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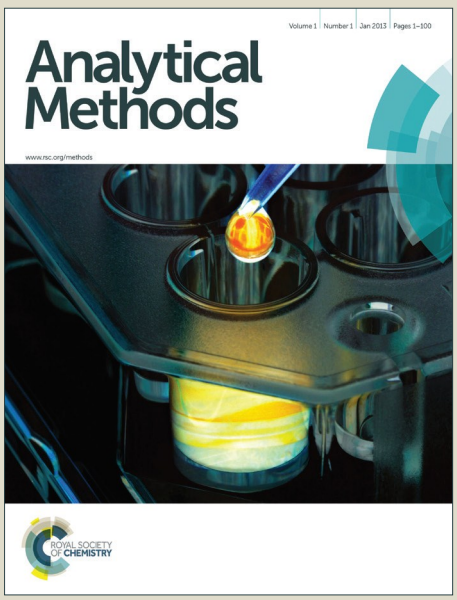

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COMMUNICATION

The use of PAMAM dendrimers as a dynamic coating for cyclodextrin mediated enantioseparation of selected basic drugs

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A dynamic coating using polyamidoamine (PAMAM) dendrimers was used to change the direction and magnitude of the electroosmotic flow. The influence of PAMAM on electroosmotic mobility was studied in the concentration range from 0.005 to 0.100 % (w/v) and at different electrolyte pH from 2.5 to 8.5. PAMAM dendrimers as additive to the background electrolyte were able to reverse the electroosmotic flow at low concentration level (0.01 % w/v). The presented dynamic coating was then applied on the enantioseparation of selected amines such as *R,S*- and *S,R*-tapentadol, *R*- and *S*- isoproterenol and *R,S*- and S,R-ephedrine with the use of 2-hydroxypropyl-β-cyclodextrin as chiral selector.

Alteration of the electroosmotic flow (EOF) can bring the positive effect on the separation of analytes in capillary electrophoresis (CE). With the change of the velocity and direction of EOF it is possible to modify the efficiency and time of analysis or reverse the migration order of compounds, which is essential for separation of minor components in the mixture. Moreover, the commonly known issue in CE is that the positively charged species have tendency to adsorb to negatively charged fused silica capillary wall (through electrostatic, hydrophobic and hydrophilic interactions). This generates problems with separation repeatability and hence difficulties with quantification. The wall – analyte interactions also lead to degradation of peak resolution, which is the key criterion in chiral CE. Several approaches have been recently published to minimize the wall-analyte interactions using the permanent or dynamic capillary coating[1–4] or working at extreme pH and/or ionic strength[5].

A cationic surfactant cetyltrimethylammonium bromide (CTAB) is commonly used for dynamic coating of the capillary and reversal of the EOF[6–8]. However, when CTAB is used with the presence of cyclodextrins (CDs), it is not able to reverse the EOF[9]. Moreover, Funasaki et al.[10] reported that CTAB can compete with the analyte of the complexation sites of CD selector. Due to above mentioned reasons the permanent coating of capillaries is mostly recommended for chiral separation with CDs[11]. Nevertheless, the use of commercial coated capillaries is limited by their high costs and their preparation in laboratory is time consuming and laborious. The only used dynamic coating for CD mediated chiral separation proposed in the literature is based on the CElixir™ kit[12–15], however, its chemical composition is patent protected.

Polyamidoamine (PAMAM) dendrimers proposed here as a dynamic coating agent for CE are highly branched and symmetrical macromolecules[16,17]. Their structure comprises cores, interior repeating units, and terminal groups. The dendrimer structure used in the study is presented on the Fig.1. and is characterized by 14 tertiary amine groups and 16 primary amine groups. The molecular weight of compound is 3256 Da, with diameter of 2.6 nm (Ref. 18).

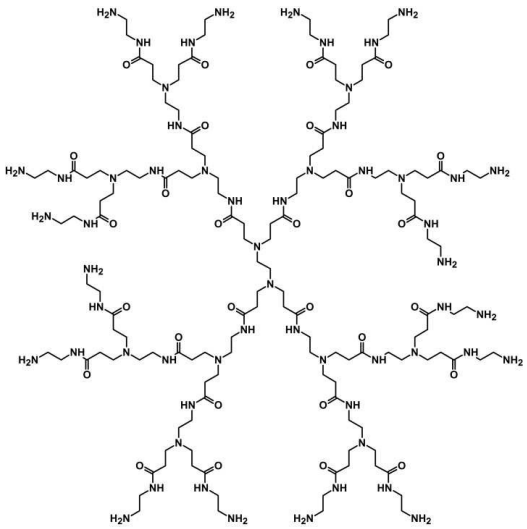

Fig.1. Chemical structure of PAMAM dendrimer G 2.0

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Since 1980s' when the first dendrimers were synthesized and characterized[16,19–22] they were applied in different fields of human activities, e.g. in medicine[23,24], petroleum industry[25,26] or material science[27–30]. Various type of dendrimers were reported useful for micellar electrokinetic chromatography (MEKC) for analysis of hydrocarbons[31], parabens (polyacid dendrimers)[32] and positional isomers of neutral phenols (sulfonic acid-modified starburst dendrimer)[33]. PAMAM dendrimers were also used for dansyl-Ala and dansyl-Val separation[34] and for proteins separation (bovine serum albumin, lysozyme, myoglobin and trypsin inhibitor)[35]. Recently, the coating properties of dendrimers were characterized for detection of albumin in biological samples[36].

The presented study introduces PAMAM dendrimers generation 2.0 (G 2.0) as a dynamic coating additive of the BGE for the enantioseparation of selected chiral amines with the use of 2-hydroxypropyl-β-CD (2-HP-β-CD) as a chiral selector (CS). According to our best knowledge this is the first use of PAMAM for that purpose.

Detailed information about instrumentation, experimental conditions and chemicals is stated in the Experimental part of the Electronic Supplementary Information (ESI).

For the dynamic coating the PAMAM G 2.0 was chosen as the additive due to its branched structure and thus limited probability to interact with the inner cavity of the CD. The diameter of G 2.0 PAMAM dendrimers in 10 % (v/v) methanol solution is 2.6 nm (Ref. 18), whereas the diameter of β-CD inner cavity is 0.78 nm (Ref. 37). It can be supposed that the PAMAM G 2.0 will not compete with interaction of the analytes with the 2-HP-β-CD cavity.

Preliminary, the influence of the dendrimer concentration on the electroosmotic mobility was studied in the 50 mM sodium acetate pH 4.5 in the range from 0.005 – 0.100 % (w/v) of the PAMAM G 2.0. Sodium acetate pH 4.5 was chosen due to strong EOF in an uncoated capillary and positive charge of the studied PAMAM[38] at acidic conditions. The aim of the study was to obtain the reproducible reversed EOF (from cathode to anode) with the lowest concentration of the additive. The influence of increased PAMAM G 2.0 concentration on the electroosmotic mobility in sodium acetate pH 4.5 is presented in the Table 1.

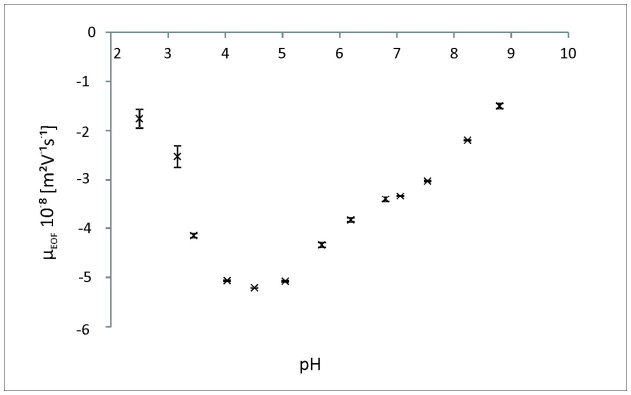
Table 1. Influence of the PAMAM G 2.0 concentration on the electroosmotic mobility in 50 mM sodium acetate pH 4.5 (see details in ESI).

	PAMAM G 2.0 concentration [% (w/v)]				
	0 .000	0.005	0.010	0.050	0.100
Electroosmotic mobility [$10^{-8}m^2s^{-1}V^{-1}$], n = 10	4.13	-4.99	-5.21	-5.44	-5.45
RSD [%]	0.49	0.14	0.12	0.91	0.58

The experiments conducted at pH 4.5 revealed that already low concentration (0.005 % (w/v)) of PAMAM G 2.0 in the BGE is able to change the direction of the EOF from strong anodic to strong cathodic. Due to similar effect of the concentration of studied additive on the magnitude and direction of the EOF, the 0.01 % (w/v) (0.031 mM) of PAMAM G 2.0 was chosen as an optimal and was used for further experiments. The RSD value of the migration time for EOF marker peak (DMSO) did not exceed the 0.92 % (n = 10). For a comparison, commonly used CTAB additive had to be used at 0.1 mM concentration, which is around 3 times higher than for PAMAM[8].

As a next step, the influence of pH of the BGE with constant PAMAM G 2.0 concentration (0.01 % (w/v)) on the EOF, in the pH range from 2.5 to 8.5, was studied. The results are presented on the Fig.2. The EOF was already reversed for the pH 2.5, however with low magnitude, which was similar also for pH 8.0 and 8.5. Two plateaus were observed for the pH 4.0, pH 4.5, pH 5.0 and for the pH 7.0 and pH 7.5. It can be speculated that is a consequence of the silanol group ionization as well as the protonation of amine groups in the dendrimer structure[38,39]. The strongest anodic EOF was obtained for pH 4.5.

Fig. 2 Influence of the pH of the BGE on the electroosmotic mobility. BGEs: sodium phosphate pH 2.5-3.0, sodium acetate pH 3.5-5.5, sodium MES pH 6.0-6.5, sodium MOPS pH 7.0-7.5, sodium TAPS pH 8.0-8.5. See ESI for details.



The presented results show that PAMAM G 2.0 dendrimers are able to modify the inner capillary wall in wide pH range. The capability of the PAMAM dynamic coating together with enantioseparation was demonstrated on selected basic drugs (ephedrine, isoproterenol, tapentadol) with 2-HP-β-CD as CS. The non-ionic 2-HP-β-CD was considered accordingly to minimize the quantity of charged components of the BGE and to avoid electrostatic attraction of the CS with positively charged species. The choice of the CS was based on the published results for ephedrine[40] and tapentadol[41]. In the case of isoproterenol the preliminary experiments were conducted without PAMAM, but with different concentration of 2-HP-β-CD (data not included).

The ephedrine, isoproterenol and tapentadol have the pK_a as follows 9.65 (Ref. 21), 9.81 (Ref. 43), 9.34 (Ref. 44). The enantiomers were separated using 2-HP-β-CD in 50 mM sodium acetate pH 4.4 with and without addition of PAMAM G 2.0 dendrimers. The corresponding electropherograms shown

on Fig.3 document the change in the resolution of the studied enantiomers with different concentration of 2-HP-β-CD with and without PAMAM G 2.0 additive (0,01 % (w/v))

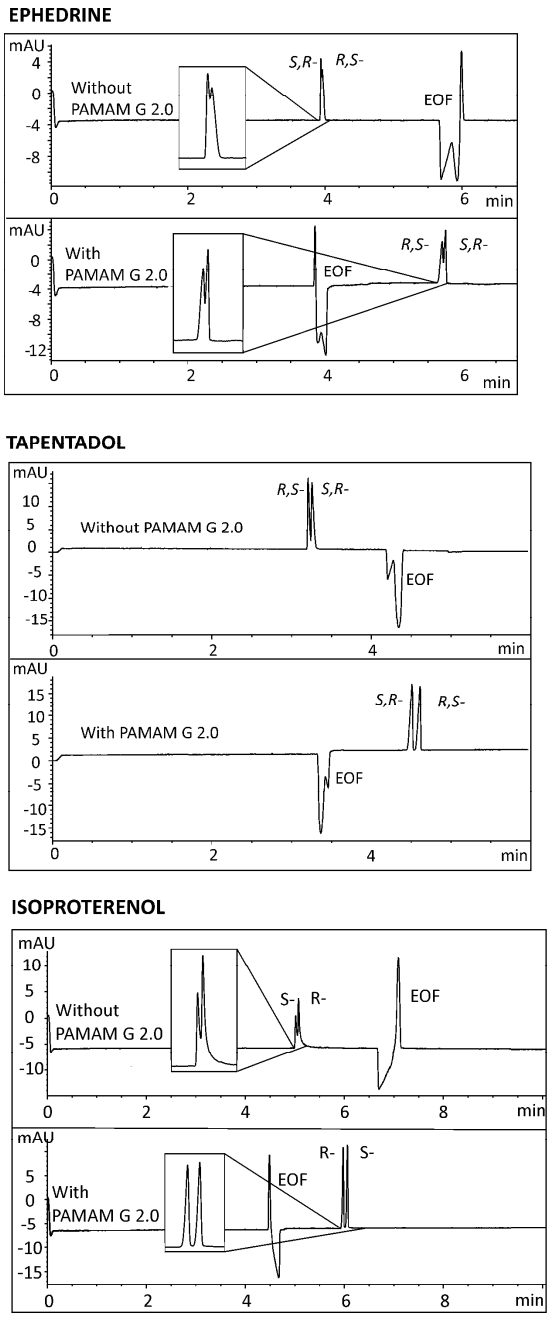


Fig.3. Chiral separation of basic drugs at pH 4.4 with (0.01 %, w/v) and without PAMAM addition. BGE: 50 mM sodium acetate pH 4.4, for ephedrine 5.0 % of 2-HP-β-CD, for tapentadol 0.1 % of 2-HP-β-CD and for isoproterenol 10.0 % of 2-HP-β-CD.

According to the different structure of analytes the presence of dendrimers differently influenced their resolution (see the Table 2). For all studied chiral amines higher resolution was obtained with PAMAM dynamically coated capillary. At these conditions the CS is carried by the EOF that is in the opposite direction of the migration of positively charged analytes (counter-current mode)[45]. In order to detect the studied drugs in the PAMAM G 2.0 modified capillary, negative voltage was applied.

Optimal resolution of the drugs was achieved with different amount of the CS. In the case of ephedrine the maximum resolution was obtained at 5 % (w/v) of 2-HP-β-CD. At 20 % (w/v) of CS the resolution values of ephedrine with and without dynamic coating were similar. For the isoproterenol the chiral separation was already achieved at a lower concentration of 2-HP-β-CD (1 % (w/v)) and was notobtained without PAMAM. The maximum resolution of isoproterenol enantiomers was obtained for 10 % (w/v) concentration of CS with PAMAM in the BGE, whereas for an uncoated capillary was at 20 % (w/v) concentration. Enantioseparation of tapentadol presents the similar decreasing trend in resolution with increasing concentration of the CS for both coated and uncoated capillary. However, the resolution was better in the coated capillaries. The RSD of migration times at studied pH 4.4 for tapentadol was up to 1.05 % (at 0.1% (w/v) of 2-HP-β-CD), for ephedrine up to 0.12 % (at 5% (w/v) of 2-HP-β-CD) and for isoproterenol the RSD value did not exceed 0.30 % (at 10 % (w/v) 2-HP-β-CD).

Table 2. Effect of 2-HP-β-CD concentration on resolution of studied chiral drugs with (0.01 % (w/v)) and without PAMAM as BGE additive.

Analyte	Concentration of 2-HP-β-CD [%]	Average resolution without PAMAM G 2.0 (±RSD)	Average resolution with PAMAM G 2.0 (±RSD)
Ephedrine	5.0	0.55 (±0.03)	0.72 (±0.01)
	10.0	0.57 (±0.03)	0.69 (±0.01)
	20.0	0.55 (±0.01)	0.58 (±0.01)
Isoproterenol	1.0	-	0.76 (±0.02)
	2.5	0.69 (±0.01)	1.47 (±0.01)
	5.0	0.76 (±0.02)	1.90 (±0.01)
	10.0	1.05 (±0.03)	2.03 (±0.01)
	20.0	1.23 (±0.03)	1.95 (±0.01)
Tapentadol	0.05	0.79 (±0.01)	1.49 (±0.02)
	0.1	1.06 (±0.01)	1.65 (±0.01)
	1.0	0.62 (±0.02)	0.59 (±0.01)

Next, the influence of the dynamic coating on the migration order of the analytes was evaluated. The standard addition method was applied for the peak identification. Without addition of PAMAM the *R,S*-tapentadol migrates before *S,R*-tapentadol, *S,R*-ephedrine before *R,S*-ephedrine and *R*-isoproterenol before *S*-isoproterenol at positive polarity of separation voltage. As was mentioned, the addition of PAMAM G 2.0 to the sodium acetate pH 4.4 generate the strong cathodic EOF and the reversal of migration order for all studied analytes was observed. The change in the migration order is not caused by the change of the enantioselectivity, but only by the manipulation with the EOF direction. The

alteration of the migration order is desirable in the pharmaceutical analysis, for determination of the minor component in front of the major constituent.

Additionally, for all studied drugs, pH 2.5 was studied to determine if an improved resolution could be obtained, see the Fig. 1 in the ESI. At pH 2.5, the resolution of isoproterenol was improved at even lower CS concentration, . The drawback of the separation was the prolongation of the analysis time, isoproterenol peaks migrates at 5.98 min, 6.06 min with PAMAM at pH 4.4 and at 10.86 min and 11.18 min with PAMAM at pH 2.5. Similarly, for the ephedrine lower pH enhanced the resolution, but higher concentration of 2-HP-β-CD and 30 kV were needed. The analysis time changed from 5.71 min and 5.76 min to 13.98 min and 14.17 min. For tapentadol enantiomers the resolution also increased as well as time of analysis, from 4.51 min and 4.61 min to 9.78 min and 10.27 min. At pH 2.5 the addition of PAMAM reversed EOF however, its magnitude was too low to transport the analytes to the detector, thus all analyses were performed at positive voltage. The low magnitude EOF at pH 2.5 allowed better resolution of all drugs, but without the possibility of reversal order of studied enantiomers.

Conclusions

Presented results proved that the use of PAMAM dendrimers limits or eliminates the analyte adsorption (isoproterenol), enables better resolution (ephedrine, isoproterenol, tapentadol) or changes of the migration order of the enantiomers (tapentadol, isoproterenol, ephedrine) compared to the uncoated capillary. The time of analyses for both coated and uncoated capillaries was comparable.

The proposed dynamic coating is easily prepared, quickly applied and does not disturb the chiral separation, even enhances the resolution. Such coated capillaries provide a better choice to the laborious and more expensive permanently coated capillaries.

Acknowledgements

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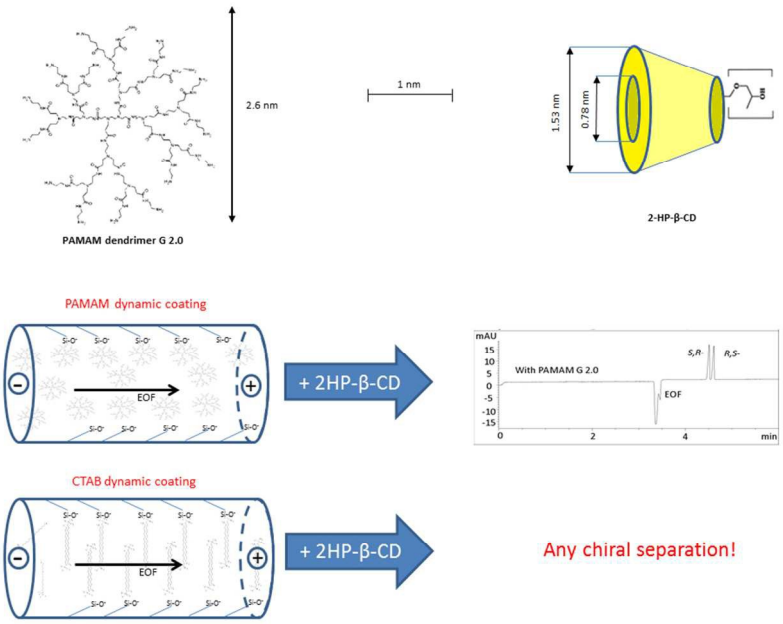

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