Capillary electrochromatography (CEC): recent materials for capillary column preparation, applications, and future perspectives

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Abstract

Capillary electrochromatography (CEC) is a variation of the electrophoresis technique that employs a capillary column filled with a stationary phase. Similarly, as liquid chromatography, the development of new columns started to receive attention from scientists interested in instrumentation, and some materials have been tested in open tubular (OT) and monolithic columns. Among the materials that have received particular attention in CEC column fabrication, are highlighted: graphene oxide (GO)/graphene, ionic liquids (ILs) and molecularly imprinted polymer (MIP). The present review discusses some historical aspects of CEC, column technologies, and new materials (GO/graphene, ILs, and MIP) in column preparation focusing on their synthesis. Additionally, properties and applications in pharmaceutical, proteomic, environmental, and food analysis covering the period of 2012-2020.

Keywords: graphene, graphene oxide, ionic liquid, MIP, column technologies, capillary electrochromatography

Highlights: new materials used in column development - graphene, graphene oxide, ionic liquids, MIP - open tubular and monolith column technologies – applications in pharmaceutical, food, proteomics, and environmental separations.

Abbreviations: BSA, bovine serum albumin; CEC, Capillary electrochromatography; CNTs, carbon nanotubes; CPTMS, chloropropyltrimethoxysilane; EDMA, ethylene dimethacrylate; EOF, electroosmotic flow; G, graphene; GO, graphene oxide; HETP, height equivalent theoretical plate (HETP); IL, ionic liquid; LC, liquid chromatography; LMA, lauryl methacrylate; MIP, molecularly imprinted polymer; MNPs, magnetic nanoparticles; ODS, octadecylsilane; OT, open tubular; PAHs, polycyclic aromatic hydrocarbons; PANI, polyaniline; PDA, polydopamine; poly-TRIM, polytrimetilpropane trimethacrylate; RSD, residual standard deviation; SAX, strong anionic exchange; TMOS, tetramethoxysilane; XPS, x-ray photoelectron spectroscopy; XRD, x-ray diffraction.
1. CEC Historical aspects

The concept of capillary electrochromatography emerged with the Pretorious’ experiments in 1974 by applying a electrical potential instead of a pressure to pump the mobile phase. [1] The main problem found was column heating, a consequence of the use of an HPLC column with poor heat dissipation. In 1983 Jorgenson e Lukacs [2] conducted the first separation using graphite electrodes applying 30kV of voltage in a capillary column with 170 µm (internal diameter) packed with silica Partisil-10 ODS-2, with on-column fluorescence detection. The main advantage of this work was the possibility of applying high voltages, which contributes to faster separation. This was possible because of the use of a capillary column that dissipated better heat caused by the Joule heating effect. Following this work, Knox e Grant [3] studied the fundamental aspects of CEC, such as electroosmotic flow profile (EOF). EOF relation with particle size and a decrease in Joule heating effect when the capillary column is used were evaluated. All these works showed that better separation could be achieved using flow generated by potential difference instead of pressurized flow.

2. Column technologies and new materials used as stationary phases

It is well known that capillary columns in CEC are required due Joule effect. There are three column technologies used nowadays: packed columns, open tubular columns, and monolithic columns. These columns have vantages and advantages among them, and of course, materials used in column design are receiving special attention in the last years. The critical point is that in the period that this review comprises (2012-2020) almost all publications evaluated in several application fields (pharmaceutical, proteomic, food, environmental, etc.) employed either monolith or open tubular columns. This is because these columns do not use frits, so bubble formation in the system will decrease, as will be discussed in the next topic. Besides, heat-dissipation is better in these column types.

New materials used in column packing can improve selectivity, peak capacity, height equivalent theoretical plate (H), and, consequently, obtain better resolution. It was also noted that some materials had received particular attention in column development. Among these materials graphene (G), graphene oxide (GO), ionic liquid (IL), and also MIP, with few articles published in this period, are significant materials to obtain selective columns for particular compounds class. This subject will also be discussed in this review.

2.1. Packed columns

There is no commercially available frit for capillary columns, so it is necessary to use lab-made approaches. In situ, sintering is a useful alternative for frits if the material presents Si-OH groups. However, frit fabrication is a concern that deserves special attention because this process will affect column performance and also can be responsible for bubble formation in the CEC system. [4] The photopolymerization in UV is another alternative. Using suitable monomers, however, radiation can change the silica properties near the frit, which will also contribute to bubble formation. [5] The use of magnetic nanoparticles (MNPs) ferrite-based and reduced capillary column diameter in column extremity is another strategy. [6]

2.2. Open tubular columns

Open tubular (OT) columns are produced by forming a thin film of stationary phase on the column capillary walls. This column configuration has a better heating dissipation compared with a packed and monolithic column, but has lower sample capacity. The stationary phases used in OT columns are classified into four types: chemically bonded, physically or dynamically adsorbed, organic polymers based, and coated by the sol-gel process.
The organic polymer chemical bond onto the capillary silica wall forms the first type; the second phase-type use electrostatic interactions between the capillary and with a binder group; the third type involve a polymerization process during the capillary column preparation; a sol-gel process is the typical one representing the fourth and last type of phase. These processes are similar to monolithic column fabrication, but only a small amount is deposited in the capillary wall [7,8].

2.3. Monolith columns

Monolithic capillary columns consist of a porous bed of a solid stationary phase with network channels in which mobile phase flows. This column type has macrospores (diameter 2 to 50 nm) and mesopores (around to 2 nm diameter). The use of monolith is a good alternative for replacing the packing material because it does not require frit. These columns are prepared by in situ polymerizations. The phases that form the monolith are divided into two categories: silica-based monoliths and organic polymers based monoliths. [9,10]

2.4. Advanced materials used in capillary column preparation, properties, and synthesis strategies.

2.4.1. Graphene and graphene oxide

Graphene was discovered in 2004; this carbon nanomaterial has shown excellent properties for analytical sample preparation, columns preparation, and other applications. [11] This includes extraordinary electronic, thermal and mechanical properties, ultrahigh specific surface area, good thermal conductivity, electric mobility of charged carriers, excellent optical transparency, high Young’s modulus values, and fracture strength. Furthermore, graphene is inexpensive and easily synthesized. [11]

The nanosheet graphene morphology allows adsorption in both surfaces, and this can contribute to a better separation, been an advantage in comparison with carbon nanotubes and fullerene, for example. Nanotubes and fullerenes can present steric hindrance, blocking the possibility of the target compounds access their inner surface [12]. The main important application of graphene in separation using CEC is the separation of molecules with a delocalized π-electron system. This provides strong affinities for molecules with aromatic rings such as organic pollutants, drugs, and some biomolecules. There is also the possibility to functionalize graphene through GO. [11]

Many synthetic routes can be used to obtain graphene and GO starting with graphite. Gengle et al. [13] published a review addressing this theme. The primary method to obtain is by reducing graphene oxide; the most useful method to obtain graphene oxide is the Hummers 7 method, which follows a chemical exfoliation process. The main synthesis steps involve graphite oxidation by KMnO4, H2SO4 and NaNO3, followed by chemical exfoliation of the formed graphite oxide to obtain graphene oxide. Finally, the oxide reduction to graphene, using most commonly hydrazine, completes the sequence. A concern that must be considered is that graphene can agglomerate if dried in the wrong way (atmosphere dry). To prevent this, it is necessary to use freeze-drying under vacuum; this will prevent graphene nanosheets agglomeration. [11] If there is a need for separation of more polar compounds, GO can be used after being prepared using the same method proposed by Hummers [14], but escaping the last step (GO reduction). The same dry process can be used to prevent GO agglomeration, but if dried at room temperature, the GO agglomeration is expected to be lesser than for graphene nanosheets due presence of carbonyl and hydroxyl groups in the GO structure.

2.4.2. Ionic Liquids (ILs)

ILs are liquid salts at room temperature (25°C), whose melting point is lower than 100 °C. The ILs stand out due to the physicochemical properties derived from their molecular structure. Their molecular structure consists of organic cations or/and anions derived from Lewis bases (nitrogen and phosphorus) donor atoms. Some
examples of cations ILs are imidazolium, pyrrolidinium, and polyatomic anions containing different inorganic or organic anions as tetrafluoroborate, hexafluorophosphate, bromide). [15,16]

ILs have attracted interest in sample preparation and analytical separations due to the possibility of adding different substituents into the IL structure, thus tailoring their hydrophobic or hydrophilic abilities. The approaches used in column fabrication are supported, immobilized, attached, and surface-confined ILs.

IL supported can combine properties of IL with solid supports; the most usable are graphene, CNTs, fused silica or particles, and magnetic nanoparticles. This supported ILs allow different types of interactions such as ion-exchange (inorganic anions), hydrophobic interactions, electrostatic interactions, strong hydrogen bonding, and π-π, which contributes and improve the separation process. [16] The main limitation of this approach is to bond the IL onto the support. The most used support is silica due to the difficulty to immobilize ILs in the surface of inert materials; this is an advantage if taken in consideration that capillary column used to fabricate CEC columns are made of silica. [16]

2.4.3. Molecularly imprinted polymers (MIP)

The use of molecular imprinting arose from the molecular recognition defined as the ability of a molecule that binds to another to complement the first form, based on selective interactions such as antigen-antibody and enzyme-substrate. [17] This is responsible for the main advantages of using MIP as a stationary phase. The existence of selective sites is fundamental for the interaction between the monomer and the template molecule during the polymer synthesis. [18] For these aspects, MIPs exhibit a very high specificity for target compounds or compounds class.

Wulff and Sarham developed the first approach to molecular recognition in 1972; these authors described the synthesis of a covalent organic polymer with selective sites for glyceric acid enantiomers. In 1981, Arshady and Mosbach synthesized a non-covalent organic polymer different from the process developed by Wulff. [19] Since then, the imprinted materials have been widely studied and applied in many areas of chemistry. In CEC column preparation it is not different, although the publications in the last five years shows a clear preference for the use of graphene and ILs derivatives.

Three approaches can be used in MIP syntheses: covalent, non-covalent, and semi-covalent. The covalent method forms reversible covalent bonds between the monomers and the template. However, to remove the template from the resulting MIP, cleavage of the covalent bonds is required, and this can damage the polymer formed, and consequently, reduction of selectivity can be observed. In this type of material, the application becomes restricted as there are not many functional monomers that can be used in the syntheses. [20] The non-covalent approach involves the formation of non-covalent interactions between monomers and the template. These interactions are ionic, hydrophobic, or hydrogen bond types. This method for the preparation of MIPs is the most commonly used due to the incorporation and removal of the template without the formation and breakage of chemical bonds, an advantage over the first method. Besides, a wide variety of functional monomers can be used for selective interactions with the sites of the molecules of interest. However, this method also has lower selectivity because a balance occurs during the interaction of the monomer with the template in the synthesis process. Thus, it is necessary to use an excess of monomer to ensure the complex formation, and this factor leads to the formation of the MIP selective sites due to the presence of free functional monomers. The semi-covalent approach combines the advantages of two methods just described. Initially, covalent and non-covalent bonds are formed during the molecular recognition of the analytes. [19]
3. Applications of CEC focusing materials used in capillary columns and applications covering the period of 2012-2020.

The applications that were focused in this review comprise the period of 2012-2020 (pre prints and accepted papers as well) and intend to show possible applications of the discussed materials (G, GO, IL, and MIP) in CEC. The division of topic was done focusing on pharmaceutical, proteomic, environmental, and food applications.

3.1. Pharmaceutical application

CEC’s relevant applications that comprise pharmaceutical applications using different materials in CEC capillary columns are shown in Table 1. It can be noted that the works in this area of application focus on monolithic and OT columns. The highlighted material in this section is the use of graphene and MIP. Unfortunately, in this review it was not find any work that uses ILs for these applications.

Table 1. Recent column/material fabricated for CEC applications in pharmaceutical field.

<table>
<thead>
<tr>
<th>Column/materials</th>
<th>Applications</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolithic MIP column</td>
<td>Chiral separation of amlodipine</td>
<td>2012</td>
</tr>
<tr>
<td>Silica monolithic column chemically modified with L-pipeolic</td>
<td>Chiral separation of dansyl amino acids</td>
<td>2012</td>
</tr>
<tr>
<td>OT-BSA conjugated with GO/Fe₃O₄</td>
<td>Enantioseparation of L-tryptophan used in antidepressant agent</td>
<td>2013</td>
</tr>
<tr>
<td>Monolith BSA functionalized</td>
<td>Recognition between two of enantiomers pantoprazole and atenolol</td>
<td>2014</td>
</tr>
<tr>
<td>OT-GO functionalized</td>
<td>Benzhydryl compounds separation</td>
<td>2014</td>
</tr>
<tr>
<td>OT, GO-MIP</td>
<td>Determination of dopamine, epinephrine, and norepinephrine</td>
<td>2014</td>
</tr>
<tr>
<td>OT, silica sol-gel containing porphyrin-brucine conjugate</td>
<td>Evaluation of oligopeptides, nucleosides, nucleobases and nucleotides</td>
<td>2017</td>
</tr>
<tr>
<td>Polydopamine/Au nanoparticles/DNA on Chip</td>
<td>Separation of tryptophan enantiomers</td>
<td>2017</td>
</tr>
<tr>
<td>OT, GO modified with Maltodextrin</td>
<td>Separation of nefopam, amlodipine, citalopram hydrobromide, econazole, ketoconazole and cetirizine hydrochloride</td>
<td>2020</td>
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</table>
Lei et al. [30] explored the combination of monolithic design grafted MIP. Polytrimetilpropane trimethacrylate (poly-TRIM) was used as a template, performing the grafting polymerization. To evaluate the monolithic perform, the authors test the column for chiral separation of rac-amlodipine, zopiclone, and ofloxacin. The authors investigated the temperature effect in the column preparation, concluding that temperatures higher than 60 °C produces an occluded column. The use of different solvents in column preparation (ACN, toluene, and mix of toluene and isooctane) produced columns able to separate enantiomeric mixtures. However, the best results were obtained using toluene/isooctane. The results were also reproducible, showing an RSD lower than 5% for all compounds. This work contributes to showing a secure method to prepare a porogen column for chiral separation and also suggested that more than one solvent can be used in this kind of monolithic column preparation.

Liang et al. [23] proposed an OT column containing bovine serum albumin (BSA)-conjugated graphene oxide–magnetic nanocomposites (GO/Fe3O4) as stationary phase for enantioselective separation of L-tryptophan used as an antidepressant agent. The authors optimized the following CEC conditions: the influence of detection potential, separation voltage, buffer concentration, and pH buffer. These are the most relevant factors to be evaluated when developing a CEC method, taking into account that all these factors must be optimized to achieve better separation conditions. The RSD % obtained before method optimization was less than 6%. The main result of this work discussed by the authors was the possibility to conclude that stationary phase GO/Fe3O4/BSA can be packed at any desired position in the microchannel by an externally applied magnetic field, which simplifies the immobilization methodology of proteins in OT-CEC. Furthermore, the GO/Fe3O4/BSA can be regenerated easily, which helps method reproducibility.

Ye et al. [26] prepared an OT capillary column GO-MIP coated. **Figure 1** represents the schematic procedure to prepare this column. The method used in OT column preparation was the same used to coat CE columns using methacrylic acid and dopamine as a template monomer [31]. Results showed good reproducibility (RSD% lower than 6,2) for all analytes, with an excellent linear range (20,0–200,0 µg/mL). The material characterization was done using x-ray diffraction (XRD); most of the works that synthesize graphene for sample preparation and other applications use this method, sometimes in combination with Raman spectroscopy or x-ray photoelectron spectroscopy (XPS) [32]. The authors of this work also confirmed that EOF is stable in different pH ranges so that this study can contribute to the fabrication of other
MIP templates coated with graphene for other analytical proposed.

### 3.2. Proteomic applications

Recently scientists have moved their attention to proteomic fields identification, quantification, and characterization of the proteins and peptides of a specific biological fluid, cell, or tissue, and the study of their structure, function, and interactions. This interest is due to the expectation that the results obtained in this field can contribute to clinical diagnosis, treatment, and biomarker discovery, helping to prevent, diagnose, and monitor the evolution of many diseases. [33]

CEC can contribute to proteomic analysis, as shown in Table 2, which summarizes recent applications using materials highlighted in this review and other materials used in capillary column preparation. The material that clearly received particular attention in the revised period was ILs, which has more publications in this area, preferably using monolithic columns; some relevant works will be discussed below.

| Table 2. Recent column/material fabricated for CEC applications in environmental. |
|--------------------------------|---------------------------------|-------|
| Column materials | Applications | Year |
| Monolithic - IL (tetramethoxysilane and 3-mercap-topropyltrimethoxysilane) | PAHs, four alkylbenzenes and five phenols | 2012 [34] |
| Silica particles entrapped with monolithic frits | Alkyl benzenes separation | 2012 [35] |
| Monolithic- IL (1-vinyl-3-octylimidazolium chloride, VOICl(+):Cl(-)) with lauryl methacrylate (LMA) | Alkyl benzenes separation | 2012 [36] |
| Monolithic -GO incorpored in column | Separation of alkyl benzene and PAHs | 2012 [37] |
| Monolithic - organic-silica hybrid | Polycyclic aromatic hydrocarbons (PAHs), alkylbenzenes, phenols, inorganic anions and organic acids separation | 2013 [38] |
| Monolithic - (glycidyl methacrylate-co-ethyleneglycol dimethacrylate) | Alkylbenzene separation | 2014 [39] |
| OT – graphene | Alkylbenzene separation (in this case was evaluated different coated strategy) | 2014 [40] |
| Monolithic IL (1-butyl-3-vinylimidazolium-bis[(trifluoromethyl)sulfonyl]limide) | Separation of alkylbenzenes, phenols, anilines and polycyclic aromatic hydrocarbons | 2018 [41] |
| Monolithic, 1-vinyl-3-octylimidazolium bromide (VOI) and styrene | Separation of alkylbenzenes, polycyclic aromatic hydrocarbons, proteins and amino acids | 2020 [42] |
Wang et al. [36] proposed an IL monolithic capillary column prepared by thermal free-radical copolymerization of IL (1-vinyl-3-octylimidazolium chloride, ViOcIm\textsuperscript{+}Cl\textsuperscript{−}) together with lauryl methacrylate (LMA) as the binary functional monomers, and ethylene dimethacrylate (EDMA) as the cross-linker in binary porogen. The proportions of reagents used in column the fabrication were optimized and showed, as expected, a remarkable influence in EOF. Some factors that affected the polymerization mixture of cationic monomers, as in IL case, include: the distance of the imidazole groups and the radical position, charge density, ionic strength, and hydrophobic interactions. So, it is also important to evaluate different proportions among reagents to find better conditions. In this work the authors suggested the optimal ratio of ViOcIm\textsuperscript{+}Cl\textsuperscript{−} to EDMA was 60–40% considering of the visual appearance of peak shape (of course a better option is the use of a factorial design to evaluate this). A monolithic column with cationic imidazole groups of ViOcIm\textsuperscript{+}Cl\textsuperscript{−} can generate a stable reversed EOF in a wide pH range. It was demonstrated that the separation mechanism of charged compounds could be a mixed-mode of SAX, and hydrophobic interaction and neutral compounds separations were based on hydrophobic interaction.

Xiau Wang et al. [43] prepared a monolithic column using pyridinium immobilized in silica surface by in situ polymerization in 3-chloropropyl-silica and 4,4′-dipyridyl for hydrophilic interaction. The hybrid material has stable microstructures with a hydrophilic retention mechanism using a mobile phase with a CAN concentration higher than 50%. Stable EOF was observed in 3.0-9.0 range of pH. The combination of this IL supported on a silica surface also can supply other separations mechanisms (hydrogen bond, \(\pi-\pi\) interactions, and anion exchange). The column was employed in amino acids and nucleotides separation (and also phenol, but this is not the focus in this section), and the number of theoretical plates achieved for nucleic acids was near 164 000. This capillary column approach has great potential to be used for the analysis of polar compounds in CEC. Of course, the comparison with other approaches is necessary to evaluate the best methodology.

Gao et al. [44] produced an OT-CEC column coated with GO using a tentacle-type polymer coating (Figure 2). This approach is also used in other capillary column preparation for CEC [45,46]. This column showed a stable EOF in a wide pH range (4.0-9.0), having a good separation of small neutral molecules, amino acids, and ephedrine–pseudoephedrine isomers with a resolution higher than 2.30 and column efficiency up to over 170 000 plates/m. The reproducibility in column preparations showed an RSD lower than 7.5%, which is suitable considering that many variables can influence this kind of column preparation. This column was compared with a polymer and GO capillary coated and showed higher theoretical plate numbers and resolution than both. The results showed that this column could be used to provide separation of peptides and other small molecules. The results will be better for polar compounds because GO is better for this kind of separation than graphene (G) due to the presence of hydroxyl groups in the GO surface.
Han et al. [34] fabricated a monolithic column based on mercaptopropy-functionalized (MP-silica), prepared by in situ co-condensation tetramethoxysilane and 3-mercaptopropyltrimethoxysilane exploring sol-gel process. The authors used FT-IR and XPS to characterize

Table 3. Recent column/material fabricated for CEC applications in proteomic.

<table>
<thead>
<tr>
<th>Column materials</th>
<th>Applications</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>OT - bare gold nanoparticles (GNPs)-based stationary phase</td>
<td>Tryptic peptide fragments of native and glycated proteins, bovine serum albumin (BSA), and human transferrin (HTF)</td>
<td>2012</td>
</tr>
<tr>
<td>Monolithic - IL IL (1-vinyl-3-octylimidazolium chloride, ViOClm(+)[Cl(-)]with lauryl methacrylate (LMA)</td>
<td>Amino acids separation</td>
<td>2012</td>
</tr>
<tr>
<td>Monolithic - (ILs) based on 1-vinyl-3-octylimidazolium</td>
<td>Four proteins separation</td>
<td>2013</td>
</tr>
<tr>
<td>Monolithic-ILs pyridinium-based</td>
<td>Nucleotides, nucleic acid bases, and nucleosides and nicotines</td>
<td>2013</td>
</tr>
<tr>
<td>OT - polydopamine (PDA) coating as an adhesive</td>
<td>Proteins separation in biological sample</td>
<td>2013</td>
</tr>
<tr>
<td>OT - histone deacetylase</td>
<td>Apigenin, naringenin, luteolin, and kaempferol characterization</td>
<td>2013</td>
</tr>
<tr>
<td>OT - APAT-DVB heptakis(2,3-di-O-acetyl-6-O-sulfo)-β-CD</td>
<td>D-and l-aspartic acid, d- and l-tyrosine and d-and l-lysine</td>
<td>2014</td>
</tr>
<tr>
<td>monolith BSA functionalized</td>
<td>Tryptophan enantiomers separation</td>
<td>2014</td>
</tr>
<tr>
<td>OT - BSA/ PDA/GO</td>
<td>Separation of chiral amino acids tryptophan and threonine</td>
<td>2014</td>
</tr>
<tr>
<td>OT - N-phenylacrylamide-styrene copolymer-</td>
<td>Separation of anomers of glucose and structural isomers of maltotriose</td>
<td>2015</td>
</tr>
<tr>
<td>OT – GO</td>
<td>Amino acids separation</td>
<td>2015</td>
</tr>
<tr>
<td>OT, ctaglycidyldimethylsilyl polyhedral oligomeric silsesquioxanes and 4-aminophenyl disulfide</td>
<td>Tryptic digest of mouse liver protein</td>
<td>2017</td>
</tr>
<tr>
<td>Monolithic, 1-vinyl-3-octylimidazolium bromide (VOI) and styrene</td>
<td>Separation of alkylbenzenes, polycyclic aromatic hydrocarbons, proteins and amino acids</td>
<td>2020</td>
</tr>
</tbody>
</table>
the column, which generate a stable EOF in a wide range of pH (3.0–8.0). The column’s evaluation was done separating 4 alkylbenzenes, and 5 phenols derivatives and 3 PAHs, with an RSD lower than 6% for different columns, showing the excellent reproducibility of the synthesis method. Unfortunately, the authors did not test these aspects for other compounds (alkylbenzenes and phenols derivate), which could supply better information on the column behavior for different compounds.

Zhang et al. [40] fabricated an OT column for CEC coated with graphene, with a fascinating strategy used, different from that one just cited in this review. The authors first functionalized the capillary surface with polydopamine (PDA), then GO introduced reacting covalently with PDA. PDA’s modification efficiency, a conductive polymer, was improved, introducing polyaniline (PANI) to be a sub-layer; PDA was then introduced followed by GO to generate a multilayer capillary column coated with GO-PDA-PANI. The PANI used as a sub-layer improved the capillary separation column. The EOF of the different columns was evaluated by changing the mobile phase pH using thiourea as the EOF marker. For GO-PDA-PANI, the EOF is stable in a pH ranging 4.0-7.0 as shown in Figure 3. The separation efficiency was done evaluating alkylbenzenes separation, and theoretical plate numbers were higher than 130 000 for benzene, and RSD was lower than 6,2% for different columns.

Thang et al. [38] developed a CEC monolithic column with two separation mechanisms (mixed mode) using sol-gel tetramethoxysilane (TMOS) and c-chloropropyl-trimethoxysilane (CPTMS) as monomers followed by a post-modification using 4,4-dipyridine. The mechanism of separation exhibit hydrophobic, π-π and anion-exchange interactions, which can help in an improvement in selective mainly for the compounds used in this case: PAHs, alkylbenzenes, phenols, inorganic anions, and organic acids. The column generated a stable EOF and reversed at acidic pH (3-6) due to the pyridinium groups on the surface of the stationary phase; RSD for different monolithic columns were less than 5% for all compounds. Furthermore, the monolithic column showed a uniform, monolithic matrix tightly bonded onto the inner capillary wall, and the dipyrindinefunctionalized monolithic column showed multiple separation capabilities to be used in neutral and anionic compounds.

3.4. Food

There are still only a few publications about the determination of contaminants or other compounds in food by CEC in the period covered by this review, as shown in Table 4. The work that deserves special attention here is the application developed by OuYang et al. [54]. The innovative approach used for these authors used a pressurized CEC system that reminds a pressure pump used in HPLC. The significant advantage that the use of pressure can provide is to improve the EOF reproducibility, which is cited as one of the main problems when using CEC systems. Another point here is that the use of a packed capillary column was not cited yet during the discussion because of the disadvantages that it has when compared to OT and monolithic columns, considering the practical difficulties of reproducing frits sintering and flow reduction. The method proposed by these authors is beneficial in terms of lesser solvent consumption, high separation efficiency,
and rapid analysis than flow generated by the potential differences.

Table 4. Recent column/material fabricated for CEC applications in food contaminants analysis.

<table>
<thead>
<tr>
<th>Column/materials</th>
<th>Applications</th>
<th>Year</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monolith, core-shell silica nanoparticles Fe(3)O(4)@SiO(2)/NH(2)</td>
<td>Organic acids separation in complex samples</td>
<td>2012</td>
<td>[30]</td>
</tr>
<tr>
<td>Packed, (Electropak™, C18, ODS, 3 μm column)</td>
<td>Determination of Flumequine and Oxolinic Acid Residues in Aquatic Products</td>
<td>2014</td>
<td>[54]</td>
</tr>
<tr>
<td>Monolith, organic-inorganic hybrid MIP</td>
<td>Organophosphorus pesticides determination in food sample</td>
<td>2014</td>
<td>[55]</td>
</tr>
<tr>
<td>Monolith, Poly(vinylimidazole-CO-ethylene dimethacrylate)</td>
<td>Nitrate and nitrite in vegetables</td>
<td>2019</td>
<td>[56]</td>
</tr>
</tbody>
</table>

4. Concluding Remarks

Regarding the aspects of new analytical techniques, CEC has the most considerable importance to the pharmaceutical industry, especially in the analysis of neutral and acidic compounds, being limited for the separation of strongly alkaline compounds. This may be a paradox since many pharmaceutical industry products contain basic functions such as amines, which are often strongly alkaline, being undoubtedly an obstacle to the evolution of technique. Although some progress has been achieved by adding additives to the mobile phase, there is still a need for continued progress.

In the past, most capillary columns used were made from fused silica, but we can observe in this review that this is a trad off. The use of OT and monolithic column shows an advantage mainly because there is no need for sinterized frit in this type of column, which prevents bubble formation in the system. The study of different materials used for column fabrication has been showing a significant interesting field for researches, with ILs and graphene/GO as the most studied materials in the period covered.

A concerning point in the CEC technique is related to instrumentation. Although CE equipment can be adapted, control of EOF is difficult in these conditions. One way of solving this problem would be to use a hydraulic flow to overcome flow generated electrically, improving the reproducibility of the technique as just cited in this review. [54] This issue can be part of a μ-LC-CEC, but manufacturers have not shown interest in this system. [57].

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