Enhancing affinity-based electroanalytical biosensors by integrated AC electrokinetic enrichment—A mini review

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Abbreviations: ACEK, alternating current electrokinetic; **DEP**, dielectrophoretic; **ACEO**, alternating current electroosmosis; **ACET**, alternating current electrothermal; **LOD**, limit of detection; **BPA**, bisphenol A; **cf-DNA**, circulating cell-free DNA; **ELISA**, enzyme-linked immunosorbent assays; **REP**, rapidly electrokinetic patterning; **EDL**, electric double layer; **PPy**, polypyrrole; **EHD,** electrohydrodynamics; **ICEK**, induced-charge electrokinetic; **ICEO**, induced-charge electroosmosis; **ROT-ICEO**, induced charge electroosmosis in a rolling electric field; **SERS**, Surface-enhanced Raman scattering

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Abstract

Biosensors play a central role in moving diagnostics to being on-site or decentralized. Affinity biosensor, an important category of biosensors, has important applications in clinical diagnosis, pharmaceuticals, immunology and other fields. Affinity biosensors rely on specific binding between target analytes and biological ligands such as antibodies, nucleic acids or other receptors to generate measurable signals. Oftentimes the target analytes in practical samples are of low abundance in a complex matrix. Traditional affinity biosensors mainly rely on random diffusion of analytes in solution to conjugate with biorecognition elements on the sensor surface of electrodes. The process may take hours or even days, which is not conducive to rapid and sensitive detection of biosensors. Therefore, it is strongly desired to incorporate an enrichment mechanism for target analytes into biosensor-based detection. AC electrokinetic (ACEK) effect can realize rapid enrichment of analytes by application of AC electric fields, which holds great promise for achieving high sensitivity, low detection limit and rapid turnaround. This paper reviews the studies of affinity biosensors integrated with ACEK enrichment in the past decade, and summarizes the latest detection methods, detection devices and applications, hoping to provide some insight and references for researchers in related fields.

"Color online: See article online to view Figs.1-8 in color."

Introduction

Affinity biosensor

Biosensors have wide applications in clinical diagnosis, environment, pharmaceuticals and safety monitoring, as they can realize sensitive and selective detection of a range of analytes with small form factor and good affordability [1,2]. Biosensors can be classified into different types according to their different transduction principles, probe molecules and applications, among which electroanalytical biosensor is an important category [3]. Electroanalytical biosensors have many advantages, including high sensitivity, rapid detection, and low cost. Further, with the use of microelectrodes as the sensing elements, electroanalytical sensors can be readily miniaturized and integrated into a portable system and form an array for parallel analysis. Same as other biosensors, electroanalytical biosensors consist of two parts, probe molecules (also known as receptors) for specific identification of target analytes and a transduction mechanism to convert the presence of

target analytes into response signals. Based on the signal conversion process, electroanalytical biosensors can be divided into two categories, biocatalytic sensors and affinity biosensors [4].

Biocatalytic sensors employ enzymes, whole cells, or tissue sections that recognize target analytes and produce an electroactive substance. Biocatalytic sensing technology is a mature sensing technology, which has important applications in practice due to the high biocatalytic activity and specificity of enzymes [5,6]. However, because the types of enzymes available for sensors are limited at present, many analytes cannot be detected by enzyme electrodes. So affinity biosensors become an effective alternative for analysis [7]. Affinity sensors rely on selective and strong binding between the target analytes and recognition components such as antibodies, nucleic acids or other receptors to generate measurable electrical signals, and then to achieve sensitive and specific detection [8]. This review will discuss a few examples of affinity biosensors and their integration with AC electrokinetic (ACEK) enrichment. Some of the design concepts may very well be applied for biocatalytic sensors.

ACEK effects

Affinity biosensors are based on the specific binding between receptor probes and analytes to realize the detection of target analytes. Most of traditional affinity sensors rely on the random diffusion movement of target analytes to meet the biorecognition elements. At low abundance of target analytes, it may take hours or even days for the number of conjugates to reach detectable levels [9,10]. This is not conducive to the sensitivity and turnaround time desired for on-site detection.

ACEK effects are a group of microfluidic phenomena under the action of non-uniform alternating electric fields. They can be used to induce directed movement of microflows and particles in the solution, so that the analytes in the solution can be quickly routed toward the surface of biosensors, thus improving the binding efficiency between the analytes and the probes on the surface of the sensor [11].

ACEK effects mainly include the induced particle movement, known as dielectrophoretic (DEP) effect, and the movement of colloidal suspension surrounding polarized solid surfaces, i.e. alternating current electroosmosis (ACEO) effect and alternating current electrothermal (ACET) effect [12]. ACEK is a branch of AC electrohydrodynamics(EHD), which broadly covers the manipulation of colloidal particles and fluid transport phenomena under the influence of electric fields. To be more specific, AC DEP force is an ACEK phenomenon since it arises from the induced dipole on particles. ACEO and ACET flows are AC EHD phenomena if they concern only with fluid flows, while they become ACEK regime when interacting with particles. The differences between AC

EHD and ACEK are very subtle and minuscule, so they are often used interchangeably when dealing with particulate suspensions. Since ACEK effects are realized by applying AC field, they scale favorably with miniaturization and offer the ability to modulate analyte transport in microfluidic devices. Studies have showed that ACEK forces are much greater for particles in solution than natural forces such as Brownian motion diffusion [13]. There have been many reports on the investigation and application of ACEK effects in recent years. For example, Ramos et al. [14] conducted a comprehensive study of the movement and behavior of sub-micron particles induced by AC electrical fields over a model electrode pair, with comparison with Brownian motion, diffusion and the buoyancy force.

DEP effect is based on the difference in the polarizabilities of the particles and the solution under a non-uniform alternating electric field. Based on their polarizabilities, DEP can move particles towards high field regions (a.k.a. positive DEP) or low field regions (a.k.a. negative DEP), thus achieving the enrichment of particles [15,16]. Since DEP effect is proportional to particle size, DEP force on submicron size or larger particles tends to dominant [17,18]. ACEO flows are induced by the movement of induced charges at electrode surfaces in a dilute electrolyte. When the solution is in contact with the electrode surface, an electrical double layer (EDL) will be generated. In a non-uniform AC electric field, the electric field tangential to the electrode surface will drive the charges in the EDL to move in a directional way, thus generating eddy electroosmosis and forming ACEO effect [19]. The EDL is the basis for the generation of ACEO, and the thickness of EDL is inversely proportional to the electrical conductivity of electrolyte. For liquids with high conductivity, the thickness of the EDL is small, so the ACEO effect is weak. Therefore, ACEO effect is only important in the analysis of the solution with low conductivity [20]. When the solution conductivity increases, as ACEO effect on the electrode surface becomes reduced, ACET effect will become dominant. The temperature gradients generated in the electrolyte solution by non-uniform electric fields lead to perturbation of dielectric constant and conductivity of the solution, inducing mobile charges due to the electric current continuity and Gauss law. These mobile charges make directional movement under the influence of electric fields and drive the viscous fluid directionally to form ACET effect [21-24].

There is another type of electrokinetic phenomena known as induced-charge electrokinetic (ICEK). They are similar to, but not to be confused with ACEK effects. ICEK is also based on the interaction between an external applied electric field and an induced double layer surrounding a polarizable solid submerged in saline solution, forcing the induced double layer into induced-charge electroosmosis (ICEO) flow. ICEO micro pumping, typically induced with DC fields, has become a new operating tool in microfluidic devices [25,26]. Liu et al. proposed a microfluidic method for continuous transport and local collection of nanoparticles through mixed electrodynamics. On application of a DC-biased AC electric field, the nanoparticles can be continuously transmitted through DC bulk electroosmotic flow, and then selectively trapped on the metal strips due to the ICEK phenomenon [26]. Ge et al. proposed an ICEO in a rolling electric field (ROT-ICEO) method,

which is used to enhance the specific binding reaction between free antigen and immobilized antibody. The method can stir the analyte in all directions in three-dimensional space near the functional surface of the ideal polarizable floating electrode [28].

Although ICEO is a promising analytical method, it is not in the scope of this review since it mainly uses DC (or weak AC) signal. This review focuses on ACEK effects, which use AC signals as stimuli. The use of AC is preferred over its DC counterpart for a biofluid diagnostic microsystems, due to its ability to operate at relatively low voltage in order to avoid electrolysis, electrode damage or fouling, and its local control of streamlines owing to the use of patterned microelectrodes.

Scope and organization of the review

Recent research literature shows that an important application of ACEK effects is to improve biosensor performance. Various approaches have been investigated to take advantages of the directional particle movements by ACEK effects. One strategy is to assemble nanoparticles so as to improve the functionalized sensor surface and increase the sensor response. Dies et al. developed a new method for ACEK assembly of gold nanoparticles into Surface-enhanced Raman scattering (SERS) frequency-dependent structures, nanowires and branched "nanotrees", and demonstrated its application in biological and chemical sensing [29,30]. Nosrati et al. used ACEK to assemble colloidal silver nanoparticles into extended SERS active structures with high surface density of hotspots, which could be used to detect explosives, drugs and pesticides, with higher sensitivity and 100% accuracy compared with commercially available products [31].

The focus of this review is to discuss and summarize the role of ACEK in enhancing the mass transport of analytes for affinity-based electroanalytical biosensors in the past decade and put forward the current problems and development prospects in this research field. The above-mentioned work on particle assembly, while of strong interest in sensor preparation, does not fall in the scope of accelerating analyte transport by ACEK. Therefore, the discussion in this review is limited to ACEK schemes for enriching analytes at the sensor surfaces.

The structure of the paper is as follows: Section 2 to Section 4 discuss how ACEK effect works in immune sensor, nucleic acid sensor, adapter sensor and biotin sensor. Section 5 summarizes the whole paper and puts forward some opinions.

Immunosensors

Immunosensors are antibody-based affinity biosensors in which the detection of analytes, antigens or haptens is achieved by their binding to the antibody region [32]. Traditional immunoassays, such

as microarrays or enzyme-linked immunosorbent assays (ELISA), rely on molecular diffusion of the analytes to bind to the fixed receptors for detection. Because the small diffusion flux cannot match the rapid rate of the surface reaction, there is insufficient antigen source for the antigen-antibody binding reaction in the functionalized region, which may result in long incubation times, ranging from minutes to hours, and may limit the sensitivity and flux of immunoassays [33-35]. The ACEK effect can be used to accelerate the movement of macromolecules towards the sensing region by inducing particles or liquids through an externally applied AC electric field, which has been reported by several groups [36-39]. The application of ACEK effects in immunosensors has attracted extensive attention of researchers in recent years.

Devices and methods

In recent years, immunosensing methods integrated with ACEK effects have been emerging. There exist diverse designs of electrodes, innovative chip structures, sensing protocols, and sometimes accompanied with the development of portable automated detection instruments.

Yang et al. described an immunoassay lab-chip based on ACET effect to detect serum samples from cows infected with Johne's disease [40]. This ACET-based immune-chip can perform sandwich immunoassay, including sample delivery, incubation and flushing. The reagents and analytes were pumped into the reaction channel by ACET effect, which was realized by applying a low-voltage AC signal to the interdigitated electrode arrays at the microchannel bottom. In the reaction chamber, the ACET flow created vortices over the electrodes that helped transport the biomolecules to the binding site. The incubation time was shortened from 30 min to about 1 min, and differentiation between positive and negative sera for the disease was achieved. The experimental results of the chip agreed well with numerical simulation and theoretical prediction. In addition, the immunoassay chip was low-cost and disposable, which was desirable for the development of field deployable analytical instruments. The immunosensing chip is shown in Figure 1.

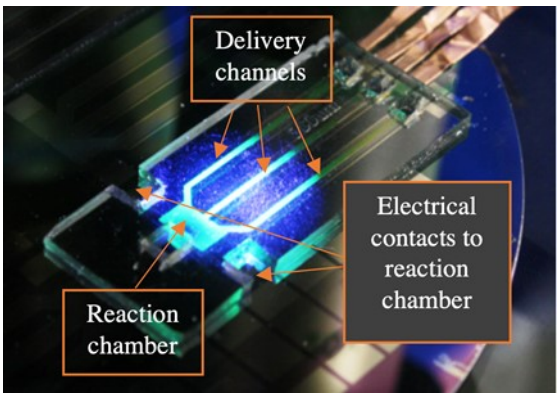

Figure 1. ACET immunosensing chip adapted for sandwich immunoassay. Reprinted with permission from [40], copyright (2017) Springer-Verlag Berlin Heidelberg.

Lin et al. introduced a chip-based concentric multi-double ring electrodes and single central disk electrode structure. The Electrode diagram is shown in Figure 2. A long-range DC-biased AC electrokinetic flow (ACEKF) was induced by applying a +1V-biased 6 V_{PP} AC voltage to a set of multi-double ring electrodes. The results showed that the ACEK vortex could effectively reduce the saturation immunoreaction time to 8 minutes, rather than 60 minutes for the unstirred immunoreaction. The increment of electron transfer resistance was 2.26 times and the sensitivity of the calibration curve obtained by Electrochemical Impedance Spectroscopy (EIS) detection was 1.51 times larger than that of unstirred solution. The data proved that ACEK could improve the efficiency of immunoreaction [41].

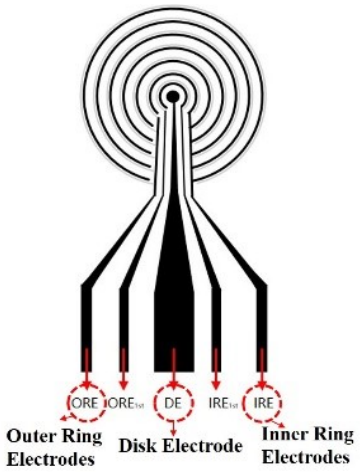

Figure 2. The concentric multi-double ring electrodes and single central disk electrode structure. Reprinted with permission from [41], copyright (2018) AIP Publishing.

Cui et al. [42] investigated an ACEK enhanced capacitive biosensor technology to detect specific serum antibody, which applied a low-voltage AC voltage on the electrode sensor and detected the change of the interface capacitance after dropping the test solution on the electrode surface. As such, this sensor integrated the ACEK enrichment and capacitance readout into a single step. The detection time was greatly shortened. Figure 3 shows the schematic diagram of ACEK enhanced capacitance biosensor and the equivalent circuit of the "electrode-fluid" system.

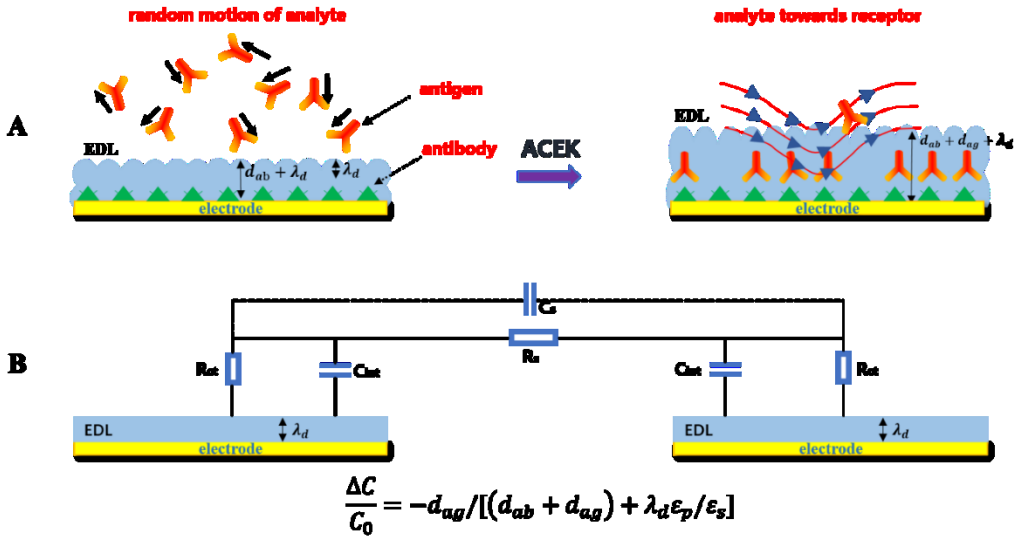

Figure 3A. Schematic diagram of ACEK enhanced capacitance biosensor and interface capacitance changes caused by the combination process. **B.** The equivalent circuit of the "electrode-fluid" system: C_{int}: interface capacitance, R_{ct}: charge transfer resistance, R_s: electrolyte resistance, C_s: electrolyte capacitance. The dashed line C_s is regarded as open circuit under the frequency. Reprinted with permission from [50], copyright (2014) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

Liu et al. developed a low-cost, high-precision portable analyzer which could be field deployable for quantitative determination of protein biomarkers in biological matrices such as serum. This work introduced the development of a board-level capacitance readout system and the protocol modification for ACEK capacitance sensing. The results showed that the detection limit of the system was around 10 ng/ml, which was equivalent to a precise desktop instrument. The system was small and light as a smartphone and could be used for point-of-care diagnostics. In addition, the readout system could be extended for multi-channel monitoring and telecommunication [43].

Inoue et al. developed a semi-automatic, occupationally safe immunofluorescence microtip sensor which was capable of rapidly detecting mycobacterium cells in sputum. The functional microtip was immersed in an aluminum well containing 1 mL of treated sputum, and a vibration motor placed under the aluminum well generated circulating flows that led the cells through the solution volume toward the microtip. Cells nearby the microtip were polarized by a function generator with AC electric field of 5 MHZ and 20 Vpp for 2 minutes, producing DEP forces to attract cells to the edges of the microtip. Mycobacterium tuberculosis complex cells bound to the fluorescence labeled specific antibodies, and fluorescence detection were used to detect cells captured on the microtip. The schematic diagram of the working principle of a microtip sensor is showed in Figure 4. The detection limit of sputum was 100 CFU/ ml, the success rate was 96%, and the detection time was 25 minutes [44,45].

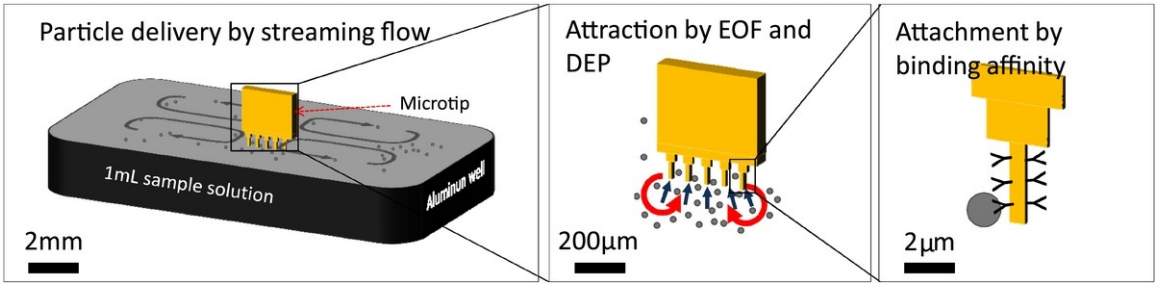

Figure 4. Schematic diagram of the working principle of a microtip sensor. Reprinted with permission from [45], copyright (2014) IOP Publishing Ltd.

Chuang et al. [46] proposed a bead-based immunosensor for tear diagnosis of diabetic retinopathy (DR) based on a rapidly electrokinetic patterning (REP) optoelectronic platform. An IR laser beam was used to generate a temperature gradient along the Z-axis of the micro-droplets to induce ACET flow. When both an electric field and a focused laser beam were applied to the microchip, the immune complexes in the droplets are subjected to a combination of various forces, which play their roles on the specific frequency domain and different particle sizes, and affect particle behavior together. When all the forces are balanced over time, the particles will be stabilized in the laser illuminated region, and the immune complexes could be further concentrated to enhance the fluorescent signal. The method has shown good performance in clinical diagnosis and provided a non-invasive method for the early diagnosis of diabetic retinopathy.

Applications

With the continuous emergence of new immune sensing methods and devices combined with ACEK effects, the application of ACEK-based immunosensors is also extensive increasingly, involving various fields such as disease diagnosis, immune analysis, molecule detection ranging from small molecules (e.g. bisphenol A, progesterone, etc.) to bacteria, proteins (e.g. D-dimer, antigens, antibodies, etc) [47-49] and so on.

Li et al. used the antigen and antibody from Johns' disease (JD) as an example to prove that positive and negative serum samples of the disease can be distinguished within a few minutes by ACEK enhancing immunoassay. In order to further clarify the importance of DEP effect and ACET effect on the binding reaction, two different electrode patterns for capacitive immunoassay were studied. Due to the DEP effect, asymmetric arrays and symmetrical electrodes showed very similar responses at lower electric fields, while asymmetric arrays had significantly higher responses at higher electric fields, because convection became more important at higher electric fields [50]. Figure 5 is the schematic diagram of capacitance immunoassay device based on ACEK effect.

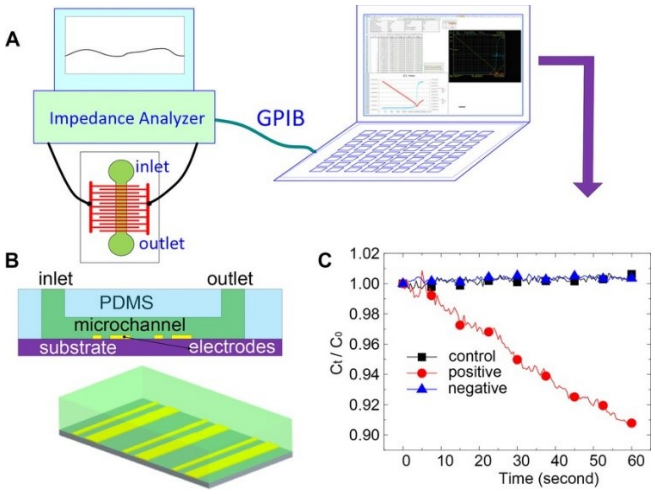

Figure 5. Schematic diagram of capacitance immunoassay device based on ACEK effect. Reprinted with permission from [50], copyright (2014) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

To further understand the relative importance of DEP and ACET effect, Cui et al detected proteins (bovine IgG) and small molecule analytes (Bisphenol A (BPA)) separately on several different electrode designs. The results showed that the sensitivity and detection limit of protein sensor were improved when the large electrode with a characteristic length of 5 µm was used, which was attributed to the stronger ability of the larger electrode to induce ACET microflows. For low abundance analytes, the benefit of ACET effect became more important because of the need for analyte enrichment over a large range of sample volume. In addition, it was found that the characteristic electric field intensity of 45 mV/µm was close to the optimal AC field for ACEK capacitance detection. The detection limits were 1 ng/ml of IgG and 10 µg/ml of BPA, lower than most existing technologies. In general, ACEK capacitive sensing method has great potential to be a point-of-care diagnostic tool in the future [51].

To explore low-cost fabrication of biosensors, Li et al. have demonstrated a low-cost, disposable sensor for circulating D-dimer biomarker. The key sensing element of the device, the interdigitated electrode array, was manufactured by using commercial optical disks and homemade gadgets. The disc-based electrode array was wet-etched to form a pattern and then electroplated with polypyrrole (PPy). The nanoporous structure of PPy enhanced the immobilization of the protein probes on the electrode surface. The porous surface of PPy electrodes in combination with ACEK capacitive sensing protocol further improves the performance of the sensor. The sensor could be used for quantitative detection of D-dimer biomarkers within one minute with a detection limit of 1 pg/ml [52].

To summarize the recent studies on ACEK enrichment in immunosensors, it was found that ACEK effects played an important role in improving the sensitivity and reducing detection limit as well as the assay time of sensors. As a result, new detection methods and devices capitalizing on ACEK manipulation of analytes continue to appear, providing new solutions for immunodetection. Nevertheless, although ACEK technology has shown high promise in the field of immune sensing, there is still much unknown to be explored for these types of biosensors to be mature for commercialization.

Nucleic acid sensors

Nucleic acid sensors, by detecting DNA/RNA hybridization with immobilized complementary DNA/RNA oligonucleotide probes, have been widely used in life science research [53], environmental monitoring [54], drug toxicity screening [55], molecular diagnosis of diseases [56] and other fields. However, traditional nucleic acid sensors rely mainly on the diffusion of the test specimens to the fixed ligand, where hybridization occurs. This diffusion has a strong limiting effect on DNA/RNA molecules with a short half-life (such as ctDNA with a half-life of 2.5 hours) and also limits the sensitivity of the sensor. ACEK effects can be used to enrich nucleic acid molecules by one or more effects in ACEO, ACET or DEP to accelerate the hybridization efficiency between DNA/RNA and complementary DNA/RNA and improve the sensitivity of the sensor.

Devices and methods

There have been research efforts on utilizing ACEK effects concentrating nucleic acid molecules to enhance nuclei acid sensors' performance. Some approaches focused on enhance DEP attraction of nuclei acid sequences. Using an AC frequency range of 3kHz ~ 10kHz and a peak voltage of 10V, Krishnan et al. used DEP to concentrate 60-nm DNA-dericatized nanoparticles (the sequence – 50-biotin-TCA GGG CCT CACCAC CTA CTT CAT CCA CGT TCA CTC AGG GCC TCA CCA CCT-3') into the region of high electrical field in a buffer solution with electrical conductivity over 1.68 S/m [57]. Sonnenberg et al. [58] prepared 1000 platinum microelectrodes with diameters of 60μm on a silicon substrate, insulated them with silica and covered with a porous hydrogel layer. The chip was used to generate DEP for the separation of blood or plasma samples containing circulating cell-free DNA (cfDNA). At a specific AC frequency and voltage level, cfDNA was more polarizable than the surrounding medium and was enriched by positive DEP into the high field region.

Some reports used ACEK microflows combined with DEP. Pak et al. [59] developed a combination of electrophoresis, DEP and ACEO to enrich single-stranded DNA fragments. They first used ACEO to transport DNA fragments from a volumetric fluid to an area near the electrode. Then they applied AC signal with a DC bias to generate DEP and electrostatic attraction to trap the charged DNA molecules and concentrate them on the surface of the electrode. Wu et al. [60] capitalized on ACEO effect by using a low-ionic-strength buffer solution of 6.1m S/cm 1mM Tris(pH9.3). By designing an electrode set with a ring width to disk diameter ratio of 1:4, a large ACEO flow rate was generated on the electrode surface, and the specific detection of target DNA (20-base ssDNA modified with the

SH-(CH2)6 group at the 5'-end with the sequences of 5'-SH-(CH2)6-CACACCTGACTTGACAGACC-3') concentration was achieved within 117 seconds. The DNA enrichment diagram by ACEO on electrode surface is shown in Figure 6. Wu et al. [61] designed a coaxial double-loop single-disk microarray and developed a DNA (20-base tDNA fragments) detection chip using DC bias ACEO stirring and electroanalytical impedance spectroscopy. The DNA sensor chip presented good linearity and repeatability over the 1 aM–10 pM concentration range and had the ultra-sensitive detection limit of 0.5 aM.

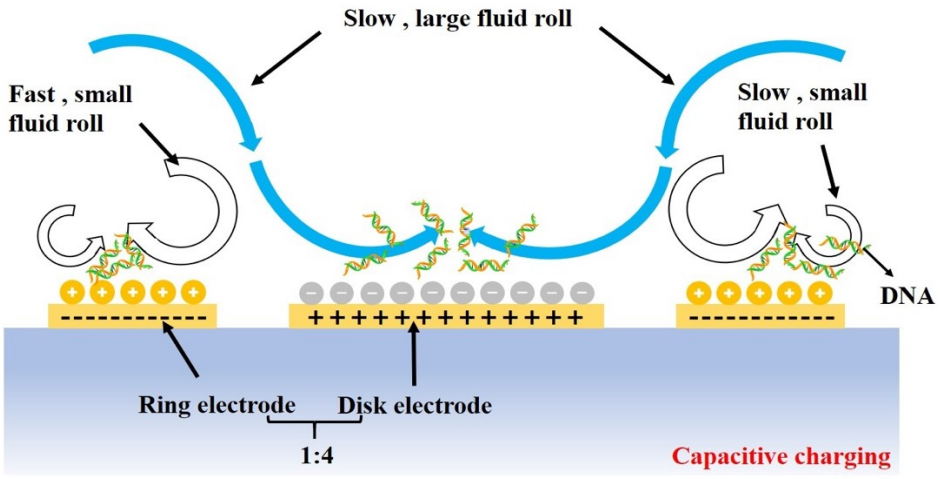

Figure 6. DNA enrichment diagram by ACEO on electrode surface. Reprinted with permission from [60], copyright (2013) Elsevier B.V.

Applications

At present, ACEK effect has been widely used in clinical diagnosis [62] and biomedical [63]. Its fast and label-free characteristics provide ideas for solving the technical problems of traditional nucleic acid sensors. Cheng et al. [64] induced positive DEP to accelerate DNA hybridization by applying a non-uniform AC electric field on the sensing electrode and realized the detection of the whole genome DNA of human herpes virus within a response time of 30 seconds. As common with testing clinical samples, serum samples are diluted with standard buffer prior to testing. The results of two dilution factors were compared. Interestingly, HSV-1 DNA serum at 1:20 dilution (LOD was 20.23pg/mL) had a lower detection limit than that of 1:10 dilution (LOD was 29.73pg/mL), which could be attributed to DEP enrichment since DEP is more significant in more dilute electrolytes. The experimental results are shown in Figure 7.

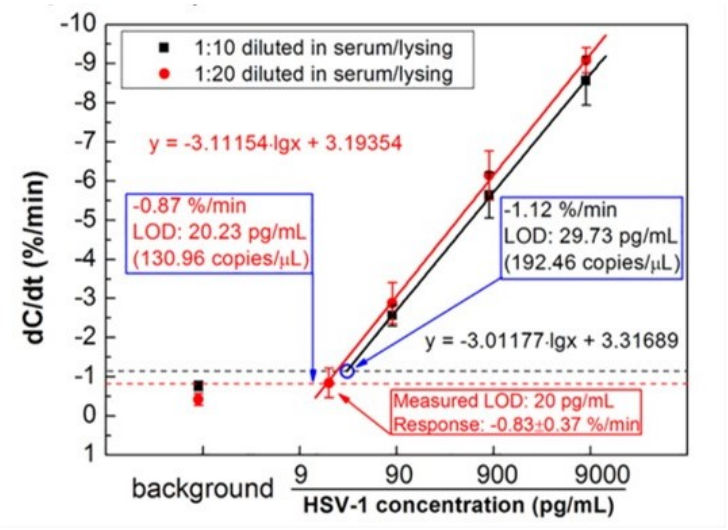

Figure 7. Dose response of HSV-1 DNA serum samples with 1:20 and 1:10 dilution with LOD of 20 pg/mL (129.47 copies/μL or 0.214fM) in serum with 1:20 dilution. Reprinted with permission from [64], copyright (2017) WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

The ACEK effect was integrated into the oxidize nanostructured porous silicon (pSI) optical biosensor by Rita et al. [65], and the target DNA molecules were enriched onto the pSI by the ACEO effect. Compared with the standard detection method using the same biosensor, the detection limit was improved by 1000 times. In another report, Cheng et al. [66] used DEP to rapidly enrich ssDNA molecules in flowing solution and make them form spike-like nano-colloid on the chip, thus achieving the detection of target single-stranded DNA within 1 minute, and the detection limit can reach picomolar level.

Other sensors

Aptamer sensors

Aptamer is a small piece of oligonucleotide fragment screened by vitro exponential enrichment technology [67,68]. Due to its high sensitivity, low cross-reactivity to small molecules and high stability, it gains popularity as a probe for biosensors. Aptamer-based biosensors are suitable for detection of many target analytes ranging from small molecules (e.g.BPA, cocaine, metal ions, pesticides) to larger particles such as LPS, bacteria and so on [69].

Although aptasensors based on traditional sensing mechanisms have good performance, there are some disadvantages such as complicated operation, long detection time, expensive equipment with them [70,71]. It is reported that combining ACEK effect with aptamer sensor could reduce the detection time and improve sensor performance effectively. The sensing method integrated with ACEK operates by using a carefully chosen symmetric or asymmetric AC signal to detect the

interfacial capacitance change of aptamer-modified fixed-size electrodes in PBS or deionized water diluted sample, and the AC signal will also simultaneously induce enrichment of molecules by ACEK effects during the detection [72].

Zhang et al. developed a broad-spectrum aptasensor specific to four high-toxic organophosphorus pesticides (profenofos, isocarbophos, omethoate and phorate) based on an interdigitated microelectrode chip, which integrated with ACET effect to enrich the trace target molecules without extra pretreatment for target detection. Due to the small size of organophosphates, DEP would not be an effective enrichment mechanism. So the AC signal was optimized to induce ACET directional flows carrying organophosphates, hence increased chance of binding. This aptasensor achieved a LOD of (0.24–1.67) fM, and a wide linear range (fM ~ nM) with simple preparation, and the turnaround time from sample to result was as short as 30s. Moreover, it showed an acceptable storability (about 14 days) which was essential to practical applications [73].

Oueslati et al. developed a highly sensitive and specific on-site detection method of serum cocaine by a low cost aptasensor. The cocaine-specific aptamer was immobilized on the electrode, and an AC signal at a fixed frequency was applied over the electrode sensors in sample fluids. The AC signal caused ACEO flow and enhanced the binding efficiency of cocaine and aptamer. The sample-to-result time was 30 seconds, the limit of detection was 7.8 fM, and the linear response for cocaine ranged from 14.5fM to 14.5pM in standard buffer, which were great improvements from other reported cocaine sensors [74]. The working principle of the aptamer sensor is shown in Figure 8.

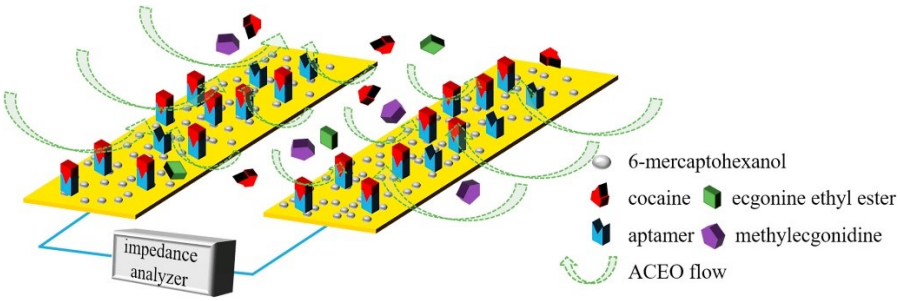

Figure 8. ACEO stream brings cocaine molecules to the functionalized sites and the specific detection of cocaine by aptamer sensors. Reprinted with permission from [74], copyright (2018) 2018 Elsevier B.V.

Lin et al. detected BPA from serum matrix by using the ACEK capacitive sensing method. The BPA aptamer specific to BPA was immobilized onto the platinum planar microelectrodes which were fabricated on silicon wafers. An AC signal at a fixed frequency was applied over the array of planar microelectrode sensors in sample fluids. The AC signal induced ACEO microflows to promote BPA-

aptamer binding and the interfacial capacitance change caused by molecular binding at the interface of sensor electrode and sample fluid could be read directly at the same time. They successfully detected BPA at fM level in biological fluids with good specificity in a short period of time [47].

Wu et al. used the similar method to detect larger bioparticles, such as lipopolysaccharide (LPS) and bacteria. LPS-specific aptasensors were prepared by immobilizing LPS specific aptamer on interdigitated microelectrodes. An inhomogeneous AC electric field was applied on the sensor, which induced positive dielectrophoresis (pDEP) to attracte LPS particles to the sensor electrodes. The binding process was detected by the same AC signal (500mVrms, 100kHz) via measuring the change of interfacial capacitance at the surface of micro-electrodes. The same sensor can be used to detect Gram-negative bacteria, since LPS is present in the outer membrane of Gram-negative bacteria. The AC signal was adjusted to 5 mVrms for bacteria detection, since bacteria are much larger than free LPS particles and 5 mVrms can produce sufficient DEP force on bacteria. The detection limit was 4.9 fg/mL for free LPS molecules and 53 #/mL of bacteria within a 30s response time [75].

Biotin sensors

Biotin consists of a tetrahydrothiophene ring containing a valeric acid side chain, which is joined to an imidazolidone ring to form eight possible stereoisomers. Biotinylated antibodies/antigens are currently used in many immunoassay formats of clinical settings for diversified analytes and biomarkers to offer high detection selectivity and sensitivity [76].

There are few studies on biotin sensors based on ACEK effect. Crivellari et al. studied a nanoparticle-based biosensing method using interfacial electrokinetic transduction. The sensor was based on the fluidic dielectrophoresis (fDEP). Biotin binding on nanoparticle was shown to increase the interfacial electrical conductivity of the suspending electrolyte, which was sensitively transduced as a change in electrokinetic interfacial deflection. The experimental device employed a combination of microfluidic flow, electrokinetics, and colloid-based biomolecular surface chemistry. They created a liquid interface for binding by co-flowing two different fluid streams through a microfluidic T-channel and integrated an array of parallel point microelectrodes within the main flow channel. Biotin concentrations could be detected as low as 500 aM by using interfacial electrokinetic transduction [77].

Feldman et al. studied ACET enhancement of heterogeneous assays in microfluidics. They investigated experimentally the effectiveness of electrothermal stirring by using a biotin–streptavidin heterogeneous assay. They used biotin as the probe which was immobilized on the bottom of a microwell device, and streptavidin was suspended in a high conductivity buffer.

Microfabricated electrodes were integrated within a microwell and driven at a frequency of 200 kHz and 10 Vrms, which could cause ACET. Fluorescent intensity measurements showed that for a five-minute assay, the binding rate increased by almost nine times through electrothermal stirring [78].

Conclusion

Affinity-based electroanalytical biosensors have great potential in the field of on-site real-time detection for a variety of applications. This review aims to briefly summarize the investigation and implementation of ACEK strategies for analyte enrichment that can be integrated with affinity biosensors over the past decade. Table 1 summarizes the affinity biosensors covered in this review, based on the enrichment mechanisms, the types of biorecognition, transduction methods and the LODs.

Table 1. Affinity-based electroanalytical biosensors integrated with ACEK enrichment organized by ACEK mechanisms

ACEK effect	Biomarker	Method	LOD	Ref
DEP	Antibody-antigen	Immunofluorescence	100 CFU/mL	[44,45]
	Antibody-antigen	Capacitive sensing		[50]
	DNA	Electrochemistry		[59]
	Antibody-antigen	Capacitive sensing	10 ng/mL	[42]
	DNA	Fluorescence labeling		[58]
	DNA	Capacitive sensing	20.23 pg/mL	[64]
	DNA	Fluorescence labeling	1 pM	[66]
	Aptamer	Capacitive sensing	4.9 fg/mL	[75]
	Biotin	Electrochemistry	500 aM	[77]
ACEO	Protein	Electrochemical Impedance spectroscopy		[41]
	Protein	Immunofluorescence	110 pg/mL	[46]
	Human D-dimer	Capacitive sensing	1 pg/mL	[52]
	DNA	Electrochemistry		[60]
	DNA	Electrochemical Impedance spectroscopy	0.5 aM	[61]
	DNA	Capacitive sensing		[65]
	Aptamer	Electrochemistry	14.5 pM	[74]
	Aptamer	Capacitive sensing	1 fM	[47]
ACET	Antibody-antigen	Sandwich Immunoassay		[40]
	Protein	Capacitive sensing	10 ng/mL	[43]
	Aptamer	Capacitive sensing	IgG:1ng/mL BPA:10μg/mL	[51]
	Antibody-antigen	Electrochemistry	0.24 fM	[73]
	Biotin	Electrochemistry		[78]

As we can see, ACEK enrichment can be integrated with a variety of biorecognition and transduction mechanisms, resulting in superior limits of detection. Breaking the limit by diffusion-controlled binding reaction will lead to high sensitivity and shortened assay time. Furthermore, the integration of ACEK effects with electroanalytical sensors is particularly advantageous, due to the fact that they are both operated by electrical signals. The small factor and programmability intrinsic to ACEK methods and electroanalytical sensors allow the realization of sensor portability, automation and rapid detection.

The above features are becoming important development directions, as they are critical to the realization of point-of-care diagnostics. Consequently, the application of ACEK microfluidic functions in electroanalytical biosensors is expected to increase, attracting attention and effort towards its research and development. New detection methods and devices will continue to appear, including the improvement of traditional methods, the combination with multiple detection methods, and the development of integrated biosensor chips. It is in this context that we hope this review could provide some insight and reference for researchers in related fields.

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Conflict of interest

The authors have declared no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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