitted on 4 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1 **Cross-frontal mode: an alternative methodology for Taylor** 2 **Dispersion Analysis of monomodal sample**

3 *Jérémie Gouyon^{a*}, Ariane Boudier^{a,b}, Arnaud Pallotta^{a,b}, Célia Boukoufi^{a,c}, Igor*
4 *Clarot^{a,b}*

5
6 ^a Université de Lorraine, CITHEFOR, F-54000 Nancy, France

7 ^b Nanocontrol, F-54000 Nancy, France

8 ^c Pharmacy Department, University Hospital, 54511 Vandœuvre-lès-Nancy, France

9
10 *Corresponding author: jeremie.gouyon@univ-lorraine.fr (ORCID number: 0000-0002-
11 8834-9009), +33 3 72 74 73 10

12 **Keywords**

13 Capillary, Hydrodynamic radius, Methodology, Taylor Dispersion Analysis

14 **Abstract**

15 Taylor dispersion analysis (TDA) is a technique dedicated to the determination of the
16 molecular diffusion coefficient (D) of species, using band broadening of an analyte in
17 a laminar flow. Two modes are commonly used to perform TDA: pulse and frontal
18 modes. In each case, a fitting of the signal is required. We propose here a third mode
19 denoted as cross-frontal mode, combining two crossed sample fronts without
20 modification of a classical CE device for the rapid and accurate determination of D of
21 caffeine, reduced glutathione (GSH), insulin from bovine pancreas, bovine serum
22 albumin (BSA) and citrate-capped gold nanoparticles (AuNP). Theoretical aspects and
23 methodology are described, showing a good correlation between the so-called cross-
24 frontal mode and usual frontal mode. Limitations of the techniques are also assessed,
25 and are similar to regular modes while no fitting is required. This new methodology
26 allows improving the sensitivity toward low concentrated sample compared to pulse
27 mode, and an alternative mathematical treatment compared to regular TDA modes.

28 **1. Introduction**

29 Taylor dispersion analysis (TDA) is known as a characterization technique for size
30 determination in the nm range (0.1 – 100 nm)[1]. It has been used for a wide variety of
31 analytes size, from small organic molecules[2] to biomolecules characterization[3] or
32 inorganic nanoparticle size determination[4]. Using capillary electrophoresis (CE)
33 instrument[5], TDA is easily accessible and complementary to other characterization
34 techniques such as Dynamic Light Scattering (DLS) or Transmission Electronic
35 Microscopy (TEM)[6]. Basically, TDA consists in the measurement of the peak
36 broadening of a solute injected in a laminar Poiseuille's flow for sub-nm size range
37 determination. Peak width is, in this particular case, inversely proportional to the
38 diffusion coefficients of the analyte. Such a laminar flow is feasible with unmodified CE
39 devices[7] due to capillary dimensions, pressure control and UV-Visible detector
40 coupling.

41 Two modes are nowadays commonly used for Taylor dispersion: pulse and frontal
42 modes[8]. The pulse mode consists in the injection of a finite plug of sample before
43 mobilization through the capillary[9] (typically, the plug is inferior to 1% of the volume
44 of the capillary between the inlet and the detection window). The resulting Taylorgrams
45 being described by a Gaussian curve, the peak width is linked to the analyte size.
46 Nonetheless, the sensitivity of the pulse mode is limited (due to low sample volume),
47 which can be counteracted using different types of detectors[10,11], or using the frontal
48 mode. It consists in the continuous injection of the sample[12], leading to a half-wave
49 signal reached after saturation of the capillary with the sample. Compared to pulse
50 mode, frontal mode allows reaching higher sensitivity since there is no limitation in
51 terms of introduced quantity of analyte. Each mode leads to different Taylorgram
52 profiles, which are summarized in Figure 1.

53 Mathematical interpretation has already been described in the literature[12,13] for both
54 modes, and is not the object of this paper. Nonetheless, it has to be mentioned that
55 fitting of the signal (either Gaussian or half-wave) using appropriate software is
56 required for the determination of the temporal variance σ^2 of the elution profile, directly
57 proportional to the size of the analyte (see following review[14] for more details).
58 Specific software and mathematical treatment are required for this operation, which
59 can be unappealing for the non-initiated.

60 In this paper, we aimed at presenting a third mode, denoted as “cross-frontal mode”,
61 so named because it combines crossed sample fronts. Mathematical demonstration
62 and applications are described for monomodal sample. A comparison with regular
63 frontal mode is also presented, since both modes are comparable in terms of sensitivity
64 and methodology. The main interest of the proposed methodology relies on the
65 absence of fitting required to interpret the data. Similar methodology was applied using
66 capillary zone electrophoresis[15] and is here adapted to TDA application. This method
67 is promising to simplify the interpretation compared to frontal mode while being as
68 simple to perform, since no device modification is required. Demonstration is applied
69 in this paper for analyte size ranging from 0.2 to 5 nm hydrodynamic radius (r_H), from
70 small organic molecule to biomolecules. Feasibility of the cross-frontal methodology is
71 thus expressed for a broad range of analytes.

72 **2. Material and methods**

73 **2.1. Materials and reagents**

74 All reagents and solvents were of analytic grade and were used without further
75 purification. Ultrapure deionized water ($> 18.2 \text{ M}\Omega \text{ cm}$) was used for the preparation of
76 all solutions. Sodium phosphate monobasic monohydrate $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (for analysis,
77 99 %) was purchased from Merck (Darmstadt, Germany). Sodium phosphate dibasic
78 dodecahydrate $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (for analysis, 98 %) was purchased from Carlo Erba
79 Reagents (Val de Reuil, France). Sodium citrate dihydrate ($\geq 99\%$), sodium
80 borohydride NaBH_4 (ReagentPlus[®], 99 %), gold(III) chloride trihydrate $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$
81 (ACS reagent, $\geq 49.0 \%$ Au basis), caffeine (SigmaUltra), L-glutathione reduced (GSH,
82 $\geq 98.0 \%$), Insulin from bovine pancreas (BioReagent, suitable for cell culture), Bovine
83 serum albumin (BSA, 98 % electrophoresis), hydrochloric acid, HCl, and nitric acid,
84 HNO_3 , were purchased from Sigma Aldrich (St Louis, MO, USA). Solutions
85 preparation, citrate-capped gold nanoparticles (AuNP) synthesis[16,17] and the
86 related characterizations are detailed in Supplementary Information.

87 **2.2. Apparatus**

88 Taylor dispersion analyses were performed using a P/ACE MDQ CE system (Beckman
89 Coulter) equipped with a diode array detector (DAD, wavelength ranging from 200 to
90 600 nm). Measurements were performed at $25 \pm 1 \text{ }^\circ\text{C}$ using a 40 cm bare fused silica
91 capillary (effective length: 30 cm, Polymicro Tech.) with inner diameter of 100 μm .

92 **2.3. Cross-frontal TDA experimental methodology**

93 Depending on the analyte, mobilization pressure P and injection time t_{inj} and duration
94 of the experiment were adjusted but the concept remains similar for each tested
95 condition:

96 (i) The capillary was flushed with water, 0.1 M NaOH, and water.

97 (ii) the capillary was filled with the sample. Each sample was prepared in a specific
98 solvent, depending on its properties as mentioned in (iii) below (see Supplementary
99 Information for more details).

100 (iii) the analyte-free solvent, denoted as “empty plug” (a solution of 1.5 mM sodium
101 citrate for citrate-capped AuNP analysis, 20 mM phosphate buffer for caffeine and BSA
102 analysis, 10 mM HCl for insulin analysis or 1 mM HCl for GSH analysis) was injected
103 at P for a duration t_{inj} (P being equal to 0.4, 0.3 or 0.2 psi and t_{inj} varying from 5 to 100
104 s, depending on the analyte).

105 (iv) Starting the UV-Visible acquisition (at 514 nm for AuNP[18], 272 nm for caffeine,
106 214 nm for GSH and 220 nm for BSA and insulin), the empty plug was pushed
107 hydrodynamically with the sample using the same P.

108 (v) 1.5 mM sodium citrate, 20 mM phosphate buffer, 1 mM HCl or 10 mM HCl
109 (depending on the analyte) were pushed using the same P after measurement of the
110 negative peak.

111 Figure 2 shows a schematic view of a cross-frontal Taylorgram obtained using the
112 experimental methodology, indicating how the mean solute concentration across the
113 cross-section of the capillary \bar{C} and the concentration of the front \bar{C}_0 were measured.
114 Each measurement was repeated 3 times for each described condition. Experimental
115 conditions are summarized in Supplementary Information section (Table S1). It is to
116 be noted that higher P values were not tested since Taylor’s conditions would not be
117 fulfilled (as mentioned in Experimental results section).

118 **3. Results and discussion**

119 **3.1. Theoretical aspects of cross-frontal TDA mode**

120 Considering fundamental aspects of Taylor dispersion, it has been
121 demonstrated[19,20] that the concentration profile (Taylorgram) using frontal mode for
122 monomodal sample is given by eq. (1):

123
$$\frac{\bar{C}}{\bar{C}_0} = \frac{1}{2} \pm \frac{1}{2} \operatorname{erf} \left[\frac{t-t_R}{\sigma\sqrt{2}} \right] \quad (1)$$

124 where t is time, t_R is the mean residence time, σ is the square root of the temporal
 125 variance of the elution profile and erf is the error function. This equation is adequate
 126 for symmetric profile.

127 The new proposed methodology consists in filling the capillary with the sample,
 128 followed by injection of variable volumes (for different t_{inj}) of an “empty plug”. Using
 129 same velocity (related to P), the empty plug will then be pushed by re-introducing the
 130 sample, resulting in the following theoretical Taylorgram which is the sum of two front
 131 Taylorgrams i and j (Figure 3.B):

132 Considering the continuous laminar flow (Poiseuille’s flow) occurring during the
 133 experiment and for a precise value of t_{inj} , both sample profiles (shown by the * on the
 134 Figure 3.A) can be described independently using eq. (1). The profile i being centred
 135 on $t_R + t_{inj}/2$ and j being centred on $t_R - t_{inj}/2$, eqs. (2) and (3) give mathematical
 136 description of profiles i and j .

$$137 \quad \frac{\bar{C}_i}{\bar{C}_0} = \frac{1}{2} + \frac{1}{2} erf \left[\frac{t - (t_R + \frac{t_{inj}}{2})}{\sigma\sqrt{2}} \right] \quad (2)$$

$$138 \quad \frac{\bar{C}_j}{\bar{C}_0} = \frac{1}{2} - \frac{1}{2} erf \left[\frac{t - (t_R - \frac{t_{inj}}{2})}{\sigma\sqrt{2}} \right] \quad (3)$$

139 The Taylorgram is equal to the sum of both eqs. (2) and (3). At $t = t_R$, *i.e.* the intersection
 140 of both Taylorgrams i and j (but also the minimum of the peak), the equation can be
 141 simplified and give eq. (4):

$$142 \quad \frac{\bar{C}}{\bar{C}_0} = 1 - erf \left[\frac{t_{inj}}{\sigma 2\sqrt{2}} \right] \quad (4)$$

143 It is possible to express the previous equation as follow, giving eq. (5):

$$144 \quad erf^{-1} \left[1 - \frac{\bar{C}}{\bar{C}_0} \right] = \frac{t_{inj}}{\sigma 2\sqrt{2}} \quad (5)$$

145 For different t_{inj} , the previous expression will fit a linear curve with a slope equal to
 146 $1/(\sigma 2\sqrt{2})$. The proposed methodology remains in the possibility to inject variable
 147 volumes of empty plug (using variable t_{inj}) and to plot eq. (5) in order to calculate σ
 148 using slope of the linear curve. Using UV-visible detector, the absorbance is directly
 149 proportional to the concentration. It can be approximated that \bar{C} is correlated to
 150 absorbance value at t_R and \bar{C}_0 is correlated to absorbance value of the plateau. An
 151 illustration of this hypothesis is presented in Figure 3.C.

152
 153 Fulfilling Taylor’s conditions[12], the diffusion coefficient D of the analyte can be
 154 determined using eq. (6):

155
$$D = \frac{r_c^2 t_R}{24\sigma^2} \quad (6)$$

156 with r_c , capillary radius. If no particle interactions occur and analytes are considered
157 as spheres, D can be linked to r_H using Stokes-Einstein equation (eq. (7)):

158
$$D = \frac{k_B T}{6\pi\eta r_H} \quad (7)$$

159 with k_B the Boltzmann constant ($\text{m}^2 \cdot \text{kg} \cdot \text{s}^{-2} \cdot \text{K}^{-1}$), T the temperature (K) and η the
160 viscosity of the medium (Pa.s).

161 **3.2. Experimental results**

162 Using cross-frontal TDA methodology for caffeine (194.2 g/mol), GSH (307.3 g/mol,
163 tripeptide), insulin (~ 5 kDa), BSA (~ 66 kDa) and AuNP (inorganic NP) size
164 determination, D and r_H were obtained and results are reported in Table 1. Peclet (Pe)
165 and dimensionless residence time τ are also indicated for the cross-frontal mode, to
166 insure Taylor's conditions were satisfied[12] (Pe > 69 and $\tau > 1.4$, see Supplementary
167 Information). These conditions are dependent on P and capillary dimensions. Every
168 tested values of P meet the criteria for applicability of TDA modes, and are not
169 supposed to influence size determination.

170 As summarized, caffeine r_H was measured using cross-frontal mode and values are
171 comparable with reports from the literature for the lowest tested pressures (0.32 and
172 0.35 nm for 0.2 and 0.3 psi respectively against 0.32 to 0.49 nm[21–23]). Figure S6
173 shows the plots for caffeine analysis. These results tend to show the potential of cross-
174 frontal TDA mode for small organic molecules size determination. Concerning the
175 lower value of r_H obtained at 0.4 psi (0.25 nm), the deviation can be attributed to the
176 low injection time applied, which cannot be fully approximated and undergoes
177 misinterpretation of the final result. It is therefore important to only use the cross-frontal
178 mode with higher t_{inj} only (> 10 s), since ramp time to reach the pressure becomes
179 negligible. Results were also calculated using frontal mode, with values close to the
180 cross-frontal mode and the one reported in the literature (0.32 nm for frontal mode and
181 cross-frontal mode at 0.2 psi).

182 For GSH, values obtained with the cross-frontal mode (0.43, 0.39 and 0.40 nm for 0.2,
183 0.3 and 0.4 psi) are comparable with reports from the literature (0.36 nm[24]) and with
184 the frontal mode measurement (0.42 nm). Figure S7 shows the plots for GSH analysis.
185 These results tend to show the potential of cross-frontal TDA mode for organic
186 molecules size determination. Moreover, it is the first time TDA was used to measure
187 GSH hydrodynamic radius.

188 For insulin, values from cross-frontal mode (1.21, 1.16 and 1.09 nm for 0.2, 0.3 and
189 0.4 psi respectively) are comparable with reports from the literature (1.05 nm[25]) and
190 with the frontal mode measurement (1.04 nm). Figure 4 shows the plots for insulin
191 analysis. These results support the potential of cross-frontal TDA mode for small
192 protein size determination.

193 Concerning BSA, results on r_H are comprised between 5.00 and 3.99 nm (see Figure
194 S8 for cross-frontal Taylorgrams). These values are coherent with previously reported
195 works (see Table 1). In this case, working at $P = 0.2$ psi, the injection time increased
196 up to 100 s, and gave a deviation from the linear fitting model (visible in Figure S8.B).
197 Since viscosity of the sample is different than the 'empty plug' (due to high
198 concentration of BSA), we suppose that a local change in media properties occurs and
199 leads to such a deviation. Another explanation is based upon adsorption of the protein
200 which will be explained later (see Limits and perspectives). With no consideration for
201 this t_{inj} , r_H becomes equal to 4.13 nm, closer to results obtained using other pressure
202 levels. Still, variations between r_H from different P are to be mentioned.

203 For citrate-capped AuNP, results for each tested P are closed (Figure S9) and coherent
204 with DLS measurement: a slightly inferior value is reported for TDA (2.82 nm at 0.3 psi
205 using cross-frontal mode against 3.32 nm using DLS). As already stated in the
206 literature for this type of NP (citrate-capped AuNP), DLS measurements are sensitive
207 to potential aggregates, thus leading systematically to a superior value than with
208 TDA[4] since DLS is an intensity-based method, while TDA is a mass-based method.
209 Comparing cross-frontal mode with frontal mode, r_H estimated via TDA also gave
210 similar results (2.74 nm using frontal mode). No significant differences comfort the use
211 of cross-frontal mode as an alternative of frontal mode for inorganic NPs size
212 determination. Moreover, pulse analysis of citrate-capped AuNP is hardly feasible in
213 our case since the sample is not concentrated ($< \mu\text{M}$). It leads to low signal barely
214 exploitable since injected volume is limited in pulse mode. It is even more convenient
215 to use cross-frontal mode since there is a gain in terms of sensitivity, which makes it a
216 potential alternative for both pulse and frontal modes.

217 Since results between cross-frontal mode, frontal mode and reported values of the
218 literature are coherent, one can assume that the proposed methodology is adequate.
219 One advantage of such a methodology relies in the easy data handling, since no fitting
220 is required. Moreover, regular TDA conditions (such as values of P , t_{inj} , ...) can be kept,

221 while non-initiated can start using similar conditions for other molecule/nanoparticle
222 size determination.

223 **3.3. Limits and perspectives**

224 Few aspects need to be discussed concerning limitations of the cross-frontal mode,
225 inherent to TDA. It is known that, depending on the operating conditions, measurable
226 size range is variable. In the case of cross-frontal mode, several precisions are
227 required.

228 For instance, during the development of the methodology, very high injection volume
229 (related to t_{inj}) led to low \bar{C}/\bar{C}_0 ratio. The function erf^{-1} being strongly impacted for ratio
230 values close to 0, strong variations of the ratio occur and deeply impact the final results,
231 with high standard deviation. In order to avoid such an effect, $1-(\bar{C}/\bar{C}_0)$ should not
232 exceed 0.96 (with $\text{erf}^{-1}(0.96) = 1.45$) since variation of 0.01 on $1-(\bar{C}/\bar{C}_0)$ leads to a
233 change of more than 0.08 on $\text{erf}^{-1}(1-(\bar{C}/\bar{C}_0))$ (> 5 % variability). In other words,
234 preliminary analysis using variable t_{inj} should be performed to determine the maximum
235 t_{inj} to be used for TDA before reaching the specified value. As for frontal or pulse
236 modes, optimization of TDA conditions is required before use.

237 Moreover, low t_{inj} should be avoided to decrease the probable variation inherent to the
238 CE device (due to ramp time to reach the pressure for instance), as illustrated for
239 caffeine analysis at 0.4 psi. In our case, 10 s minimum was the best option. This value
240 is dependent on the capacity of the apparatus to deliver a stable pressure profile during
241 the injection.

242 Another aspect which needs to be considered is the sample adsorption. In the case of
243 BSA, no results were indicated for frontal mode (Table 1) since the Taylorgrams were
244 asymmetric and no suitable fittings were obtained for determination of σ . It suggests
245 that interactions between the protein and the inner surface of the capillary may occur,
246 which is detrimental for TDA application[5], no matter the mode used. Even if results
247 for BSA size determination using cross-frontal mode seem to agree with the
248 literature[26], no conclusion can be adopted since Taylor's conditions are not entirely
249 fulfilled. This adsorption can also explain the variations observed using the cross-
250 frontal mode for BSA characterization. Use of coating (dynamic or covalent/semi-
251 covalent) is suggested, but can be detrimental for detection of the analyte and was not
252 explored here for this reason.

253 As a prospect, simplification of the interpretation and similarities between cross-frontal
254 and frontal mode results suggest the possibility to consider the cross-frontal mode as
255 an alternative if following conditions are respected:

256 (i) Usual TDA conditions (on Pe and τ) are respected.

257 (ii) No adsorption of the sample occurs on the inner capillary wall.

258 (iii) t_{inj} is sufficiently high to avoid strong variation due to the CE device and
259 misinterpretation (> 10 s). If volume is too important, a decrease in P should be
260 considered.

261 (iv) t_{inj} is not too high to insure $1 - (\bar{C}/\bar{C}_0) < 0.96$.

262 (v) Sample is monomodal / monodisperse for the equation to be applicable.

263 Still, cross-frontal mode is more sensitive than pulse mode since the limitation in terms
264 of injected volume of sample is not to be considered ($< 1\%$). It may allow to measure
265 less concentrated samples or analytes with limited absorption properties, as illustrated
266 with citrate-capped AuNP. Moreover, cross-frontal mode requires no fitting of the
267 signal, in contrary to frontal mode which facilitates data manipulation, even if similar
268 sensitivity is expected. No particular software is necessary to estimate σ due to a
269 simplified data treatment, which is more appealing for non-initiated and can makes
270 TDA more accessible.

271 **4. Conclusions**

272 To conclude, cross-frontal mode TDA was successfully applied for r_H determination of
273 caffeine (0.32 nm), GSH (0.43 nm), insulin (1.21 nm) and citrate-capped AuNP (2.93
274 nm), with similar results compared to frontal mode TDA and literature. As well as for
275 the pulse and frontal modes, cross-frontal mode methodology is still limited by several
276 requirements. For instance, adsorption of analytes is incompatible with TDA
277 measurements. Still, this new methodology requires no fitting of the Taylorgram,
278 leading to an interesting simplification of the signal treatment. Moreover, the
279 applicability of the method seems feasible for a wide range of analytes (r_H from 0.3 to
280 5 nm), as stated in the paper with small organic molecules, biomolecules and inorganic
281 NP measurements. Larger particles and polydisperse / multi-modal samples should
282 also be tested for validation of the methodology.

283 **Credit authorship contribution statement**

284 Jeremie Gouyon: Conceptualization, Investigation, Methodology, Writing – original
285 draft & editing. Ariane Boudier: Writing - review & editing. Arnaud Pallotta: Writing -

286 review & editing. Celia Boukoufi: Investigation, Writing - review & editing. Igor Clarot:
287 Writing - review & editing.

288 **Declaration of Competing Interest**

289 The authors declare that they have no known competing financial
290 interests or personal relationships that could have appeared to influence
291 the work reported in this paper.

292 **Acknowledgements**

293 This research did not receive any specific grant from funding agencies in the public,
294 commercial, or not-for-profit sectors.

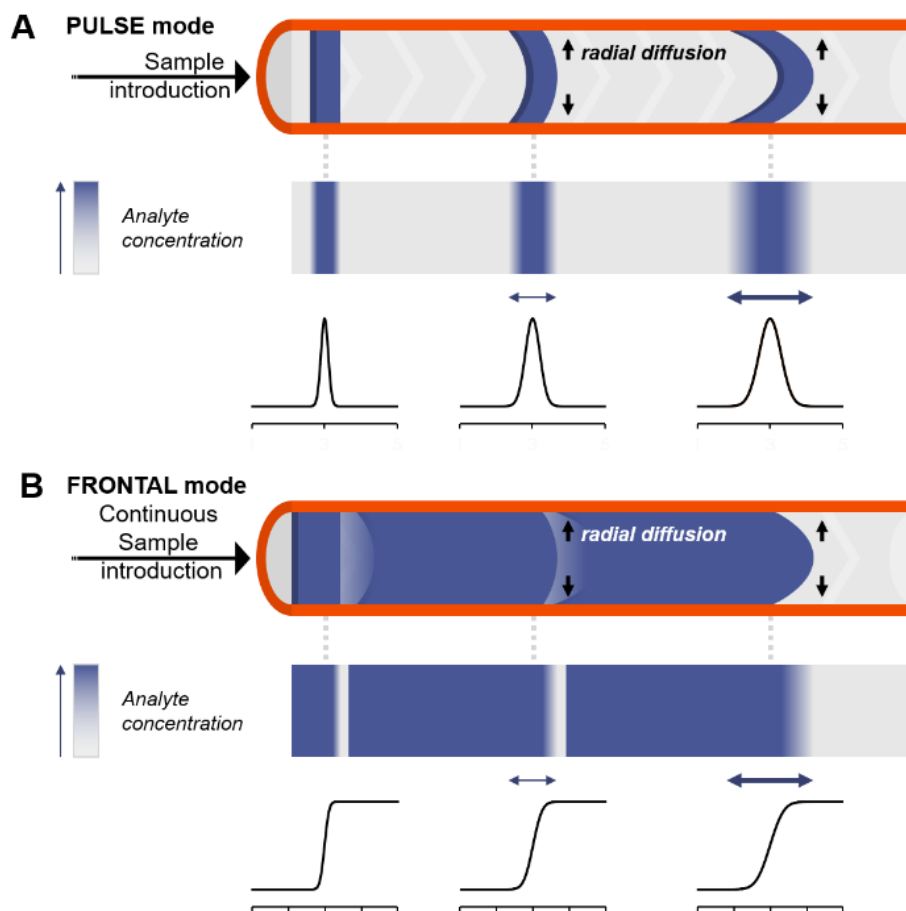
295 **Appendix A. Supplementary material**

296 Supplementary information to this article can be found online.

297 **References**

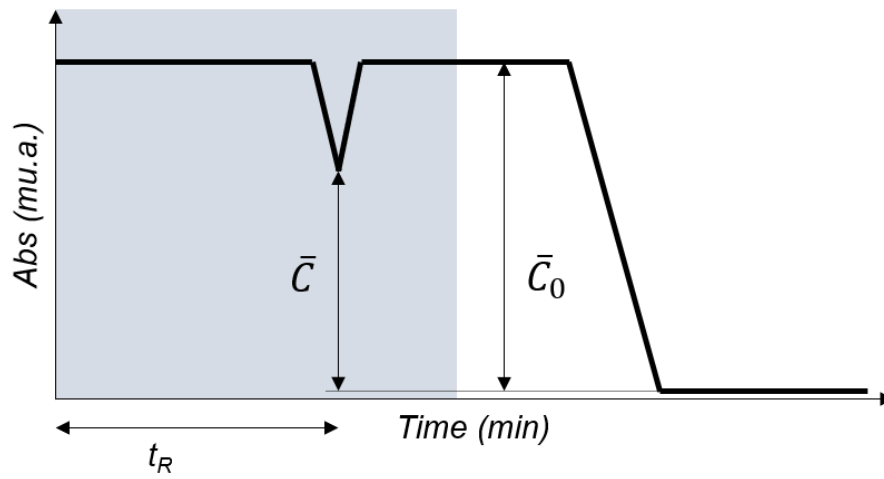
- 298 [1] Adamo, G., Campora, S., Ghersi, G. In *Nanostructures for Novel Therapy*, Ficai,
299 D., Grumezescu, A. M., Eds., Micro and Nano Technologies, Elsevier, 2017, pp.
300 57–80.
- 301 [2] Ye, F., Jensen, H., Larsen, S. W., Yaghmur, A., Larsen, C., Østergaard, J. *J.*
302 *Pharm. Biomed. Anal.* 2012, *61*, 176–183.
- 303 [3] Hong, J., Wu, H., Zhang, R., He, M., Xu, W. *Anal. Chem.* 2020, *92*, 5200–5206.
- 304 [4] Pyell, U., Jalil, A. H., Pfeiffer, C., Pelaz, B., Parak, W. J. *J. Colloid Interface Sci.*
305 2015, *450*, 288–300.
- 306 [5] R. Moser, M., A. Baker, C. *Anal. Methods* 2021, *13*, 2357–2373.
- 307 [6] Urban, D. A., Milosevic, A. M., Bossert, D., Crippa, F., Moore, T. L., Geers, C.,
308 Balog, S., Rothen-Rutishauser, B., Petri-Fink, A. *Colloid Interface Sci. Commun.*
309 2018, *22*, 29–33.
- 310 [7] Saetear, P., Chamieh, J., Kammer, M. N., Manuel, T. J., Biron, J.-P., Bornhop,
311 D. J., Cottet, H. *Anal. Chem.* 2017, *89*, 6710–6718.
- 312 [8] Leclercq, L., Reinhard, S., Chamieh, J., Döblinger, M., Wagner, E., Cottet, H.
313 *Macromolecules* 2015, *48*, 7216–7221.
- 314 [9] Cottet, H., Biron, J.-P., Martin, M. *Anal. Chem.* 2007, *79*, 9066–9073.
- 315 [10] Casto, L. D., Do, K. B., Baker, C. A. *Anal. Chem.* 2019, *91*, 9451–9457.
- 316 [11] Moser, M. R., Smith, C. M., Gutierrez, G. G., Baker, C. A. *Anal. Chem.* 2022, *94*,
317 6089–6096.
- 318 [12] d’Orlyé, F., Varenne, A., Gareil, P. *J. Chromatogr. A* 2008, *1204*, 226–232.
- 319 [13] Labied, L., Rocchi, P., Doussineau, T., Randon, J., Tillement, O., Lux, F.,
320 Hagège, A. *Anal. Chem.* 2021, *93*, 1254–1259.
- 321 [14] Gouyon, J., Boudier, A., Barakat, F., Pallotta, A., Clarot, I. *ELECTROPHORESIS*
322 2022, *43*, 2377–2391.
- 323 [15] Jin, W., Chen, H. *Chromatographia* 2000, *52*, 17–21.
- 324 [16] Beurton, J., Lavalle, P., Pallotta, A., Chaigneau, T., Clarot, I., Boudier, A. *Int. J.*
325 *Pharm.* 2020, *580*, 119244.
- 326 [17] Berthou, M., Pallotta, A., Beurton, J., Chaigneau, T., Athanassiou, A., Marcic, C.,
327 Marchioni, E., Boudier, A., Clarot, I. *J. Chromatogr. B* 2022, *1198*, 123244.

- 328 [18] Tournebize, J., Sapin-Minet, A., Schneider, R., Boudier, A., Maincent, P., Leroy,
329 P. *Talanta* 2011, 83, 1780–1783.
- 330 [19] Taylor, G. I. *Proc. R. Soc. Lond. Ser. Math. Phys. Sci.* 1953, 219, 186–203.
- 331 [20] Sharma, U., Gleason, N. J., Carbeck, J. D. *Anal. Chem.* 2005, 77, 806–813.
- 332 [21] Hulse, W. L., Forbes, R. T. *Int. J. Pharm.* 2011, 411, 64–68.
- 333 [22] Leaist, Derek., Hui, Lu. *J. Phys. Chem.* 1990, 94, 8741–8744.
- 334 [23] Chamieh, J., Oukacine, F., Cottet, H. *J. Chromatogr. A* 2012, 1235, 174–177.
- 335 [24] Stricks, W., Kolthoff, I. M. *J. Am. Chem. Soc.* 1952, 74, 4646–4653.
- 336 [25] Yousefi, R., Taheri, B., Alavi, P., Shahsavani, M. B., Asadi, Z., Ghahramani, M.,
337 Niazi, A., Alavianmehr, M. M., Moosavi-Movahedi, A. A. *J. Biomol. Struct. Dyn.*
338 2016, 34, 362–375.
- 339 [26] Hulse, W., Forbes, R. *Int. J. Pharm.* 2011, 416, 394–397.
- 340 [27] Mazloum-Ardakani, M., Sheikh-Mohseni, M. A., Mirjalili, B.-F. *Electroanalysis*
341 2013, 25, 2021–2029.
- 342 [28] Jensen, S. S., Jensen, H., Cornett, C., Møller, E. H., Østergaard, J. *J. Pharm.*
343 *Biomed. Anal.* 2014, 92, 203–210.
- 344 [29] Jachimska, B., Wasilewska, M., Adamczyk, Z. *Langmuir* 2008, 24, 6866–6872.
- 345 [30] Le Saux, T., Cottet, H. *Anal. Chem.* 2008, 80, 1829–1832.
- 346
- 347



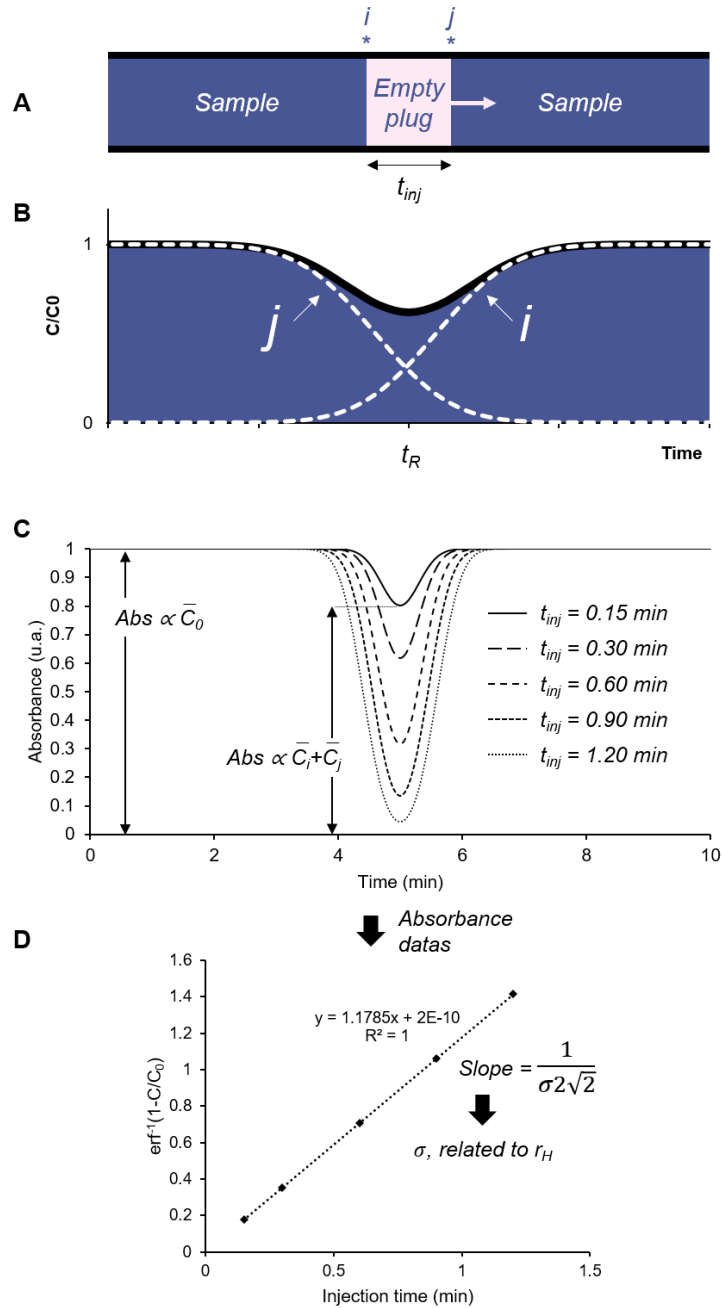
348
 349 **Figure 1 - Taylor dispersion analysis representation of (A) pulse and (B) frontal mode. [Reprinted,**
 350 **with permission, with modifications from ref [14]. Copyright © 2022 WILEY-VCH Verlag GmbH &**
 351 **Co. KGaA, Weinheim]**

352



353
 354
 355
 356
 357

Figure 2. Schematic view of a cross-frontal Taylorgram. The coloured part indicates the time at which the sample is hydrodynamically pushed, before continuous injection of the solvent in order to decrease the signal to obtain \bar{C}_0 and \bar{C} .

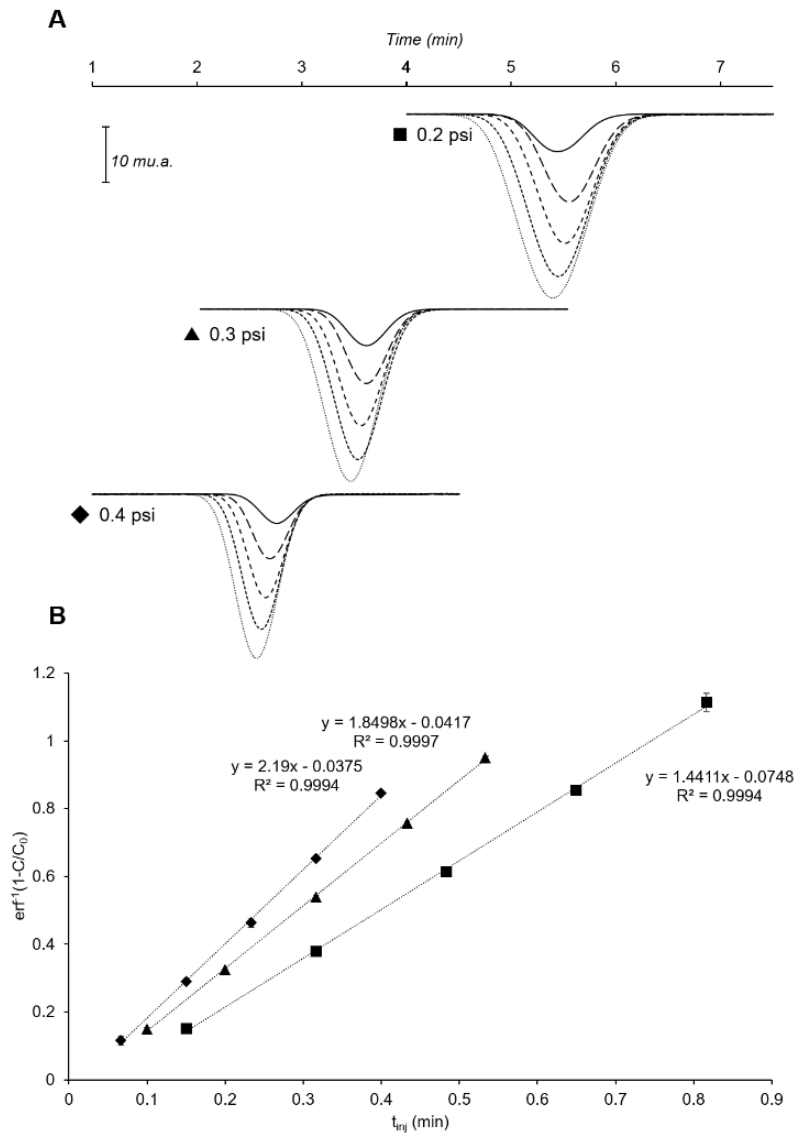


358

359 **Figure 3. (A) Schematic capillary profile of the proposed cross-frontal TDA mode and (B) example**
 360 **of resulting Taylorgram centred on t_R . Sample (blue) contains the analyte to be measured, and**
 361 **empty plug (clear) is composed of solvent free of analyte. (C) Simulated cross-frontal**
 362 **Taylorgrams using variable t_{inj} for $t_R = 5 \text{ min}$ and $\sigma = 0.3 \text{ min}$ and (D) eq. (5) plot for determination**
 363 **of σ , used to calculate diffusion coefficient D and r_H .**

364

365



366
367
368

Figure 4. (A) Typical cross-frontal Taylorgrams obtained for insulin using different P and t_{inj} and (B) resulting plot for determination of σ ($n=3$ for each level)

369

370 **Table 1 – Results obtained using cross-frontal mode TDA and comparison with frontal mode TDA**
 371 **and literature data. Values were statistically evaluated by the Student's Test ($p < 0.05$).**

Sample	P (psi)	Cross-frontal mode			Frontal*	r_H th. Ref.
		0.2	0.3	0.4		
Caffeine	σ (min)	0.125 ± 0.013	0.106 ± 0.021	0.077 ± 0.013	-	
	D ($\cdot 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$)	6.26 ± 0.66	5.65 ± 1.11	8.10 ± 1.38	-	
	r_H (nm)	0.32 ± 0.03	0.35 ± 0.07	0.25 ± 0.04	0.32 ± 0.00	0.32^a - 0.491 ± 0.008^a [21–23]
	τ / Pe	85.1 / 94	49.9 / 160	54.9 / 146	-	
GSH	σ (min)	0.150 ± 0.005	0.113 ± 0.007	0.099 ± 0.004	-	
	D ($\cdot 10^{-10} \text{ m}^2 \cdot \text{s}^{-1}$)	4.64 ± 0.17	5.16 ± 0.32	4.98 ± 0.18	-	
	r_H (nm)	0.43 ± 0.02	0.39 ± 0.02	0.40 ± 0.01	0.42 ± 0.00	0.36^b - 0.81 ± 0.05^c [24,27]
	τ / Pe	66.6 / 120	47.3 / 169	33.6 / 238	-	
Insulin	σ (min)	0.241 ± 0.009	0.191 ± 0.002	0.161 ± 0.004	-	
	D ($\cdot 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$)	1.66 ± 0.06	1.72 ± 0.02	1.84 ± 0.04	-	
	r_H (nm)	1.21 ± 0.04	1.16 ± 0.01	1.09 ± 0.03	1.04 ± 0.01	1.05 ± 0.1^d - 1.7^a [25,28]
	τ / Pe	22.0 / 364	15.0 / 535	12.2 / 654	-	
BSA	σ (min)	0.494 ± 0.032	0.367 ± 0.017	0.307 ± 0.016	-	
	D ($\cdot 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$)	4.01 ± 0.26	4.51 ± 0.21	5.02 ± 0.27	-	
	r_H (nm)	5.00 ± 0.32	4.44 ± 0.21	3.99 ± 0.21	**	3.3^d - 4.84 ± 0.03^a [21,26,29]
	τ / Pe	5.4 / 1477	3.8 / 2114	3.3 / 2432	-	
AuNP	σ (min)	0.385 ± 0.021	0.298 ± 0.010	0.266 ± 0.017	-	
	D ($\cdot 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$)	6.84 ± 0.31	7.10 ± 0.24	6.54 ± 0.42	-	
	r_H (nm)	2.93 ± 0.14	2.82 ± 0.10	3.06 ± 0.20	2.74 ± 0.10	3.32^d (2.9 ± 0.5) [16]
	τ / Pe	9.6 / 833	6.2 / 1292	4.2 / 1914	-	

372 *Results for frontal analysis were obtained by fitting the experimental curve with eq. (1), see Supplementary
 373 Information for more details[30]

374 **Results for frontal analysis of BSA are not provided since no suitable fitting of the experimental curve were
 375 possible, due to asymmetric signals

376 ^a Result obtained by TDA / ^b Result obtained by polarography / ^c Result obtained by chronoamperometry / ^d
 377 Result obtained by DLS

378