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Knowing more from less: miniaturization of ligand-binding assays and electrophoresis as new paradigms for at-line monitoring and control of mammalian cell bioprocesses

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Monitoring technologies for Process Analytical Technology (PAT) in mammalian cell cultures are often focusing on the same hand full parameters although a deeper knowledge and control of a larger panel of culture components would highly benefit process optimization, control and robustness. This short review highlights key advances in microfluidic affinity assays and microchip capillary electrophoresis (MCE). Aiming at the miniaturization and integration of PAT, these can detect at-line a variety of metabolites, proteins and Critical Quality Attributes (CQA’s) in a bioprocess. Furthermore, discrete analytical components, which can potentially support the translation of increasingly mature microfluidic technologies towards this novel application, are also presented as a comprehensive toolbox ranging from sample preparation to signal acquisition.

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Introduction
More efficient tools to optimize, understand, monitor and control the manufacturing processes of biopharmaceuticals, for increased production yield of the product of interest (PoI), controlled PoI quality (CQA’s) and production robustness are desirable. In this context, PAT aims at supporting design, analysis and control of biopharmaceutical manufacturing by real-time parameter measurements [1,2]. Culture process development relies on identifying the medium composition and process parameters by trial and error approach, supported by statistical tools used for example, for Quality-by-design (QBD) and process characterization. Nowadays on-line/at-line measurements in the culture still remain limited however broader possibilities would represent significant benefits in several aspects; (i) Most medium components are tuned based on a hand full parameters, that is, cell density/health, main metabolism, PoI production and CQA’s, rather than directly quantified, implying that deeper mechanistic understanding and thorough control are lacking. Depletion of a medium component can provoke cell apoptosis if it is essential and metabolism shift if non-essential, both detrimental for the process performance. (ii) Manufacturing process performance, depending on ‘alive cell factories’, are varying by nature and require improved monitoring and controls. (iii) Besides lactate and ammonia, other by-products can affect the culture [3,4] for which monitoring would be an asset. (iv) Miniaturized bioreactors (2–15 mL volume) provide high throughput (HTP) for process development however their analytical support represents a major bottleneck, worsened by very small sample volumes (0.2–1.5 mL) [5]. (v) The power of mathematical models for understanding, predicting, optimizing or controlling the culture processes stays today unexploited due to lack of information about many culture components [6]. (vi) Today’s methods to quantify the culture components, others than the most common ones, and the CQA’s are slow and a heavy labor burden.

Process parameters commonly measured on-line, include pH, pO2, pCO2, and cell density, whereas few important
metabolism markers, such as glucose, lactate, glutamine and ammonia, can be quantified at-line/on-line by robot analyzers, spectroscopy probes or biosensors [7,8]. Besides these, other analytes such as amino acids, vitamins, sugars and carbohydrate precursors, lipids and proteins as detailed with concentration ranges in Supplementary Material Table S1, can influence the process, making them important targets for real-time quantification for the reasons mentioned above. The different constraints of sample volumes, tolerated costs and precision requirements of HTP versus stirred tank bioreactor, and development versus manufacturing, represent supplementary challenges [6,9]. Spectroscopic probes, for example, Raman, NIR, MIR, can provide on-line information of some components, but cannot detect low concentrations and require process specific implementation by chemometric models [8]. HPLC/UPLC and capillary electrophoresis (CE) allow the detection of many analytes even at low concentrations, but often require sample preparation, connection to mass spectrometry (MS), or NMR to distinguish the analytes [8]. These methods or affinity-based analyses can also be used to assess CQA’s [1,9]. MS is a powerful technique to analyze most analytes [1], but is hard to integrate at-line due to bulkiness and high costs (instrument costs approx. 200 000–500 000 €), requires qualified personnel and remains very challenging for HTP. A HPLC or CE instrument with optical detection, without the MS detector, costs almost a factor 10 less. Comparing the cost for a separation column for HPLC and a capillary for CE, the cost per analysis becomes significantly lower for CE (approx. 2 € versus 0.02 €). Also, the HPLC requires a roughly 20 times larger volume of chemicals for each analysis than a CE.

To avoid complex and costly instrumentation, miniaturization in microfluidic systems with automation of analytical methods represent an attractive alternative [10]. Recent achievements on miniaturized systems for sensing in bioprocesses are being driven by rapid prototyping technologies, such as soft-lithography techniques [11], precision micromachining [12] and high-resolution 3D printing [13]. These can be coupled with affinity probes, enzymatic activity or electrophoresis followed by electrochemical or optical signal transduction methods, such as amperometry, voltammetry, electrochemical impedance spectroscopy (EIS), (electro)chemiluminescence, Raman spectroscopy, colorimetry and laser-induced fluorescence [14,15]. In the present short review, we place a particular focus on microfluidic methods using (i) affinity probes for protein quantification and (ii) CE for metabolite detection (Figure 1), which are particularly promising in the context of PAT. Although HPLC is the most commonly used in PAT, CE requires much smaller sample volumes (μL versus nL in CE) and is more easily miniaturized. The detection of bioprocess proteins with affinity probes can provide highly versatile and cost-efficient bioanalytics integration into miniaturized setups, with detection limits in the range of low ng/mL and dynamic ranges up to three orders of magnitude.

**Miniaturized devices allowing multiplexed protein detection**

Currently largely unexplored in the context of PAT, microfluidic immunoassays have been under rapid development in recent years mostly for biomarker/pathogen diagnostics [16,17] and food/environmental monitoring applications [18]. Remarkably, this know-how can be readily translated to fulfill the requirements of at-line protein measurements, including product titer and host cell proteins (HCP), for bioprocess development. This section aims at providing a deconstruction of literature in the past three years towards a state-of-the-art toolbox on miniaturized protein analysis systems irrespective of their intended application.

Figure 2 and Table 1 comprise key features of microfluidic protein analysis devices focusing on (i) liquid handling on the device, (ii) type of materials used to fabricate the device, (iii) selection of probe to specifically capture the target molecule, (iv) configuration in which the probe is used, (v) type of optically active label allowing the detection of the molecular recognition event, (vi) strategies to multiplex the detection of multiple molecules from a single sample and (vii) optical to electrical signal transduction. The liquid handling on miniaturized devices is typically performed by applying either a positive pressure at the inlet or a negative pressure at the outlet using a syringe pump, a pressure flow controller or a peristaltic pump. Alternatively, to minimize the
Miniaturization for at-line sensing of bioprocess Pinto et al. 3

Toolbox of microfluidic immunoassay development towards at-line protein measurements. The compiled data is based on papers published between 2018 and 2021 [16–20,22,23,24,25–29]. Details for each reference are listed in Table 1.

equipment requirements, capillary flow can be used [16,19,20], but this requires either a careful material selection or surface modification to ensure a stable and reproducible contact angle. In addition, centrifugal flow and electrowetting-on-dielectric (EWOD) represent elegant solutions to manipulate single droplets of few microliters, minimizing equipment requirements down to a simple rotating motor [17] or an electric circuit [18]. While some authors report the use of stagnant liquid in microsystems, these are generally suboptimal due to the large surface-to-volume ratios and rapid depletion of the molecules in solution limiting the overall sensitivity and assay time [21], unless active solid phases are used and moved relative to the liquid [22]. Most devices are usually prototyped in polydimethylsiloxane (PDMS) using mold replication techniques or computer numerical control (CNC) milled into a hard plastic (e.g. poly(methyl methacrylate) (PMMA), polystyrene or cyclic olefin copolymer). After formalizing the design, scalability can then be achieved by using injection molding (lower costs, typically single use) or silicon/glass photolithography (higher costs, reusable). Monoclonal antibodies (mAb) and their fragments are typically the probes of choice providing highest affinity and batch-to-batch reproducibility. However, more stable and easily synthesizable aptamers have also been reported in a label-free assay to monitor a mAb-
<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cell type</th>
<th>Sample</th>
<th>Application</th>
<th>Liquid handling</th>
<th>Chip material</th>
<th>Probe</th>
<th>Assay design</th>
<th>Signal transduction</th>
<th>Label</th>
<th>Multiplexing strategy</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prostate-specific antigen (PSA)</strong></td>
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<td>Synthetic serum</td>
<td>Pump-free microfluidic sensor for PSA detection</td>
<td>Capillary flow</td>
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<td>Active solid phase</td>
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<td>SERS-active label</td>
<td>NA</td>
<td>[16]</td>
</tr>
<tr>
<td><strong>IgG/IgM/antigen of SARS-CoV-2</strong></td>
<td>NA</td>
<td>Human serum/ pharyngeal swabs</td>
<td>Point-of-care diagnostic of SARS-CoV-2</td>
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<td>Polycarbonate and paper</td>
<td>Antibody</td>
<td>Porous solid phase</td>
<td>Fluorometer</td>
<td>Fluorescent microsphere</td>
<td>Spatial-resolved</td>
<td>[17]</td>
</tr>
<tr>
<td><strong>Proteins, vegetative bacteria, bacterial spores, viruses</strong></td>
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<td>Droplets from aerosol</td>
<td>Detection of airborne pathogens</td>
<td>EWOD</td>
<td>Glass/chromium coated with Parylene-C</td>
<td>Antibody</td>
<td>Active solid phase</td>
<td>Photodiode</td>
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<td>NA</td>
<td>[18]</td>
</tr>
<tr>
<td><strong>Ferritin</strong></td>
<td>NA</td>
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<td>Point-of-care diagnostic of plasma biomarkers</td>
<td>Negative or positive pressure</td>
<td>PMMA and magnetic beads</td>
<td>Antibody</td>
<td>Active solid phase</td>
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<td>Acridinium ester</td>
<td>NA</td>
<td>[26]</td>
</tr>
<tr>
<td><strong>Host cell proteins (HCPs)</strong></td>
<td>CHO</td>
<td>Cell culture supernatant</td>
<td>Automated monitoring of HCPs during biopharmaceutical production</td>
<td>Negative or positive pressure</td>
<td>Glass</td>
<td>Antibody</td>
<td>Planar solid phase</td>
<td>Fluorometer</td>
<td>Organic fluorophore (DyLight650)</td>
<td>Spatial-resolved</td>
<td>[24]</td>
</tr>
<tr>
<td><strong>HCPs, IgG, lactate dehydrogenase (LDH)</strong></td>
<td>CHO</td>
<td>Cell culture supernatant</td>
<td>Multiplexed protein monitoring during biopharmaceutical production</td>
<td>Positive pressure</td>
<td>PDMS and agarose beads</td>
<td>Antibody</td>
<td>Porous solid phase</td>
<td>Flatbed scanner</td>
<td>(Colorimetry)</td>
<td>HRP</td>
<td>Spatial-resolved</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td>Peripheral blood mononuclear cells (PBMC)</td>
<td>Culture medium</td>
<td>Immunophenotyping of patients with immune-related diseases</td>
<td>Positive pressure</td>
<td>PDMS and microbeads</td>
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<td>Porous solid phase</td>
<td>CCD camera (Microscopy)</td>
<td>Phycoerythrin</td>
<td>Spatial-resolved</td>
<td>[27]</td>
</tr>
<tr>
<td><strong>p24 antigen</strong></td>
<td>NA</td>
<td>Buffer</td>
<td>Automated detection of pathogens</td>
<td>Stagnant flow</td>
<td>PMMA and magnetic beads</td>
<td>Antibody</td>
<td>Active solid phase</td>
<td>Smartphone camera (Colorimetry)</td>
<td>HRP/H2O2/TMB</td>
<td>NA</td>
<td>[22]</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td>NA</td>
<td>Human serum</td>
<td>Semi-automated multiplexed detection of biomarkers</td>
<td>Negative pressure</td>
<td>PDMS</td>
<td>Antibody</td>
<td>Planar solid phase</td>
<td>CCD camera</td>
<td>HRP/H2O2/TMB</td>
<td>Spatial-resolved</td>
<td>[25]</td>
</tr>
<tr>
<td><strong>Fab fragment</strong></td>
<td>E. coli</td>
<td>Culture broth</td>
<td>In-line monitoring of Ranibizumab concentration in a bioreactor</td>
<td>Positive pressure</td>
<td>PDMS/glass and gold electrode</td>
<td>Aptamer</td>
<td>Planar solid phase</td>
<td>Impedimetric</td>
<td>Label-free</td>
<td>NA</td>
<td>[23]</td>
</tr>
</tbody>
</table>
based product in bioreactors, achieving a limit-of-detection of ~1 \( \mu \text{g/mL} \) without need of sample pretreatment [23*].

Specific probes can be immobilized via covalent modification, by affinity interactions (e.g. streptavidin-biotin) or via physisorption on three main categories of solid phases, namely a planar surface such as the surface of a microfluidic channel (passive, liquid moves relative to the surface) [20,23*,24*,25], the surface of a magnetic bead (active, bead can move relative to stagnant liquid) [16,18,22,26], or a porous solid phase (e.g. cellulose paper or cross-linked agarose) [17,19,27,28], providing a substantial increase in surface (i.e. binding sites) to volume ratio. A few assays based on aptamers also report the feasibility of one-step detection without solid phase via change in conformation upon binding to the target molecule [29]. To generate a signal proportional to the amount of captured molecules, optically active labels are most commonly used, such as enzymes that catalyze the conversion of a non-active substrate into a fluorescent or light absorbing compound [22], fluorophores (organic molecules [24*], quantum dots [29]) or bleaching-resistant fluorescent microparticles [17,20]) or Raman tags [16]. Label-free detection can also be achieved by measuring impedance changes induced directly by the captured target molecule [23*]. Moreover, multiplexed detection of different targets in a single sample can be achieved in two main ways, namely using discretely immobilized probes (spatial-resolved) [17,25,27], or resorting to homogeneously immobilized probes in which the captured molecules are discriminated by optically distinct labels (label-resolved) [29]. Finally, the selection of the signal transduction method depends on the device design and target assay sensitivity. For instance, if high sensitivities are required, a photomultiplier tube allowing single photon detection can be used [26], otherwise, a silicon photodiode may be sufficient [18]. On the other hand, if high spatial multiplexing needs to be discriminated, a CCD camera or smartphone camera coupled with a lens system would provide the most suitable performance [19].

One of the few reports of miniaturized devices for protein monitoring in a cell culture process is the Ella® platform, which allows automated quantification of Chinese hamster ovary (CHO) HCPs using a cartridge-based immunoassay coupled with fluorescent detection [24*]. While the price of a microfluidic cartridge to run 72 samples is fairly comparable to a conventional plate-based ELISA kit (only ~1.5x higher), the striking advantages are (i) the reduction of sample volume required (~4-fold) and quantities of antibodies used, (ii) the reduction of assay time (~4-fold) with virtually no manual steps involved, since all required molecules and buffers are self-contained in the cartridge, and (iii) the higher level of reproducibility.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Chip material</th>
<th>Probe</th>
<th>Assay design</th>
<th>Signal transduction</th>
<th>Label</th>
<th>Multiplexing strategy</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A Virus-like particle</td>
<td>PMMA</td>
<td>Planar solid phase</td>
<td>CCD camera and LEF</td>
<td>Fluorescent label</td>
<td>Nanomaterial flourescent dye</td>
<td>[16]</td>
<td></td>
</tr>
<tr>
<td>Human serum</td>
<td>PMMA</td>
<td>Cross-linked agarose</td>
<td>PMT and laser</td>
<td>Nanomaterial flourescent dye</td>
<td>Quantum dots</td>
<td>[20]</td>
<td></td>
</tr>
<tr>
<td>Cancer biomarkers</td>
<td>PDMS</td>
<td>Cross-linked agarose</td>
<td>PMF and laser</td>
<td>Label-free</td>
<td>Nanomaterial flourescent dye</td>
<td>[23*]</td>
<td></td>
</tr>
</tbody>
</table>

While most of the listed microfluidic immunoassays are applied in the field of point-of-care diagnostics, their translation and adaptation to at-line monitoring of relevant protein analytes in cell culture are still in the early stages. The potential of miniaturized devices specifically related to protein quantification (PoQ) and impurities in bioprocesses are highlighted with (*). NA – Not Applicable.

Table 1 (Continued)
Capillary electrophoresis and microchip electrophoresis

CE offers different separation modes that exploits differences in for example, charge, size, hydrophobicity or stereospecificity of molecules to achieve separation. The separation is performed in narrow fused-silica capillaries filled with background electrolyte (BGE) under the influence of an applied voltage. Major advantages with CE are the high separation efficiency, short analysis time, and negligible waste generation, while the main disadvantage is the loss in sensitivity due to the narrow capillaries, that is, short detection light path. However, this can be overcome by pre-concentrating samples before analysis, or increasing the detection sensitivity [30]. Despite all these advantages, CE has not yet been extensively used in PAT. One reason to this could be that CE is not as established as HPLC.

During the recent decades, further development to reduce the size of CE has led to the advancement of microchip CE (MCE), which is much more compatible with PAT [31,32]. The microchips can be fabricated using several materials for example, silicon wafers, polymeric substrates, paper, glass, or quartz, with various designs of the separation channels [33]. A comparison of instrument configuration between CE and MCE can be seen in Figure 3. MCE possesses the same advantages as conventional CE together with higher throughput due to the even faster analysis, integration capabilities for sample preparation, separation, and detection. A challenge using MCE is the shorter available separation length when analyzing complex samples containing a high number of analytes. Still, MCE requires a smaller volume of chemicals per analysis than a CE (approx. 20 times less), and the cost per run could be estimated to a factor of 20 less than for HPLC. The possibilities to design fully automated systems for direct coupling to bioreactors are promising and MCE increases portability due to its small size, potentially allowing in-line analysis. Several commercial chip systems for automated analysis of for example DNA, RNA, and proteins are available from for example, Agilent, BioRad, Perkin Elmer, and Shimadzu. The price for such systems is roughly 15,000 €. These systems are, however, developed for application specific analysis, while generally, electrophoresis as a separation technique is much more versatile.

CE has been applied for the analysis of a wide variety of compounds, including small ions, amino acids, carbohydrates, vitamins [34], lipids, nucleic acids, proteins, and even whole cells [35]. In Table 2, examples of the utilization of CE for analysis of samples from bioprocesses or substances relevant for this application are listed, reflecting an increased interest for on-line/at-line monitoring. For some analytes and techniques, detection at nanomolar levels can be achieved (see Table 2), which is below the lower concentration range for several compounds given in Table S1. Direct fluorescence detection is often applied since it enables high concentration sensitivity, but typically requires analyte derivatization. For PAT, sample preparation such as derivatization has to be fast, simple and amenable to automation. That implies

Figure 3

Conceptual workflow for analysis of derivatized amino acids from a mammalian cell culture bioreactor using (a) capillary electrophoresis, or (b) microchip capillary electrophoresis system.
that elevated temperatures and supplementary operations such as shaking, ultrasound and centrifugation should be avoided.

Analyte derivatization can be avoided using capacitive coupled contactless conductivity detection (C1D) or MS detection, however the latter notably increases the instrumentation complexity. Alhusban et al. developed an automated on-line monitoring of glucose, glutamine, leucine/isoleucine and lactate in Jurkat human T lymphocyte cell culture [36]. In another contribution, the consumption of serine, tryptophan, proline and valine in *Escherichia coli* culture was quantified using MCE-high pressure MS within 3 min analysis time [37*].

Recently, there has been an increased interest in using CE for analysis of mAb glycosylation and charge heterogeneity (Table 2). Although CE-MS is extensively used [38*], other alternatives have also been presented. For glycosylation characterization, CE with laser induced fluorescence (LIF) could be used after derivatization [38*,39]. The use of capillary gel electrophoresis (CGE) [39], introduces separation regarding size and shape, a benefit that also could be utilized for aggregate analysis [40]. The CE mode capillary isoelectric focusing (CIEF) is often chosen for charge profiling, together with either UV detection [41], or imaging [42]. MCE for mAb analysis has been only occasionally reported (e.g. Ref. [43]); and a combination of imaging CIEF with MS

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample</th>
<th>Application</th>
<th>Sample preparation</th>
<th>Technique</th>
<th>LoD</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 AA, lactic acid</td>
<td>Cultivation media</td>
<td>Monitoring of analytes during cultivation of <em>L. helveticus</em> D75 and D76 strains</td>
<td>Centrifugation, filtration, frozen to –20°C before analysis, dilution</td>
<td>CE-UV</td>
<td>2–27 µM</td>
<td>[45]</td>
</tr>
<tr>
<td>19 AA, taurine, Ala-Gln</td>
<td>Cultivation media</td>
<td>Determination of the metabolic activity of human embryos</td>
<td>Protein precipitation, centrifugation, derivatization</td>
<td>CE-LED induced F</td>
<td>12.6–39.3 nM</td>
<td>[46]</td>
</tr>
<tr>
<td>8 AA</td>
<td>Cultivation media</td>
<td>Monitoring of analytes released by the <em>C. glutamicum</em> in microbial fuel cell</td>
<td>Microwave aided derivatization</td>
<td>High speed CE-LIF</td>
<td>10–60 nM</td>
<td>[47]</td>
</tr>
<tr>
<td>Glucose, Gln, Leu/Ile, lactate</td>
<td>Suspension cultures</td>
<td>Monitoring of analytes during cultivation of Jurkat cells</td>
<td>Online sampling, cell density measurement, and filtration</td>
<td>CE-C5D</td>
<td>0.17–0.32 µM</td>
<td>[36]</td>
</tr>
<tr>
<td>Fructose, galactose, glucose, lactose, sucrose</td>
<td>Standards</td>
<td>MCE separation of carbohydrates</td>
<td>NA</td>
<td>MCE-C5D</td>
<td>150–740 µM</td>
<td>[48]</td>
</tr>
<tr>
<td>20 AA</td>
<td>Cultivation medium</td>
<td>Monitoring consumption of analytes during cultivation of <em>E. coli</em></td>
<td>Centrifugation, filtration, dilution</td>
<td>MCE-MS</td>
<td>NA</td>
<td>[37*]</td>
</tr>
<tr>
<td>Recombinant proteins</td>
<td>Humanized IgG1k expressed in murine suspension</td>
<td>Interlaboratory validation of the methods for analysis of N-glycosylation in mAbs, and determination of consensus medians for the glycosylation of the Humanized IgG1 mAb produced in NS0 cells</td>
<td>Cell lysis, commercial assay kit</td>
<td>MCE-LIF</td>
<td>10 pg/µL</td>
<td>[57]</td>
</tr>
<tr>
<td>mAb glycosylation</td>
<td>hlgG1 and human serum samples</td>
<td>Serum sample preparation protocol that is scalable up to dozens of µLs of serum sample</td>
<td>Magnetic bead-based glycoprotein trapping, protein denaturation and digestion, vacuum centrifugation, derivatisation</td>
<td>CE-LIF, CE-MS</td>
<td>NA</td>
<td>[39]</td>
</tr>
<tr>
<td>mAb aggregates</td>
<td>mAb-1, mAb-2</td>
<td>Optimization of a CE method for protein therapeutics</td>
<td>Denaturing of proteins</td>
<td>CGE-UV</td>
<td>NA</td>
<td>[40]</td>
</tr>
<tr>
<td>mAb charge profiling</td>
<td>Drug products, MabThera™, Humira™, Remicade™</td>
<td>Distinction of charge variants based on their different pls for mAbs therapeutics</td>
<td>Digestion, spin filtration, centrifugation</td>
<td>CIEF-UV</td>
<td>NA</td>
<td>[41]</td>
</tr>
<tr>
<td>mAb charge heterogeneity</td>
<td>IgG1 anti-VEGF, mAb-1, mAb-2</td>
<td>Interlaboratory validation of mAb analysis method</td>
<td>Dilution, centrifugation</td>
<td>CIEF-UV</td>
<td>0.9–6.9 µg/mL</td>
<td>[42]</td>
</tr>
<tr>
<td>mAb charge variant</td>
<td>Trastuzumab, biosimilar mAb</td>
<td>Development of a prototype integrated CIEF-MS system</td>
<td>Reconstitution, desalting by spin filtration</td>
<td>MCE-CIEF-MS</td>
<td>&lt;1% of mAb mass</td>
<td>[44*]</td>
</tr>
<tr>
<td>mAb glycan profiles</td>
<td>Cultivation medium</td>
<td>Analysis of glycan species</td>
<td>Centrifugation</td>
<td>MCE-LIF</td>
<td>NA</td>
<td>[43]</td>
</tr>
</tbody>
</table>
has been presented for identification of charge variants [44].

In our opinion, CE or, in particular, MCE offers a high potential for at-line monitoring to aid manufacturing process control and optimization. In addition, several of the sample preparation actions listed in Table 2 could potentially also be performed in a microfluidic setup integrated with analysis.

**Perspectives**

In view of the complexity of a mammalian cell culture, today’s monitoring of the usual parameters represents only the emerged tip of the iceberg. Miniaturization of analytical methods such as reviewed here opens up for fast information about metabolites and proteins. This can be instrumental for process development and manufacturing robustness, by bringing solutions to items (i)–(vi) listed in the Introduction; deeper mechanistic understanding, control of the culture component, solutions for analytical challenge, and booster of mathematical modelling. Together with automation and sample preparation using microfluidics or liquid handling, these techniques enable PAT at reduced cost and complexity in comparison with sophisticated instrument such as MS. Furthermore, sample volumes required are very small, thus supporting the ideal of minimal invasiveness to reduce the risk of process perturbations and contamination. Ideally, the miniaturized analytical technologies are integrated to the cultivation process as modules harboring one or several multiplexed quantification tool(s) (Figure 1). Modularity enables flexibility in function of the needs and easy inclusion of new detection methods. Here the very small size is highly suitable for disposable equipment, alleviating cleaning and carry-over issues. Moreover, a database of the large monitoring information, for example, monitored information database (MID), also connected to control actuators, is necessary.

Finally, beyond manufacturing of biopharmaceuticals, these tools are highly relevant for the production process of Advanced Therapy Medicinal Products (ATMPs), with similar needs in terms of detection of metabolites and even larger needs for protein detection such as growth or stimulation factors. Automated monitoring can be instrumental for autologous productions where the multiplicity of individual cultures appeals for streamlined controls.

**Conflict of interest statement**

Nothing declared.

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**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.copbio.2021.06.018.

**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest


By using microchip capillary electrophoresis for analysis of product quality during E. coli fermentation, the authors have shown that implementing at-line analyses from multi-well plate format using liquid handling stations is possible.


The authors report the development of an aptamer-based affinity assay in a microfluidic chip for in-line monitoring of a therapeutic Fab fragment. The label-free detection was based on electrochemical impedance spectroscopy (EIS) and the bioreactor samples could be directly measured without any pre-treatment step.


An automated instrument that utilizes microfluidic cartridges for Chinese hamster ovary (CHO) host cell protein (HCP) quantification is reported. The miniaturized immunoassay uses a fluorescent probe for detection and provides results comparable to a traditional sandwich ELISA in 96-well plates using less sample volume and reduced assay time.


An interlaboratory study including universities, biopharma companies, government agencies and industries worldwide where glucan distributions of the primary sample of NISTmAb using several analytical methods was reported to determine a community-based consensus median.


The authors use a novel approach to image a capillary electrophoretic focusing microfluidic system coupled to a mass spectrometer for acquisition, quantitation, and identification of highly resolved intact mAb charge isoforms along with their critical N-linked glycan pairs.

