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1	Droplet-interfacing strategies in microscale electrophoresis for sample treatment,
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4	Théo Liénard-Mayor ¹ , Myriam Taverna ^{1,2} , Stéphanie Descroix ³ and Thanh Duc Mai ^{1*}
5	
6	¹ Université Paris-Saclay, CNRS, Institut Galien Paris-Saclay, 92296, Châtenay-Malabry,
7	France
8	² Institut Universitaire de France
9	³ Laboratoire Physico Chimie Curie, Institut Curie, PSL Research University, CNRS
10	UMR168, 75005 Paris, France
11	
12	Correspondence: E-mail: thanh-duc.mai@u-psud.fr;
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15	Keywords: droplets; microfluidics; capillary electrophoresis; microchip electrophoresis
16	
17	List of abbreviations:
18	CE/MCE: capillary electrophoresis / microchip capillary electrophoresis; EOF:
19	Electroosmotic flow; DMF: digital microfluidics; EWOD: Electrowetting on dielectric
20	technology; IL: ionic liquid; QC: quality control; LIF: laser induced fluorescence; SPE: solid
21	phase extraction; PCR-RFLP: PCR-restriction fragment length polymorphism; PDMS:
22	polydimethylsiloxane; PSS: poly(styrene)sulfonate; HTS: high-throughput screening; CGE:
23	capillary gel electrophoresis; PTFE: polytetrafluoroethylene; NGS: next-generation
24	sequencing; MEKC: micellar electrokinetic chromatography; EMMA: mediated
25	microanalysis; PMMA: poly(methyl methacrylate); PEEK: Polyether ether ketone

26 Abstract

In this study, for the first time we report on a comprehensive overview of different strategies to hyphenate droplet-based sample handling and preparation with electrophoretic separation in different formats (i.e. microchip and capillary electrophoresis). Droplet-interfaced electrophoresis is an emerging technique in which micro/nanometric droplets are used as a bridge and carrier of target analytes between sample treatment and electrokinetic separation steps, thus being expected to overcome the challenges of working dimension mismatch and low degree of module integration. This review covers all works on this topic from 2006 (the year of the first communication) up to 2020, with focus being given to three principal interfacing strategies, including droplets in immiscible phases, digital microfluidics with electrowetting-on-dielectric principle and inkjet droplet generation. Different instrumental developments for such purpose, the viewpoints on pros and cons of these designs as well as application demonstrations of droplet-interfaced electrokinetic strategies are discussed.

- 2 -

51 **1. Introduction**

52 Electrokinetic separations in a microchannel (i.e. microchip or capillary) offer several 53 advantages, notably high separation efficiency, low power requirements and limited 54 consumption of sample/chemicals [1-3]. To improve separation resolution, efficiency and 55 detection sensitivity for trace analysis in a complex matrix sample (e.g.biological samples), 56 sample pretreatment is often needed for removal of the sample matrix and target analytes 57 preconcentration prior to their electrokinetic separation and subsequent detection. Readers can 58 refer to various sample treatment techniques as forefront of microscale electrophoresis in 59 different reviews [4-9]. One should note that injection of tiny sample volumes (nL ranges) is 60 normally employed prior to electrokinetic separations in a microchannel whereas much larger 61 volumes (> 10 μ L up to sub mL ranges) are expected when using conventional (batchwise) 62 forefront sample treatment modules. Without an efficient coupling strategy, the majority of 63 the preconcentrated and purified sample volume (in µL-mL range) at the outlet of the sample 64 treatment module is wasted without being exploited for sensitive and selective determination 65 of the target analytes at the separation step (nL range). To overcome these challenges, 66 different sample pretreatment strategies were developed to couple with microscale 67 electrophoresis, including in-line liquid phase microextraction, on-line electrophoretic 68 preconcentration and sample pretreatment by integrated immobilized enzyme microreactors 69 [10]. In-line solid phase extraction (SPE)-CE with microcartridges using different solid-phase 70 materials such as monolith, sorbent particles and magnetic beads is another well-investigated 71 technique to cope with the problem of working volume incompatibility [11]. Besides the pros 72 and cons for these in-line / on-line techniques that were well discussed in these reviews, they 73 share the same inherent consideration that typically only a limited number of steps (i.e. 74 extraction and / or preconcentration) can be performed within the unique microchannel which is also used for electrophoresis. Furthermore, the sample matrix flow percolating the 75

76 microchannel during in-capillary extraction and preconcentration may lead to adsorption of 77 unwanted interferents present in the sample matrix to microchannel's walls, resulting in possible degradation of subsequent separation efficiency. If several steps are needed for 78 79 sample processing (e.g. sample digestion, analyte capture, chemical labelling, purification, 80 preconcentration etc.), these approaches are not relevant anymore. In this case, the sample 81 treatment protocol should be dissociated from the microchannel used for electrophoresis in order to gain operational flexibility and to avoid undesired modification of the capillary / 82 83 microchip internal surface.

84

85 In another context, droplet microfluidics has witnessed exponential growth over the past 86 decade as a powerful tool for bioanalysis and high-throughput screening purposes [12-15]. 87 Droplet microfluidics can be considered, at least to some extent, as the science and 88 technology of generation, manipulation, reaction, analysis and screening of micro to 89 femtoliter microreactors used as discrete functional units (e.g. droplets, particles or bubbles) 90 in micrometric to nanometric channels [14, 15]. In such systems, the liquid is segmented into 91 discrete droplets which are independent of each other, using typically immiscible phases, 92 which in turn prevent cross-contamination between different droplets. Technologies and 93 methods for droplet generation and manipulation can be found in recent reviews [12, 13] 94 whereas many applications exploiting droplet generation, fusion, sorting, splitting and in-95 droplet solid-phase handling can be gleaned to [14, 15]. Droplet microfluidics is a matching 96 candidate for high-throughput screening and assays such as drug screening, cell sorting, 97 digital PCR as well as biological analysis at single cell or single molecule levels. Following 98 the boom of droplet-based technologies and applications, the use of droplets to empower and 99 facilitate microscale separations has found its increasing interest and significance in recent 100 years. Droplet microfluidics was employed for bridging 2D liquid-phase separations [16, 17],

as well as for chemical separation for cellular analysis [18] and biochemical analysis [19]. 101 102 Among all strategies to exploit the power of droplets in separation science, droplet-interfaced 103 microscale electrophoresis is an emerging technique in which micro/nanometric droplets are 104 used as a carrier of target analytes between sample treatment and electrokinetic separation 105 steps. This particular marriage between droplets and microscale electrophoresis is expected to 106 overcome the challenges of working dimension mismatch and low degree of module 107 integration. Limitations encountered in aforementioned batchwise and on-line/in-line sample 108 treatment approaches are expected to be solved with droplet-interfacing strategies. Readers 109 can find some (inexhaustive) applications of droplet microfluidics in microseparation systems 110 elsewhere [20, 21].

111

112 In this review, we report on for the first time a comprehensive overview of different strategies 113 to hyphenate droplet-based sample handling with microchip and capillary electrophoresis. 114 Different instrumental developments for such purpose, the positive features and limitations of 115 these designs, as well as application demonstrations of droplet-interfacing strategies in 116 microscale electrophoresis are discussed. There have been almost 40 research articles on this 117 topic, with several ones released in the last two years, confirming the interest of research 118 community in this emerging and active approach to provide a promising tool to reliably and 119 accurately address the analysis of trace components in complex samples.

120

121 **2.** Microscale electrophoresis coupled with droplets in immiscible phases

A summary of all strategies for interfacing droplets to microscale electrophoresis is given in table 1. This overview shows that droplet encapsulation in oil (an immiscible fluid) has been the most frequently practiced (see strategy 1 in table 1), and therefore accounts for the majority of the communicated works on droplet interfacing strategies. This approach employs

segmented flow analysis where the sample droplet / plug is carried out by an immiscible fluid 126 127 to the separation capillary or microchannel, where it is extracted and injected. Isolating 128 samples into droplet present several advantages, notably prevention of axial dispersion during 129 transportation [22], elimination of contamination between samples and evaporation [23], 130 exclusion of valves or other switching mechanism between samples [24] and possibility of 131 internal flow recirculation within a plug for enhanced mixing [24]. One major challenge when 132 using isolation of sample into droplets suspended in oil is that de-segmentation and oil 133 removal are required in order to inject only the aqueous portion of the flow into the separation 134 capillary/microchannel for separation. Indeed, the oil-based carrier fluid is usually non-135 conductive and may cause instability of the electroosmotic flow (EOF) and plug formation in 136 the separation channel, inducing shortcuts or dielectric device breakdown [25]. Different 137 designs to couple CE/MCE to droplets-in-oil therefore had to come with their distinct instrumental and / or physio-chemical approaches to overcome the challenge of oil intrusion 138 139 into the separation microchannel. Some typical strategies for oil removal include: i) 140 extraction of aqueous droplets from an oil flow into a methanol stream with an on-chip de-141 emulsification interface [26], employment of a hydrophilic extraction / separation channel to 142 extract / inject the aqueous fraction [24, 27], oil draining via on-chip micrometric pillars [23], 143 oil soaking with a hydrophobic and oleophilic membrane [28], oil removal via density 144 difference between the CE buffer and the carrier oil [25]. Another strategy which has not been 145 applied yet for CE/MCE but holds a high potential to our opinion is the emulsification/ 146 demulsification with microgels (*i.e.* from microgel in oil phase to microgel in medium phase) 147 [29]. At the present state of the art, more efforts have been spent for instrumental conception 148 and optimization for high-throughput sample injection to CE/MCE, using model analytes in 149 many cases. Less attention was given to methodology development for such approaches, 150 which should be expected to come in the next research phase once the instrumental

development has been established. Accordingly, the following discussion focuses more on thetechno-aspects of the reviewed droplet-interfacing strategies.

153

154 2.1. MCE coupled with aqueous droplets in oil

155 The direction of droplet-interfacing to microscale electrophoresis was first opened by Edgar *et* 156 al. in 2006 with the method for integrating droplet generation and MCE [30]. They were the 157 first to report the use of a stable immiscible boundary (also called "virtual wall") to divide the 158 microfluidic chip into a droplet generation part and a separation part (see Fig. 1 for their 159 setup). In systems using a virtual wall, a stable aqueous/oil interface has to be maintained via 160 optimization of the hydrophobicity of the surfaces, the surface tension, the viscosity of the 161 fluids and / or the channel dimensions [24]. When the sample reaches the interface, the 162 aqueous fraction coalesces with the interface and goes preferably into the hydrophilic channel. 163 Edgar et al. reported the use of a simple T-junction to produce droplets that were injected into 164 a straight separation channel. They also found it is possible to inject sample directly into the 165 separation channel by reversibly breaking and sealing the immiscible boundary. Different 166 approaches to stabilize this boundary were investigated using hydrophobic (native 167 polydimethylsiloxane, PDMS) and selectively hydrophilic patterned (poly(styrene)sulfonate, 168 PSS) separation channels. A hydrophobic separation channel with no surface modification 169 required precise regulation of the oil flow to prevent the oil from entering the separation 170 channel. Unsatisfactory repeatability was observed with hydrophobic channels using micro 171 injectors for pressure control and oil handling. Indeed, as oil preferably wets the hydrophobic 172 walls, when it enters the separation channel it will remain wet on the surface, rendering 173 impossible the measurement of EOF. On the other hand, a hydrophilic separation channel, 174 despite a non-trivial selective surface patterning, was found to efficiently prevent the entrance of oil into it. In this case, the oil preferably wets only the hydrophobic walls of the T-junction. 175

The droplet interfacing approach proposed by Edgar *et al.* was applied for the separation of an amino acid mixture segmented into 12 pL droplets as the proof of concept demonstration.
While the authors did not mention the achieved throughput, the employed mode of pressure control was found not yet compatible for high throughput. Changing samples with this method would also require several rinsing steps to avoid cross-contamination.

181

182 Following this work, Roman et al. presented a system where a shallow channel 183 (electrophoresis channel, 7.5 µm) and a deeper channel (droplet channel, 75 µm) were etched 184 on different glass plates and bonded together [24]. The channels were then aligned in order to 185 overlap, leading to a small stable interface after surface treatment of the droplet channel. The 186 droplet is led into the separation channel due to difference in surface energy and capillary 187 forces. This flow de-segmentation system required two microchip plates to be well aligned. In 188 an effort of simplifying microfabrication, Pei et al. later presented a tuned version of this 189 system where the separation channel was placed close to the droplet generation channel (both 190 with 80 µm depth) and these channels intersected via the etching of the glass, creating a small 191 gap (5-10 µm) between them [31]. In order to increase the throughput, three MCE channels 192 were integrated in the same chip. The droplet channel was further treated to become 193 hydrophobic, allowing the creation of an aqueous/oil interface. Wang *et al.* then reported an 194 alternative design with the use of an intermediate hydrophilic and shallow "extraction 195 channel" that connects the hydrophobic droplet channel to the hydrophilic separation channel 196 [22]. Similar to the previous systems, the sample is extracted into the extraction channel due 197 to both difference in surface energy and capillary forces. In this bridge system the whole 198 content of the droplets was injected into the separation channel, whereas in the other ones 199 only a fraction was injected. The droplet-interfaced MCE systems by Roman et al. and Wang 200 et al. (coupling to microdialysis for the latter) were applied for monitoring concentration 201 changes of derivatized amino acids dissolved in artificial cerebral spinal fluid [22, 24]. The 202 setup by Pei et al. was applied for high-throughput screening (HTS) of a GTPase assay, using 203 an array of three electrophoresis channels in the same microchip to achieve a throughput of 204 120 samples in 10 min [31]. One limitation of this system is the instability of the surface 205 coating, which loses effectiveness after 3 to 4 hours of operation. Improvement of the surface 206 treatment is thus needed for longer trials. From our viewpoint, these approaches relying on a 207 stable aqueous/oil interface between the (hydrophobic) droplet generation channel and the 208 (hydrophilic) separation channel are not facile for routine use, especially for users with no / 209 little skill in microfluidics and / or microfabrication. Several parameters have to be carefully 210 optimized to maintain the operational reproducibility, notably long-term hydrophobicity of the 211 total or partial channel's surfaces, as well as the channels' dimensions and intersections. 212 These factors are indeed not trivial to master at the micrometric / nanometric scales, and 213 therefore would not see widespread exploitation by non-expert users in the very near future.

214

215 In a related context, a design was proposed to take advantage of the native properties of glass 216 and PDMS for extraction, without recourse to surface modification [27]. The design was 217 separated in two parts. First, a straight PDMS channel was used to transport the segmented 218 flow from a syringe. This hydrophobic droplet channel was connected via a hydrophilic 219 extraction capillary to a natively hydrophilic MCE glass chip. This strategy was applied for 220 the screening of 140 compounds against protein kinase A. Compared to the 'virtual wall' 221 approach, this method demonstrated a 10-fold improvement of the sample analysis rate and 222 10-fold reduction of the reagent amounts needed for separation. The same system was then 223 improved to allow HTS and used for the identification of sirtuin inhibitors, allowing 1408 224 samples to be analyzed at a frequency of 0.5 Hz in 46 min [32]. For each sample, eight MCE injections were made using the injection cross, generating more than 10 000electropherograms during analysis.

227

Rather than extracting the sample droplet from the main oil-based flow, other options focused 228 229 on removing the oil around the aqueous droplet to inject the sample into the separation 230 channel. Niu et al. proposed the use of pillars made of native PDMS in front of a separation 231 microchannel, which were positioned perpendicular to the main flow to remove the oil from 232 the stream [23]. When an aqueous droplet came in contact with the pillars, the surface tension 233 kept the droplet stable and allowed it to flow past the perpendicular pillars without entering it, while the oil was drained through them to a waste reservoir. This method required only simple 234 235 soft lithography process and relied on channel geometry rather than surface modification to 236 extract the oil passively. It allowed complete injection of a droplet into the separation channel 237 without introduction of any residual oil. For application, the outlet effluent of a peptide mixture from a HPLC-based purification step was segmented into droplets that were 238 239 subsequently injected into a pillar-interfaced MCE channel. A positive feature of this 240 approach is the possibility to adjust droplet volumes (fL-nL) thanks to the tunable oil flow. Ye 241 et al. used this system to fragment the chromatographic effluent of urinary protein digest into 242 353 droplets of 40 nL each, allowing individual HPLC peaks to be fractioned by 4-40 times 243 for subsequent MCE operations [16]. The same group then presented another oil-extraction 244 method using a hydrophobic and oleophilic membrane to soak the oil from the main flow 245 [28]. In this method, a portion of PDMS was removed from the flat PDMS layer at the 246 junction with the separation channel, and hydrophobic oleophilic а and 247 polytetrafluoroethylene (PTFE) membrane was placed instead. When the droplet arrived at 248 the junction, it was injected by capillary forces into the separation channel while the oil was 249 absorbed by the PTFE membrane. While this method does not require surface treatment, the

250 working droplet size (or volume) had to be predefined before experiments, as the geometry of 251 the separation channel and the size of the delivery tubing must be carefully selected for each 252 range of droplet volume. To our opinion, these physical approaches using either on-chip 253 pillars or oil-soaking membrane, thanks to the possibility of prospective massive production, 254 would hold higher potential than the aforementioned chemical treatment alternatives. Users 255 with little expertise in microfabrication could purchase these ready-to-use materials and insert 256 them in front of the electrophoretic module for oil removal and aqueous sample 257 extraction/injection. Before these approaches can be used for routine operation, further 258 instrumental development has to be made to allow facile integration of the pillars or membranes in between the droplet generation and separation parts, as well as their 259 260 detachment in case of replacement.

261

262 Recently, Ouimet et al. presented a method based on the difference of density between the CE 263 buffer and the carrier oil to remove oil from segmented flow [25]. In this system, the 264 separation glass chip was etched at the end of the sampling channel with a gap of 300 µm 265 between the sampling channel and the separation channel. As the segmented flow advanced 266 into the chip, the oil arrived and pooled in the buffer reservoir, whereas formulated sample 267 droplets (~ 30 nL with heavier density than oil) fell at the bottom of the chamber due to a net 268 buoyancy force higher than gravity. The oil came in contact with the separation channel but 269 was prevented from entering due to the native hydrophilicity of glass. When a sample droplet 270 arrived at the outlet of the sample channel, it came in contact with the inlet of the separation 271 channel where it coalesced due to a high voltage continuously applied at the oil removal 272 reservoir. After the samples entered the separation capillary, it migrated to an injection cross 273 where the amount of injection can be controlled by gated injection. This chip was suitable for 274 gel electrophoresis, where the gel matrix was loaded onto the glass chip using vacuum. It was

275 found to sustain up to 630 separations without reconditioning, with a throughput of about 10 276 s/sample). Considerations for this system include dilution of the sample when it goes through 277 the buffer reservoir to the separation capillary and the limited choice of carrier phases for the 278 segmented flow. Note that high-density oils are often preferred in droplets microfluidics for 279 their inert properties and low partitioning of analytes. The low-density oils required for this 280 approach are nevertheless often viscous, rendering them less suitable for droplet 281 microfluidics. We would expect to see more applications of this density-based approach for 282 discrete collection of sample droplets, which can be batchwise transferred to a CE/MCE 283 device for separation. If a high throughput is not required, this sample collection mode can be 284 practiced by any operator thanks to its simple and straightforward protocol.

285

286 Rather than segmenting the aqueous flow in oil and then remove one phase or the other, Quan 287 et al. [33] presented a method where the sample was segmented between two plugs of an 288 immiscible, hydrophobic, conductive ionic liquid (IL). The system was composed of a simple 289 PDMS linear channel interfaced to a silica capillary tube in the way that most of the capillary 290 stayed outside of the PDMS one. One drop of IL, one drop of sample and one drop of buffer 291 were placed on a PDMS slab and connected to the ground. A plug of IL was first 292 electrokinetically injected into the capillary by immersing the tip of the capillary into the IL 293 drop. The operation was then repeated for the sample, and then for the IL. For separation, the 294 capillary tip was immersed in the buffer drop and a high voltage was applied for separation. 295 With this system, the injected sample volume was easily controllable because it depended 296 only on the time and voltage of injection. It requires no de-segmentation of flow, as the carrier 297 fluid was conductive. Nevertheless, surface treatment was required in order to inject IL into 298 the capillary. For application, a model mixture of flavin adenine nucleotides (FAD) and flavin 299 mononucleotides (FMN) was separated using this method, allowing a signal improvement by 300 6.3-folds compared to conventional electrokinetic injection. The authors also showcased the 301 use of this method for the on-line labeling of myoglobin and cytochrome for CE-LIF, with the 302 whole assay involving the sample compartmentalization, labeling and CE analysis completed 303 within 4 min. The widespread use of IL plugs is nevertheless hindered by the fact that this 304 approach can be used only with electrokinetic injection. The more practiced hydrodynamic 305 injection mode has not been investigated with this approach. Furthermore, this method may 306 encounter the same problem with instability of the surface treatment, as already discussed 307 above.

308

309 In a recent study, Serra et al. developed an integrated droplet microfluidic device for magnetic 310 particles handling used as slid support for DNA size selection in NGS libraries preparation 311 [34]. In this work, a train of aqueous droplets in fluorinated oil was used to carry out several 312 rounds of sample processing steps, including DNA precipitation on magnetic microparticles, 313 on-bead DNA washing and DNA release to the target matrix (see Fig. 2 for the setup). A high 314 number of steps was made possible thanks to magnetic beads that carried target analytes 315 through a series of droplets without cross-contamination and with high efficiency. Compared to the batchwise procedure for DNA size selection in NGS libraries preparation, this droplet 316 317 protocol is simpler (thanks to reduction of the number of washing steps), much faster (with 318 on-bead DNA precipitation or in-droplet DNA release in approximately 1 min) and less 319 sample-consuming (using 100 nL droplets in the protocol) while maintaining an excellent 320 quality of purified DNA fragments. The final droplet containing desired DNA fragments was 321 then offline introduced to MCE (BioAnalyzer instrument) via manual droplet collection. 322 Compared to other microchip-based setups for sample handling in droplets prior to MCE, this 323 design exhibited higher flexibility and the possibility to carry out an extended number of 324 sample treatment steps and in particular solid phase extraction. While oil removal was not 325 required for MCE, droplet dilution was the limitation of this setup as it worsened the detection326 limits and did not allow full integration and automation.

327

328 2.2. CE coupled with aqueous droplets in oil

329 Different approaches to interface water-in-oil droplets to CE have been reported in efforts to 330 seek for a more robust and automatable alternative to the droplet-MCE format. For instance 331 with a practically identical method as already described for droplet-MCE coupling, Niu et al. 332 used their oleophilic-PTFE-membrane system for coupling CE with a segmented flow [28]. 333 By inserting a capillary into the separation channel in the design previously described, the 334 authors showcased the possibility to use this method for capillary gel electrophoresis (CGE), 335 where a polymer matrix is used to separate large biomolecules by size or mass. By inserting a 336 capillary pre-filled with the sieving matrix into the separation channel, separation of a 50 bp 337 dsDNA standards could be achieved where 14 out of 16 fragments could be unambiguously 338 identified. Note that, as most matrices for CGE are highly viscous, the loading of the buffer 339 into a CE channel required high pressure, which is not fully compatible with droplet-340 interfaced chip-based systems.

341

342 De La Marre *et al.* proposed an alternative design to remove the oil from the main flow prior 343 to CE operations [35]. In this method, two patterned slabs of PDMS were aligned and bonded 344 together in order to intersect out of plane with a cross geometry. The bottom slab contained a 345 straight 75 µm-wide sample channel, which ended with a punched waste reservoir. The 346 segmented droplets were prepared off-chip and brought into this sample channel by an 347 inserted capillary. The top slab contained a narrower straight separation channel (30 µm 348 wide), with a grounded buffer reservoir punched at its inlet and a silica capillary inserted at 349 the outlet. The top slab's surface (made of hydrophobic PDMS) was selectively modified with 350 a Corona treatment to make it hydrophilic. At the channels cross-section, a stable oil/buffer 351 interface was created. When a droplet arrived at the intersection, it coalesced with the buffer 352 interface and was sucked into the separation channel by surface tension, while the oil was 353 prevented from entering the separation channel. As Corona treatment is quite easy to use, this 354 method offers simplification of the fabrication process, with only two straight channels being 355 used for the chip. This approach allowed injection of almost the whole droplet content into the 356 separation channel, with only some droplet residues (called satellite droplets) observed after 357 the injection. The handling of the segmented flow in this method was performed using a 358 pressure reservoir actuated manually by a thumb, which would be non-optimal and could 359 hinder operation speed. For characterization of the system performance, droplets of 750 pL 360 containing Riboflavin was used allowing temporal resolution of its peak down to 15 s. 361 Basically, this droplet-interfacing setup for CE relied on channel's surface treatment, 362 therefore may find similar challenges to overcome as its counterparts for MCE (see section 363 2.1), before routine use can be envisaged.

364

365 Instead of relying on flow segmentation with oil, Li et al. proposed a system where the 366 samples were placed into a nanoliter well array and covered by a non-volatile oil to prevent 367 evaporation [36]. This array was placed on an x-y-z translation stage, and along with vials 368 filled with buffer and various solutions needed for CE separations. The inlet end of a silica 369 capillary was mechanically ground into a tapered tip. The capillary was bent in to a "∩" shape 370 and fixed vertically with the inlet and outlet at the same level. After the capillary was filled 371 with separation buffer, the sample injection occurred spontaneously by plunging the capillary 372 inlet through the cover oil into a sample well, and then removing it quickly. As the surface of 373 the capillary plunged in the sample is uncoated and thus hydrophilic, some sample solution 374 adhered to the capillary sidewall and tip during the capillary withdrawal. The removing speed 375 was well controlled to ensure satisfied injection reproducibility. A pL volume of the sample 376 solution thus remained at the tip of the capillary and was subsequently sucked into the 377 capillary by surface tension, while the oil scraped the sample residual present at the sidewall, 378 limiting cross-contamination between samples (only 0.14 %). The capillary was then placed 379 into the buffer vial connected to electrode for CE separation. For a proof of concept using 380 three amino acids, 25 separations could be implemented in less than 15 min with this system. 381 The system was also used for monitoring the in-droplet derivatisation of amino acids. FITC 382 and three amino acids were added into a 500 nL droplet and separations were carried out 383 every 5 min for 3h. The same group recently used this approach for the detection of multigene mutations using PCR-restriction fragment length polymorphism (PCR-RFLP) [37]. A 384 385 lowest mutation frequency of 0.37% was achieved without cross-contamination between 386 samples. This system has the advantages of being relatively easy to build, requiring no 387 microfabrication. As each droplet is contained within a well, each sample is easily re-388 accessible via its identified position. The size of injection can be tuned by varying the speed at 389 which the capillary is removed from the sample well [38]. The reloading of the sample wells 390 nevertheless could pose some problem, as it required thorough cleaning of each well, and has 391 to be carried out manually. Variation of sample matrix viscosity should be taken into account, 392 as it may influence the sample quantity to be attached at the tip and sucked into the capillary. 393 This approach hold high potential for widespread use, thanks to its automation feature. 394 Modification of the CE instrument to allow integration of the dedicate sample tray is 395 nevertheless required, which is not yet feasible with existing commercial CE instruments. 396 Utilization of dedicate capillaries is another point to be considered, as the commonly used 397 fused silica capillaries are not adapted to the purpose-made system at the initial state.

399 Diverted from de-segmentation of sample flow prior to CE injection, Abdul Keyon et al. 400 proposed a method for the compartmentalization of analytes after CE separation, allowing 401 selective processing and detection of target analytes in the fraction of interest which is 402 purified by CE [39]. The droplet-based sample handling is implemented as a downstream of 403 the separation step rather than a forefront operation as in other cases. A micrometric cross 404 was used to create an intersection between four capillaries (see Fig. 3 A and B for their setup). 405 The separation capillary was positioned towards the top whereas at the bottom capillary is 406 located a salt bridge with a Pt electrode connected to the ground to maintain electrical 407 connection during separation. A train of droplets was introduced from the left capillary. The 408 capillary on the right was used for collecting the droplets and postcolumn detection. As the 409 buffer-filled droplets (14 nL each) came in front of the separation capillary outlet and the salt 410 bridge, analytes from the CE separation were electrophoretically transferred into the droplets. 411 By adjusting the frequency at which the droplets were formed, a continuous contact from the 412 separation capillary via droplets to the salt bridge could be maintained to allow a stable 413 separation current. For demonstration, a mixture of two paralytic shellfish toxins was 414 separated by CE and then segmented into droplets where they were oxidized in a post column 415 reaction and detected by fluorescence (see Fig. 3C for a typical electropherogram). The 416 droplets were created at a rate of 1 Hz, with one separation lasting for 10 minutes. When 417 compared to previous precolumn oxidation from the same group [40], the separation was 418 quicker (10 min versus 30 min). Postcolumn derivatization also allowed selective oxidation of 419 only one toxin at a time, avoiding the problem with simultaneous oxidation that complicates 420 product identification encountered with precolumn oxidation. In another strategy for droplet-421 based detection after CE separation, Mai et al. proposed to couple capillary isoelectric 422 focusing (CIEF) to single-step magnetic-beads based immunoassays in a microfluidic droplet 423 for sequential determination of amyloid-beta (A β) peptide biomarkers for molecular diagnosis

424 of Alzheimer's disease [41]. Aß peptides possessing different isoelectric points were 425 compartmentalized into different fractions along a capillary filled with a pH gradient under a 426 high electrical field prior to detection using a droplet-based enzyme-linked immunosorbent 427 assay (ELISA). Using pressure mobilization, these fractions (A β 1–40 in the first (pI = 5.33), 428 A β 2–40 in the second (pI = 5.98) and A β 5–40 in the last one (pI = 6.46)) were then collected 429 and subsequently specifically quantified by the in-drop-ELISA. This platform was composed 430 of a syringe pump and a motorized pipettor arm used for droplet generation, as well as a 384-431 well plate (for the storage of sample and reagents) placed on a holder that is adjustable in the 432 x-y-z directions. By using oil in the pipetting tubing, the sample and reagents needed for 433 ELISA were first segmented into 200 nL droplets inside the tubing. Four pairs of magnetic 434 tweezers activated by a magnetic coil were placed around the tubing to capture and released 435 antibody-grafted magnetic beads, which were re-suspended in different droplets containing 436 various reagents for the ELISA protocol. Compared to conventional ELISA, this droplet-437 based alternative offered automation, reduction of working volume and significant diminution 438 of operation time. A throughput of 8 analyses per hour could be achieved with this droplet-439 based protocol, compared to a duration of 2 hours per analysis required for conventional 440 ELISA. Significant reduction of sample volumes (200 nL / analysis for the droplet protocol 441 vs. 50-100 µL for conventional ELISA) is advantageous for such type of biomarker detection 442 application as biological sample volumes are often limited. Detection sensitivity with this 443 approach for determination of A β 1-40 and A β 1-42 was still inferior to that obtained with 444 conventional ELISA, and therefore needs to be further improved. Manual transfer between 445 CIEF and droplet-based ELISA protocols is still a point for improvement before full 446 automation and high-throughput is possible.

448 An overview of aforementioned approaches revealed that they all generate droplets of a 449 constant-volume (in the pL-nL range), mostly for high throughput sample injection and 450 analysis. Only few studies explored the potential of droplet trains for down-scaling sample 451 treatment protocols. Large room therefore still remains to be exploited in this direction. From 452 our viewpoint, the power and flexibility of droplet microfluidics could be further exploited by 453 employing droplets of different sizes for different sample processing purposes. For example, a 454 large sample droplet can be trapped onto a carrier support and transferred into a much small 455 droplet for preconcentration by solid-phase extraction. In-droplet chemical reactions for 456 fluorescent labeling of target analytes for CE-LIF for instance, or in-droplet enzymatic 457 reactions can be triggered by simple fusion of two separate droplets into a bigger one. The 458 droplet merging or splitting on-demand operations can be implemented simply by modifying 459 the microchannel geometry, oil nature and / or surfactants to be added into droplets for interfacial tension modification [42, 43]. Eventually, sample pre-treatment in a down-leveling 460 461 droplet sequence in oil would be foreseen as an optimal setup for this oil-based strategy of 462 droplet-interfacing to microscale electrophoresis. For instance, a typical protocol for down-463 leveling droplet train can start with a large sample (µL range) droplet; then passing through 464 sample processing via solid supports in droplets and/or droplet fusion/splitting; and finally 465 focalizing target analytes into a tiny (nL) droplet for CE injection and separation.

466

467 3. Microscale electrophoresis coupled to digital microfluidics with electrowetting-on468 dielectric principle

Another strategy to manipulate droplets in the forefront of CE employed digital microfluidics (DMF) in which the droplets are actuated by Electrowetting On Dielectric (EWOD) technology (see strategy 2 in table 1 for the summary). The EWOD principle is based on electrical potentials to enable manipulation of fluid shape and flow via control of the 473 wettability of liquids on a dielectric solid surface. Indeed, the application of an electric 474 voltage leads to modification of the free energy on the dielectric surface, inducing a change in 475 the wettability on the surface and the contact angle of the droplet. Readers can refer to a 476 fundamental work by Cho et al. for more detailed explanation and demonstration of EWOD 477 principle [44]. The popular scheme of DMF-EWOD allows creation and actuation of 478 individual droplets from a reservoir and their independent manipulation (e.g. transport, 479 division, addition) over a planar electrode array via application of electrical fields. Its 480 principle eliminates the need of complex networks of tubing or microvalves but at the price of 481 more complex microfabrication [45]. While microchannels were typically used to manipulate 482 droplets flowed in an immiscible fluid stream, DMF introduced a distinct paradigm as a basic 483 method for moving droplets on a surface [45-47]. The DMF-EWOD principle was first 484 applied by Abdelgawad *et al.* to develop a digital-to-channel interface for sample processing 485 and MCE separations [48]. This hybrid digital-channel microfluidic device, comprised of an 486 electrode array for sample preparation by digital microfluidics and a network of MCE 487 microchannels (see the side-on design in Fig. 4), was used for on-chip NDA labelling of 488 amino acids and cell lysate as well as for on-chip digestions of singly labelled FITC-insulin. 489 The µL-sized droplet contents were driven into the separation channel electrokinetically for 490 pinched injections with assistance of EOF, followed by separations performed in micellar 491 electrokinetic chromatography (MEKC) mode. The same authors then improved the DMF-492 MCE side-on design by a multi-layer one, in which droplets are manipulated by DMF in the 493 two-plate format (see the multi-layer design in Fig. 4) [49]. The second DMF-MCE 494 generation, which facilitated sample dispensing from reservoirs, droplet splitting and storage 495 for subsequent analysis, was applied for an on-chip serial dilution experiment as well as 496 multistep enzymatic digestion prior to electrokinetic separations. While DMF platforms were 497 thought to be ideally suited to electrokinetic separations at first sight, these pioneering works

498 on DMF-MCE were then discontinued. The widespread use of DMF-EWOD for droplet-499 interfaced MCE indeed is still hindered by the typically complicated process of making DMF 500 devices (i.e. the fabrication of microchip-integrated electrodes and coating with layers of 501 dielectric and hydrophobic materials that requires microfabrication and electrical workshop).

502

503 To bring low-cost DMF to microscale electrophoresis, Kaljurand et al. exploited the DMF-504 EWOD phenomenon for transporting sample and buffer droplets in succession under the CE 505 capillary inlet end and allow the capillary to be immersed into the sample/buffer droplet [50]. 506 In this portable CE analyzer coupled with a DMF device, the actuation of droplets was 507 achieved via an electrode system prepared from the copper substrate of the common printed 508 circuit coated by food wrap whereas CE separation was performed by applying a high voltage 509 between the (grounded) buffer droplet and CE outlet reservoir (Fig. 5). The system was 510 demonstrated with monitoring of sample concentration kinetics during evaporation of the 511 droplet containing model thiamine, pyridoxine and nicotinamide, using capacitively coupled 512 contactless conductivity detector (C⁴D). A prototype based on this DMF-CE principle was 513 then developed to include solid-liquid extraction of amino acids from sand matrices prior to 514 their determination with CE-C⁴D [51]. Alternatively, the design was rearranged to allow the 515 use of the DMF platform not as a sampler but rather as a collector and transporter of CE 516 fractions after electrokinetic separations [52]. This CE coupled with downstream EWOD 517 actuation of droplets, using air plugs instead of a carrier liquid for encapsulation of the 518 selected fractions, was applied for off-line electrospray ionization mass spectrometry (ESI-519 MS) characterization of some vitamin standards [52], and MALDI-MS analysis of peptide 520 fractions [53]. In a parallel work, Patel et al. developed a system integrating a droplet-based 521 DMF platform with a capillary-based reagent delivery unit and a quantitative CE module for automated quality control (QC) platform for next-generation sequencing (NGS) library 522

523 characterization [54]. This system consisted of three key modules, including i) fluidic delivery 524 element using fused-silica capillaries connected to an 8-port syringe pump for high-precision 525 fluid metering, ii) DMF unit for sample mixing and fluid routing and iii) CE-LIF separation 526 and detection module. Using this system, double-stranded DNA samples were 527 electrokinetically injected and quantified for quality control prior to NGS with the Illumina 528 Genome Analyzer sequencing platform. This setup allowed detection of double-stranded 529 DNAs in the range of 5–100 pg/ μ L, which is suitable for the commercial sequencing 530 platform, while consuming ten-fold less sample volume than the current Agilent Bio-Analyzer 531 QC technique. This DMF-CE system therefore helps preserve precious samples while providing necessary sensitivity and accuracy for optimal sequencing performance. 532

533 While these DMF-CE and CE-DMF setups could be of interest to laboratories without access 534 to well-equipped infrastructure (e.g. clean-rooms or lab robots), some electronic skills and 535 facility are still needed to prepare the EWOD boards with etched electrodes for droplet 536 manipulation. The aforementioned challenges encountered with the DMF-CE/MCE coupling 537 would have to be overcome before this droplet-interfacing strategy for microscale 538 electrophoresis could gain more popularity. From this point, 3D printed microfluidics and 539 microelectronics seem to be the matching solution, as they allow microfluidic platforms to be 540 fabricated with fully integrated microelectronics, which are required for electrowetting-on-541 dielectric (EWOD) phenomena and (on-chip) electrophoresis [55]. Another consideration 542 when using this approach is a relatively large droplet volume generated (typically 2-5 µL 543 droplets). Downscaling of the working droplet volume will be needed to minimize volume 544 mismatch in the DMF-CE/MCE coupling.

545

546 **4. Microscale electrophoresis coupled with inkjet droplet generator**

547 Among all droplet-interfaced strategies for CE/MCE, inkjet injection could be the one that 548 allows a total compatibility of working volumes (i.e. both in nL ranges) between droplet-549 based sample handling and electrokinetic separation (see strategy 3 in table 1 for an overview 550 of this approach). This mode is based on inkjet printing principle, which is a type of computer 551 printing to propel droplets of ink onto paper and plastic substrates in order to recreate a digital 552 image. This is the most commonly used type of printers, ranging from small inexpensive 553 consumer machines to expensive professional ones. Inkjet injectors have found their 554 applications not only for office printing technology but also for various industrial fabrication 555 processes [56] thanks to their desirable properties, notably droplet spatial and volume (pL-nL) controllability, high speed, and accurate spotting on the surface of a wide variety of 556 557 substrates. The coupling of inkjet injection to MCE was first developed by Yasui et al. in 558 2012 for microchannel array electrophoresis analysis of DNA droplets [57]. The inkjet 559 injector in this system (Fig. 6) allowed precise control of the injection volume of DNA 560 samples down to pL-nL ranges and the use of a simple straight microchannel for MCE, which 561 otherwise are not trivial with conventional cross injection method in the cross- or T-shaped 562 microchannels. This droplet-interfaced approach offered some positives features to MCE, 563 including suitability for high density array of microchannels, ability to separate biomolecules 564 by one voltage programming and rapid analysis duration as no sample loading time is needed 565 with the inkjet injection method as in conventional MCE.

566

A similar droplet generation concept was then developed for CE setups by Uchiyama *et al.* [58-62]. In their first inkjet-CE design (Fig. 7A), they employed precise control of waveform driving piezoelectric crystal inside the inkjet head to trigger by a drop-on-demand approach stable droplet generation from various solutions. The piezoelectric droplet generator was used for injection of well-defined amounts of sample (179 pL droplets with excellent 572 reproducibility) in capillary electrophoresis, with the droplet size and delivery frequency 573 adjustable via waveform turning. The authors demonstrated its first application with analysis 574 of theobromine, caffeine and theophiline using MEKC [58]. This inkjet-CE setup was then 575 adapted to drop-by-drop introduction process for electrophoretically mediated microanalysis 576 (EMMA), allowing (1) on-line multi-segment injection pattern by alternately ejecting small 577 plugs of sample and reagents, (2) in-capillary incubation for fluorescent labelling reaction 578 with an overlapping region of the plugs for mixing the reactant solutions by electrophoresis 579 and (3) CE-LIF determination of amino acids tagged with a fluorophore (4-fluoro-7-580 nitrobenzofurazan) [59]. In another application, the system was used to eject a large sample 581 volume onto the inlet end of the capillary and then introduce it into the capillary under gravity 582 and Laplace pressure, allowing quantitative on-line concentration of methylxanthines in 583 bottled green tea via stacking and sweeping in MEKC [60]. The application of the inkjet-CE instrument was then extended for online EMMA-based immunoassays (using ~ 200 pL 584 585 droplets of fluorescein-labeled anti-human IgG antibody and human IgG), followed by CE 586 separation of the antigen-antibody complex formed in the merged zone in the capillary [61]. The method showed a wide linear range of calibration (10-2000 ng.mL⁻¹) with satisfied 587 linearity ($R^2 = 0.9912$). The detection limit (5 ng.mL⁻¹) was substantially lower than those 588 589 obtained for conventional immunoassays (including CE-based methods). The most recent 590 application of this approach was for CE separation of mammalian cells encapsulated in 591 picometric droplets, in which calibration linearity was obtained by varying the number of 592 droplets (25-400 drops) injected into the capillary for electrokinetic separations [62] (see Fig. 593 7B for a typical electropherogram). Compared to conventional sampling techniques, this 594 inkjet sampling setup allowed precise sample manipulation with spatial and temporal control 595 which is important for sampling at the single cell level. Furthermore, it can realize 596 quantitative analysis of cell by adjusting the cell concentration and the number of droplets,

597 which is otherwise not trivial with the conventional modes. An overview on this inkjet-598 CE/MCE approach indicated that its applications are still limited despite its power on precise 599 control and generation at high-frequency of CE-volume-matching droplets. Dedicate and 600 complicate electronic and optical instrumentation required for such setup for droplet-capillary 601 alignment and movement would be the reason for the hindering of its replication and 602 widespread use. As modification of commercial CE instruments to include the inkjet module 603 is not favored yet by their manufacturers, only purpose-made CE systems could allow 604 integration of inkjet injectors. To the authors' point of view, this mode could open more doors 605 for utilization / applications only if ready-to-use (off-the-shelf) inkjet injection modules that 606 can be plugged to CE instruments are available.

607

608 5. Other strategies for droplet introduction to microscale electrophoresis

609 Besides the main strategies using conventional droplet microfluidics, DMF or inkjet injection, 610 some other approaches were developed for generation and actuation of upstream droplets for 611 CE/MCE. Kaneda et al. gave an account of sequential operations of droplet-based reaction 612 process followed by MCE separation realized in a single microfluidic chip with pneumatic 613 handling of liquid [63]. In this system, automated liquid handling (i.e. introduction of liquid 614 samples, generation and merging of 420 pL droplets) was implemented by pressurization (40-615 100 kPa) through microcapillary vent structures, allowing air to pass and stop liquid flows. 616 The setup was applied for a binding reaction of a single-stranded DNA with a peptide nucleic 617 acid oligomer followed by denaturing electrophoresis to discriminate a single-base 618 substitution. Despite interesting results, no further works using this approach was reported.

619

As an alternative to the train of droplets, the SlipChip developed in 2009 by Ismagilov group
was adapted to allow parallel and quantitative MCE [64]. This SlipChip, called GelChip,

622 consisted of two poly(methyl methacrylate) (PMMA) plates, *i.e.* a droplet plate containing 623 droplet wells and a separation one on which separation channels were built in parallel. Once 624 the droplet wells were filled with the sample solution, the droplet plate was slid to allow the 625 sample to overlap with the separation channel. Upon contact with the buffer, immediate 626 merging of the sample with the buffer occurred, allowing the total volume of the droplet to be 627 injected into the separation channel. The SlipChip system was applied for rapid separation of 628 DNA ladders, with 30 separations taking place in 120 seconds. The same group also used a 629 modified SlipChip format for isoelectric focusing (IEF) of different proteins (trypsin inhibitor, 630 β -lactoglobulin A, carbonic anhydrase isozyme II, myoglobin, and lectin) [65]. When aligned 631 and clamped together with magnets, the two plates form a zig zag channel where the sample 632 can be loaded and separated by IEF. The chip was then slid to compartmentalize the focalized 633 analytes into 140 wells, allowing the user to collect target droplets for further processing. The 634 SlipChip principle was then coupled with a GelChip for development of a modified platform, 635 which consisted of two plastic plates, the one for droplet wells and the other for separation 636 channels with preloaded/cured gel [66]. The SlipChip-GelChip setup was demonstrated for 637 separations of 30 sub-nL sample droplets containing fluorescent dyes or DNA fragments. The 638 SlipChip design deemed user-friendly, and no flow control was required. Nevertheless the 639 injection volume for a given design cannot be varied, and manual reloading of the chips and 640 plate sliding after separations would hinder full automation of the protocol.

641

642 Opekar *et al.* introduced a syringe-based technique, using a needle of an automated 10 μ L-643 syringe located directly opposite to the capillary inlet at a defined distance, to produce 125 nL 644 droplets for direct sample injection into the CE capillary via negative pressurization [67]. The 645 setup utilized off-the-shelf microfluidic components (e.g. Hamilton syringe, Supelco 646 connectors, KD Scientific 200 micro-pump) to allow re-fabrication, and was applied for CE- 647 C^4D separation of inorganic cations. The authors then modified the design with an air-assisted 648 flow-gating interface (FGI), replacing the micro-syringe with a delivery capillary to generate 649 75 nL sample droplets for hydrodynamic injection [68]. This droplet interfacing strategy via 650 FGI was then used for on-line connection of CE with a dialysis unit to allow sample dialysis 651 into an acceptor solution (sub μ L) trapped in a dialysing hollow fibre, prior to CE-C⁴D 652 separations of extracted analytes. This approach was applied for simultaneous determination 653 of the majority minerals in unflavored yoghurts [69], as well as determination of basic amino 654 acids (histidine, lysine and arginine) in a blood serum sample [70]. The most recent approach 655 was communicated by Ngamakarn et al., using a moving drop setup with electrokinetic 656 sample injection for CE [71]. A polyether ether ketone (PEEK) tube functioned as a drop-657 head tube for solution dropping. For droplet guiding, the system employed a tile path 658 supporter coated with hydrophobic Teflon tape and arranged in a 45° slope compared to the 659 CE capillary position. It can be seen that the large droplet volume (25 µL) renders this 660 approach less favorable for droplet-interfaced CE due to pronounced working volume 661 mismatch. Note also that these syringe-based, FGI and moving-drop setups, despite their instrumental simplicity to allow facile reproduction and employment, are more adapted to 662 663 droplet-based injection rather than sample pre-treatment. Design and protocol improvement 664 would be needed before they can be used in a sample processing protocol with extended steps.

665

666 **6. Conclusion remarks and perspectives**

667 Several droplet-interfacing strategies have recently been developed for microscale 668 electrophoresis, allowing the use of tiny working volumes for sample handling and/or high-669 throughput injection prior to electrokinetic separations of target analytes with CE/MCE. 670 These approaches are gaining more and more interest and both academic and industrial 671 communities have put efforts to develop integrated systems for seamless sample processing 672 and electrophoretic separation with little or no working dimension (volume in particular) 673 incompatibility. Different companies (for instance Inorevia - Innovative Bioassays, Fluigent 674 or Elvesys in France) are exploiting droplet-based operations for sample treatment which can 675 be subsequently connected to separation instruments to our opinion. As existing commercial 676 CE instruments do not allow facile integration of external sample treatment modules, the 677 droplet-interfacing would be promoted at the first time with purpose-made CE instruments. 678 The recent introduction of open-source CE [72] and modular CE [73] may allow flexible 679 instrumentation with a high degree of standardization, which is required to develop automated 680 droplet-CE/MCE systems and further widespread these designs. Of course it is too early at 681 this stage to foresee the utilization of droplet-CE/MCE systems for routine analyses outside 682 the research context, as inherent issues of microscale electrophoresis (notably migration time 683 fluctuation and / or adsorption of unwanted molecules on capillary wall) need to be overcome 684 first. One analytical niche in the future for droplet-CE/MCE systems could be compact, cost-685 effective and transportable instrumentation allowing both sample processing and 686 electrophoretic separations of target analytes. Towards this direction, fabrication of 687 commercially available prototypes for dedicated uses could be possible by exploiting the 688 commercial microfluidic droplet modules and modular / open-source CE, to our opinion. The 689 droplet-interfacing is also important when working with precious samples of limited volumes 690 (for example cerebrospinal fluids for analysis of neurodegenerative diseases' or brain cancers' 691 biomarkers). Combining CE/MCE with droplet-interfaced sample pretreatment techniques 692 would represent a powerful tool for bioanalytical laboratories for analysis of molecules often 693 present in trace amounts in biological matrices. From these points, the droplet-CE/MCE 694 systems, once they are at a more mature instrumental stage, could see their first applications 695 in biological and clinical domains where the challenges with limited sample volumes and 696 trace amounts of target analytes could be overcome with this droplet interfacing strategy.

697

698 At the present state of the art, droplet-interfacing for CE/MCE is still at its infancy, focusing 699 more on the high-throughput injection / screening aspect, and much less on its potential for 700 down-scaling and automating sample treatment protocols. All instrumental designs presented 701 in this review stop at the utilization of constant-sized droplets. The power of droplet volume 702 variation, droplet fusion and splitting functions is still to be explored. Among all strategies 703 presented in this review, we envision that the coupling of CE with droplet trains flowing in 704 immiscible phases will further emerge and could be transferred to industrial use. Its 705 straightforward and flexible instrumental setups that can be adapted to various types of 706 sample processing and preconcentration (e.g. immune-enrichment on magnetic beads, 707 fluorescent labeling, chemical precipitation, enzymatic reaction etc.) make this droplet-708 sequence-in-oil approach a particularly important toolset for researchers working in analytical 709 science, especially in the bioanalytical, biomedical, and clinical diagnostic fields.

710

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715 **Table 1:** Strategies to interface droplets to microscale electrophoresis

Droplet interfacing strategy	Approach	Setup	Oil removal method	Droplet volume	Application	Remarks (pros / cons)	Reference
	Parallelized virtual wall and K-shaped interface	MCE- Fluorescence Microscope	Coalescence with immiscible interface	9 nL	GTPase assay	Passive removal, parallel analysis	[31]
	Virtual wall and K-shaped interface	MCE-LIF	Coalescence with immiscible interface	12-17 nL	Separation of fluorescently labeled amino acids	Passive removal, only a fragment of the droplet is injected	[24]
	Segmenting effluent from HPLC for 2 dimension separation	MCE-UV	Pillars to filter the oil	10 nL	Two-dimension separation of a peptide mixture	Passive removal, adjustable droplet volume	[23]
	Sample from a microdialysis probe is segmented and injected into a MCE	MCE-LIF	Extraction bridge	8-10 nL	In vivo chemical monitoring of amino acids	Passive removal, monitoring	[22].
Strategy 1 : Droplet train in immiscible	Open channel with hydrophobic and oleophilic membrane	MCE-LIF	Passive absorption by membrane	4 nL	Separation of 50 bp dsDNA molecular weight standard	Passive removal, suitable with gel CE, high throughput (5 droplet/sec)	[28]
phases	A PDMS chip is linked to a glass MCE using a hydrophilic capillary	MCE-LIF	Hydrophilic capillary	8 nL	Screening a test library of 140 compounds against using protein kinase A	Passive removal, high- throughput potential	[27]
	A PDMS chip is linked to a glass MCE using a hydrophilic capillary	MCE-LIF	Hydrophilic capillary	8 nL	Screening of 1280 compounds against SIRT5	Passive removal, high- throughput potential	[32]
	Sample are electrokinetically injected into a separation channel while the oil experiences buoyancy and is drained upward	MCE-LIF MGE-LIF	Difference of density between carrier phase and running buffer	5 nL	Separation of protein- protein complexes and enzymatic reactions	Passive removal, some dilution of sample	[25]
	EOF-based	MCE-LIF	-	30 pL	Separation of a mixture	On-chip labeling,	[33]

compartmentalized sampling/labeling using hydrophobic ionic liquid to segment flow				of flavin adenine nucleotides (FAD) and flavin mononucleotides	electrokinetical injection	
Integrated droplet microfluidics for magnetic particles handling	MCE-LIF	Manual collection and fusion of outlet droplets via a micro-pipette	100-200 nL	DNA size selection in NGS libraries preparation		[34]
Array of nano-scaled, oil covered sample wells fixed on a x-y-z translation stage with a fixed capillary	CE-LIF	-	200 pL	Separation of samples made of 3 amino acid	Easy to build, tunable plug volume	[36]
Selective surface patterning of the channel to create stable interface	CE-LIF	Coalescence with immiscible interface	10 fL	Separation of 3 amino acid	Sample can also be injected directly into the separation channel	[30]
Out of plane intersecting channels with independent droplet formation	CE-LIF	Surface patterning and geometry	750 nL	Changes in riboflavin concentration	Satellite droplets, manually operated, passive removal	[35]
Array of nano-scaled, oil covered sample wells fixed on a x-y-z translation stage with a fixed capillary	CE-LIF	-	200 nL	Detection of multi-gene mutations from colorectal cancer samples	Easy to build, tunable plug volume	[37]
Segmenting effluent from HPLC for 2 dimension separation	CE-UV	Pillars to filter the oil	40 nL	Two dimensional separations of human urinary protein digest	Passive removal, adjustable droplet volume	[16]
Droplet-based immunoassay with off-line CIEF	CIEF-UV	Manual collection of outlet droplets and dilution prior to CE	200 nL	Detection of amyloid- beta peptide-based biomarkers of Alzheimer's disease	Extended steps of sample processing in a droplet train with back and forth movement. Design flexible for various operations. High throughput CE was not yet	[41]

						possible as droplets need	
						to be collected manually.	
	Effluent from CE are				Separation followed by		
	segmented into droplets for	CE-LIF	-	14 nL	oxidation of paralytic	-	[39]
	post column detection				shellfish toxins		
					DNA labelling of amino		
	Digital-to-channel interface	MCE		251	acids and cell lysate; on-		[49]
	for MCE (side-on design)	WICE-	-	2.5 µL	chip digestions of singly		[40]
					labelled FITC-Insulin		
	Digital to shannal interface				On-chip serial dilution		
	for MCE (multi layer design)	MCE-	-	~3 µL	and multistep enzymatic		[49]
	for wee (multi-layer design)				digestion		
Strategy 2:	Digital microfluidia compler			5-10 μL	Sample concentration		
Digital	for portable CE	CE-C ⁴ D	-		kinetics of thiamine,		[50]
microfluidics	for portable CE				pyridoxine, nicotinamide		
with	Digital microfluidic sampler for portable CE		-	3 µL	Solid-liquid extraction of		
electrowetting-		CE-C ⁴ D			amino acids from sand		[51]
on-dielectric					matrices		
principle	Collector and transporter of	CE MS		31	Characterization of some		[52]
	CE fractions for ESI-MS	CE-MI5	-	5 μL	vitamin standards		[32]
	Collector and transporter of	CE-MS	_	31	Analysis of peptide		[53]
	CE fractions for MALDI-MS	CL-MI5		5 μΕ	fractions		[33]
	DME platform with capillary	CELIE	-	2-3 µL	Quality control platform		
	based reagent delivery unit				for next-generation		[54]
		CL-LII			sequencing (NGS) library		[]4]
					characterization		
	Droplet injection for				Electrophoretic analysis		
	microchannel array	MCE-		pL-nL	of DNA droplets		[57]
Strategy 3:	electrophoresis				of Divit dioplets		
Inkjet droplet	Piezoelectric droplet injector	CE	-	179 pL	Analysis of theobromine,	Droplet size and delivery	
injector	in the inkiet head				caffeine and theophiline	frequency adjustable via	[58]
					using MEKC	waveform turning	
	Drop-by-drop introduction for	CE-LIF	-	181-183 pL	Determination of amino		[59]

	electrophoretically mediated microanalysis (EMMA)				acids tagged with a fluorophore (4-fluoro-7- nitrobenzofurazan)		
	Quantitative CE by inkjet with on-line concentration	CE-UV	-	(sub)-nL	Quantitative on-line concentration of methylxanthines in bottled green tea		[60]
	Inkjet injection in immunoassays by quantitative on-line EMMA	CE-	-	~ 200 pL	Online EMMA-based immunoassays and CE separation of the antigen- antibody complex		[61]
	CE coupled with inkjet printing system.	CE-	-	~ 200 pL	CE separation of mammalian cells encapsulated in droplets	Calibration linearity via variation of droplet numbers (25-400 drops)	[62]
	Single microfluidic chip with pneumatic handling of liquid	MCE-	-	420 pL	Reaction of a ss DNA with a peptide nucleic acid oligomer and denaturing electrophoresis to separate a single-base substitution	Introduction of liquid samples, generation and merging of droplets via pressurization (40-100 kPa), allowing air to pass and stop liquid	[63]
Other	Syringe-based direct sample injection to CE capillary via negative pressurization	CE-C ⁴ D	-	125 nL	Separation of inorganic cations	Use of off-the-shelf microfluidic components	[67]
su ategies	Air-assisted flow-gating interface with a delivery capillary	CE-C ⁴ D	-	75 nL	Separation of inorganic cations	Use of off-the-shelf microfluidic components	[68]
	Dialysis of one sample drop on-line connected with electrophoresis in short capillary	CE-C ⁴ D	-	100 nL	Separation of model inorganic cations (K ⁺ , Ba ²⁺ and Na ⁺) and organic molecules (creatinine, histidine and arginine). Determination	Dialysis in micro-litre sample volumes into submicro-litre volumes of an acceptor solution in a dialysing fibre	[69, 70]

				of basic amino acids (histidine, lysine and arginine) in a blood serum sample. Rapid determination of majority cations in yoghurts.		
Moving drop setup with electrokinetic injection	CE-C ⁴ D	-	25 µL	Separation of inorganic cations and anions	Pronounced working volume mismatch	[71]
Slipchip: two patterned plates are moved to generate droplets	MGE-LIF	-	800 pL	Separation of fluorescent dyes, DNA fragments	Suitable for gel CE, easy to use, sample reloading, parallel analysis	[66]
Collecting segmented sample separated by IEF with a SlipChip	M IEF	-	50 nL	Separation of five standards proteins: trypsin inhibitor, β- lactoglobulin A, carbonic anhydrase isozyme II, myoglobin, and lectin	Easy to use	[65]

718 Figures:





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738	Fig. 2: (A) Setup overview of droplet train generator for DNA purification. (B) The particles
739	confined in the parent droplet experience a magnetic force that induces their
740	deflection, extraction and trapping in the capture region (inset). Subsequently, they
741	can be either released in a second flowing droplets or discarded through the
742	secondary channel, according with the desired protocol. (C) Picture of the
743	microfluidic device integrating the micrometric magnetic structures. Red dye is used
744	to underline the channel design. Reprinted from [34] with permission. Copyright
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(A)













Fig. 4: *Comparison of the side-on hybrid microfluidic device configuration and the multilayer* hybrid microfluidic device configuration. (A) The side-on configuration comprises a one-plate digital microfluidic device mated to a PDMS microchannel on a common substrate. The design is straightforward to fabricate; however, it suffers from the requirement of dispensing by pipet and material limitations of PDMS. (B) The multilayer device design comprises a DMF array patterned on a top substrate mated to a network of microchannels in a glass substrate below. Although more complex to fabricate, this configuration allows for dispensing droplets from reservoirs and splitting droplets on-chip, and in addition glass is more favorable for microchannel separations. Reprinted from [49] with permission. Copyright (2010) ACS.

(A)







Fig. 5: Interfacing the DMF sampler into the portable CE analyzer. (A) Portable CE analyzer
with DMF sampler. (B) Instrumentation scheme: 1, capacitively coupled contactless
conductivity detector (C⁴D); 2, grounded piece of syringe needle with the inlet end of
separation capillary; 3, spring-loaded contact pins; 4, ground electrode during
droplet actuation; 5, rectangular opening for exposing the electrode array. Reprinted
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811 Fig. 6: (A) Illustration of inkjet injection of DNA droplets for microchannel array 812 electrophoresis. There were two electrodes, a microchannel array, a detection part, 813 and an inkjet injector. (B) Photograph of the PulseInjector with a cartridge. (C) 814 Schematic illustration of the PulseInjector, which is a piezo element-based plastic 815 drop-on-demand type inkjet head. Epoxy resin composite material was used as the 816 structural material; this made it possible to integrate various components, such as a 817 pressure chamber, nozzle with water repellent coating, flow channel, vibrating 818 membrane, bracket, and PZT element into one body, resulting in superior chemical 819 resistance. (D) Photograph of a COC microchip with three microchannels (size: 3 cm 820 \times 7 cm) and magnified image of the injection ports for DNA samples; scale bar, 100 821 μ m. The microchannel width was 100 μ m. (E) Schematic diagram of a cross-sectional 822 view of the COC microchip. The diameters of the reservoirs and injection ports were 1 823 mm and 100 µm, respectively. The height of the microchannels was 40 µm. 824 Thicknesses of both the microchannel-patterned and nonpatterned COC substrate 825 were 0.5 mm. Reprinted from [57] with permission. Copyright (2012) ACS. 826

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Droplet-interfaced Microscale Electrophoresis

Graphical abstract