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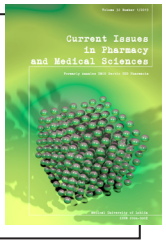

An underestimated technique. Does pressurized and pressure-assisted capillary electrochromatography have potential in drug and pharmacological-active compounds analysis?

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ABSTRACT

Pharmaceutical analysis guarantees patient safety all over the world. Thus, continuous development of existing analytical techniques is still very important. Pressurized capillary electrochromatography and pressure-assisted capillary electrochromatography are hybrid separation techniques that combine the selectivity of liquid chromatography and the high separation efficiency of capillary electrophoresis. They use a smaller amount of reagents and samples, hence, reducing the total cost of analysis. Therefore they have found application in a number of pharmaceutical and biomedical analysis. This review article focuses on the use and importance of pressurized and pressure-assisted capillary electrochromatography in pharmaceutical and biomedical analysis, taking into account types of detectors and capillaries used. Despite the fact that pressurized capillary electrochromatography and pressure assisted capillary electrochromatography offer many possibilities and have been available for over a dozen years, they are still underdevelopment and not fully explored.

INTRODUCTION

Patient health and safety is a very important issue. For this reason, methods for determining drug composition and evaluating possible impurities are in permanent development. Thus, there is a clear need for continuous improvement of current analytical techniques or for the creation of alternative ones for qualitative and quantitative analysis.

Methods routinely used in pharmaceutical analysis are liquid chromatography (LC) or gas chromatography (GC) as separation techniques or coupled with ultraviolet-visible (UV-VIS), mass spectrometry (MS) or nuclear magnetic resonance (NMR) detection [1,2]. There are, however, alternative methodologies with significant potential that are economically attractive and environmentally friendly. These variants include miniaturized separations through electromigration techniques such as capillary electrophoresis (CE) and capillary electrochromatography (CEC), nano-liquid chromatography (nano-LC), capillary liquid chromatography (CLC), micro-liquid chromatography (micro-LC) and their microchip versions. Such techniques offer a variety of advantages over conventional techniques, for example, shorter analysis time or lower reagent and sample consumption [3]. Although the number of studies or lab practices employing these techniques is still marginal in comparison to classical LC or GC, a literature research evidences that their role in pharmaceutical analysis may become significant.

Despite the fact that HPLC and GC play dominant roles in the field of analytical chemistry, pressurized capillary electrochromatography (pCEC) and pressure-assisted capillary electrochromatography (PACEC) have found a special place. This situation has come about mainly because of the combined advantages coming from LC and CE. In pCEC, due to molecular interactions between substances being separated and components of the separation system (stationary and mobile phases located in capillary interaction), greater separation selectivity is obtained in comparison to HPLC and CE. Herein, the use of electroosmotic flow provides low solute dispersion in the capillary – which increases the efficiency of separation in comparison to other, traditional separation techniques. In addition the high surrounding pressure suppresses the effect of the bubble formation that disturbs separation processes and especially solute detection in the flow cell (see a schematic view of typical pCEC equipment in Fig. 1a and 1b).

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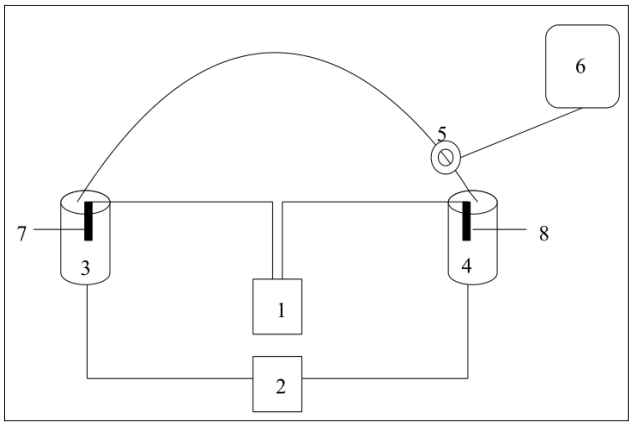
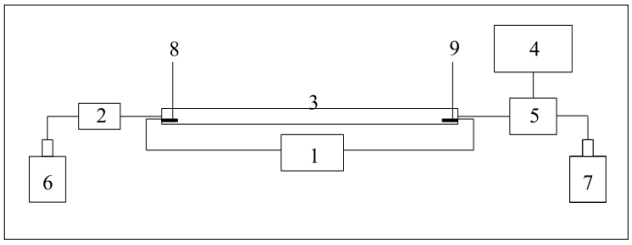


1 - high-voltage power supply, 2 - pump, 3 - inlet vial, 4 - outlet vial, 5 - detector, 6 - computer, 7 - anode, 8 - cathode

Figure 1a. Schematic of a pCEC system on a typical CE device



1 - high-voltage power supply, 2 - pump, 3 - column, 4 - computer, 5 - detector, 6 - mobile phase, 7 - waste, 8 - anode, 9 - cathode

Figure 1b. Schematic of a pCEC system on a liquid chromatography instrument

Although pCEC has many advantages, it also has some limitations. Disadvantages include capillary fragility; peak tailing of basic compounds; low column capacity (which affects the efficiency of separation); Joule heating; the need to generate an internal standard while using pressure injection and that electrokinetic injections are highly dependent on sample matrix.

It is easy to find reviews about the overall application of CEC in food, proteomics, peptidomics, natural products or pharmaceutical analysis, but some are not up to date. Moreover, no author has focused precisely on pCEC/PACEC and their different modes (packed or monolithic columns/capillaries), the types of detectors used and the possibility of connection with mass spectrometry (MS). Therefore, the aim of this work is to fill out the above-mentioned gap and emphasize that pCEC and PACEC have considerable potential. The purpose of this review article is to conduct an accurate and comprehensive study of possibilities in pharmaceutical and biomedical analysis for pCEC/PACEC and to pay attention to new prospects in the development of planar electrochromatography.

FUNDAMENTALS OF CAPILLARY ELECTROCHROMATOGRAPHY

History

The history of CE and CEC can be found in Paul D. Grossman and Joel C. Colburn – "Capillary Electophoresis: theory and practice" [4] or Keith D. Bartle and Peter Mayers – "Capillary Electrochromatography" [5].

Despite the many advantages of the above-mentioned techniques, researchers were still feeling the need for their improvement, especially in respect of reduction of Joule heating causing bubble formation. The first attempts were made by Tsuda *et al*. [6], who used both pressure and electrosmotic flow in the columns. Here, the authors, after careful study, concluded that bubbles do not form in the effective part of capillary, but rather in the outlet line. To minimize this, they applied constant electric current during the experiment. Their technique, pseudo-electrochromatography, is considered a useful method for separating electrically neutral substances from charged components.

The solution advanced by Knox and Grant [7] to mitigate capillary tube bubbling was to incorporate thermosets and to pressurize the apparatus, particularly, in the inlet and outlet valve areas [8,9]. Other researchers applied additional pressure to only the inlet valve [10,11]. Criticism, however, rose about complicating a simple apparatus and technique [12].

The pCEC technique is another step in advancing electrochromatographic methods. Here, to prevent bubble formation, equal pressure is applied to both ends of the capillary tube. In this variant, it is also possible to apply pressure to just one side of the capillary/column or to apply non-equal pressure. This mode is called 'pressure-assisted capillary electrochromatography' and gives new possibilities, for example, applying pressure flow opposite to the direction of electroosmotic flow. In pCEC, the pressure applied is independent of electroosmotic flow and not only minimizes bubble formation, but also changes the selectivity. Another alternative is a hybrid of two techniques: capillary electrochromatography and high performance liquid chromatography. This reflects more the idea of PACEC. The fusion provides high efficiency and comprehensive selectivity [13].

Instrumentation

Pressurized capillary electrochromatography (pCEC) may be performed using modified CE devices (Figure 1a) or capillary liquid chromatography instruments (Figure 1b) with voltage applied over the glass column or with tubing switched to tetrafluoroethylene (such material guarantees insulation against current of electricity). The capillary liquid chromatography instrument also gives possibility of utilizing gradient elution, which helps to solve the general elution problem – a desirable feature in chromatographic techniques [14]. One of the most commonly used pCEC instruments is TriSep 3000, offered by Unimicro. The item provides "ultra-efficiency, ultra-speed, ultra-peak capacity and ultra-reproducibility", and results can be easily verified, because pCEC may be compared to other techniques while utilizing the same equipment. TriSep 3000, for example, gives opportunity to perform separations by means of three techniques using one instrument i.e. pCEC, micro-high-performance liquid chromatography (micro-HPLC), and CEC [15]. Another product designed for pCEC is the 7100 CE System from Agilent. This also offers the advantage of several operation modes using one instrument [16].

Although there is commercially available equipment for pCEC, researchers also have found other, new solutions. Vickers and Smith performed their experiments with in-house modified Unicam and Lauerlabs (Prince CE and ABI instruments) devices [17]. Moreover, Wu *et al*.

have modified a TriSep 2100 GV CEC instrument for laser induced fluorescence detection [18].

General principles of pCEC

Pressurized capillary electrochromatography enables the separation of neutral compounds due to their chromatographic partitioning, similar to micro-HPLC. For charged compounds, the separation mechanism is the result of electrophoretic mobility and chromatographic partitioning. This approach is analogous to both CE and micro-HPLC. The hyphenation of the separation mechanisms results in high separation speed, high resolution, high efficiency and high peak capacity, mainly due to the combination of hydrodynamic laminar flow (Figure 2a) and the mobile phase electroosmotic induced flat profile of flow (Figure 2b) [19].

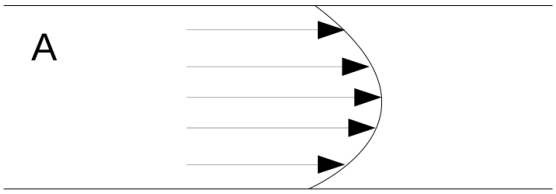


Figure 2a. Scheme of laminar flow profile

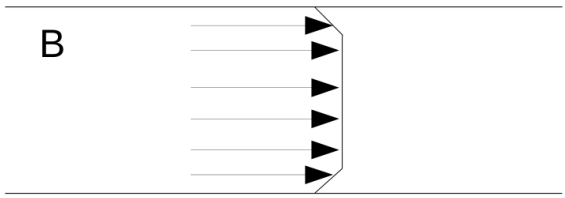


Figure 2b. Scheme of electroosmotic flow profile

THE ROLE OF PCEC IN DRUG ANALYSIS

A significant number of articles can be found in the bibliography of this paper that reveals that pCEC can be successfully used in pharmaceutical analysis. Moreover, existing publications demonstrate that this technique has a wide range of application.

Applications of pCEC with packed columns

Almost one third of the works summarized in Table 1 concern packed columns, probably because these are commercially available or can may be easily prepared in the laboratory. In [20-28], the capillaries used ranged in length from 30 to 45 cm. In [20], the longest capillary is applied for alkaloid separation from cortex *Phellodendri amurens* so as to obtain the highest resolution for such complicated mixture. In [20], probably a similar length was used to separate flumequine and oxolinic acid residues in aquatic products. Still, such long capillary is not needed if the pH of the mobile phase and the ratio mobile phase/buffer is properly selected, and it should be noted that the length of the packed stationary phase is of greater importance. For example, in [22], Wistuba *et al*. prepared several variants of capillary length, the majority of packed capillaries being of 100 μm inner diameter, while only few were 75 μm. Herein, the researchers were aware that inner diameters larger than 100 μm may cause band broadening and may be very significant in a separation system that is without an adequate cooling system. It is easy to notice that pCEC gives opportunity for using small bed diameters. Indeed, the diameter of the stationary phase can be from 1 to 5 μm, the most often employed are those of 3 or 5 μm. Smaller particles are rarely used because they are difficult to packed and bubble formation may occur [20-28]. In contrast, stationary phases of largest diameter are used for enantioseparation. Herein, silica is bonded with permethyl-β-cyclodextrine built of even 8 molecules of glucose [22,23].

Almost all the stationary phases in capillaries are capable of being used as reversed phase systems. These are employed for separation of organic drugs with high molecular weight. The only exception is the Pirkle type stationary phase Whelk-O 1, which produces a normal-phase system [23]. Most of the particles used in experiments are of the octadecylsilica (ODS) type. They are commercially available and their usefulness has been proven in both CEC and μHPLC. Other types are employed in specific circumstances, for example: hydride- based octadecyl stationary phase or perphenylcarbamoylated β-cyclodextrin bonded silica particles for enantioseparation [20-28].

Table 1. Applications of pCEC with packed capillaries in pharmaceutical analysis

Compound	Matrix	Sample preparation	Total length, (effective length), inner diameter	Stationary phase	Detector	Article
Alkaloids from *Cortex Phellodendri amurens*	Biological sample - cortex	Extraction with 80% ethanol	45 cm (20 cm), 100 μm i.d.	Octadecylsilica (3 μm)	Photodiode array dectector 230 nm	20
Steroids (Prednisolone, corticosterone, norgestrel, progesterone)	Solutes		30 cm (20 cm) i.d. 100 μm; 35 cm (20 cm), i.d. 50 μm	Hydride- based C18 (4 and 1,8 μm)	UV - 254 nm	21
Barbitals	Enantiomers solutes		40 cm (25, 23.5cm) 100 μm i.d.	Permethyl- β- cyclodextrin (?)	UV - 230 nm	22
Warfarin	Enantiomers solutes		45 cm (30 cm), 100 μm i.d.	Spherisorb silica, R,R Whelk-O 1 (3 or 5 μm)	UV - 254 nm	23
Ephinephrine, synephrine, alprenolol, isoproterenol, propranolol	Solutes		35 cm (18 cm) 75 μm	Perphenylcarbaoylated β- cyclodextrin bonded- silica (5 μm)	UV - 230 nm	24
Propranolol and its enantiomers	Biological sample - urine		30 cm (18 cm), i.d. 100 μm	Perphenylcarbaoylated β-cyclodextrin bonded- silica (5 μm)	UV - 214 nm	25
Trimethoprim impurities	Solutes		40 cm (20 cm), 75 μm i.d.	octadecylsilica (1 μm)	UV - 254 nm	26
Coumarins	Extract from plant	Ultrasonic extraction with ethanol	40 cm (15 cm), 100 μm i.d.	octadecylsilica (3 μm)	UV - 320 nm	27
Flumequine, Oxolic acid residues	Fish tissiues	Solid phase extraction	(20 cm), 100 μm i.d.	octadecylsilica (3 μm)	UV - 180-800 nm	28

The columns used in such work have been subdivided into two categories: packed and monolithic, but Chen *et al*. prepared a packed column with a monolithic outlet frit, which proves that various technical solutions may be applied in pCEC practices. The procedure has been validated; therefore it is easy to compare the solution mentioned with other approaches [27].

Diode Array Spectrometers are mostly used as detectors. They give the opportunity to measure a wide range of spectrum during a single analysis, thus making it possible to identify the compound by its absorption spectrum [20-28].

In addition to technical parameters, the usefulness of pCEC is also characterized by its application. Although there are not many articles about packed columns in pCEC, a great variety of article themes can be noticed. Scientists have utilized the instrument to separate both plant and synthetic drugs, for example, alkaloids from *Phellodendri amurens* [20], coumarins from *Cinidium monnieri* [27], steroids [21], barbitals [22], β-blokers [25], symphatomimetics [24], chemotherapeutics [28] and anticoagulants [23]. The subjects of the analysis were matrixes from cortex [20], fruits [27], urine [25], fish [28], plants [27] or even enantiomer solutes [22]. Such complicated mixtures cannot be separated directly; therefore, a prior extraction is necessary. In such cases, extraction with organic solvent is the most popular [20,27]. This is sometimes coupled with ultrasonification and solid phase extraction [27,28].

Yang *et al*. [20] provide one of the most interesting applications of pCEC]. The team developed two analytical RP-HPLC and pCEC methods for the separation and determination of total alkaloids from the cortex of *Phellodendri amurens.* The scientists compared the results they received from utilizing both methods and the conclusion is that alkaloids from *Phellodendri amurens* were both well separated by pCEC and RP-HPLC, yet the results obtained by pCEC were clearly better than that by RP-HPLC. Moreover, the analysis was more than two times shorter, the consumption of reagents was smaller and the column efficiency was higher than that in RP-HPLC. Of note, the results may be very important for potential drug development, as the recovered alkaloids may be useful in treating hypertension, diarrhea, inflammations and arrhythmia.

Another application worth mentioning in the field of pharmaceutical analysis is that presented by Ou Yang *et al*. [28]. Here, the authors separated flumequine and oxolinic acid residues in fishes. The separation required solid phase extraction, as animal tissues are a complicated matrix. The conclusions of this method were very similar to the one mentioned above, that the method has a good potential, high separation efficiency, short analysis time and the consumption of solvents is low. That is why this technique is beneficial for the environment.

Applications of pCEC with monolithic columns

Within the last ten years, due to the development of technology, there has been a significant breakthrough in synthesizing monolithic columns (see Table 2). In comparison to applications with packed columns, there are almost two times more papers about the use of monolithic capillaries in pCEC drug analysis [29-42].

The length of the monolith capillaries adopted was more diverse than in case of packed columns. The range was from 30 to 66 cm, and it was difficult to find two capillaries with the same length. If we compare internal diameter (ID), monolith capillaries look similar to packed columns – the range was from 75 to 100 µm, and capillaries with a 100 µm ID are most often used.

Even though the first monolithic capillaries were prepared in the mid-1990s, scientists are still looking for new insights

Table 2. Applications of pCEC with monolithic capillaries in pharmaceutical analysis

Compound	Matrix	Sample preparation	total length, (effective length), inner diameter	Stationary phase	Detector	Article
Antiparasitic drugs: ornidazole	Tablets	Extraction with acetonitrile	52 cm (30 cm) I.d. ?	Moleculary imprinted polymer	UV - 310 nm	29
β2-agonists	Biological sample - urine	Extraction with methanol	66 cm (?), 100 µm i.d.	Self- made silica- based monolith	MS - positive ionization	30
Hydroquinone	Solutes		45 cm (30 cm), 100 µm i.d.	Sulfonated stearyl methacrylate	UV - 214 nm	31
Dopamine, epinephrine	Solutes		45 cm (30 cm), 100 µm i.d.	Sulfonated stearyl methacrylate	UV - 214 nm	31
β2 -agonists (salbutamol, terbutalina), benzocaine, cocaine, heroin	Biological sample - urine	Extraction with methanol	60 cm, 100 µm	(1-hexadecene-co-TMPTMA)	ESI - MS	32
Water- and fat- soluble vitamins	Tablets	Extraction with ethanol	30 cm (12,5 cm) 100 µm i.d.	Methacrylate-based	UV - 210 nm	33
Phenols: Thymol, hydroquinone	Solutes		55 cm (25 cm), 75 µm i.d.	Phenylaminopropyl silica	UV - 214 nm or 254 nm	34
Nitroimidazole drugs: metonidazole, tinidazole, dimetridazole, ronidazole, secnidazole	Solutes		51 cm (30 cm), 100 µm i.d.	Moleculary imprinted polymer	UV - 320 nm	35
Narcotics: methadone, pethidine, fentanyl, morphine, diamorphine	Human urine	Extraction with methanol	56 cm, 100 µm i.d.	Silica- based MTMOS, MTMS	ESI - MS	36
Phenols: hydroquinone, catechol, resorcinol	Solutes		64 cm (44 cm) 75 µm i.d.	3-(2-aminoethyloamino)propyl ligands	UV - 254 nm	37
Flavonoids	Biological sample - corn	Extraction with methanol	35 cm (200 µm i.d.)	octadecylsilica	UV - 270 nm	38
Phenols: pyrogallol, pyrocatechol	Solutes		55 cm (35 cm), 100 µm i.d.	Ion liquid bonded multifuntcional stationaryphase	UV - 202 nm	39
β- blockers: propranolol, carteolol, bisoprolol, celiprolol, esmolol, metoprolil, atenolol	Human urine	Extraction with methanol	58 cm, 100 µm i.d.		ESI - MS	40
Resorcinol, catechol	Solutes		50 cm (25 cm) 100 µm i.d.	Polymethacrylate-base	UV - 214 nm	41
Acidic, basic, neutral drugs: benzylamine, terbutaline, remacemide, nortriptyline, biphenyl, anisole,	Solutes		33,5 cm (25 cm), 100 µm i.d.	TMOS and MTMS	UV - 210 nm	42

into the refinement of monolithic columns. That is why in such articles, such large difference in the method of stationary phase preparation can be found. Herein, researchers have investigated molecularly imprinted polymers [29,35], sulfonated and cationic stearyl methacrylate [31], methacrylate based [33], phenylaminopropyl silica based [34] columns. Most are proprietary ideas or modifications of ideas of other authors [29-42].

In this field, a larger number of works has been recorded. This results in considerable differentiation of the molecules studied. Through monolith capillary pCEC, it is possible to distinguish medicinal substances of plant origin, as well as synthetic. Scientists have used the device to determine phenols (catechol, thymol, hydroquinone, resorcinol) [31,41], flavonoids (morin, daidzein, naringenin, chrysin) [38], narcotics (methadone, fentanyl, pethidine, morphine, diamorphine) [36], catechol amines (dopamine, epinephrine) [31], water- and fat-soluble vitamins (A, D, E, K, C, PP, B_1, B_2, B_6) [33] and chemotherapeutics (nitroimidazole, tinidazole, dimetridazole, ronidazole, secnidazole) [35]. The matrices were biological samples such as corn [38], human urine [30,32,33,36,38,40] or tablets [33]. Naturally, earlier preparation of the samples was necessary, but the most common methods applied were simple extraction with organic solvents (acetonitrile, methanol) and then filtration [29-42].

In one fourth of the works, the CEC device was matched with a mass spectrometer for use in detecting, for example, certain β_2-agonists, β-blokers and narcotics [30,32,36,40]. All of the experiments in such studies were performed with equipment from Agilent Technologies. This is because their CEC product can be readily matched with MS instrumentation. The ability to combine CEC with such a sensitive detector is very important, because the intent of the experimentation is to devise ways to test samples in anti-doping and forensic analyzes. The authors of these experiments were unanimous in claiming that the proposed methods are rapid, sensitive, simple and versatile [30,32,36,40]. In other studies, the researchers used typical DAD detectors or simple UV detectors, but Liu *et al*. [31] coupled pCEC with off-column amperometric detection. The connection was achieved through a porous polymer joint, and the scientists were able to successfully separate and detect dopamine and epinephrine. Here, the detection limit was $1{,}0 \times 10^{-7}$ mol L^{-1} and the results were repeatable.

It is easy to notice that most interesting studies were those involving doping and illicit drug testing, because they deal with controversial issues met with on a daily basis. However, the registry also includes other intriguing articles [32,36]. In one of the experiments, carried out by Sulan Liao *et al*., capillaries with molecularly imprinted monoliths were employed for chiral separation of antiparasitic drugs [29].

It must be underlined that molecularly imprinted polymers are very specific materials that are comparable to the binding sites of antibodies and receptors, therefore, these items may be a potential bed for enantioseparation. In the work mentioned, the scientists were able to separate a racemic mixture of ornidazol. Such experiments are of importance, since reacemic drugs have different therapeutic effect and sometimes even undesirable action. The method enabled a reasonable separation (14 min) and was successfully used for analyzing pharmaceutical formulation. The capillary mentioned above has also been used to separate a larger group of nitroimidazole drugs. This method may be adapted in the pharmaceutical and in the food industry to monitor the illegal use of substances such as feed additives for food producing animals, because the drugs mentioned have human carcinogenic and mutagenic properties [29, 35].

The intent of Yamada *et al*. [33] was to simultaneously separate water- and fat-soluble vitamins from pharmaceutical formulations. While the analysis of water-soluble vitamins can be easily performed by capillary electrophoresis and the fat-soluble vitamins by micellar electrokinetic chromatography, both items can be separated by high performance liquid chromatography with gradient elution. Thus, the authors assumed that it is also possible to do so via pCEC with isocratic elution [33]. The research turned out to a partial success, because it was impossible to determine all the vitamins, as their concentration was too low for UV detection. It might have been possible, however, if the pCEC device was coupled to MS detection. The authors, hence, drew attention to a potential application of pCEC and MS technology that might be useful in the pharmaceutical industry and in the biochemical sciences [33].

FUNDAMENTALS OF OPLC AND PPEC AND THEIR APPLICATION IN DRUG ANALYSIS

OPLC – overpressured layer chromatography or optimum performance laminar chromatography is a planar analog of HPLC. Here, the mobile phase is driven through a planar stationary phase by external pressured pumped flow. The technique also gives an opportunity to analyze a sample in a two-dimensional mode [43].

PPEC – pressurized planar electrochromatography is a planar equivalent of CEC in which the separation process takes place on a TLC plate instead of the capillary. The driving force for the mobile phase is an electric field that generates electrophoresis and electrosomotic flow. Apart the chromatographic solute partitioning, the electrophoretic effect also influences the separation [44].

Theoretically, both techniques may seem promising, but they have some disadvantages that force their further development. It can be supposed that a combination of OPLC and PPEC should emphasize their advantages and result in high resolution. In theoretical considerations, the hyphenation of the two models of mobile phase flows and their independent regulation seem promising.

OPLC and PPEC, however, have possibility to be used in pharmaceutical analysis. Wiszkidenszky *et al*., for example, have developed a purity test for allylestrenol bulk drug substance and tablet by means of OPLC analysis. The method has been validated and enables marking all impurities of allylestrenol except one isomer [45]. Furthermore, researchers from India have developed a validated method for separation and quantification of colchicine from tubers of *Gloriosa superba*. They concluded that this method is a powerful tool that can be of use in the pharmaceutical industry [46].

With regard to PPEC, Hałka *et al*. have used the technique to successfully separate acetylsalicylic acid, caffeine and acetaminophen from tablets. This was impossible using thin layer chromatography (TLC). According to the authors, the analysis had short separation time and unique selectivity [47].

Science is based on analogy and drawing conclusions, therefore following the history of pCEC raises the question of why there is no planar equivalent of this technique. Indeed, planar analogs of HPLC and CEC have been successfully applied in pharmaceutical or biochemical assays. The evaluation of the combination of CEC and HPLC into pCEC suggests that a similar fusion is possible with planar separation techniques. A new technique could evolve with the combination of OPLC and PPEC.

CONCLUSIONS

Despite the fact that pCEC has been available for over a dozen years, it is still a somewhat marginal technique. The reason can be found in the issue of preparing the capillaries and their small commercial availability. That is why we can find so many works on developing new columns, especially the monolithic. Moreover, few specialists have explored this technique and therefore pCEC seems complicated.

One-third of the articles refer to packed capillaries and two-thirds to monolithic capillaries, probably because of the greater number of advantages of the latter. It is possible that even more interest is necessary to produce stationary phases suitable for pCEC experiments, and to put them on the market.

It is surprising that there are a large number articles dealing with pCEC and MS connection. This evidences the willingness to increase the resolution and sensitivity in an already high-resolution technique.

Although the fact that a lot of analytical techniques are available, continuous development is still very important. Maybe it is also time to increase the interest in and put effort into developing the field of planar electrochromatography techniques. Maybe it is worth developing a combination of PPEC and OPLC approaches to create a completely new technique – the planar equivalent/analog of pCEC.

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