

Cite this: *Chem. Sci.*, 2026, 17, 2028

# The progress of the cyclic strategy in separation and detection

Baisen Chen, Ziwang Liu, Xiaoshuai Yang, Xiaolu Zhou, Ling Xia and Gongke Li \*

Analytical chemistry is undergoing a transformation from the traditional linear model to a cyclic model, driving separation and detection technologies toward greater efficiency and sustainability. The core lies in introducing the temporal dimension into the separation and detection process through the cyclic strategy. This review discusses the implementation of the cyclic strategy in separation and detection technologies, beginning with an explanation of two fundamental modes including macroscopic flow cycling and microscopic reaction cycling, along with an analysis of the energy and key device requirements. Furthermore, it explores the application of the cyclic strategy in sample preparation, chromatographic separation, electric field-driven separation, as well as in spectrum, electrochemical and mass spectrometry detection. Typical cases demonstrate that by incorporating the temporal dimension, the cyclic strategy significantly enhances separation efficiency, detection sensitivity, information dimensionality, dynamic monitoring capabilities and process sustainability within confined spaces and limited resources. Then the review outlines future challenges and potential directions for cyclic strategies in separation and detection, offering forward looking perspectives for further research. This review aims to clarify that the cyclic strategy represents not merely a collection of technical methods, but a methodological framework that leverages spatiotemporal synergy to address future challenges in complex analytical systems.

Received 23rd October 2025  
Accepted 26th December 2025

DOI: 10.1039/d5sc08208k

rsc.li/chemical-science

## 1. Introduction

From molecular motion in the microscopic world to celestial motion in the macroscopic universe, circulation is a fundamental law that exists universally in nature. In recent years, the concept of cycle has been increasingly integrated into chemistry fields, giving rise to transformative approaches in sustainable chemistry. Since Keijer *et al.* proposed the 12 principles of circular chemistry in 2019,<sup>1</sup> the integration of green chemistry, circular economy and sustainable development concepts has rapidly gained widespread recognition. Subsequently, Mohan *et al.* introduced intelligent monitoring and digital twin technology into the circular chemical system,<sup>2</sup> promoting the transition from laboratory-scale to industrial-scale closed-loop production. In this context, Slootweg further integrated the principles of green chemistry with the sustainable design framework in a systematic manner, providing theoretical support for the management of the entire life cycle of chemical

products.<sup>3</sup> Parallel to these developments, circular mechanisms have permeated analytical chemistry. In 2024, a concept termed circular analytical chemistry was introduced.<sup>4</sup> This emerging paradigm integrates circular economy principles, sustainable chemistry, and green development goals. Separation and detection technologies are core tools in analytical chemistry. Accurate and efficient separation and detection methods are vital for both fundamental research and industrial application. In this review, we explore the developments of various separation and detection technologies involving cyclic strategies in analytical chemistry from another perspective. We systematically summarize research progress in the application of cyclic strategies for separation and detection from 2014 to 2025 and provide an outlook on future development paths. The aim of this review is to elucidate how cyclic strategies can drive separation and detection technologies toward greater efficiency and sustainability.

The core mission of analytical chemistry lies in achieving accurate and sensitive analysis of target substances within complex systems. However, the traditional analytical model has long relied on linear or single-pass operation procedures, with

School of Chemistry, Sun Yat-sen University, Guangzhou, 510006, China. E-mail: cesgkl@mail.sysu.edu.cn

Baisen Chen is a PhD candidate at Sun Yat-sen University. Her research interest focuses on cyclic chemiluminescence methods for the rapid detection of targets in complex samples.

Ziwang Liu is a PhD candidate at Sun Yat-sen University. His research interest focuses on spectral analysis of trace targets in complex samples.



samples undergoing only one time processing in the separation or detection units. While straightforward, separation efficiency and detection sensitivity enhancement in such models often comes at the cost of increased reagent consumption, expanded instrument scale, and higher energy consumption, leading to inefficient utilization of space and resources, progressive limitations in sensitivity enhancement, and growing environmental burdens. In light of the global consensus on green and sustainable development, overcoming the limitations of traditional linear analytical models and developing novel methodologies and strategies that harmonize analytical performance with resource efficiency has become a critical challenge in advancing analytical chemistry. The emergence of cyclic strategies is driving new breakthroughs in analytical chemistry. The core innovation of cyclic strategies lies in incorporating the temporal dimension into the design of separation and detection processes. By establishing (semi)closed cyclic pathways, it enables samples, reagents, separation media or active sites to undergo multiple rounds of cycling within the system, thereby transforming conventional single use linear processes into intelligent, iteratively optimized procedures. This model shift promotes a transition from static reliance on physical space and chemical reagents toward the systematic utilization of spatio-temporal synergy and dynamic process control.

Briefly, in separation processes, the cyclic strategy employs the trading time for space mechanism, extending effective separation paths or increasing interaction frequency within confined device spaces, thereby significantly enhancing separation efficiency. For instance, cyclic pressurization extraction

utilizes alternating gas pressure to drive cyclic solvent penetration through the material matrix,<sup>5</sup> thereby enhancing extraction efficiency and target compound yield. Twin-column continuous chromatography employs a multi-column parallel configuration and flow-path switching technology, to enable directional cycling of incompletely separated target components between columns,<sup>6</sup> significantly improving utilization and separation performance of chromatographic systems. Cyclic ion mobility spectrometry (cIMS) achieves synchronous enhancement of separation efficiency and resolution through electric field-driven ion cycling that cumulatively amplifies mobility-based differentiation.<sup>7</sup> These examples collectively demonstrate how the cyclic strategy, by introducing the dimension of time, liberates the separation efficiency from its dependence on fixed physical dimensions and transforms it into a process that can be dynamically controlled. In detection processes, it leverages the introduction of the temporal dimension through cyclic signal amplification and dynamic monitoring, achieving exponential improvements in detection sensitivity, information dimensionality and signal-to-noise ratio while maintaining existing reagent consumption levels. For instance, nucleic acid amplification (NAA) typically achieves accurate detection of low-concentration target substances through cyclic amplification reactions.<sup>8</sup> In cyclic chemiluminescence (CCL), multistage signals can also be gradually generated by continuously consuming reactants through catalysis or redox reactions.<sup>9</sup> Cyclic Voltammetry (CV) drives reversible redox reactions through periodic potential scanning, enabling the cyclic regeneration of signals at the electrode

*Xiaoshuai Yang is a PhD candidate at Sun Yat-sen University. His research interest focuses on the fluorescence spectrometry method for the rapid detection of trace targets in complex samples.*

*Ling Xia received her PhD degree in 2014 from the University of Wyoming, USA, under the supervision of Prof. D. Dutta. After postdoctoral training at the University of Wyoming, she joined the Sun Yat-sen University of China, as a research associate in Prof. Gongke Li's group in 2017. Her research focuses on separation science, sample preparation and micro/nanofluidic instrumentation.*

*Xiaolu Zhou is a master's degree candidate at Sun Yat-sen University. Her research interest focuses on the cyclic cataluminescence method for rapid detection of targets in complex samples.*

*Gongke Li obtained her PhD in Analytical Chemistry from Sun Yat-sen University in 1992. She then joined Sun Yat-sen University and has been a professor since 2000. Her major research interests are focused on sample preparation techniques, analytical techniques for trace analysis of complex samples, such as food, environmental, and biological samples, cyclic techniques in separation and detection, and coupling devices for online analysis. She is currently the Associate Editor of Journal of Chromatography A. She is also a member of the editorial boards of Microchimica Acta. She is currently a permanent scientific committee member of the International Symposium on High Performance Liquid Phase Separation. She was awarded the Chinese Women's Analytical Chemist in 2015. She was named one of the 2016 Power List and the Top 50 Most Influential Women in the Analytical Sciences. She was also named one of the 2019 and 2021 Power List and the Top 100 Most Influential Analytical Scientists in the World by the Analytical Scientist.*



surface. This approach enhances detection sensitivity, provides information on reaction kinetics, and supports real-time monitoring.<sup>10</sup> Moreover, the cyclic strategy inherently aligns with green chemistry principles, offering a viable technical pathway towards sustainable development in analytical chemistry through resource recycling. In addition, separation and detection technologies driven by the cyclic strategy have many similarities in terms of fluid handling, integration and real-time performance, and often complement each other in overall analytical systems to form an all-in-one solution.<sup>11</sup> For example, a microfluidic system that integrates separation and detection can effectively reduce reagent consumption and manual operation time.<sup>12</sup> This integrated strategy allows separation and detection to achieve qualitative leaps in efficiency and accuracy.

While previous reviews have focused on individual aspects such as green chemistry, continuous-flow technologies, or specific cyclic methods (such as chromatography or voltammetry),<sup>13,14</sup> a unifying theoretical framework that reveals the common design principle behind these disparate techniques is still lacking. This review bridges this gap by proposing the cyclic strategy as a methodology that transcends conventional linear modes. Its central contribution lies in introducing the temporal dimension *via* the cyclic strategy, which breaks the physical space or resource constraints, and how the cyclic strategy synergistically resolves the long-standing conflict between different analytical performances (separation efficiency, sensitivity, information dimensionality and green sustainability), paving a new path for efficient and environmentally benign analytical chemistry.

This review aims to systematically summarize recent advances in the cyclic strategy for separation and detection, while outlining future development pathways. Beginning with the implementation of the cyclic strategy, the review explains two fundamental operational modes including macroscopic flow cycling and microscopic reaction cycling, and analyzes the energy and device

requirements. Subsequently, it focuses on representative applications of the cyclic strategy in separation and detection processes, covering key techniques including sample preparation, chromatographic separation, electric field-driven separation, as well as spectrum detection, electrochemical detection, and mass spectrometry detection (Fig. 1), and delves into the essential contributions of these technologies based on the cyclic strategy for enhancing system selectivity, sensitivity, stability, accuracy, automation, application scope and sustainability. Finally, the review addresses current challenges and proposes future research directions, offering new perspectives for advancing the development of green, intelligent and highly efficient analytical methodologies. We anticipate that these discussions not only present the technical framework of the cyclic strategy, but also inspire further exploration of spatiotemporal synergistic models in analytical chemistry, thereby promoting the broader application and development of this innovative approach across diverse domains.

## 2. Cyclic strategy implementation

### 2.1 Cyclic mode

The implementation of the cyclic strategy in analytical chemistry is primarily based on two core modes, flow path cycling and reaction cycling. The two approaches advance synergistically from the dimensions of macroscopic system control and microscopic molecular interactions, respectively, collectively constructing a complete cyclic analysis technology system.

Flow path cycling mode is grounded in equipment level path cycling. By constructing (semi)closed precision fluid paths and combining them with the precise coordination of fluid control components such as pumps and valves, this configuration enables the cyclic flow of samples or reagents within the system, effectively enhancing process efficiency through external flow paths. For example, in the CCL system, through the ingenious design of the flow path, the reaction system can achieve a dynamic cycle reaction mode where the carrier fluid and the sample can interact.<sup>15</sup> Through the accumulation of the cycle number, it is equivalent to extending the separation path, amplifying retention behavior differences between components and significantly improving separation resolution. The development of microfluidic technology has further expanded the applicability of flow path cycling, enabling controlled and efficient cyclic separation at the microscopic scale through the construction of complex microchannel networks on chips.<sup>16</sup> The advantage of this mode is in the ability to prolong the residence time of samples in the system and increase interaction frequency, significantly enhancing separation efficiency and process selectivity. It is noteworthy that integrating microfluidics with detection methods demonstrates significant advantages. Wang *et al.* proposed a novel dynamic CO<sub>2</sub> sensing method based on cyclic microfluidic fluorescence response (Fig. 2).<sup>17</sup> By constructing a gas-liquid cyclically coupled microfluidic chip, closed-loop fluorescent reagent cycling was achieved, significantly enhancing system sustainability. The study established a microflow permeation model that elucidated the quantitative relationship between the CO<sub>2</sub> permeation flux and concentration/flow rate, and introduced



Fig. 1 Schematic illustration for the implementation of the cyclic strategy in separation and detection.



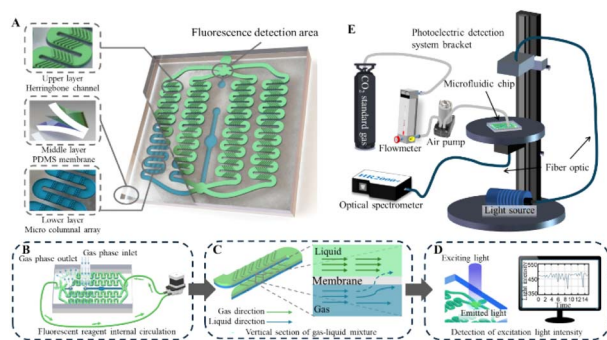


Fig. 2 High-precision, dynamic CO<sub>2</sub> sensing via cyclic microfluidic fluorescence: (A) a cyclic chip, (B) flow directions, (C) phase mixing, (D) intensity detection and (E) a full system platform.<sup>17</sup> Reprinted and adapted with permission from ref. 17. Copyright 2024 Elsevier B.V.

a strategy to dynamically adjust gas–liquid cyclic operations according to concentration levels, which improved the overall sensitivity across the concentration range of 1–10<sup>6</sup> μL L<sup>-1</sup>. This method achieves a response time of only 2 seconds, and the system can operate continuously and stably for 40 weeks.

Reaction cycling mode focuses on cyclic mechanisms at the molecular level, achieving continuous signal amplification or efficient transformation of target substances by designing self-sustaining or externally triggered chemical reaction pathways. This mode relies on the intrinsic characteristics of the reaction system. For instance, in enzymatic cyclic amplification, a single enzyme molecule can catalyze hundreds to thousands of reactions, achieving exponential signal growth through cascade networks.<sup>18</sup> NAA drives exponential template replication through temperature cycling.<sup>19</sup> Cyclic assembly based on molecular recognition gradually enhances the signal response through layer-by-layer alternating binding on sensor surfaces (Fig. 3).<sup>20</sup> The core value of this mode lies in transforming limited recognition events into continuous signal output, and it can effectively overcome the sensitivity bottleneck caused by the consumption of reactants or the limited signal output in a single reaction system.

Although the two cyclic modes differ in their implementation mechanisms, flow path cycling relies on external fluid path design and system control, while reaction cycling is based on

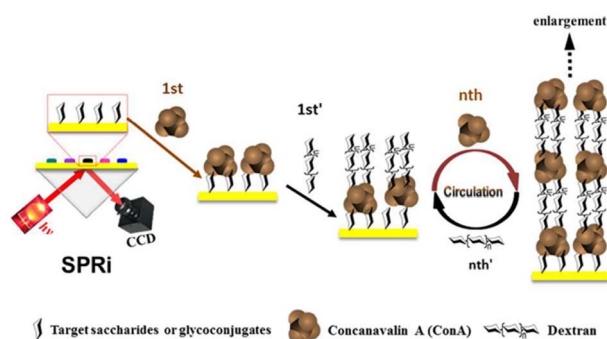


Fig. 3 Scheme of the signal stepwise amplification in surface plasmon resonance imaging detection of saccharides and glycoconjugates.<sup>20</sup> Reprinted and adapted with permission from ref. 20. Copyright 2016 American Chemical Society.

intrinsic molecular interactions and reaction pathway design. But they both significantly enhance the comprehensive performance of analytical methods in terms of separation efficiency, sensitivity and sustainability by introducing the temporal dimension and constructing cyclic processes under conditions of limited physical space and reagent consumption. This reflects the unified concept of the cyclic strategy for optimizing analytical performance.

## 2.2 Energy introduction

The stable operation and performance optimization of cyclic analysis systems rely on effective energy input and precise regulation. As the core driving force of the cyclic strategy, energy plays a decisive role in material transformation, reaction regulation, and separation detection processes, not only providing operational power but also directly influencing reaction rates, mass transfer efficiency, and process selectivity. Based on the forms and mechanisms of action, energy inputs can be categorized into two main types, primary driving forces (mechanical, electrical and chemical energy) that directly power the system, and auxiliary fields (sound, magnetic and microwave) that enhance process efficiency. From chemical bond reorganization at the molecular level to material cycling in macroscopic systems, directed energy input provides the fundamental basis for constructing controllable cyclic systems. Different energy forms exhibit distinct characteristics, mechanical energy offers wide applicability, electrical energy enables high precision control, and chemical energy provides excellent selectivity, while auxiliary fields further enhance system performance through synergistic effects. This energy matter synergy significantly reduces the resource consumption of traditional linear processes, driving a shift in research focus from high energy consumption unidirectional operations to intelligent cyclic systems, thereby opening important technical pathways for establishing resource saving and environmentally sustainable development models.

**2.2.1 Driven force.** The effective operation of cyclic processes relies on the sustained support of specific driven forces, which not only provide the necessary energy input to the system but also profoundly influence its overall performance and process efficiency. As a fundamental form of driven force, mechanical-driven force converts mechanical energy into directional fluid motion through mechanical equipment such as pumps and centrifuges. In a CCL system,<sup>21</sup> the flow and mixing of reagents are typically regulated by pumps (Fig. 4), enabling the chemical reaction to occur and generate a measurable light signal. Mechanical energy is introduced in the form of pressure, specifically using the pump valve apparatus, to facilitate the circulation of reactants within the pipeline. This circulation is crucial for achieving efficient CCL reactions. This type of driving method is characterized by its simple operation and wide applicability. However, there is still room for improvement in terms of control accuracy and energy efficiency.

Electrical-driven force utilizes electric potential drop to achieve precise regulation of charged particles or fluids. In the field of electric-driven separation, asymmetric electric fields can guide ions to cyclically migrate along circular paths, while



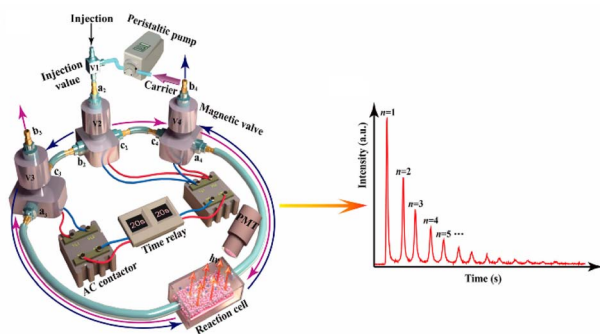


Fig. 4 Schematic of a gas-phase CCL system regulated by pump valves.<sup>21</sup> Reprinted and adapted with permission from ref. 21. Copyright 2014 American Chemical Society.

symmetric electric fields are employed to achieve free flow electrophoresis separation. In electrochemical cyclic detection, periodic scanning of electrode potential drives reversible redox reaction cycling, enabling in-depth analysis of electron transfer mechanisms. For example, a polyaniline sensor was developed to monitor the dynamic changes in acetaldehyde during the oxidation process of wine in real time using the CV method. The polyaniline-modified electrode undergoes reversible redox reactions within a specific potential range, and its current response is linearly related to the concentration of acetaldehyde.<sup>22</sup> The notable advantages of electrical-driven force lie in its exceptional control precision, rapid dynamic response capability, and precise manipulation characteristics at the microscale.

Chemical energy drive relies on the energy released during chemical reactions to propel cyclic processes. Enzymatic reactions represent a typical example of chemical energy drive, achieving efficient cyclic reaction progression through the specific binding and catalytic action between enzymes and substrates. For instance, Hu *et al.* developed a dual-mode biosensor based on enzymatic catalysis and chemical redox cycling, integrating photoelectrochemical and colorimetric analysis, for the highly sensitive detection of heat shock proteins. The introduction of the chemical redox cyclic strategy significantly improves the signal amplification efficiency (Fig. 5).<sup>23</sup> This type of driving method demonstrates excellent selectivity and mild reaction conditions, but its application

effectiveness is constrained by the intrinsic characteristics and operational stability of the reaction system.

**2.2.2 Assistant field.** Based on the primary driven forces, the introduction of multiple physical fields as auxiliary regulation means can further enhance the comprehensive performance of cyclic systems. Through synergistic interactions with the main driven forces, these auxiliary fields improve mass transfer, promote reactions, and enhance selectivity particularly at the microscale.

Magnetic field assistance utilizes the magnetic force to control the movement and behavior of magnetic particles. Magnetic energy can be used to assist various separation processes and highly sensitive detection methods. For instance, a magnetic separation-assisted self-circulating primer extension reaction was used to detect miRNA-31 in saliva.<sup>24</sup> Through the simple magnetic separation technology, the primers can be selectively separated and enriched from complex saliva samples, effectively remove the interfering matrix, significantly improve the local effective concentration of the primers, then enhance the recognition and capture efficiency between the primers and the hairpin, and improve the amplification efficiency and the sensitivity of the detection. Sound assistance primarily enhances mass transfer processes through cavitation effects. In microfluidic cyclic systems, acoustic field assistance has become an important means to enhance mixing and promote reactions. Zhang *et al.* applied surface acoustic waves in the microchannel. They integrated a single-layer valve for reagent distribution and used the acoustic flow produced by the sound field to assist in achieving rapid and uniform reagent mixing, and the system was used to perform protein crystallization.<sup>25</sup> Microwave assistance accelerates reaction processes through dielectric heating effects.<sup>26</sup> Microwaves can directly act on polar molecules, causing them to vibrate rapidly and generate heat, thereby achieving rapid and uniform heating of the system. In extraction and reaction processes, microwave assistance can significantly shorten processing time and improve reaction efficiency.

The scientific selection and optimal combination of energy forms are the key to implementing an efficient cyclic strategy. The mode of energy introduction depends on the specific technique and the nature of samples. Rational use of energy not only improves separation efficiency and detection sensitivity, but also saves resources and reduces environmental impact during cyclic strategy operations. However, the introduction of different energy forms may impose higher demands on the equipment and operating conditions. When choosing the energy introduction method, it is necessary to balance efficiency, cost and the match of experimental conditions.

### 2.3 Device requirement

In separation and detection, devices based on the cyclic strategy differ from traditional devices that adopt a unidirectional open-loop circuit and aim for endpoint detection in aspects such as flow path design, fluid control, monitoring capabilities and material requirements. The core objective is to achieve efficient circulation of samples or reactants, thereby enhancing both

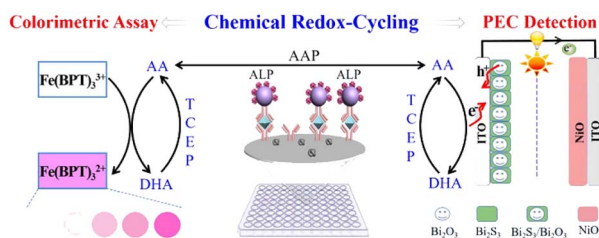


Fig. 5 Scheme of a chemical redox cycling biosensor integrated self-powered photoelectrochemical and colorimetric immunoassay.<sup>23</sup> Reprinted and adapted with permission from ref. 23. Copyright 2025 American Chemical Society.



separation efficiency and detection sensitivity. This section elaborates on the design requirements of the device for achieving efficient cycling.

For the flow path design and fluid control, the device involving circulation can adopt a closed-loop flow path design or supporting continuous or periodic flow functions, enabling samples to recirculate through the sensing units, which can significantly extend the time for mass transfer and reaction, and enhance the processing efficiency of complex samples. The integrated high-precision pump and valve system dynamically regulates the fluid direction, flow rate, and cycling periods to achieve precise control over sample flow behavior and distribution patterns. For example, by connecting pipes end-to-end, chemical substances at different stages periodically flow through the reaction tank; CCL devices are typically driven by gas-phase reciprocating pumps and liquid-phase unidirectional circulation pumps.<sup>27,28</sup> In contrast, a traditional device typically employs a one-way, open-loop flow structure. For example, in flow injection chemiluminescence detection, the sample only passes through the system once. It mostly relies on simple pump and valve systems to maintain stable flow, lacking the complex control capabilities required for circulation. This makes it limited in handling complex systems. In terms of real-time monitoring, the circulation device integrates online sensors, which can conduct real-time tracking and feedback regulation of the component changes during the circulation process. For instance in CV,<sup>29</sup> the high-frequency signal acquisition system can accurately capture the rapid transient changes of current and voltage. By recording the voltage–current relationship, high-resolution volt–ampere curves are generated, thereby providing a crucial basis for the analysis of reaction mechanisms. In contrast, single-experiment methods such as constant potential or constant current chronoamperometry using traditional three-electrode workstations have a measurement mode that is essentially endpoint or offline, lacking the ability for real-time response and process control. For materials, the circulating device requires the contact components to have high corrosion resistance, wear resistance and mechanical stability to withstand the pressure and chemical erosion caused by long-term circulation. Conventional equipment designed

based on a single or limited number of analysis processes pays more attention to chemical compatibility and short-term stability, and has lower requirements for anti-cyclic fatigue performance.

Microfluidics provides an ideal platform for cycle separation and detection through its characteristics of microscale channels, precise fluid manipulation and integrated equipment.<sup>30</sup> Key benefits include precise control of fluid flow, integration and versatility, and low sample consumption and high throughput. Microfluidics has demonstrated unique advantages in the development of circulation separation devices. For example, Shen *et al.* proposed an inertial microfluidic device that utilizes helical channels to achieve high-throughput cell manipulation (Fig. 6).<sup>31</sup> It can achieve the focusing of three different types of cancer cells at high concentrations. Meanwhile, a microfluidic system can be combined with electrochemistry,<sup>32</sup> optics<sup>33–38</sup> and mass spectrometry<sup>39</sup> detection technologies for highly integrated analysis as the main assistive technology. Briefly, microfluidics can be combined with other techniques to achieve multifunctional integration, consolidating various separation and detection steps into one system, realizing an “all-in-one” approach. This effectively breaks through the bottlenecks of the separate-detection system that relies on independent devices and has discrete steps in terms of throughput, integration and sample consumption. Microfluidics with its precise fluid control, efficient separation mechanism and real-time detection capability, is driving the development of separation and detection systems towards a more intelligent and integrated direction. Especially in the development of cyclic analytical devices, the microfluidic platform has demonstrated remarkable technical potential, providing an ideal solution for the on-site rapid analysis of complex samples.

Devices that implement the cyclic strategy necessitate several critical features, such as an accurate fluid control system, an efficient circulation path design and the selection of durable, high-performance materials. Additionally, the device should incorporate real-time monitoring capabilities and flexible configurations to accommodate various separation and detection needs. Collectively, these requirements ensure that samples can be efficiently cycled through the device, leading to enhanced separation efficiency and detection sensitivity. Although the development of devices based on the cyclic strategy has significant advantages in separation and detection, there remains considerable room for improvement in automation and intelligent control in future devices. Enhancing these aspects not only optimizes performance but also increases efficiency and user-friendliness in various applications.

### 3. Cyclic strategy enhanced separation

Separation technology is a cornerstone of analytical chemistry, and has long relied on the traditional linear operation mode, which faces bottlenecks such as high consumption of separation media, low processing efficiency and suboptimal resource

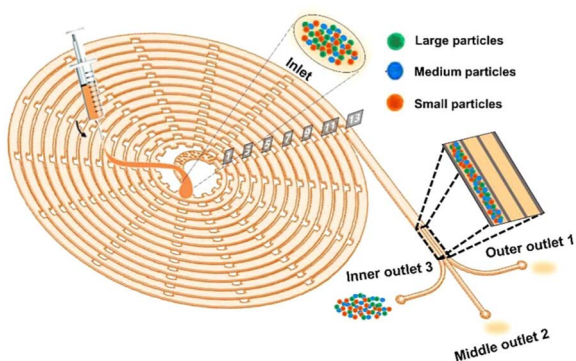


Fig. 6 A scheme of the spiral channel for high-throughput cell manipulation.<sup>31</sup> Reprinted and adapted with permission from ref. 31. Copyright 2024 American Chemical Society.



utilization. The introduction of the cyclic separation strategy has effectively overcome the aforementioned limitations by transforming single-pass processes into cyclic operations within closed systems, leading to significant enhancement in separation efficiency, resolution, and sustainability of the process. This section examines innovations and applications of the cyclic strategy across three major separation fields, including sample preparation, chromatographic separation, and electric field-driven separation. Covered methodologies range from the classic Soxhlet extraction and its miniaturization and functional improvement, to efficient and green cyclic pressurized extraction, and from the cyclic chromatography technology based on phase-to-phase distribution to advanced methods using electric fields to achieve ion and molecule cyclic enrichment and separation in annular or microfluidic channels (such as cIMS and continuous free-flow electrophoresis (cFFE)). Although these technologies differ in principle and implementation, they all embody the core idea of trading time for space. By introducing a cyclic mechanism in the limited device space, they effectively extend separation paths or increase the number of interactions, thereby breaking through the limitations of traditional linear separation in terms of efficiency, accuracy and resource consumption. This section systematically analyzes the fundamental principles, green advantages, and emerging trends across these cyclic separation technologies, highlighting their shared characteristics and context-dependent applicability, thereby providing a theoretical foundation and practical guidance for high-efficiency, sustainable analysis of complex samples.

### 3.1 Sample cyclic preparation

The sample cyclic preparation technology surpasses the single immersion static mode. At its core, this approach facilitates orderly solvent cycling, continuous renewal, and efficient mass transfer by leveraging cyclic solvent operation within a (semi) closed system, supported by a carefully engineered spatial layout and the application of physical forces such as gravity, temperature gradients, or pressure-driven flow. Operating on the principles of automation and (semi)closed cycles, the technology enables efficient and sustainable extraction of target substances from matrices. It not only significantly enhances extraction efficiency and the degree of automation, but also offers considerable value in supporting green chemistry and sustainable analytical practices.

Soxhlet extraction is a classic cyclic extraction technique named after the German chemist Franz von Soxhlet.<sup>40</sup> It is based on the automatic cyclic process of evaporation, condensation, extraction, suction and reflux, achieving continuous solvent circulation and efficient extraction of target substances. Its ingenious spatial structure layout, suction tube, extraction chamber, condenser tube and flask work together through the synergistic effect of gravity and the temperature gradient, allowing the solvent to circulate autonomously in different functional areas, featuring advantages such as high efficiency, continuous operation, solvent conservation, simple operation and thorough extraction. With continuous technological

innovation, micro-Soxhlet extraction adapts to milligram-level samples through device miniaturization,<sup>41</sup> significantly saving samples and solvents. For example, a method combining micro-Soxhlet extraction with a colorimetric sensor based on  $\alpha$ -cyclodextrin-functionalized silver nanoparticles has been developed for detecting trace chlorpyrifos pesticide in fruits and vegetables.<sup>42</sup> The micro-Soxhlet extraction technique demonstrates significant advantages in this method. It enables miniaturized sample pretreatment with extremely low solvent consumption, allows solvent recovery and reuse, and offers features such as simple operation, real-time visual monitoring, and prevention of cross-contamination. The strategy can also be extended to the field of chiral separation, enabling automated purification and efficient deracemization of enantiomers through cyclic solvent removal and re-addition. For instance, van Dongen *et al.* achieved rapid deracemization of a clopidogrel precursor using a modified Soxhlet apparatus under mild conditions.<sup>43</sup> This method increased the enantiomeric excess of the sample from 10% to 99% within 18 hours. This method demonstrates excellent controllability, strong scalability potential, and promising prospects for producing high-purity chiral compounds under mild conditions.

Based on the innovative concept of dynamic solvent cycling, cyclic pressurization-assisted extraction technology achieves directional solvent flow through precise regulation of the pressure field within a closed system.<sup>44</sup> The core mechanism relies on rapid and continuous gas pressurization–depressurization cycling. During the pressurization phase, solvents penetrate deeply into the microstructure of the material, while instantaneous depressurization causes dissolved gas to violently escape, generating strong shear forces and gas explosion effects that efficiently disrupt the material matrix. This technique involves placing samples and solvents in a pressure-resistant vessel and performing multiple pressure cycles, enabling green, efficient, and automated extraction of target substances at room temperature within minutes. For example, Hong *et al.* developed a cyclic pressurization-assisted extraction method for efficient extraction of pollutants such as polychlorinated biphenyls and polycyclic aromatic hydrocarbons from sediments.<sup>45</sup> Using 10 nitrogen pressure cycles at 1.0 MPa, the method completes extraction from 15 g of sediment in just 15 min. It shows significantly higher extraction efficiency than traditional Soxhlet extraction, offering greater yield, shorter processing time and lower solvent consumption.

The core of sample cyclic preparation technology lies in achieving efficient phase separation and solvent circulation through precisely designed spatial structures. This structural advantage enables effective extension of separation pathways within limited spaces by regulating cyclic parameters, yet it also faces challenges such as enhancing mass transfer efficiency and optimizing complex parameters. Through innovative approaches including optimized spatial configurations, intelligent control algorithms, and miniaturized integrated devices, this technology can achieve a balance between high-efficiency mass transfer and energy-saving sustainability, demonstrating significant value in improving extraction efficiency, automation and environmental friendliness. In the future, with deeper



integration of micro-nano fabrication and artificial intelligence, sample cyclic preparation technology is expected to realize higher precision fluid control and more intelligent process optimization, further expanding its application boundaries in trace analysis, chiral separation, and other cutting-edge fields, thereby providing stronger technical support for green analytical chemistry.

### 3.2 Chromatographic cyclic separation

The core of traditional chromatographic separation efficiency lies in the column efficiency provided by the stationary phase, which is a static spatial resource. However, the cyclic strategy introduces a dynamic temporal dimension. The core of chromatographic cyclic separation technology lies in enabling periodic cycling of the sample within the chromatographic system. By extending the effective path length temporally rather than physically elongating the column, this approach enhances the separation path through the dimension of time, significantly improving both separation efficiency and resolution under constrained hardware conditions. Based on cyclic operation within (semi)closed systems, this technology enables efficient and precise isolation of target substances from complex mixtures. It not only substantially increases purification capacity and automation, but also offers considerable advantages in terms of sustainability and a green methodology. The typical applications include cycling preparative chromatographic techniques such as twin-column continuous chromatography and recycling counter-current chromatography (CCC), which respectively demonstrate innovative applications of the cyclic strategy across different separation principles and system architectures.

Briefly, recycling preparative chromatography builds upon traditional liquid–solid adsorption principles, by allowing the sample to undergo multiple cycles within a chromatographic column to amplify the minute retention differences, and it is an effective strategy for achieving high-resolution separation, especially suitable for purifying components with extremely similar properties. Twin-column continuous chromatography is a high efficiency preparative strategy based on multi-column synergy and dynamic flow path switching.<sup>46</sup> The core principle lies in transforming traditional single column batch operations into a continuous multi-column parallel process, enabling target components to cyclically migrate between columns, thereby significantly enhancing chromatographic system utilization and separation efficiency. Represented by technologies such as N-Rich<sup>47</sup> and multi-column counter-current solvent gradient purification (MCSGP),<sup>48</sup> this approach integrates four stages such as loading and elution, cyclic enrichment of target substances, removal of interfering components and high-resolution elution. It enables selective enrichment of trace impurities while effectively eliminating main product interference, demonstrating significant advantages in the purification of oligonucleotides, chiral drugs and biomacromolecules. Compared with traditional batch chromatography, it achieves orders of magnitude improvements in target purity and concentration with shorter processing times and less solvent

consumption, while minimizing manual operation intensity. This provides a scalable and efficient solution for precise separation. For instance, Weldon *et al.* presented a study on the efficient enrichment and purification of angiotensin II peptide impurities using N-Rich twin-column continuous chromatography.<sup>49</sup> Two application examples demonstrated its significant advantages. In example 1, the technique achieved simultaneous enrichment of multiple impurities, increasing production efficiency by more than 9-fold compared to analytical high-performance liquid chromatography. In example 2, targeting a critical impurity co-eluting with the main product, it enabled the preparation of 1 mg of the impurity at 88% purity, with a 79-fold increase in production efficiency and a 69-fold reduction in solvent consumption. The study demonstrates that N-Rich technology effectively resolves the conflict between resolution and loading capacity inherent in traditional chromatographic methods for impurity separation, providing a scalable solution for the efficient preparation of peptide impurities.

In contrast, CCC represents a fundamental innovation by eliminating the solid stationary phase. Instead, it relies solely on the differential partitioning of solutes between two immiscible liquid phases. This approach overcomes key limitations such as irreversible adsorption, sample degradation, and difficulties in high-load purification. The technology employs long helical column tubes, establishing a unidirectional fluid dynamic equilibrium under the influence of gravity or centrifugal force fields. The sample cycles continuously through this coil, undergoing thousands of partitioning steps. It is worth emphasizing that the recycling elution mode in CCC further expands its separation capabilities.<sup>50</sup> This mode enables multiple cycles of the sample within a limited column length, effectively simulating a longer separation path in a single instrument. Consequently, it significantly enhances both resolution and peak capacity without increasing hardware complexity. This cycling mechanism is particularly suitable for the efficient purification of complex and separation-sensitive systems, such as chiral enantiomers<sup>51</sup> and natural products.<sup>52</sup> For instance, Zhang *et al.* used recycling high-speed counter-current chromatography (HSCCC) to separate amlodipine besylate (ADB) enantiomers (Fig. 7).<sup>53</sup> Cyclic elution gradually improved the separation efficiency, and the enantiomer purity exceeded 97.5% through five cycles. He *et al.* proposed a method called unlimited recycling counter-current chromatography (URCCC),<sup>54</sup> which integrates concentration technology with multi-stage recycling elution to address the limitations of conventional CCC, such as restricted recycling cycles and inadequate separation efficiency caused by peak broadening. This approach was successfully applied in the preparative separation of naphthoquinones. Through two rounds of concentration and three stages of recycling, efficient separation of isobutyrylshikonin,  $\beta,\beta$ -dimethylacrylshikonin, and iso-valerylshikonin was achieved, with resolution values reaching 1.38 and 1.26, respectively. The purity of each compound exceeded 98%, with an overall recovery rate of 89.6%. URCCC not only significantly enhanced separation efficiency and theoretical plate numbers but also reduced the dependency on solvent system optimization, thereby expanding the application





Fig. 7 Schematic of the recycling HSCCC for ADB enantiomer separation.<sup>53</sup> Reprinted and adapted with permission from ref. 53. Copyright 2015 Elsevier B.V.

potential of CCC in separating structurally analogous compounds.

Chromatographic cyclic separation technology introduces a temporal dimension by utilizing cyclic mechanisms to extend the effective separation path. Techniques such as twin-column continuous chromatography and CCC significantly enhance separation efficiency and resolution, while the closed-loop system design markedly reduces solvent and energy consumption, embodying the principles of green and sustainable development. However, this technology faces challenges such as band broadening during cycling and limited separation efficiency, particularly when dealing with structurally similar compounds where peak capacity constraints become evident. To address these issues, strategies such as solvent gradient modulation can be applied to generate band compression effects that suppress band broadening, concentration combined with multi-stage cyclic strategies can enhance separation capability, and dynamic monitoring with intelligent switching technologies can optimize cyclic parameters. The future development of this technology will focus on achieving higher separation efficiency, lower solvent consumption and improved system adaptability. Through deeper integration with advanced technologies such as intelligent algorithms and novel materials, it will further expand its application potential in the precise separation of complex systems.

### 3.3 Electric field-driven cyclic separation

Electric field-driven cyclic separation technology achieves significant improvements in separation efficiency and resolution by precisely controlling the cyclic motion of charged particles or fluids within a confined space through the skillful application of electric field forces. This technology primarily includes representative methods such as asymmetric field-driven cIMS and symmetric field-driven techniques like cFFE. By constructing cyclic pathways within limited physical space, these methods transform traditional linear unidirectional separation processes into cyclic operational modes, effectively overcoming the conventional limitations between device size and separation performance. This technological paradigm not only markedly enhances separation performance but also reduces resource consumption through system miniaturization

and process intensification, providing a new technical pathway for efficient and green analysis of complex samples. It represents an important direction in the development of modern analytical technology toward miniaturization, high-throughput operation, and sustainable development.

cIMS utilizes a cycle path to drive ions through multi-cycling.<sup>55</sup> This approach effectively converts the separation path length into a function of the number of cycles, leading to a linear increase in resolution with each cycle. By significantly extending the effective ion migration path without enlarging the physical dimensions of the instrument, cIMS markedly enhances separation precision. Benefiting from the cumulative separation effect of multiple cycles, this method is particularly suited for the high-resolution separation of isomeric ions and species with highly similar structures, which are challenging to distinguish using conventional techniques.<sup>56</sup> In 2019, Giles *et al.* modified the ion mobility separation region to accept a cyclic ion mobility (cIM) device,<sup>57</sup> and combined with mass spectrometry formed a cIMS system. In this work, when conducting 100 cycles of testing on the CIM device using reverse sequence peptides, its movement resolution reached approximately 750, and the resolution will increase with the square root of the number of scans around the device. And through the cIMS system, it is possible to effectively separate three isomeric pentasaccharide species and the 6+ ion of ubiquitin. Benzenberg *et al.* systematically evaluated the use of cIMS for the separation and identification of phosphorothioate (PS) diastereomers in siRNA systems (Fig. 8).<sup>58</sup> The results demonstrate that cIMS effectively resolves diastereomers in short (5 mer) to medium length (9 mer) oligonucleotide systems.

cFFE achieves seamless scaling from analytical to preparative scales and uninterrupted separation by establishing a steady-state closed cycling.<sup>59</sup> Its core advantage lies in the continuous cyclic operation, which effectively avoids the operational interruptions and resource wastage inherent in batch-processing modes, thereby significantly improving material and energy utilization efficiency. Building on this, microfluidic free-flow electrophoresis further leverages miniaturization and integration technologies to overcome traditional heat dissipation challenges.<sup>60</sup> It fully capitalizes on the platform advantages of continuous, label-free operation and online coupling with detection systems, providing an efficient analytical tool for complex biological samples. For example, Xu *et al.* developed an

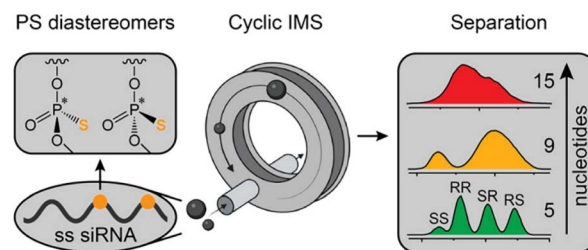


Fig. 8 Schematic of cIMS for phosphorothioate diastereomer separation in siRNA.<sup>58</sup> Reprinted and adapted with permission from ref. 58. Copyright 2025 American Chemical Society.



efficient protein purification method utilizing a micro-free-flow electrophoresis platform combined with a cyclic injection strategy.<sup>64</sup> By cyclic recovery and reinjecting the target proteins from the effluent, they achieved a removal rate of up to 94.7% for high abundance proteins (HSA and IgG), along with a 95.3% recovery rate and an approximately 32-fold purity increase for the low abundance protein (GFP). This strategy effectively handles complex human plasma samples without generating additional liquid waste, significantly enhancing purification efficiency and controllability, demonstrating great potential in proteomics and medical diagnostics.

The electric field-driven cyclic separation technology based on the core design concept of trading time for space, achieves continuous accumulation of separation paths within limited device dimensions, demonstrating systematic advantages of high resolution, high separation efficiency and low consumption. However, the practical application of the technology still faces challenges such as stability in cyclic control, high complexity in system integration and reliability in continuous operation. To address these issues, strategies such as optimizing electric field regulation, advancing microfluidic integration, and incorporating closed-loop control with intelligent monitoring units can enhance the stability and operational controllability of the separation process. In the future, electric field-driven cyclic separation technology is expected to enable more efficient and environmentally precise separation applications in fields such as single-cell omics and online quality control in biopharmaceutical manufacturing.

This section elaborates on various cyclic separation techniques ranging from classical sample preparation to modern high throughput separation. These case studies collectively demonstrate the broad applicability and innovative value of cyclic strategies in separation science. By reconstructing the relationship between time and space, the cyclic strategy realizes the concept of trading time for space. Through the design of cyclic pathways within limited device dimensions, the constraints of physical space are transformed into extensibility in the temporal dimension, significantly extending the effective separation path. This shift breaks through the inherent limitations of traditional linear separation processes in terms of device size and separation precision. Space is no longer the sole constraining dimension in separation processes. By introducing the cyclic strategy, time becomes a key variable that can be exchanged with space to overcome its limitations. The cyclic strategy unifies separation performance with green objectives, exhibiting characteristics such as high automation, (semi) closed operation and excellent reproducibility. They not only significantly enhance separation efficiency and resolution but also substantially reduce resource consumption through solvent recycling and sample miniaturization. Furthermore, continuous operation modes ensure high throughput and reproducibility, laying a solid foundation for intelligent analysis. This strategy promotes a shift in separation instrument design philosophy from scale expansion to efficiency optimization, driving the development of miniaturized, integrated and high-throughput devices. With deep integration of advanced technologies such as microfluidics and artificial intelligence,

the cyclic strategy is guiding separation science toward a new stage of greater precision, efficiency and sustainability.

## 4. Cyclic strategy enhanced detection

Detection technology is a key link in analytical chemistry, aiming to achieve highly sensitive identification and precise quantification of target substances in complex samples. Conventional static detection methods are constrained by limitations such as single-use reagent consumption, insufficient signal stability, and lack of dynamic monitoring capability, leading to high analytical costs and suboptimal reproducibility. The cyclic strategy has been introduced by systematically integrating the temporal dimension into the detection process, enabling signal amplification and in-depth information extraction through cyclic operations. This strategy transcends the static limitations of traditional single measurement approaches by transforming the time variable into a key resource for enhancing detection performance. Under constrained physical space and reagent usage, cyclic pathway design, dynamic signal acquisition, and multi-round information accumulation collectively achieve breakthroughs in three aspects such as exponential growth in signal intensity, expansion of information dimensions, and improved resource utilization efficiency. This progress promotes the evolution of detection capability from being sufficiently sensitive to a higher level characterized by ultrasensitivity and information richness. From spectrum sensing and electrochemical analysis to mass spectrometry, cyclic strategy leveraging their unique ability to regulate temporal dimensions is driving a transformative shift in analytical science from single point static measurement to dynamic process analysis. Although they differ in implementation mechanisms, these technologies share a common core principle, by introducing a cyclic strategy during the detection process, thereby breaking through the limitations of traditional detection in terms of sensitivity, information dimension and resource efficiency. This section systematically introduces cutting-edge applications of the cyclic strategy in spectrum, electrochemical and mass spectrometry detections, with a focus on elucidating their operational mechanisms, performance advantages, and future prospects, thereby providing a theoretical foundation and technical support for the development of next generation analytical detection technologies.

### 4.1 Spectrum cyclic detection

The application of the cyclic strategy in spectrum detection is driving an important transformation in the analytical models. By transforming the conventional static model of single-measurement endpoint detection into a dynamic cyclic reaction-dynamic monitoring mode, spectrum detection technology has achieved remarkable breakthroughs in sensitivity, information dimensionality, and sustainability. From the perspective of technological development, the transformation primarily advances along two key directions. On one hand, the



implementation of cyclic reaction mechanisms enables exponential signal amplification, substantially enhancing detection sensitivity. On the other hand, dynamic monitoring processes facilitate the extraction of multidimensional information from continuous reaction cycling, achieving comprehensive characterization of sample properties. These technologies further exemplify the concept of green analysis through optimized reagent utilization efficiency. In the context of growing demands for high sensitivity and multi parameter detection in analytical science, signal amplification and information extraction strategy based on reaction cycling have emerged as crucial technological paths for overcoming traditional analytical limitations. This section analyzes the fundamental principles and application characteristics of various spectrum cyclic detection technologies, providing a theoretical foundation and technical reference for developing next-generation spectrum detection methodologies.

**4.1.1 Cyclic reaction-signal amplification.** Signal amplification is a critical pathway for overcoming sensitivity limitations in detection systems. Traditional detection methods are limited by the single-use efficiency of the recognition components, making it difficult to achieve effective signal amplification. By designing cyclic reaction paths that enable limited recognition elements to participate in multiple reaction cycling, linear signal responses can be transformed into exponential gains, thereby breaking through the sensitivity barriers of traditional detection approaches. This cyclic amplification strategy not only significantly enhances detection sensitivity but also optimizes reagent utilization through well-engineered reaction pathways. This section analyzes the signal amplification mechanisms based on cyclic reaction paths, focusing on the technical characteristics and performance advantages in achieving highly sensitive detection, thereby providing theoretical guidance and technical reference for the development of novel detection methodologies. Among the numerous signal cyclic amplification techniques, NAA, enzymatic reaction systems, and cyclic amplification based on molecular recognition and assembly serve as representative examples.

Typically, NAA is a method for amplifying a specific DNA or RNA sequence through multiple cyclic reactions.<sup>62</sup> The cyclic strategy is the core concept of cyclic NAA, which realizes exponential amplification and sensitive detection of nucleic acids by precisely controlling the reaction cycle. According to different detection requirements, different NAA can be combined with spectrum techniques such as fluorescence and SERS. For instance, using DNA enzymes as a recognition element and chain shift reaction as a signal amplification technique, Chen *et al.* proposed an up conversion luminescent biosensor based on nucleic acid functionalization between a up conversion nanoparticle donor and a tetramethylrhodamine receptor to achieve quantitative analysis of  $\text{Cr}^{3+}$  through changes in ratio fluorescence.<sup>63</sup> Meanwhile, the microfluidic platform can be utilized to carry out the reaction on the picoliter scale, significantly reducing the consumption of reagents and energy, embodying the concept of green analysis. For instance, Zhang *et al.* integrated the CRISPR/CAS system with the CHA, and used SARS-COV-2 RNA as the model analyte to detect it in

combination with a SERS sensing chip.<sup>64</sup> Within 60 min, ultra-sensitive detection of  $5.18 \times 10^2$  copies per mL was achieved.

Enzymatic signal amplification operates by constructing highly efficient enzyme reaction networks, where the product of one enzyme is converted into the substrate of another, establishing a cascade cycling path that enables cyclic use of key signaling molecules within the system.<sup>65</sup> A single target molecule can initiate hundreds to thousands of catalytic cycles, resulting in exponential signal amplification. The signal intensity is directly determined by the cycling frequency and number of reaction cycles, demonstrating excellent temporal controllability. For instance, an ultrasensitive ratiometric fluorescence platform based on semiconducting polymer dots (Pdots-Pt) coupled with an enzymatic cascade reaction has been developed for L-lactate detection.<sup>66</sup> This system utilizes a cyclic reaction involving lactate oxidase and lactate dehydrogenase to continuously consume oxygen and NADH in the presence of lactate, thereby enhancing the oxygen-sensitive red fluorescence of Pdots-Pt at 650 nm while simultaneously diminishing the blue fluorescence of NADH at 422 nm. This dual-signal response enables highly selective and sensitive lactate detection, achieving a detection limit as low as  $0.18 \text{ nmol L}^{-1}$ . Furthermore, the enzymes and cofactors introduced initially can participate in multiple catalytic events. This not only significantly reduces reagent consumption and byproduct generation but also aligns with green analytical principles, providing an ideal approach for highly sensitive and sustainable biosensing applications.

Cyclic amplification based on molecular recognition and assembly represents a uniquely distinctive technological pathway. Unlike methods that rely on template replication of the target or enzymatic catalysis, this approach achieves progressively enhanced exponential signal intensity through the cyclic, alternating, and layer-by-layer assembly of biomolecules (such as lectin polysaccharide pairs, antibody-antigen complexes, and nucleic acid hybridization partners) at the sensor interface. For instance, in the surface plasmon resonance imaging (SPRi)-based saccharide detection system developed by Chen's group,<sup>20</sup> precise control over the number of cyclic assembly steps (up to 20 cycles) between concanavalin A and dextran enabled the detection limit for glucose to be pushed to  $2.5 \mu\text{mol L}^{-1}$  and that for carcinoembryonic antigen to  $50 \text{ pg mL}^{-1}$ , demonstrating exceptional controllability over the amplification process and tunability of the dynamic range. Similarly, in the detection of nucleic acid biomarkers such as microRNA, signal multistage amplification and effective background suppression can be achieved by designing cyclic hybridization and displacement reactions of DNA probes. For example, by combining miRNA-triggered surface DNA cyclic crosslinking with DNA-initiated upward cyclic polymerization, an SPRi chip with gold islands isolated by a hydrophobic CYTOP boundary was constructed.<sup>67</sup> This approach significantly enhanced the detection contrast, achieving a detection limit of  $0.56 \text{ fmol L}^{-1}$  and a quantification limit of  $5 \text{ fmol L}^{-1}$  for miRNA-15a, with approximately 107-fold higher sensitivity compared to conventional SPRi techniques. Furthermore, in 2025, Chen's group introduced an SPRi method combined with



a chemically selective stepwise signal amplification (CS<sup>3</sup>A) strategy for chemical imaging and quantitative analysis of fingerprints (Fig. 9).<sup>68</sup> This technique leverages the cyclic recognition reaction between concanavalin A and dextran, achieving high-definition fingerprint images of glucose and related substances through 5–7 rounds of signal amplification, and can be extended to simultaneous detection of carboxyl-containing substances such as amino acids. The method not only clearly reveals the three level structural characteristics of fingerprints but also enables quantitative analysis of glucose and serine. It has been successfully applied to dynamic monitoring of metabolic processes during exercise. A unique advantage of this strategy lies in its ability to deeply integrate the inherent high sensitivity of SPRi with the spatiotemporal resolution of microscopic imaging. This enables not only single-molecule-level detection sensitivity but also the preservation of spatial distribution and dynamic binding information of biomolecules on the sensor surface. This signal amplification and spatial imaging simultaneous realization capability allows researchers not only to determine the presence or absence of targets but also to resolve their spatial localization and quantitative abundance, offering an analytical dimension unattainable by traditional homogeneous amplification techniques for studying biological processes such as the distribution of cell membrane receptors and molecular interaction networks. With the ongoing development of novel recognition elements and optimization of assembly pathways, this class of interface-based cyclic assembly amplification strategies holds great promise for advancing applications in single-cell analysis, spatial multi-omics, and other cutting-edge fields.

The cyclic amplification strategy utilizing pathways such as NAA, enzymatic cyclic reactions and molecular recognition-based assembly, converts linear signals into exponential gains, achieving ultra-high sensitivity detection while significantly reducing reagent consumption. To address challenges such as multi-system integration interference, insufficient dynamic regulation precision, and the trade-offs in miniaturization, solutions including orthogonal reaction pathway design, the development of intelligent control algorithms, and the

synergistic integration of microfluidic chips with novel materials have been proposed. In the future, the cyclic amplification strategy is expected to further expand its application depth in areas such as early disease screening, environmental monitoring and food safety, continuously advancing analytical chemistry toward greater sensitivity, precision and sustainability.

**4.1.2 Cyclic reaction-dynamic monitoring.** Traditional optical detection methods usually rely on static signals obtained from a single measurement, which inherently limits access to dynamic information during reaction processes. The cyclic reaction strategy overcomes this limitation by extending the observation time and enabling multiple signal acquisitions, transforming the detection paradigm from static measurement to dynamic monitoring, and substantially enriching the obtainable information dimensions. This section focuses on CCL as the representative technology to elucidate the unique advantages of cyclic reaction-based dynamic monitoring in signal acquisition and complex system analysis.

CCL represents an innovative detection paradigm that exemplifies the transition from static detection to dynamic monitoring. As a typical cyclic detection technology based on mechanically driven reaction cycling, our group first proposed CCL detection in 2014.<sup>21</sup> The core lies in constructing an integrated cyclic detection system comprising peristaltic pumps, high-pressure pumps, and other fluid driven units for continuous reagent delivery, multi-port valves for flow path switching, reactors with immobilized catalytic materials for signal transformation, and photomultiplier tube detectors for signal acquisition, collectively forming a closed-loop detection circuit. Compared with traditional chemiluminescence, CCL demonstrates significant advantages across three dimensions. In the temporal dimension, the precise analysis of reaction kinetics was achieved by extending the observation window. In the information dimension, it achieves the transition from static detection to dynamic monitoring by acquiring multistage signals from a single injection. In terms of efficiency, it substantially reduces reagent consumption through reactant cyclic usage. Particularly noteworthy is the CCL system with innovative incorporation of an exponential decay equation to quantify reaction kinetics. By establishing correlations between signal decay characteristics and sample composition, the system effectively discriminates chemiluminescence behavioral differences that are challenging to identify using conventional methods, enabling identification and precise differentiation of multiple components in complex systems. This approach provides novel solutions for quality control and research involving complex systems.

Gas-phase and liquid-phase CCL detections are two important modes. Gas-phase CCL illustrates that CCL can be a powerful technology for quality assurance in the future. CCL can be used to identify corrupted and adulterated samples. Alcohol compounds are the characteristic components of alcoholic beverages, based on the principle that alcohols produce CCL signals on the surface of inorganic catalysts (MgO, SrCO<sub>3</sub>, and Al<sub>2</sub>O<sub>3</sub>), combined with a gas-phase CCL system.<sup>15</sup> By studying the multi-level signals of alcoholic beverages and



Fig. 9 Schematic of the chemical imaging of fingerprints with SPRi combined with a CS<sup>3</sup>A strategy.<sup>68</sup> Reprinted and adapted with permission from ref. 68. Copyright 2022 American Chemical Society.



cosmetics, our group realized rapid identification of liquor, beer, toner and baby powder. Zhang *et al.* employed a cyclic cataluminescence (CCTL) sensor array to assess the gasoline quality.<sup>27</sup> The set of decay coefficient-values produced by different gasolines on different catalysts yields a unique digital code for further differentiating gasolines. Zhong *et al.* prepared a catalyst MgO/HKUST-1 *via* the hydrothermal method,<sup>69</sup> combined with the CCL detection method, and put forward a method for capturing, catalyzing and detecting esters, which can be applied for the rapid identification of cigarette and essential oil. Ji *et al.* used CCTL to analyze ketones, combined with linear discriminant analysis and hierarchical cluster analysis stoichiometric techniques, and successfully realized the distinction of 82 coffee samples from 8 producing areas.<sup>70</sup> CCL possesses strong discriminatory capabilities in identifying complex samples, making it a promising technique for quality assurance.

Liquid-phase CCL indicates good prospects for CCL detection in chiral related fields such as asymmetric synthesis and pharmaceutical industries in the future. The decay coefficient ( $k$ ) value obtained in the CCL system can not only be used to identify alcohols, amines and acids with a single chiral configuration, but also to quickly determine the enantiomeric excess ( $ee$ ) value. The two configurations in the enantiomeric mixture will control the  $k$  value, and the  $k$  value has a linear relationship with the ratio of enantiomers in the mixture. In 2021, Zhang *et al.*<sup>28</sup> proposed a novel approach utilizing liquid-phase CCL for the rapid measurement of  $ee$ , which provides a unique method to study the interaction between chiral subjects and chiral objects. *D*-Alanine in dairy products is related to its deterioration and can be monitored as a deterioration biomarker. Zhong *et al.* prepared a chiral metal-organic framework (MOF) and applied a CCL reaction for amino acid (AA) enantiomeric analysis.<sup>71</sup> The chiral MOF was employed to distinguish AAs in the quality control of dairy products containing AAs. In addition to food analysis and quality control, CCL also shows promising prospects in clinical analysis. The levels of different amino acids in the human body will affect the generation and development of diseases, and can be used as disease markers for detection. Based on CCL, a method for amino acid enantiomer detection in biological samples was established.<sup>71</sup> When the  $k$  value of the serum sample is higher than 40, it can provide assistance for the diagnosis of cardiovascular diseases (Fig. 10). This suggests that the chiral CCL reaction can be utilized to differentiate biological samples and holds potential for clinical diagnosis, further demonstrating that CCL could serve as a valuable tool in future research. The use of the cyclic strategy in CCL has many advantages, including multistage signal acquisition, the ability to identify complex samples and resource conservation. However, it also presents several challenges, such as specific equipment requirements and issues related to reaction stability. By thoroughly understanding these advantages and drawbacks, researchers can optimize CCL for more efficient and cost-effective analyses. In the future, the research direction of CCL can focus on high-sensitivity detection, intelligent miniaturization system development, and the design of more kinds of chiral catalysts.



Fig. 10 Schematic of a liquid-phase CCL for amino acid enantiomer detection.<sup>71</sup> Reprinted and adapted with permission from ref. 71. Copyright 2023 American Chemical Society.

## 4.2 Electrochemical cyclic detection

Electrochemical cyclic detection technology transforms traditional single-step detection processes into cycling information extraction modes by introducing a temporal dimension. Represented by CV, this technique utilizes periodic changes in electrode potential to drive reversible redox reaction cycling, enabling efficient and information-rich analysis of trace samples. Its innovation lies in converting chemical reactions into information extraction tools, significantly increasing the information output per unit sample through extended detection time, thereby providing a new technical paradigm for analytical science.

CV as a typical example of electrochemical cyclic detection employs periodically applied potentials to drive reversible redox reaction cycling.<sup>72</sup> The dynamically evolving voltammetric profiles generated through cyclic scanning enable direct analysis of electron transfer processes and tracking of reaction intermediates, allowing multidimensional information, including reaction reversibility and kinetic parameters, to be obtained in a single experiment. This time-dimensional cyclic design significantly enhances information density by maximizing data extraction through repeated reaction cycles of limited sample volumes. The technique exhibits notable green analytical attributes, and the analyte acts as a messenger which participates in the reaction without being consumed under ideal conditions, thereby eliminating waste generation at the source. These characteristics underscore the unique value of electrochemical cyclic detection in fields such as clinical diagnostics and food safety. When combined with microelectrode technology, sample consumption can be reduced by 2–3 orders of magnitude compared to conventional methods. Furthermore, the method is applicable not only to routine analysis but also to the study of interfacial phenomena *via* surface process monitoring. For example, Kim *et al.* successfully captured dynamically evolving redox potential gradients at corrosion interfaces using a microelectrode (Fig. 11).<sup>73</sup> This work demonstrates the significant potential of a microelectrode in elucidating localized corrosion mechanisms in molten salts, while also providing guidance for optimizing measurement accuracy and electrode design.

By incorporating a time-dimension extension strategy, electrochemical cyclic detection technology achieves enhanced detection sensitivity while maintaining minimal sample





with miniaturization and multi-technology integration strategies, to continuously improve system stability and accessibility. As mass spectrometry instrumentation and the cyclic strategy continue to converge, this technology is expected to play an increasingly important role in complex system studies such as life sciences, environmental analysis and material characterization, driving analytical chemistry toward higher information density and sustainability.

This section provides applications of the cycling strategy across three detection domains such as spectrum, electrochemistry and mass spectrometry detection. From NAA and enzymatic reactions to signal amplification based on molecular recognition and assembly, and from CCL and CV to Orbitrap and ICR mass spectrometry, the concept of cycling serves as a core engine for enhancing detection performance throughout the entire process. Based on this, optimized regulation of cyclic parameters extends the effective interaction time, allowing for substantial enhancement in signal generation efficiency and information acquisition quality while maintaining the basic resource consumption unchanged. This approach effectively overcomes the limitations in sensitivity and information dimensionality inherent in traditional single-pass detection, establishing a new model for enhancing detection performance through temporal control. The cyclic strategy introduces the temporal dimension to enable efficient reuse of limited reagent molecules, active sites and physical processes, significantly improving resource utilization efficiency, providing a key technical pathway for the green and sustainable development of analytical chemistry. The cyclic strategy is driving profound transformation of detection technologies toward intelligence and sustainability. Emerging frontiers such as microfluidic integration, AI-optimized control and multi-mode cyclic coupling not only highlight the central role of temporal regulation but also guide instrument development toward ultra-high sensitivity, high information throughput, miniaturization and low carbon operation. This model not only resolves the inherent conflict between high sensitivity and sustainable requirements but also opens new dimensions for the advancement of analytical chemistry.

## 5. Conclusions

In summary, the cyclic strategy elaborated in this review represents a central theme that runs through the fields of separation and detection in analytical chemistry. The contribution of this review lies in constructing a unified spatiotemporal synergistic analytical framework, which distinctly differentiates it from previous reviews that focused solely on individual techniques or merely emphasized greening. We reveal that the cyclic strategy, by treating time as a tunable resource, systematically shifts the analytical process from a static, single use, and resource intensive linear paradigm to an intelligent mode characterized by dynamism, cyclic reuse and resource economy. This model shift provides a new systematic solution to the inherent challenges of throughput, separation efficiency, sensitivity and environmental compatibility in analytical chemistry.

This review discusses the advances in the cyclic strategy for separation and detection. It begins by elucidating the two

fundamental modes of the cyclic strategy, including macroscopic flow cycling and microscopic reaction cycling, and analyzes the energy and device requirements. Based on this, the application of the cyclic strategy in separation and detection is explored. In the field of separation, the cyclic strategy reconfigures the relationship between time and space, realizing the concept of trading time for space. It demonstrates outstanding performance in sample preparation, chromatographic separation and electric field-driven separation. This mechanism manifests as the transformation of separation path length from a fixed physical dimension to a function dependent on cyclic number, achieved through cycling of solvents, samples or separation media within confined device spaces. Through the expansion of the temporal dimension, the physical space limitations are overcome. This spatiotemporal conversion mechanism not only significantly enhances separation efficiency but also achieves a qualitative leap in resource utilization efficiency through process automation and system closure. In the field of detection, the introduction of cyclic designs and the temporal dimension demonstrates versatility across three major domains including spectrum, electrochemistry and mass spectrometry. The core of this concept lies in transforming limited reagent molecules, enzyme active sites, or information carriers into sustainable signal amplifiers, through temporal extension to achieve exponential accumulation of signals per unit resource and in-depth excavation of multidimensional information. This design model shifts the detection from conventional end point measurement to dynamic monitoring and from single point information acquisition to process kinetic resolution, ultimately enabling the synergistic enhancement of sensitivity, information dimensionality and dynamic monitoring capability. What is particularly important is that the introduction of the iterative strategy not only achieves resource savings in individual technical processes, but also unifies the analysis efficiency and environmental sustainability at the system level, providing an innovative solution that combines high-performance and greenness to address the challenges of complex system analysis. Currently, the cyclic strategy has demonstrated mature applications across various separation and detection technologies. The successful implementation of these techniques not only addresses the limitations of traditional non-cyclic analytical techniques that rely on fixed paths, one-way processes, and endpoint measurements but also provides innovative solutions for analyzing complex samples. Therefore, the fundamental value of the cyclic strategy lies in its systematic and universal nature as a design methodology. It provides us with a new perspective for rethinking and designing analytical systems.

Despite these impressive outcomes, challenges remain, including the new green materials or solvents and development of new methods, the complexity of technology integration, and the automation and intelligence of devices. Future research can focus on the following directions. Firstly, in novel material development, exploring and developing new separation media and catalytic materials can enhance the effectiveness and cost-efficiency of the cyclic strategy in specific applications. Secondly, in detection method innovation, cyclic detection technology can be integrated with various techniques, such as Raman spectroscopy, fluorescence spectroscopy and



colorimetric detection. By implementing cyclic detection across these diverse spectral regions, we can not only acquire multi-dimensional data but also significantly enhance the sensitivity, selectivity, and stability of the detection process. Thirdly, multi-functional integrated systems. Designing and developing multi-functional automated detection systems that can perform various separation and detection functions on a single platform improves experimental efficiency and data accuracy. Finally, in data processing and intelligence, integrating machine learning and big data analysis to enhance data processing capabilities in cyclic strategy applications, further improves the accuracy and reliability of results. Overall, the cyclic strategy presents a promising outlook and significant development potential in the field of separation and detection. With continued research and technological advancements, this innovative strategy is expected to find deeper applications across a broader range of fields. Overall, the cyclic strategy has established its universal value as a core analytical chemistry method by reconfiguring the constraints between time and space, resources and information. With the continuous integration with cutting-edge technologies, the cyclic strategy will surely drive analytical chemistry to continuously evolve towards a more precise, efficient and green systematic direction.

## Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## Conflicts of interest

There are no conflicts to declare.

## Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

## Acknowledgements

The work was financially supported by the State Key Program of National Natural Science of China (No. 22134007), the National Natural Science Foundation of China (No. 22576240) and the Guangdong Basic and Applied Basic Research Foundation of China (No. 2024A1515011077), respectively.

## Notes and references

- 1 T. Keijer, V. Bakker and J. C. Slootweg, *Nat. Chem.*, 2019, **11**, 190–195.
- 2 S. V. Mohan and R. Katakajwala, *Curr. Opin. Green Sustainable Chem.*, 2021, **28**, 100434.
- 3 J. C. Slootweg, *One Earth*, 2024, **7**, 754–758.
- 4 E. Psillakis and F. Pena-Pereira, *TrAC, Trends Anal. Chem.*, 2024, **175**, 117686.
- 5 M. Gallo, A. Formato, R. Giacco, G. Riccardi, D. Luongo, G. Formato, A. Amoresano and D. Naviglio, *Heliyon*, 2019, **5**, e01526.
- 6 G. Lievore, R. Weldon, M. Catani, A. Cavazzini and T. Müller-Späth, *J. Chromatogr. B*, 2022, **1209**, 123439.
- 7 P. Wisanpitayakorn, N. Jariyasopit, K. Duangkumpha, J. X. Goh, M. E. Palmer, Y. Sirivatanauksorn and S. Khoomrung, *ACS Meas. Sci. Au*, 2025, **5**, 109–119.
- 8 J. Zhang, J. Liu, Y. Lv, H. Sun and X. Su, *Sensor. Actuator. B Chem.*, 2023, **385**, 133637.
- 9 R. Zhang, Y. Zhong, Y. Hu, Y. Chen, L. Xia and G. Li, *Anal. Chem.*, 2024, **96**, 3933–3941.
- 10 S. Aderyani, P. Flouda, S. A. Shah, M. J. Green, J. L. Lutkenhaus and H. Ardebili, *Electrochim. Acta*, 2021, **390**, 138822.
- 11 D. Li, Z. Huo, L. Xia, X. Xiao and G. Li, *Anal. Chem.*, 2024, **96**, 5368–5374.
- 12 S. M. Keshavarz, M. J. Inanlu, K. Omidfar and V. Bazargan, *Microchem. J.*, 2025, **214**, 114061.
- 13 X. Huang, S. Ignatova, P. Hewitson and D. Di, *TrAC, Trends Anal. Chem.*, 2016, **77**, 214–225.
- 14 J. G. Roberts and L. A. Sombers, *Anal. Chem.*, 2018, **90**, 490–504.
- 15 Y. Zhong, Y. Hu, G. Li and R. Zhang, *Anal. Chem.*, 2019, **91**, 12063–12069.
- 16 Y. Cheng, Y. Wang, Z. Ma, W. Wang and X. Ye, *Lab Chip*, 2016, **16**, 4517–4526.
- 17 Y. Wang, T. Li, S. Wang, X. Du, L. Li, H. Xiao, Z. Zhang, F. Jiang, S. Li, Z. Wang, Y. Wang and N. Yang, *Microchem. J.*, 2025, **208**, 112449.
- 18 M. E. Gramajo, L. Otero Maffoni, L. M. Hernández Parra, W. A. Marmisollé, M. L. Cortez, M. E. Toimil-Molares, A. S. Peinetti and O. Azzaroni, *Chem. Commun.*, 2024, **61**, 697–700.
- 19 B. Zhou, Y. Han, R. Song, J. Hu and C. Zhang, *TrAC, Trends Anal. Chem.*, 2025, **192**, 118405.
- 20 C. Liu, X. Wang, J. Xu and Y. Chen, *Anal. Chem.*, 2016, **88**, 10011–10018.
- 21 R. Zhang, Y. Hu and G. Li, *Anal. Chem.*, 2014, **86**, 6080–6087.
- 22 P. Begum, L. Yang, T. Morozumi, T. Sone and T. Kawaguchi, *Food Chem.*, 2023, **414**, 135740.
- 23 X. Hu, Y. Wang, J. Lv, M. Zheng, S. Ma, J. Cao and Y. Liu, *ACS Sens.*, 2025, **10**, 196–203.
- 24 J. Li, W. Zhang, R. Chang, Y. Lan, D. Qiu, K. Wang, J. Huang and Q. Xu, *Biosens. Bioelectron.*, 2025, **269**, 116936.
- 25 Y. Zhang, C. Devendran, C. Lupton, A. de Marco and A. Neild, *Lab Chip*, 2019, **19**, 262–271.
- 26 X. Xu, J. Gao, D. Cao, X. Li, X. Zhao, S. Yue and L. Zhang, *J. Chromatogr. A*, 2022, **1667**, 462882.
- 27 Y. Zhong, W. Huang, C. Zhang, R. Zhang, Y. Hu, X. Xiao and G. Li, *Sensor. Actuator. B Chem.*, 2022, **364**, 131901.
- 28 R. Zhang, Y. Zhong, Z. Lu, Y. Chen and G. Li, *Chem. Sci.*, 2021, **12**, 660–668.
- 29 N. Navashree and P. Parthasarathy, *Mater. Today: Proc.*, 2023, DOI: [10.1016/j.matpr.2023.05.175](https://doi.org/10.1016/j.matpr.2023.05.175).
- 30 K. Zhang, S. Qin, S. Wu, Y. Liang and J. Li, *Chem. Sci.*, 2020, **11**, 6352–6361.



- 31 S. Shen, X. Liu, K. Fan, H. Bai, X. Li and H. Li, *Anal. Chem.*, 2024, **96**, 11412–11421.
- 32 S. Fande, K. Amreen, D. Sriram and S. Goel, *Anal. Chim. Acta*, 2023, **1237**, 340591.
- 33 B. Karakuzu, Y. Gulmez and H. C. Tekin, *Microelectron. Eng.*, 2021, **247**, 111583.
- 34 P. Rud, S. Chapek, P. Medvedev, O. Polozhentsev, S. Soldatov, A. Bagliy, A. Guda, A. Soldatov and M. Soldatov, *Microchem. J.*, 2024, **196**, 109659.
- 35 T. Uema, T. Ohata, Y. Washizuka, R. Nakanishi, D. Kawashima and N. Kakuta, *Chem. Eng. J.*, 2021, **403**, 126338.
- 36 S. Augustine, M. V. Chinnamani, C. W. Mun, J. Shin, T. Q. Trung, S. J. Hong, L. T. N. Huyen, E. H. Lee, S. H. Lee, J. Rha, S. Jung, Y. Lee, S. Park and N. Lee, *Biosens. Bioelectron.*, 2024, **248**, 115987.
- 37 N. Choi, J. Lee, J. Ko, J. H. Jeon, G. Rhie, A. J. DeMello and J. Choo, *Anal. Chem.*, 2017, **89**, 8413–8420.
- 38 B. Li, X. Liu, D. Gao, Z. Ma, J. Peng, X. Wang and Y. Jiang, *Sensor. Actuator. B Chem.*, 2023, **395**, 134510.
- 39 T. Ngernsutivorakul, D. J. Steyer, A. C. Valenta and R. T. Kennedy, *Anal. Chem.*, 2018, **90**, 10943–10950.
- 40 S. Sun, J. Zheng, Z. Liu, S. Huang, Q. Cheng, Y. Fu, W. Cai, D. Chen, D. Wang and H. Zhou, *Chem. Eng. J.*, 2023, **463**, 142368.
- 41 B. Sahu, R. Kurrey, B. R. Khalkho and M. K. Deb, *Colloids Surf., A*, 2022, **654**, 129947.
- 42 M. Vázquez-Torres, I. Cabrera-Asencio and N. Rivera-Portalatín, *Pharmacol. Res.*, 2025, 100286.
- 43 S. W. van Dongen, I. Baglai, M. Leeman, R. M. Kellogg, B. Kaptein and W. L. Noorduyn, *Chem. Commun.*, 2023, **59**, 3838–3841.
- 44 G. Batista, G. A. Surek, C. Benincá, M. L. Corazza and E. F. Zanoelo, *Fuel*, 2016, **163**, 133–138.
- 45 P. A. Hong and S. Nakra, *Chemosphere*, 2009, **74**, 1360–1366.
- 46 F. Wei, Z. Yang, Y. Zhao and Q. Wang, *AIChE J.*, 2019, **65**, 702–711.
- 47 Y. S. Lee, J. Lee, K. Fang, G. V. Gee, B. Rogers, D. McNally and S. Yoon, *J. Chromatogr. B*, 2024, **1242**, 124206.
- 48 E. Bigelow, Y. Song, J. Chen, M. Holstein, Y. Huang, L. Duhamel, K. Stone, R. Furman, Z. J. Li and S. Ghose, *J. Chromatogr. A*, 2021, **1643**, 462008.
- 49 R. Weldon and T. Müller-Späth, *J. Chromatogr. A*, 2022, **1667**, 462894.
- 50 A. E. Kostanyan and A. A. Erastov, *J. Chromatogr. A*, 2016, **1462**, 55–62.
- 51 S. Tong, Y. Guan, J. Yan, B. Zheng and L. Zhao, *J. Chromatogr. A*, 2011, **1218**, 5434–5440.
- 52 Y. Chen, X. Yan, F. Lu, X. Jiang, J. B. Friesen, G. F. Pauli, S. Chen and D. Li, *J. Chromatogr. A*, 2019, **1599**, 180–186.
- 53 P. Zhang, G. Sun, K. Tang, C. Zhou, C. Yang and W. Yang, *Sep. Purif. Technol.*, 2015, **146**, 276–283.
- 54 J. He, J. Huang, W. Wu and Q. Mu, *J. Chromatogr. A*, 2020, **1626**, 461368.
- 55 T. L. Peterson and G. Nagy, *Anal. Chem.*, 2021, **93**, 9397–9407.
- 56 C. R. de Bruin, W. J. C. de Bruijn, M. A. Hemelaar, J. Vincken and M. Hennebelle, *Talanta*, 2025, **281**, 126804.
- 57 K. Giles, J. Ujma, J. Wildgoose, S. Pringle, K. Richardson, D. Langridge and M. Green, *Anal. Chem.*, 2019, **91**, 8564–8573.
- 58 L. R. Benzenberg, M. Vincent, K. Greis, M. Zimmermann, I. Oganessian, M. Walles, J. Hall, K. Root and R. Zenobi, *Anal. Chem.*, 2025, **97**, 18670–18680.
- 59 K. L. Saar, T. Müller, J. Charmet, P. K. Challa and T. P. Knowles, *Anal. Chem.*, 2018, **90**, 8998–9005.
- 60 C. Benz, M. Boomhoff, J. Appun, C. Schneider and D. Belder, *Angew. Chem., Int. Ed.*, 2015, **54**, 2766–2770.
- 61 X. Xu, D. Wang, C. Zhou, Y. Xing, T. Cai, X. Yang, R. Yang, P. Xie, F. Liu and Z. Jia, *Anal. Chem.*, 2025, **97**, 11544–11553.
- 62 T. Wang, Y. Liu, H. Sun, B. Yin and B. Ye, *Angew. Chem., Int. Ed.*, 2019, **58**, 5382–5386.
- 63 Q. Chen, S. Chen, Z. Chen, K. Tang, L. Zeng, W. Sun, F. Wu, J. Chen and J. Lan, *Anal. Chim. Acta*, 2024, **1328**, 343161.
- 64 J. Zhang, Z. Chen, H. Lv, J. Liang, C. Yan, C. Song and L. Wang, *Biosens. Bioelectron.*, 2024, **253**, 116196.
- 65 S. H. Jang, H. W. Jeon, H. Han and J. K. Ahn, *Talanta*, 2025, 128663.
- 66 S. He, W. Liu and S. X. Wu, *Anal. Chim. Acta*, 2024, **1303**, 342523.
- 67 F. Hu, J. Xu and Y. Chen, *Anal. Chem.*, 2017, **89**, 10071–10077.
- 68 M. Li, J. Xu, Q. Zheng, C. Guo and Y. Chen, *Anal. Chem.*, 2022, **94**, 7238–7245.
- 69 Y. Zhong, Y. Chen, Y. Hu, G. Li and X. Xiao, *Anal. Chem.*, 2021, **93**, 16203–16212.
- 70 M. Ji, Y. Chen, Y. Hu and G. Li, *Talanta*, 2025, **285**, 127261.
- 71 Y. Zhong, Y. Chen, L. Chen, Y. Hu, X. Xiao, L. Xia and G. Li, *Anal. Chem.*, 2023, **95**, 6971–6979.
- 72 M. K. Homer, D. Kuo, F. Y. Dou and B. M. Cossairt, *J. Am. Chem. Soc.*, 2022, **144**, 14226–14234.
- 73 C. Kim and A. Couet, *J. Am. Chem. Soc.*, 2025, **147**, 19013–19025.
- 74 J. Snijder, M. van de Waterbeemd, E. Damoc, E. Denisov, D. Grinfeld, A. Bennett, M. Agbandje-McKenna, A. Makarov and A. J. Heck, *J. Am. Chem. Soc.*, 2014, **136**, 7295–7299.
- 75 X. Wang, Y. Chen, Z. Li, Z. Fan, R. Zhong, T. Liu, X. Li, X. Lu and G. Xu, *Anal. Chem.*, 2025, **97**, 11506–11514.
- 76 J. O. Kafader, K. R. Durbin, R. D. Melani, B. J. Des Soye, L. F. Schachner, M. W. Senko, P. D. Compton and N. L. Kelleher, *J. Proteome Res.*, 2020, **19**, 1346–1350.
- 77 H. Chan, B. Krichel, L. J. Bandura, E. A. Chapman, H. T. Rogers, M. S. Fischer, D. S. Roberts, Z. Gao, M. Wang and J. Wu, *J. Am. Chem. Soc.*, 2025, **147**, 30809–30819.
- 78 K. O. Nagornov, A. N. Kozhinov, E. Nicol, O. Y. Tsybin, D. Touboul, A. Brunelle and Y. O. Tsybin, *J. Am. Soc. Mass Spectrom.*, 2020, **31**, 2258–2269.
- 79 S. A. Pieczonka, M. J. Thomas, P. Schmitt-Kopplin and J. W. Marshall, *Anal. Chem.*, 2025, **97**, 8491–8498.
- 80 Q. You, Y. Cheng, Q. Fu, G. Cao, J. Liu, M. Fujii, L. Blaney, P. Fu and Y. Wang, *Anal. Chem.*, 2025, **97**, 10442–10451.

