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Comparative high-performance liquid chromatography enantioseparations on polysaccharide based chiral stationary phases prepared by coating totally porous and core–shell silica particles

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ABSTRACT

This article reports comparative high-performance liquid chromatographic separations of enantiomers with chiral stationary phases (CSPs) prepared by coating cellulose tris(4-chloro-3-methylphenylcarbamate) on totally porous and on core–shell type silica of comparable particle diameter. Several interesting observations were made: (1) the selectivity of separation was higher on core–shell type CSP compared to totally porous CSP at comparable content of chiral selector (polysaccharide derivative); (2) much flatter dependence of plate height on the mobile phase flow rate was observed for columns packet with CSP prepared with core–shell silica compared to the ones packed with CSPs prepared with totally porous particles; (3) at low mobile phase flow rates core–shell CSP provided lower resolving ability compared to a commercially available CSP having four times higher content of chiral selector along with higher retention of chiral analytes. However, at high flow rates core–shell type CSP performed similarly or better than the commercial column in regards of plate count (N) and peak resolution (R_s) per column length and within a given total analysis time. The advantage of CSP prepared with core–shell silica is obvious from the viewpoint of plate numbers and resolution calculated per unit time (i.e. speed of analysis).

1. Introduction

Chromatographic separation media based on core–shell silica particles are becoming very popular for high-performance liquid phase (HPLC) separations [1]. Shorter diffusion path-length and consequently higher column efficiency belong to the major advantages of these materials [1–5], as well as to a lesser extent more uniform particle-size distribution of core–shell particles [1,6] compared to their totally porous analogues. In addition to the above mentioned advantages, core–shell materials provide flatter dependence of column performance on the mobile phase flow-rate, primarily due to decreased resistance to mass-transfer compared to fully porous silica (a smaller C-term in the van Deemter equation), hence are better suited for high-speed separations [1]. While lower surface area belongs to certain limitations of these particles, lower retention and lower loadability of analytes result as a consequence. Intuitively, such material can be coated only with a limited amount of chiral selector compared to totally porous silica materials, which typically have higher surface area. To the best of our knowledge no journal article has discussed to date the use of core–shell silica materials for the preparation of chiral stationary phases (CSPs) for HPLC enantioseparations or the use of such CSPs in comparative studies with traditional chiral columns.

The goal of this preliminary study was to evaluate the feasibility of preparing polysaccharide-based CSP by coating core–shell silica and to perform initial comparative studies on this CSP and a CSP prepared by coating totally porous silica of same particle size, as well as on a commercial chiral column available with the same chiral selector.

2. Experimental

2.1. Materials

The chiral test compounds *trans*-stilbene oxide, benzoin and 2,2′-dihydroxy-6,6′-dimethylbiphenyl (Fig. 1A–C) were commercially available from Sigma–Aldrich (St. Louis, MI, USA). HPLC-grade n-hexane and 2-propanol were supplied by Karl Roth (Karlsruhe, Germany). The commercially available chiral column Lux Cellulose-4 used in this study (of 4.6 mm × 250 mm dimensions and packed with 3 μm particles) was supplied by Phenomenex Inc. (Torrance, CA, USA). The structure of the chiral selector in Lux Cellulose-4 is shown in Fig. 1D. Core–shell silica with 2.6 μm nominal particle diameter and 9 nm nominal pore size

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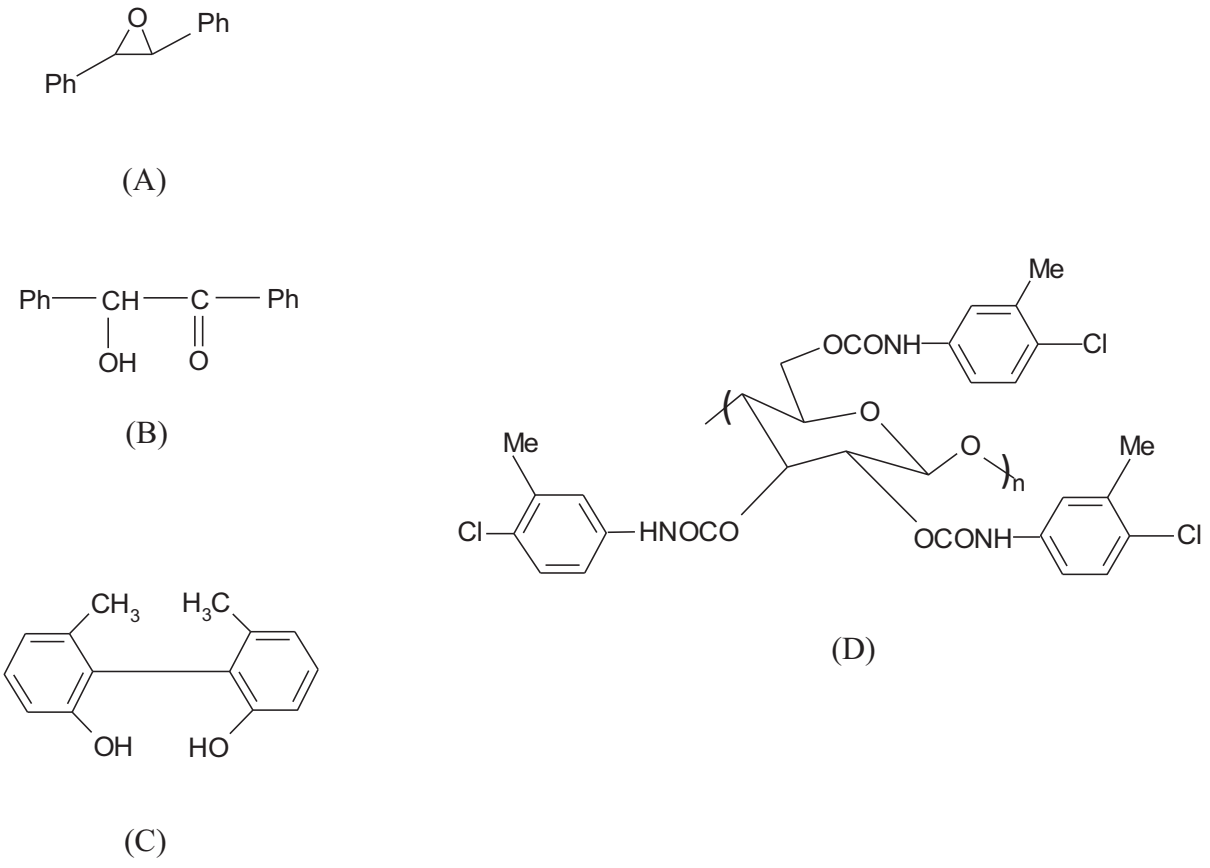

Fig. 1. Structures of *trans*-stilbene oxide (A), benzoin (B), 2,2′-dihydroxy-6,6′-dimethylbiphenyl (C) and cellulose tris(4-chloro-3-methylphenylcarbamate) (D).

was provided by Phenomenex. Totally porous silica with 3.0 μm nominal particle diameter and 10 nm nominal pore size was purchased from Daiso (Osaka, Japan). Cellulose tris(4-chloro-3-methylphenylcarbamate) used for coating core–shell and totally porous silica was synthesized according to a previously described method [7]. Coating of silica with the chiral selector was performed by dissolving of appropriate amount of cellulose tris(4-chloro-3-methylphenylcarbamate) in tetrahydrofurane, adding the weighed amount of silica to this solution, sonication applied for few minutes and evaporation of the solvent at reduced pressure, at room temperature, to dryness. The obtained powder was further dried under reduced pressure at 60 °C for 1 h and used for packing experimental HPLC columns. A slurry of the packing material was prepared in n-hexane/2-propanol mixture 9/1 (v/v), decanted two times and then packed at 500 bar in stainless-steel HPLC columns of 4.6 mm × 250 mm dimensions supplied by Phenomenex Inc.

2.2. Instruments

HPLC separations of test compounds dissolved in the mobile phase in the concentration 0.2 mg/ml were performed using a Knauer K1001 isocratic HPLC pump, a Knauer injection valve with 20 μl sample loop and Knauer K2001 fixed wavelength (220 nm) UV detector (Knauer, Berlin, Germany). The instrument control and data management were performed with Eurochrom software. Knauer K-1900 pneumatic pump was used for packing HPLC columns. Rotary evaporator used in this study was Rotavapor R-210/R-215 from BUCHI Labortechnik GmbH (Essen, Germany) with temperature control and an ultrasonic bath Sonorex RK-100 was from Bandelin (Berlin, Germany). Elemental analysis (N,C) was performed using an Elemental Combustion System CHNS-O, Model ECS4010 by Costech Analytical Technologies Inc. (Valencia, CA, USA). Pore size analysis was performed with the Tristar II Surface Area and Porosity System 3020 instrument made by Micromeritics (Norcross, GA, USA).

3. Results and discussions

3.1. Coating of core–shell silica with cellulose tris(4-chloro-3-methylphenylcarbamate)

Since the specific surface area of core–shell materials is lower and their density is higher compared to totally porous silica, coating of a polysaccharide phenylcarbamate derivative onto its surface is a challenging task due to formation of numerous particle aggregates. Due to this problem, the maximal amount of cellulose tris(4-chloro-3-methylphenylcarbamate) that could be coated with high yield (>80% of final packing material) onto the surface of core–shell silica was in the range of 5% (w/w) (Column 2). This material was used for further experiments together with the CSP prepared with totally porous silica also coated at nominally 5% cellulose tris(4-chloro-3-methylphenylcarbamate) (Column 3). Elemental analysis of CSPs packed in columns 2 and 3 showed that the actual amount of coated cellulose tris(4-chloro-3-methylphenylcarbamate) was 6.8% (w/w) onto the core–shell silica and 5.6% onto the totally porous silica.

3.2. Comparative chromatographic evaluation of experimental core–shell and totally porous CSPs of similar particle size and their comparison to the commercially available chiral column Lux Cellulose-4

The chromatographic performance of the commercial chiral column Lux Cellulose-4 (Column 1; made with 3 μm packing material) and two experimental columns prepared by coating of the same

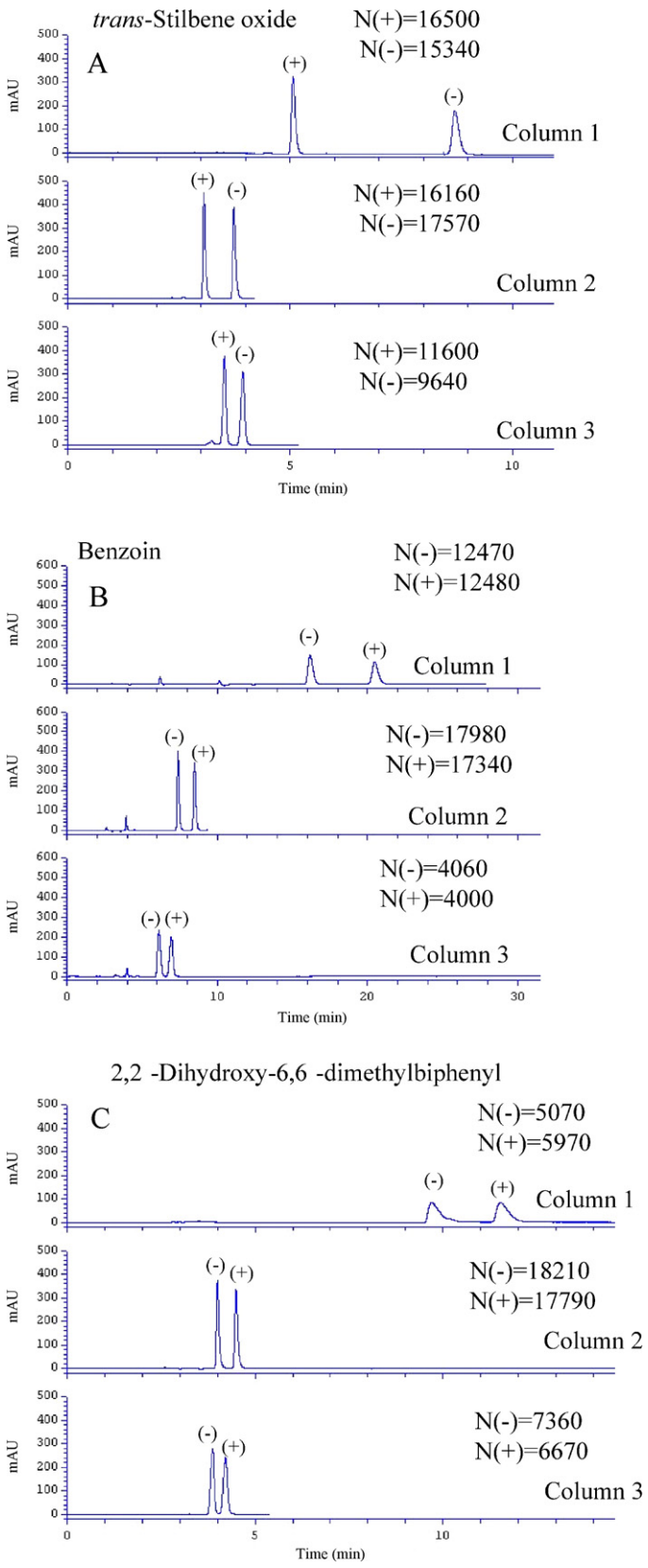

Fig. 2. Separation of enantiomers of *trans*-stilbene oxide (A), benzoin (B) and 2,2′-dihydroxy-6,6′-dimethylbiphenyl (C) using the chiral columns indicated on each figure. The mobile phase was n-hexane/2-propanol = 90/10 (v/v) with the flow rate 1 ml/min.

Table 1
Separation factors of the enantiomers of *trans*-stilbene oxide, benzoin and 2,2′-dihydroxy-6,6′-dimethylbiphenyl on 3 different chiral columns investigated in this study.

Analyte	Column 1 (t_0 = 3.06)	Column 2 (t_0 = 2.61)	Column 3 (t_0 = 3.00)
trans-Stilbene oxide	2.85	2.47	1.84
Benzoin	1.33	1.27	1.20
2,2′-Dihydroxy-6,6′-dimethylbiphenyl	1.27	1.22	1.15

chiral selector onto core–shell silica (Column 2) and totally porous silica (Column 3) as described above was evaluated for the separation of three neutral chiral analytes *trans*-stilbene oxide, benzoin and 2,2′-dihydroxy-6,6′-dimethylbiphenyl (structures shown in Fig. 1A–C), in normal phase elution mode. In view of the negative impact extra-column contributions to analyte band broadening can have on the outcome of the investigation, an important consideration for any such evaluation is the type and configuration of the HPLC instrument used. In this preliminary study the instrument was not specially adapted to high performance columns. Given the large volume of these columns significant instrument contributions to analyte band broadening are not expected. Still, selected separations were repeated on the high performance HPLC instrument Agilent 1200 which proved that there was no bias in the experimental results caused by any significant extra-column band broadening effect.

Separation selectivity (α) was highest with the commercial column Lux Cellulose-4 for all chiral analytes (Fig. 2A–C and Table 1).

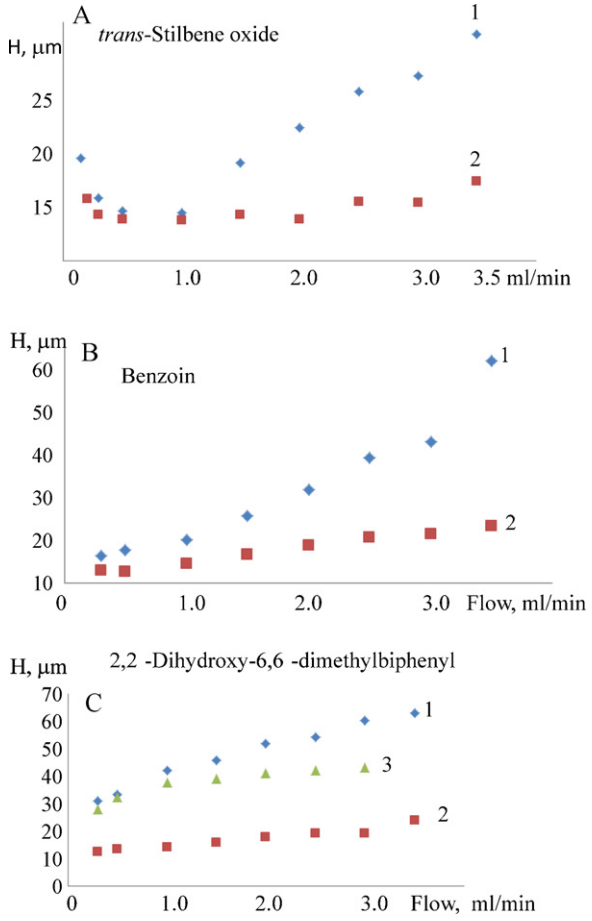

Fig. 3. Dependence of plate numbers for (−)-*trans*-stilbene oxide (A), (+)-benzoin (B) and (+)-2,2′-dihydroxy-6,6′-dimethylbiphenyl (C) on the flow rate of the mobile phase on the columns indicated on each figure.

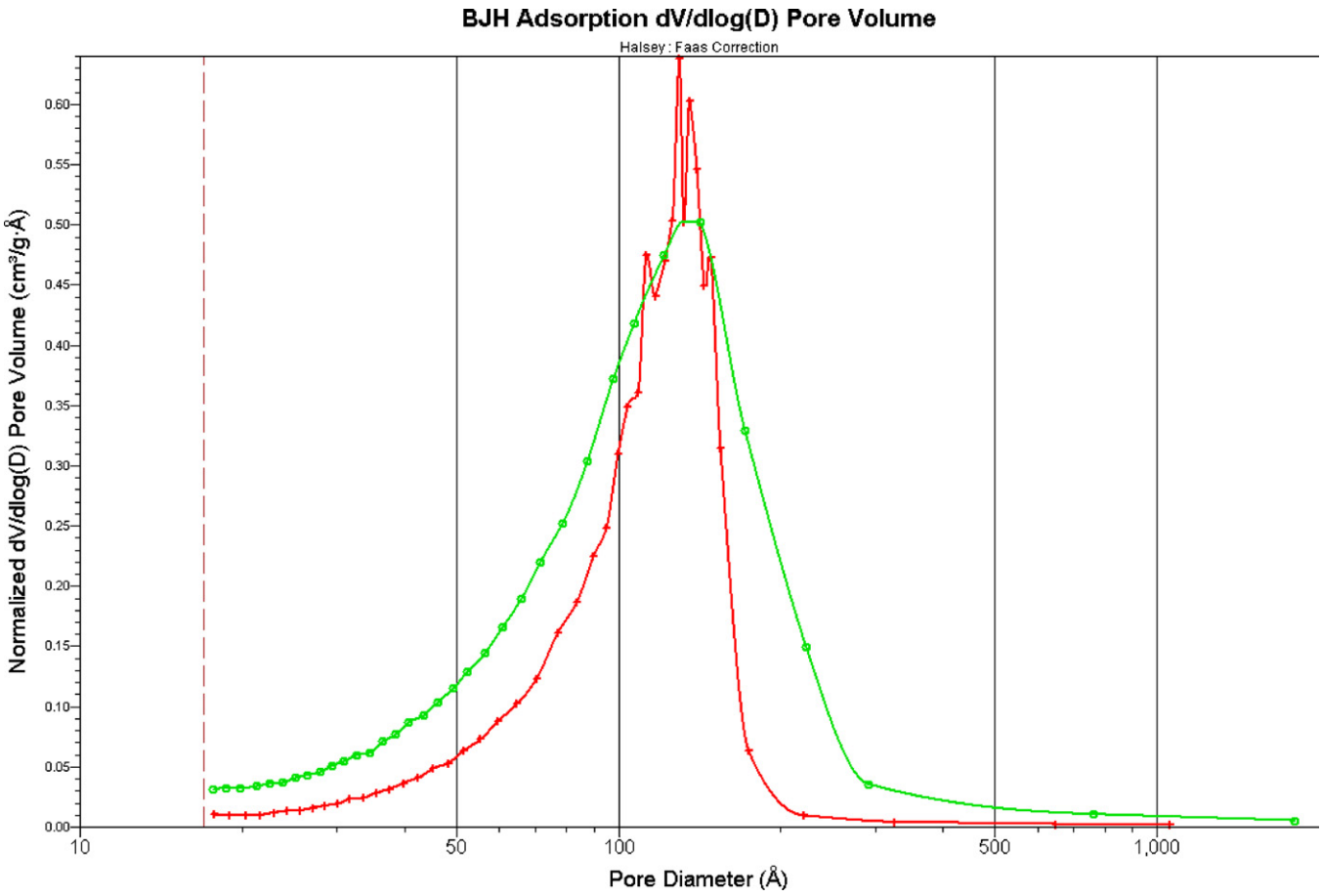

Fig. 4. Results of pore size analysis of the core–shell material (A) and the Daiso 3 μm 10 nm silica (B) used in this study for coating with polysaccharide-based chiral selector.

This result was expected since the CSP in this column contains about 20% (w/w) chiral selector, while the content of chiral selector in the experimental columns studied here varied between 5.6 and 6.8% (w/w). Although a visual inspection of the chromatograms shown in Fig. 2A–C suggest significantly larger separation factors achieved on the commercial column (Column 1) compared to the core–shell based one (Column 2), the difference in calculated α values was actually not as large as expected. This finding can be explained by the difference in dead volumes between the two columns, with the core–shell based column having about 15% less dead volume (due to the presence of the cores).

Notwithstanding the larger separation selectivity observed with the Lux cellulose-4 column, the very high chiral selector content of Lux Cellulose-4 can also play a negative role on the quality of separation in cases where very slow mass transfer is observed for particular combinations of analyte-chiral selector (as in the case of 2,2′-dihydroxy-6,6′-dimethylbiphenyl with cellulose tris(4-chloro-3-methylphenylcarbamate)). Unfortunately, such cases are not uncommon with chiral separations achieved with polysaccharide-based selectors. In such cases, either both enantiomers or sometimes only the second one elute with very broad peaks and significant tailing. Due to their significantly lower loading with chiral selector, 2,2′-dihydroxy-6,6′-dimethylbiphenyl elutes off both Column 2 and Column 3 with narrow and more symmetrical peaks.

The most striking observation from the viewpoint of chromatographic resolution (function of the plate count values shown in Fig. 2 and separation factors listed in Table 1) was the significantly higher resolving ability of the chiral selector cellulose tris(4-chloro-3-methylphenylcarbamate) when coated onto core–shell silica particles compared to the material prepared by coating it onto totally porous silica at comparable coating content. This same observation was made for all 3 chiral test compounds included in this study and the difference was most pronounced in the case of *trans*-stilbene oxide (Fig. 2A–C). The slightly higher chiral selector content in the former material compared to the latter one can be partially responsible for this result but cannot fully explain the observed improvement in the selectivity factor. A more reasonable explanation may be found in the fact that the coated chiral selector is more accessible to chiral analytes when coated onto the material with superficial rather than deep through pores.

The dependence of plate numbers on the mobile phase flow rate for the separation of the three chiral analytes is shown in Fig. 3A–C. As it can be seen from these figures, the plate numbers per column for *trans*-stilbene oxide at the optimal flow rate are quite similar for Lux Cellulose-4 and Column 2 (packed with CSP based on core–shell silica). However, the optimal flow rate range is significantly wider for the latter column allowing for faster analyses with minimal loss in performance (results shown only for the second peak although a similar trend was observed for both enantiomers). Column 3 (made with chiral selector coated onto totally porous silica with 10 nm nominal pore size) exhibited lower peak efficiency per column (data not shown). This observation can be explained by considering the narrower average pore diameter of this material compared to that of the CSP packed in the commercial column, Lux Cellulose-4. Although the core–shell silica used here also has narrow pores (of 9–10 nm nominal diameter) the above mentioned

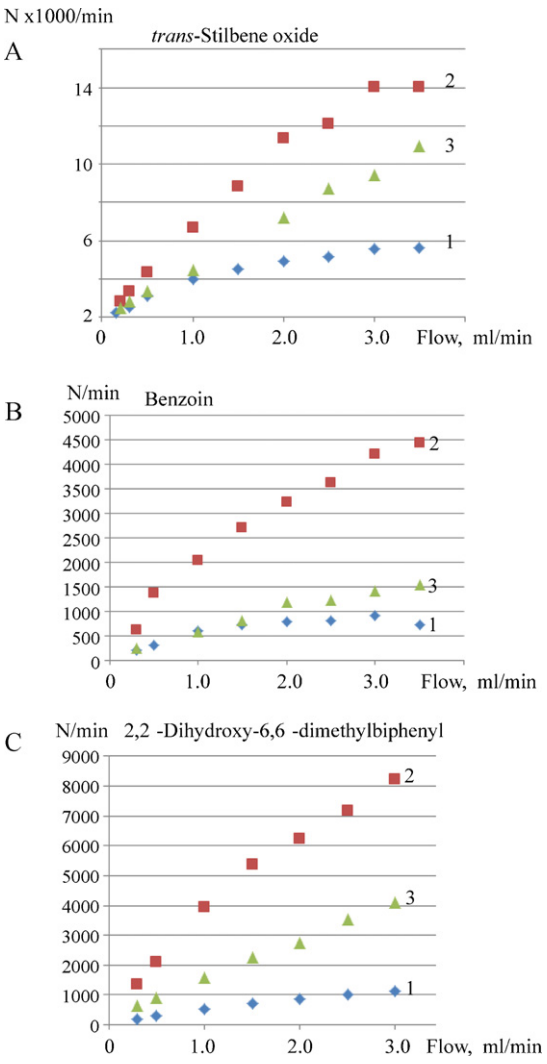

Fig. 5. Dependence of plate numbers per minute for (−)-*trans*-stilbene oxide (A), (+)-benzoin (B) and (+)-2,2′-dihydroxy-6,6′-dimethylbiphenyl (C) on the flow rate of the mobile phase for the columns indicated on each figure.

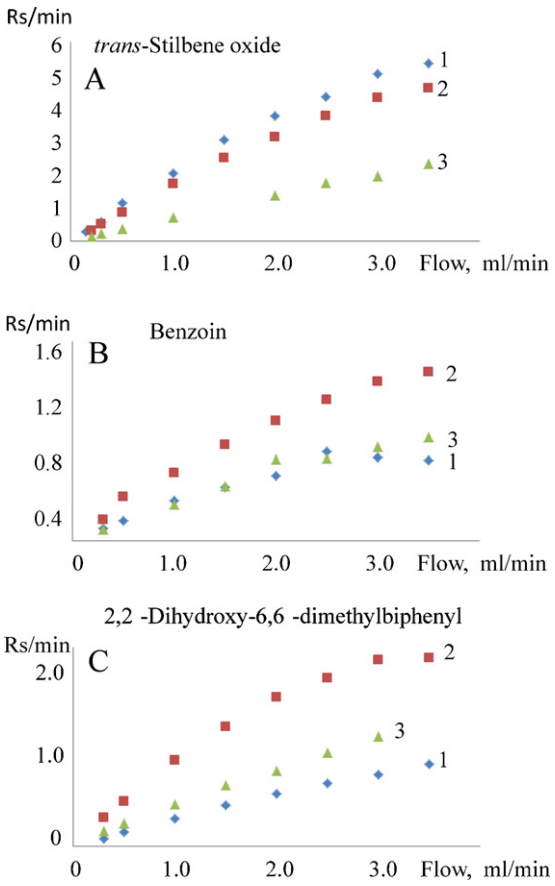

Fig. 6. Dependence of peak resolution (R_s) per minute for (−)-*trans*-stilbene oxide (A), (+)-benzoin (B) and (+)-2,2′-dihydroxy-6,6′-dimethylbiphenyl (C) on the flow rate of the mobile phase for the columns indicated on each figure.

negative effect of smaller pore size was not observed for this material. This difference in performance can be explained based on the wide pore size distribution of this material (which includes a significant fraction of wide pores, up to 40 nm in size; see Fig. 4) and by the shorter diffusion path-length limited by the thickness of the shell, which is a major feature of core–shell particles compared to totally porous silica, and which results in higher plate numbers. The important trend observed when comparing the commercial column (Column 1) to the core–shell based column (Column 2) was that the separation efficiency for Column 2 does not decrease as fast as with Lux Cellulose-4 containing 20% of chiral selector (Fig. 3A–C). This can be explained with better intra-particle mass transfer in CSPs having shorter diffusion path-length and containing a lower amount of chiral selector. Such CSPs are clearly more suitable for high speed analysis. Thus, CSP prepared by coating chiral selector onto core–shell silica combines the advantages of both wide pores (such as Lux Cellulose-4 having high plate numbers at the optimal flow rate) and lower chiral selector content (such as the experimental material used in Column 3) demonstrating less dependence of the plate numbers on the mobile phase flow rate. In addition, the CSP based on core–shell silica exhibits a wider optimal flow-rate range (Fig. 3) insuring good performance both in conventional and fast analysis. The latter case is also favored by the lower content of chiral selector providing less retention (Fig. 2). Thus, the advantages of CSPs prepared with core–shell silica become more evident by comparing plate number (N) or resolution (R_S) values obtained per unit time for the three columns (Figs. 5 and 6, respectively). Column 2 (core–shell based) is either the best, or is very close in performance to that of the commercial column for the test compounds studied here.

The results presented in this study clearly show the promise of core–shell supports for effective LC enantioseparations. Further optimization of the morphology of the core–shell particles used for this purpose has the potential of improving the benefits demonstrated above. The use of neutral analytes in this study is perfectly justifiable for the purpose of evaluating the role played by the morphology of the support particles on chromatographic performance and its dependence on the speed of analysis. Future work will extend the type of test probes used for evaluation to include ionizable analytes such as acidic and basic drug compounds. For this purpose a CSP made with core–shell support of suitable surface modification needs be prepared. To date, the best compromise in this regard has been coating polysaccharide-based chiral selectors onto amino-functionalized silica particles. An optimized particle morphology along with suitable surface modification could produce the superior core–shell CPSs of the future.

4. Conclusions

This preliminary study illustrates the potential advantages of chiral stationary phases prepared by coating core–shell silica for the separation of enantiomers by high-performance liquid chromatography. High separation factors, high plate numbers,

higher optimal flow rate and smaller loss in performance when operating at higher flow rates seem to be very valuable advantages of a core–shell type CSP, especially for fast separations. Future work will focus on the evaluation of core–shell based CSPs based on optimized particle morphology and appropriately functionalized shell surface in order to further improve their performance and minimize secondary interactions with ionizable analytes.

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