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Review of the Ph.D. thesis

Author: Anastasia Vitalevna KRAVCHENKO, Saint Petersburg State University, Institute of Chemistry, St. Petersburg, Petergof, Russia

Title: New approaches to the electrophoretic determination of drugs in samples with complex matrices using multifunctional coatings of a quartz capillary based on ionic liquids.

Reviewer: RNDr. Václav Kašička, Ph.D., Institute of Organic Chemistry & Biochemistry of the Czech Academy of Sciences, Prague, Czechia

The submitted thesis of Anastasia Vitalevna Kravchenko deals with i) the development of new capillary electromigration techniques, zone electrophoresis (CZE), electrokinetic chromatography (CEKC) and electrochromatography (CEC), using ionic liquid-based multifunctional capillary coatings, and ii) validation and application of the developed methods for high-efficient separation and high-sensitive analysis of important biologically active compounds and drugs in complex pharmaceutical and biological matrices. The topic of the thesis is very up-to-date and extremely important, because new techniques for the fast and highly sensitive analysis of biomolecules and drugs in complex matrices are very much needed.

The author has set six challenging objectives:

1. Development of synthesis of a covalent coating based on imidazolium cation with varying substituents and revealing the capabilities of such coatings in the determination of analytes of various nature (hydrophobic, hydrophilic, and enantiomers).
2. Characterization of the covalently bonded coatings and determination of the factors influencing the electrophoretic separation (pH, composition and ionic strength of the background electrolyte).
3. Synthesis of a modifier based on imidazolium cation and β -cyclodextrin followed by its usage as a pseudostationary phase in the separation of hydrophobic analytes and drug enantiomers.
4. Determination of characteristics in terms of limits of detection, separation efficiency and selectivity of the proposed approaches.
5. Search for various options for in-capillary preconcentration in order to reduce the limits of detection of analytes and development of hybrid methods for enantiomers on-line preconcentration.
6. Approbation of the regularities established on model systems in the analysis of biological liquids.

I am pleased to state that all these ambitious objectives were successfully fulfilled. Several new advanced capillary electromigration (CE) methods were developed, validated and applied for high efficient separation and high sensitive analysis of different types of compounds. The following relevant particular results were achieved:

- i) A new procedure was developed for covalent coating of the fused silica capillary with alkyl imidazolium cation with the ability to vary its substituents and thus allowing its multi-functionality in CEC.
- ii) Using β -cyclodextrin (β -CD) as the imidazolium substituent made possible CE separation and determination of various types of compounds, both hydrophilic (biogenic amines and amino acids) and hydrophobic (steroid hormones).
- iii) Application of imidazole- β -CD conjugate as a pseudostationary phase in CEKC enabled separation of corticosteroid hormones with high separation efficiency (70-90 thousand of theoretical plates) and preconcentration of non-charged analytes using field amplified sample injection of their complexes with charged β -CD derivatives.
- iv) The charged imidazole- β -CD conjugate provided both electrostatic and hydrophobic interactions and allowed CE separation of enantiomers of nonsteroidal anti-inflammatory drugs, ketorolac and ketoprofen.
- v) Combination of sweeping and pH gradient as two in-capillary preconcentration techniques provided concentration factor 295-395 and allowed determination of enantiomers of ketorolac and ketoprofen at low concentration levels.
- vi) Using the dual electrolyte system composed of imidazole- β -CD conjugate and antibiotic vancomycin resulted in change of migration order of ketorolac enantiomers.

The reviewer appreciates the detailed description of the developed methods and a clear presentation of the achieved results. High quality of these results was confirmed by the fact that they were published in six research articles in prestigious international peer-reviewed journals with impact factors according to Web of Science, such as Talanta, Journal of Separation Science, Separation Science Plus, Chemical Monthly, J. Anal. Chem. and Analytica Chimica Acta. In five of these articles, Anastasia Vitalevna Kravchenko is the first author, which documents her substantial contribution to the achieved results.

From the formal point of view, the thesis is presented in a very nice and clear graphical form, with minimal typographical errors (e.g. pages 2 and 11, point 1.1.1, Screening > screening, compound > compounds; metabolites > metabolites). The thesis is well written, with many illustrative figures and tables. The detailed introductory part of the thesis shows a deep knowledge of the author within the studied research areas.

Publication of the results in the peer-reviewed journals simplifies my role as a reviewer of the thesis. In fact, the results have been already reviewed and confirmed as correct ones. Nevertheless, fulfilling the role of the reviewer I have a few comments, questions, and topics for the discussion:

1. Ad terminology: The term "quartz capillary" should be replaced for the term "fused silica capillary". Please note that for capillary electromigration methods, the capillaries made

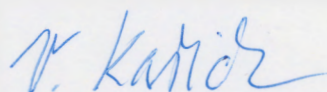
from the fused silica are used. In addition, the following changes in terminology should be performed:

Injection of modifiers into the background electrolyte > addition of modifiers to the BGE; analyzed analytes > analyzed compounds or analytes; micellar electrochromatography (MECC) > micellar electrokinetic chromatography (MEKC); molecular weight > molecular mass; 2-propanol > propan-2-ol; 1-butanol > butan-1-ol; elution time > migration time; absorbed on capillary > adsorbed on capillary.

2. Ad abbreviations: They should be presented in the alphabetical order. Some of them are not explained quite correctly, e.g. MOF – organometallic framework > MOF – metallo-organic framework, MEKC – micellar electrochromatography > MEKC – micellar electrokinetic chromatography.
3. Ad references:
 - a) Ref. [13] does not refer to isotachopheresis but to isoelectric focusing. For the separation of ketorolac enantiomers, the following paper could be cited: M. Ruzicka et al., Interactions of helquats with chiral acidic aromatic analytes investigated by partial-filling affinity capillary electrophoresis. *J. Chromatogr. A* 2016, 1467, 417-426.
 - b) Authors' surnames are missing in ref. [17], journal titles are missing in refs. [24, 36, 137 and 138] and they are presented twice in refs. [42, 57, 58, and 64] and article title is incomplete in ref. [144].
4. On page 66, behind equation (2) and in Fig. 26, the unit of EOF mobility is wrongly presented as "sm²/kV min" instead of "cm² V⁽⁻¹⁾ s⁽⁻¹⁾" or as "m² V⁽⁻¹⁾ s⁽⁻¹⁾".
5. The sensitivity enhancement factor presented on page 38 is based on the ratio of peak heights. Why peak heights are preferred to peak areas if peak areas are linear in a wider range than peak heights?
6. What is the dimension of the complexation constants in Table 16, page 97?
7. What are the further possibilities to increase the sensitivity of the developed methods?
8. What are the plans of the author in her future scientific career?

Conclusion

To summarize, my general evaluation of the thesis of Anastasia Vitalevna Kravchenko is very positive. The thesis brings new significant results and represents a valuable contribution to the development of new capillary electromigration methods and their application for high-efficient separation and high-sensitive analysis of biomolecules and drugs in complex pharmaceutical and biological matrices. The author proved a very good and deep knowledge of the studied issues. She developed new methods and showed her ability of their creative application. Based on these facts, I recommend to accept the thesis of Anastasia Vitalevna Kravchenko for the defense and to consider the thesis as a basis for awarding her with the scientific degree Candidate of Chemical Sciences.



RNDr. Václav Kašička, PhD.

Prague, 20.3.2024