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1 **Droplet-interfacing strategies in microscale electrophoresis for sample treatment,**
2 **separation and quantification: A review**

3

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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**

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

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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 μ L-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
75 is also used for electrophoresis. Furthermore, the sample matrix flow percolating the

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
78 possible degradation of subsequent separation efficiency. If several steps are needed for
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
82 order to gain operational flexibility and to avoid undesired modification of the capillary /
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],

101 as well as for chemical separation for cellular analysis [18] and biochemical analysis [19].
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**

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

126 segmented flow analysis where the sample droplet / plug is carried out by an immiscible fluid
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
138 instrumental and / or physio-chemical approaches to overcome the challenge of oil intrusion
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

151 development has been established. Accordingly, the following discussion focuses more on the
152 techno-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
175 of oil into it. In this case, the oil preferably wets only the hydrophobic walls of the T-junction.

176 The droplet interfacing approach proposed by Edgar *et al.* was applied for the separation of an
177 amino acid mixture segmented into 12 pL droplets as the proof of concept demonstration.
178 While the authors did not mention the achieved throughput, the employed mode of pressure
179 control was found not yet compatible for high throughput. Changing samples with this
180 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

225 injections were made using the injection cross, generating more than 10 000
226 electropherograms during analysis.

227

228 Rather than extracting the sample droplet from the main oil-based flow, other options focused
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,
234 while the oil was drained through them to a waste reservoir. This method required only simple
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
238 mixture from a HPLC-based purification step was segmented into droplets that were
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 a hydrophobic and oleophilic
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
259 membranes in between the droplet generation and separation parts, as well as their
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 solid 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
316 to the batchwise procedure for DNA size selection in NGS libraries preparation, this droplet
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 detection
326 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 multi-
384 gene mutations using PCR-restriction fragment length polymorphism (PCR-RFLP) [37]. A
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.
398

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 ($A\beta$) peptide biomarkers for molecular diagnosis

424 of Alzheimer's disease [41]. A β 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 μ L 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.

447

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
460 interfacial tension modification [42, 43]. Eventually, sample pre-treatment in a down-leveling
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-on-** 468 **dielectric principle**

469 Another strategy to manipulate droplets in the forefront of CE employed digital microfluidics
470 (DMF) in which the droplets are actuated by Electrowetting On Dielectric (EWOD)
471 technology (see strategy 2 in table 1 for the summary). The EWOD principle is based on
472 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
522 automated quality control (QC) platform for next-generation sequencing (NGS) library

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
532 providing necessary sensitivity and accuracy for optimal sequencing performance.

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 μ L
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)
556 controllability, high speed, and accurate spotting on the surface of a wide variety of
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
567 A similar droplet generation concept was then developed for CE setups by Uchiyama *et al.*
568 [58-62]. In their first inkjet-CE design (Fig. 7A), they employed precise control of waveform
569 driving piezoelectric crystal inside the inkjet head to trigger by a drop-on-demand approach
570 stable droplet generation from various solutions. The piezoelectric droplet generator was used
571 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
584 instrument was then extended for online EMMA-based immunoassays (using ~ 200 pL
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].
587 The method showed a wide linear range of calibration (10–2000 ng.mL⁻¹) with satisfied
588 linearity ($R^2 = 0.9912$). The detection limit (5 ng.mL⁻¹) was substantially lower than those
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

620 As an alternative to the train of droplets, the SlipChip developed in 2009 by Ismagilov group
621 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 C⁴D 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 μ L) 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
662 instrumental simplicity to allow facile reproduction and employment, are more adapted to
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
Strategy 1: Droplet train in immiscible phases	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].
	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]
	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 segmented into droplets for post column detection	CE-LIF	-	14 nL	Separation followed by oxidation of paralytic shellfish toxins	-	[39]
Strategy 2: Digital microfluidics with electrowetting-on-dielectric principle	Digital-to-channel interface for MCE (side-on design)	MCE-	-	2.5 μ L	DNA labelling of amino acids and cell lysate; on-chip digestions of singly labelled FITC-Insulin		[48]
	Digital-to-channel interface for MCE (multi-layer design)	MCE-	-	\sim 3 μ L	On-chip serial dilution and multistep enzymatic digestion		[49]
	Digital microfluidic sampler for portable CE	CE-C ⁴ D	-	5-10 μ L	Sample concentration kinetics of thiamine, pyridoxine, nicotinamide		[50]
	Digital microfluidic sampler for portable CE	CE-C ⁴ D	-	3 μ L	Solid-liquid extraction of amino acids from sand matrices		[51]
	Collector and transporter of CE fractions for ESI-MS	CE-MS	-	3 μ L	Characterization of some vitamin standards		[52]
	Collector and transporter of CE fractions for MALDI-MS	CE-MS	-	3 μ L	Analysis of peptide fractions		[53]
	DMF platform with capillary-based reagent delivery unit for quantitative CE	CE-LIF	-	2-3 μ L	Quality control platform for next-generation sequencing (NGS) library characterization		[54]
Strategy 3: Inkjet droplet injector	Droplet injection for microchannel array electrophoresis	MCE-		pL-nL	Electrophoretic analysis of DNA droplets		[57]
	Piezoelectric droplet injector in the inkjet head	CE	-	179 pL	Analysis of theobromine, caffeine and theophiline using MEKC	Droplet size and delivery frequency adjustable via waveform turning	[58]
	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]
Other strategies	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]
	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]
	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]

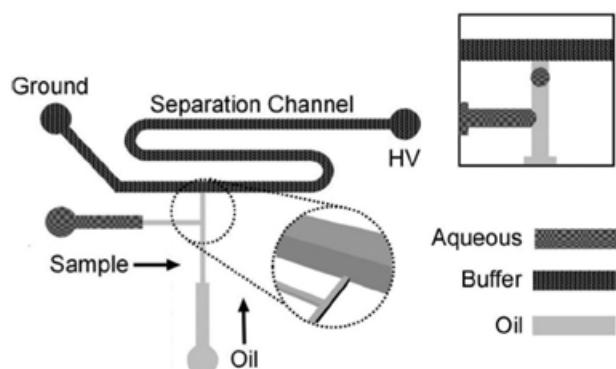
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718 **Figures:**

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(A)

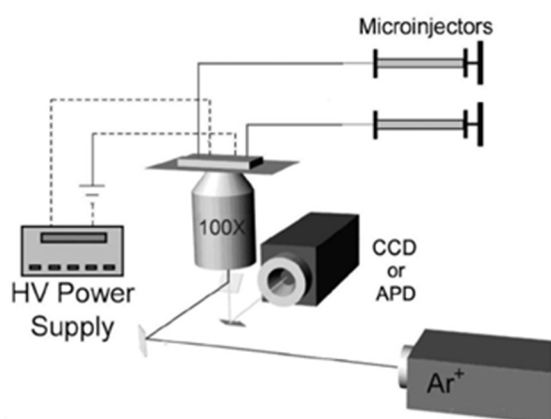


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(B)

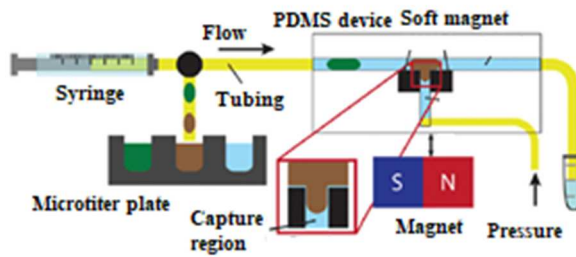


723

724 **Fig. 1:** Schematic showing the first design of droplet-interfaced MCE. (A) The separation
725 channel was $50\ \mu\text{m}$ by $50\ \mu\text{m}$ in cross section; the small T-channel where the sample
726 and oil met had a height of $3\ \mu\text{m}$ and a width of $3\ \mu\text{m}$. The inset highlights the
727 difference in dimension between these two channels. (B) The experiments were
728 performed on an inverted microscope using an Ar^+ laser for exciting fluorescence; a
729 CCD camera was used for wide-field imaging and an avalanche photodiode (APD)
730 for point detection. A set of homebuilt microinjectors and a high-voltage power supply
731 were connected to the microfluidic chip for droplet generation and MCE separation.
732 Reprinted from [30] with permission. Copyright (2006) ACS.

733

(A)



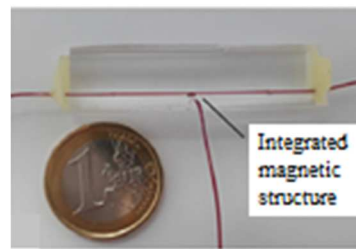
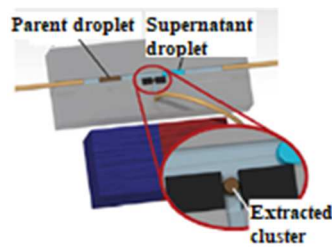
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(B)

(C)



737

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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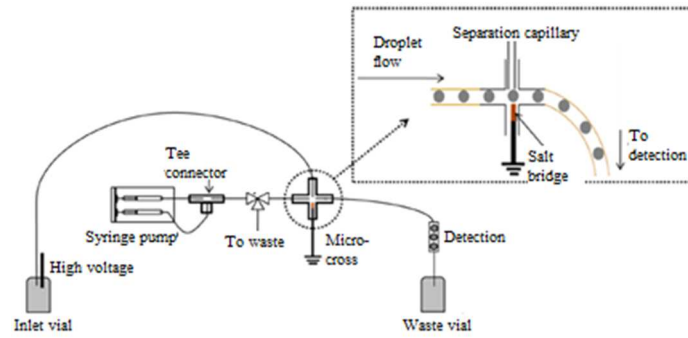
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(A)



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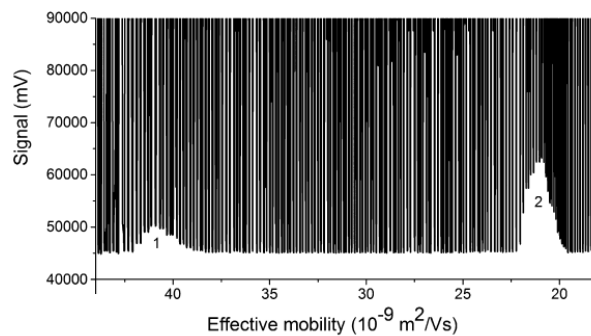
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(B)



(C)



756

757 **Fig. 3:** CE-droplet microfluidics system for post-column detection: (A) schematic outline of
758 the setup used for postcolumn compartmentalization incorporated in a CE instrument.
759 The aqueous phase was segmented by oil in the tee connector (outside CE instrument)
760 and passed the separation capillary outlet to collect the electrophoresed effluent in a
761 compartmentalized manner (within CE instrument). (B) Photograph of the micro cross
762 as the droplet microfluidics interface was positioned in a capillary cassette. Photo
763 label: connection for (1) droplet capillary, (2) separation capillary, (3) detection
764 capillary, and (4) salt bridge electrode. (C) Separation of paralytic shellfish toxins
765 mixture: electropherogram from CZE-droplets microfluidics system demonstrating
766 separation and compartmentalization of electrophoresed analytes into preformed
767 droplets for oxidation. Reprinted from [39] with permission. Copyright (2014) Elsevier.
768

769

(A) Side-on (Previous Work²⁴)		(B) Multi-Layer (New Design)	
Property	Effect	Property	Effect
1. Single-plate droplet actuation configuration	<ul style="list-style-type: none"> ➢ No dispensing from reservoirs or splitting droplets ➢ Large errors caused by pipetting ➢ Evaporation problems 	1. Two-plate droplet actuation configuration	<ul style="list-style-type: none"> ➢ Dispensing from reservoirs and droplet splitting ➢ Pipetting errors made negligible ➢ Evaporation limited
2. PDMS device construction	<ul style="list-style-type: none"> ➢ Easy to fabricate ➢ Undesirable surface properties (i.e., surface adsorption of analytes, solvent incompatibility etc.) 	2. Glass device construction	<ul style="list-style-type: none"> ➢ More difficult to fabricate ➢ Superior material properties (i.e., reduced analyte adsorption, solvent compatible etc.)
3. Shared digital and channel substrate	<ul style="list-style-type: none"> ➢ Limited space for electrodes 	3. Separate digital and channel substrates	<ul style="list-style-type: none"> ➢ Larger space for electrodes

770

771 **Fig. 4:** Comparison of the side-on hybrid microfluidic device configuration and the multilayer

772 hybrid microfluidic device configuration. (A) The side-on configuration comprises a

773 one-plate digital microfluidic device mated to a PDMS microchannel on a common

774 substrate. The design is straightforward to fabricate; however, it suffers from the

775 requirement of dispensing by pipet and material limitations of PDMS. (B) The

776 multilayer device design comprises a DMF array patterned on a top substrate mated

777 to a network of microchannels in a glass substrate below. Although more complex to

778 fabricate, this configuration allows for dispensing droplets from reservoirs and

779 splitting droplets on-chip, and in addition glass is more favorable for microchannel

780 separations. Reprinted from [49] with permission. Copyright (2010) ACS.

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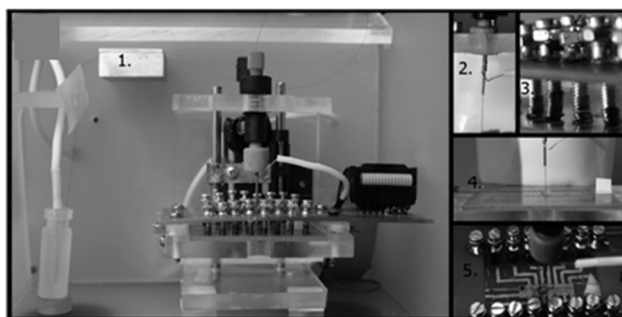
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(A)

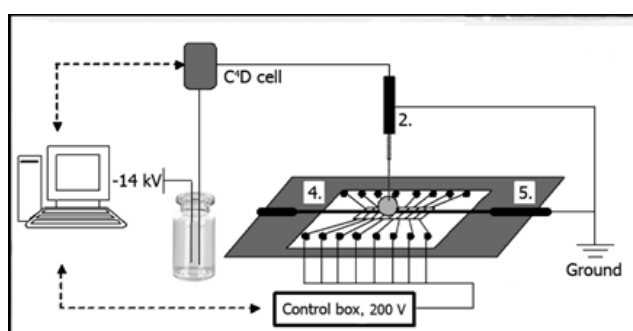


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790

(B)



791

792 **Fig. 5:** *Interfacing the DMF sampler into the portable CE analyzer. (A) Portable CE analyzer*
793 *with DMF sampler. (B) Instrumentation scheme: 1, capacitively coupled contactless*
794 *conductivity detector (C⁴D); 2, grounded piece of syringe needle with the inlet end of*
795 *separation capillary; 3, spring-loaded contact pins; 4, ground electrode during*
796 *droplet actuation; 5, rectangular opening for exposing the electrode array. Reprinted*
797 *from [50] with permission. Copyright (2009) ACS.*

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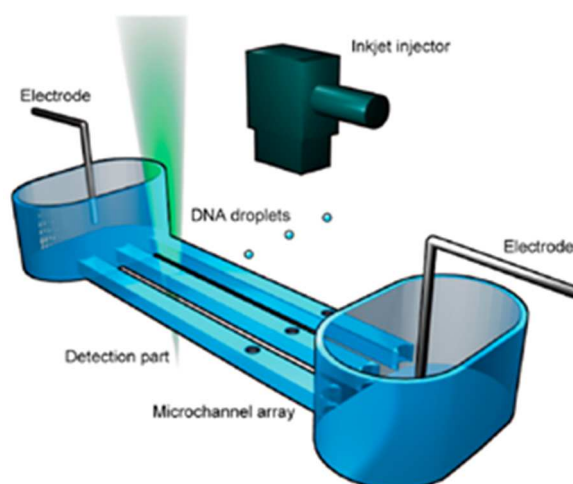
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(A)

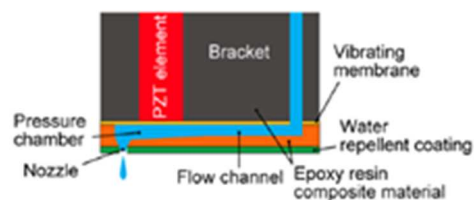


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(B)

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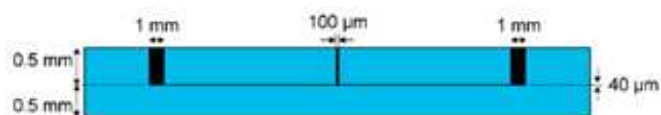
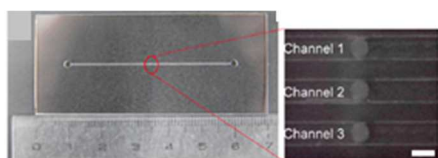


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(D)

(E)

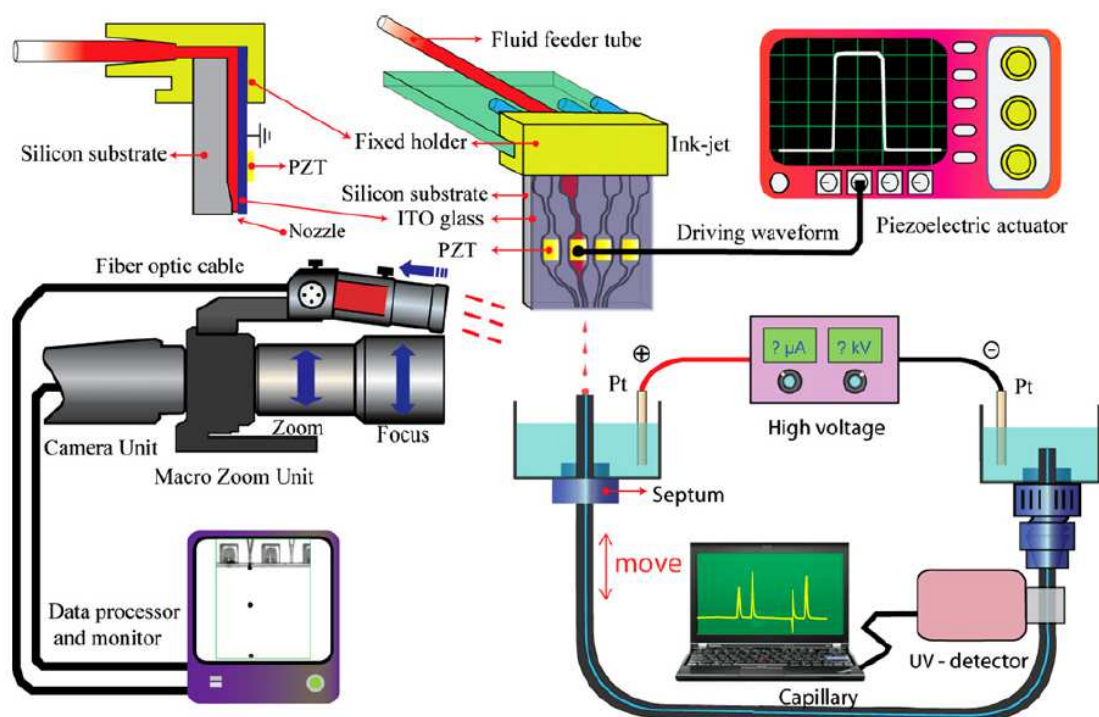


810

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 × 7 cm) and magnified image of the injection ports for DNA samples; scale bar, 100
 821 μm. The microchannel width was 100 μm. (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.
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(A)

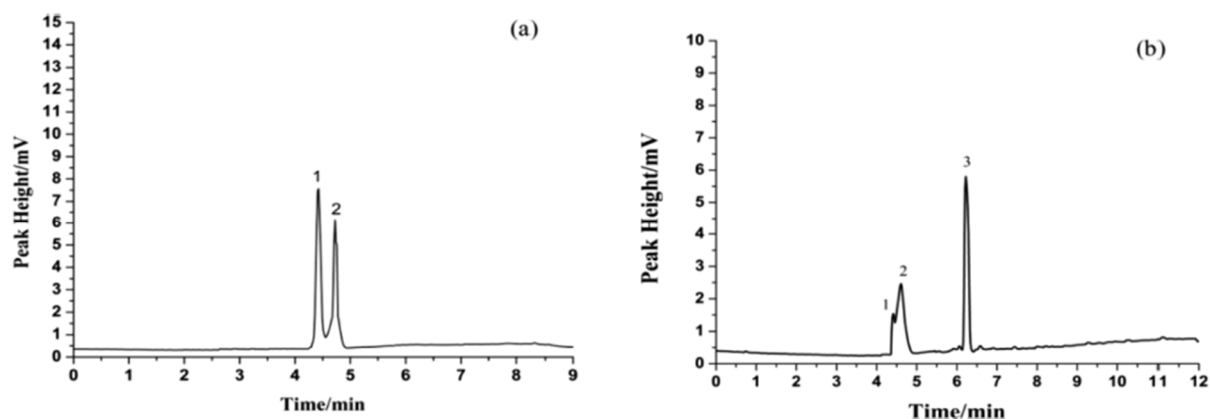


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(B)



831

832 **Fig. 7:** (A) Schematic illustration of the whole experimental setup. Inkjet printing of droplet
 833 generation, high-speed micro-observation system, and capillary electrophoresis system
 834 are presented in the figure. Reprinted from [58] with permission. Copyright (2013)
 835 Elsevier. (B) Determination of different property of cell by inkjet droplet-interfaced CE:
 836 (a) Electropherogram for normal and apoptosis HepG2 cells (Peaks (1) apoptosis cells
 837 and (2) normal cells); (b) Electropherogram of a mixture of three kind of cells - (1) Caco-
 838 2, (2) HepG2, and (3) HUVEC Cell at the concentration of 1×10^6 cells/mL (200
 839 droplets). Reprinted from [62] with permission. Copyright (2017) ACS.

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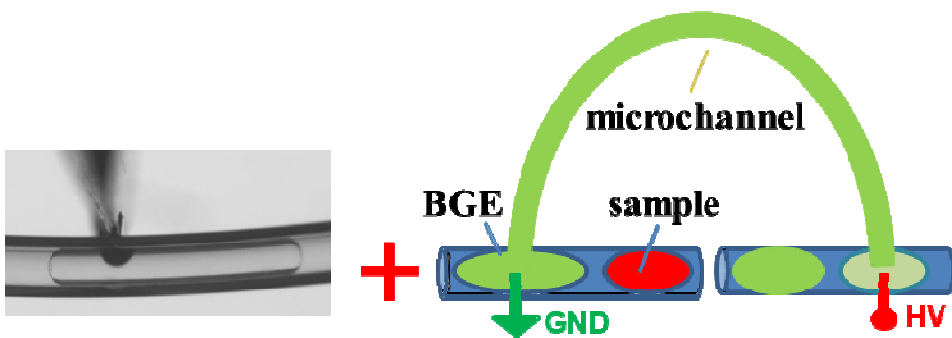
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Droplet-interfaced Microscale Electrophoresis

Graphical abstract