Enantioseparation with cationic β-cyclodextrin chiral stationary phases in supercritical fluid chromatography and high-performance liquid chromatography

Wang, Renqi

2011

Wang, R. (2011). Enantioseparation with cationic β-cyclodextrin chiral stationary phases in supercritical fluid chromatography and high-performance liquid chromatography. Doctoral thesis, Nanyang Technological University, Singapore.

https://hdl.handle.net/10356/44704

https://doi.org/10.32657/10356/44704

Acknowledgements

I am thankful to my supervisor Prof. Ng Siu Choon for his constant guidance, invaluable suggestions and warm encouragement from the start to the end of this project and during the preparation of my thesis.

I am also grateful to Asst. Prof. Arvind Rajendran and Asst. Prof. Timothy Thatt Yang Tan for their generous help and guidance to my project.

I would like to thank Dr. Ong Teng-Teng, Dr Chen Hui and Dr. Li Laisheng for their kind assistance in proof reading and suggestions on the thesis.

I am indebted to my labmates, Ph.D students Ai Feng, Xiao Yin and Wang Yong for their encouragements and suggestions.

I offer my regards and blessings to undergraduates Yuan Yixiu, Liu Maolong, Khoo Weiling Linda and Liu Qing for their fruitful contributions to the project.

Last but not least, I also want to thank Nanyang Technological University for awarding the research scholarship and providing the facilities for the research work reported herein.

Publication

Synthesis and application of mono-6-(3-methylimidazolium)-6-deoxyperphenylcarbamoyl-β-cyclodextrin chloride as chiral stationary phases for high-performance liquid chromatography and supercritical fluid chromatography

Ong Teng-Teng, Wang Ren-Qi, Ng Siu Choon*

Journal of Chromatography A, Volume 1182, Issue 1, 22 February 2008, Pages 136-140

Synthesis of cationic β-cyclodextrin derivatives and their applications as chiral stationary phases for high-performance liquid chromatography and supercritical fluid chromatography

Wang Ren-Qi, Ong Teng-Teng, Ng Siu Choon*

Journal of Chromatography A, Volume 1203, Issue 2, 5 September 2008, Pages 185-192

Synthesis of chemically bonding cationic β-cyclodextrin and application in supercritical fluid chromatography

Ren-Qi Wang, Teng-Teng Ong, Timothy Thatt Yang Tan* and Siu-Choon Ng*

Journal of Chromatography A, in preparation

Facile preparation of chemically bonded cationic β-cyclodextrin derivatives and their use as chiral stationary phases for packed column supercritical fluid chromatography

Ren-Qi Wang, Teng-Teng Ong, Timothy Thatt Yang Tan* and Siu-Choon Ng*

Tetrahedron letters, in preparation

TABLE OF CONTENTS

Executive Summary

It is estimated that around forty percent of pharmaceutical or biological entities under development are chiral. The specified stereochemistry of the substance is closely related with their pharmacological efficacy. Thus, fast and efficient analysis and preparative techniques for chiral compounds are required by the rapid development of the pharmaceutical industry. Conventional high-performance liquid chromatography (HPLC) provides diverse chromatographic conditions and versatility for most racemates' enantioseparations. On the other hand, in supercritical fluid chromatography (SFC), the analytes' higher diffusivity and eluents' lower viscosity allow faster flow rate and affords greater column efficiency in comparison with analyses using HPLC. In addition, the baseline stabilization after changing conditions in SFC is faster than in HPLC. Thus, SFC has undergone rapid development in recent years so as to afford reduced analyses time and enable higher throughput in modern pharmaceutical industry.

In all the chromatographic modalities for enantioseparations, the chiral selectors are either applied as mobile phase additives or as chiral stationary phases (CSPs). The former approach is not usually adopted for the conventional HPLC and SFC, as valuable chiral selectors are being consumed and unrecyclable. On the other hand, the development of CSPs is considered highly desirable and they are widely applied for most enantioseparations. Amongst all developed CSPs,

Cyclodextrin (CD) based CSPs have been used extensively for enantioseparations. These CSPs usually enable enantioseparations through hydrophobic inclusion or adsorption between the CD cavity and hydrophobic moieties of analytes. More specifically, in contrast to the conventional neutral CD CSPs where only hydrophobic inclusion is considered, dual interactions of electrostatic forces and hydrophobic inclusion in the CD cavity account for retention and enantioseparation of racemates in the applications of charged CD CSPs. The preparation and intensive investigations of anionic β-CD derivatives' in enantioseparation were reported during recent years where the anionic β-CD derivatives were shown to depict versatile enantioseparation capabilities. In contrast, cationic β-CD derivatives were exiguously studied. Few works on cationic β-CD derivatives in enantioseparation focused on their application as chiral mobile phase additives. Motivated by the popular investigation on imidazolium, pyridinium and ammonium based ionic liquids, we designed cationic derivatives by introducing one imidazolium, pyridinium or ammonium substituent onto the primary ring of β-CD and applied them as CSPs. To the best of our knowledge, this is the first application of introducing cationic β-CD onto stationary phase for enantioseparations in HPLC and SFC. The derived cationic β-CD CSPs are able to achieve complementary enantioseparation results with those being achieved on anionic β-CD CSPs.

The cationic β-CD CSPs are prepared via two methods: physical coating or chemical bonding. Coated CSPs were prepared based on

6^A-(3-alkylimidazolium)-6-deoxyphenylcarbamoyl-β-cyclodextrin, 6^A-(4-vinylpyridinium)-6-deoxyphenyl-carbamoyl-β-cyclodextrin and 6^A-(N,N-allylmethylammonium)-6-deoxyphenyl-carbamoyl-β-cyclodextrin. Thereafter, in order to broaden the applicable chromatography conditions, bonded CSPs were prepared via co-polymerization in the presence of small molecular monomers, as direct polymerization between chiral selector and silica gel invariably afforded diminished chiral resolutions. However, it was found that different reaction conditions and even varying the sequence of reagent additions may affect the ultimate bonding effectiveness. By optimizing all the reaction factors, cationic β-CD derivatives of 6^A-(3-vinylimidazolium)-6-deoxyphenyl-carbomoyl-β-cyclodextrin, 6^A-(*p*-vinylpyridinium)-6-deoxyphenylcarbomoyl-β-cyclodextrin and 6^A-(N,N-allylmethylammonium)-6-deoxyphenylcarbomoyl-β-cyclodextrin were chemically immobilized onto silica and three bonded CSPs were prepared.

The coated cationic CSPs prepared were applied in normal phase liquid chromatography (NPLC) and SFC. These coated cationic CSP depicted better enantioseparation abilities over commercial neutral CD CSP such as SINU-PC [129]. Three coated CSPs with precise loading of 15% (w./w.), 20% & 35% were prepared. Among them, the one with 20% loading afforded the best enantioseparation results.

In the studies on the coated CSPs, it was found that longer alkyl chain on the cationic imidazolium substituent on the chiral selectors afforded more favorable to

enantioseparations. The longer alkyl chain on the substituent may supply additional hydrophobic interaction site with the analyte and it also prevent the CD cavities from being too close to each other to afford better enantioseparation results.

Moreover, amongst all the coated CSPs prepared, the phenylcarbamate derivative has shown better enantioseparations than 3,5-dimethylphenylcarbamate derivative. The phenylcarbamate substituents may be easier to form π-π conjugation interaction with the analytes.

The bonded CSPs were applied in different chromatography modes such as normal phase liquid chromatography (NPLC), reversed phase liquid chromatography (RPLC) and SFC. Series comprising chiral flavanone derivatives, dansyl amino acids, thiazides and small molecular aromatic acids were successfully resolved. The enantioseparation abilities of the bonded cationic β-CD CSPs appears better than neutral β-CD CSPs in both HPLC and SFC.

It is notable that acid additives have significant influence on the enantioseparation results attained on the bonded cationic CD CSPs. The acid additives in the mobile phase might have interacted competitively with cationic chiral selector through electrostatic forces. Consequently, all acidic analytes depicted diminished retention time. However, only the enantioselectivities of weakly acidic analytes' were enhanced with acid additives in the mobile phase while neutral analytes and strong acids' were not changed significantly.

In order to determine the role of cationic substituents in enantioseparation

more clearly, CSPs incorporating imidazolium, pyridinium and ammonium moieties were compared. It was found the CSPs with aromatic substituents afforded better enantioseparations towards most of the racemates used. It is conjectured that the aromatic cationic substituents are more accessible due to their planer structures, thus favoring the formation of π-π conjugation which contributes to the enhanced enantioseparation abilities.

On the other hand, the importance of hydrophobic inclusion between the cationic β-CD CSPs and analytes in enantioseparations is also discussed. The cationic β-CD CSPs were found to afford different enantioseparation mechanisms in NPLC and SFC. In NPLC, the hydrophobic CD cavity is occupied by organic solvent, therefore, the enantioseparations may have arisen solely from interactions between analytes and substituents on the CD rim. In SFC conditions, small CO_2 molecules in the CD cavity can be readily displaced by the hydrophobic moiety of the analytes. Thus, hydrophobic inclusion is counted as one factor in the SFC enantioseparation processes.

Finally, as the cationic β-CD CSPs exhibited strong chiral resolution abilities in SFC, loading studies were performed. It is found that overloading the same amount of samples through overloading sample injection volume or sample concentration may give different separation results. In the comparison between the two overloading methods, the former, with samples of lower concentration but overloaded injection volume, affords broader peaks but higher chiral selectivities. During optimizations in loading study, it was also found that lower content of

organic modifier in the mobile phases would result in longer retention times but better chiral separations.

Abbreviations and Symbol

Me	Methyl
Oct	n-Octyl
PC	Phenylcarbamate
CD	Cyclodextrin
CSP	Chiral stationary phase
CMA	Chiral mobile phase additive
DMF	Dimethyl formamide
MeOH	Methanol
IPA	2-Propanol
THF	Tetrahydrofuran
AcOH	Acetic acid
TEA	Triethylamide
DMBD	2,3-Dimethylbutadiene
HD	1,5-Hexadiene
EGD	Ethylene glycol dimethacrylate
k'	Capacity factor
A	Selectivity
Rs	Resolution
BPR	Back pressure regulator
HPLC	High-performance liquid chromatography
SFC	Supercritical fluid chromatography
CE	Capillary electrophoresis
NPLC	Normal phase liquid chromatography
RPLC	Reversed phase liquid chromatography
POLC	Polar organic liquid chromatography
NMR	Nuclear Magnetic Resonance
FTIR	Fourier Transform Infrared Spectroscopy
MS	Mass Sperctrometry
TLC	Thin-layer chromatography
VIM	3-Vinylimidazolium
VPY	4-Vinylpyridinium
VAM	N-Vinyl-N-methylammonium
imi	Imidazole
Tosyl	*p*-toluenesulphonyl
allyl	Allylic group
SMM	Small molecular monomer
TEAA	Triethylamine acetate