LC Packing Materials for Pharmaceutical and Biomedical Analysis

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Abstract The author has prepared novel liquid chromatography-LC packing materials for pharmaceutical and biomedical analysis. These include LC packing materials for direct serum injection assays of drugs and their metabolites. LC packing materials for resolution of enantiomeric drugs and uniformly sized molecularly imprinted polymers for drugs and their metabolites. This review article deals with the preparation of these materials and the pharmaceutical and biomedical applications of them in recognition of the Society of Chromatographic Sciences Award.

Key words LC stationary phase, direct serum injection assay, restricted access media, chiral stationary phase, protein, molecularly imprinted polymers

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Many liquid chromatography-LC packing materials have been prepared since the introduction of HPLC. One type is for general purposes such as octadecyl-silyl silicas. The other types are for specific purposes such as chiral stationary phase-CSPs and restricted access media-RAM. The author has prepared LC packing materials for direct serum injection assays of drugs and their metabolites. LC packing materials for resolution of enantiomeric drugs and uniformly sized molecularly imprinted polymers-MIPs for drugs and their metabolites. This review article deals with the preparation of these materials and the pharmaceutical and biomedical applications of them in recognition of the Society of Chromatographic Sciences Award.

1 Restricted access media

For the LC determination of drugs and their metabolites in serum or plasma tedious and time-consuming pretreatment procedures such as the removal of proteins by precipitation-liquid-liquid extraction or solid phase extraction-SPE have been required in the past. Recently direct serum injection analysis of drugs in biological fluids by HPLC has been explored. One of the direct serum injection methods is the use of RAM. Many RAM have been prepared and used for enrichment and pretreatment of the analytes in proteinaceous samples by HPLC. With RAM large molecules such as proteins are eluted in the void volume without destructive accumulation because of restricted access to some surfaces while allowing small molecules such as drugs and their metabolites to reach the hydrophobic ion-exchange or affinity sites and to be separated. We prepared two RAM internal-surface reversed-phase ISRP and mixed functional phase-MFP materials.

1.1 Internal surface reversed-phase

The ISRP silica materials produced from porous silica gels with 8 nm average pore diameter have hydrophilic exterior and hydrophobic interior surfaces as shown in Fig. 1. Hagestam and Pinkerton prepared an ISRP support from covalently modified glycerylpropyl i.e., diol phases by attachment of the tripeptide glycine-L-phenylalanine-L-phenylalanine GFF bound via the amino groups to the glycerylpropyl phases. The phenylalanine moieties were then cleaved from the external surface of the silica with carboxypeptidase A which is too large to enter the small pores. After this enzymatic treatment the glycerylpropyl-glycine phases remained on the external surfaces while the internal surfaces remained uncleaved. Thus the external and internal surfaces consisted of hydrophilic and hydrophobic phases respectively. Further on the negatively charged GFF ana-
lytes were retained by a unique combination of mechanisms: π-electron interactions and weak cation-exchange properties. This GFF ISRP material, which is commercially available, had disadvantages in that it could not retain certain classes of hydrophilic drugs such as the amphoteroc drugs cephalosporins and penicillins. The recommended eluent pH range is limited to 6.0–7.5, and it is difficult to obtain the desired batch-to-batch reproducibility of its properties. We designed a new ISRP support having N-octanoylaminopropyl and N\textsuperscript{2} 3-dihydroxypropyl aminopropyl groups on the internal and external surfaces by using a novel enzyme, polymyxin acylas. This enzyme is a monomer with molecular mass 62 000, has an affinity for long fatty acid groups and can cleave acyl groups of C2 to C16 phenyl and substituted phenyl residues. The neutral ISRP support prepared by us is more effective than that designed by Hagestand and Pinkerton when separating hydrophilic drugs and when an eluent pH of 3 to 7 is employed. Further, the neutral ISRP support prepared by us was applied to direct serum injection assays of anticonvulsant drugs and methylxanthine derivatives combined with a column-switching technique.

1.2 Mixed functional phase MFP

We prepared MFP materials for direct serum injection assays of achiral and chiral drugs having a β-cyclodextrin-CD-diol phase and a hydrophobic phenyl butyl or octyl-diol phase bonded to porous silica particles. The MFP materials were synthesized from porous silicas in three steps into a hydrophobic or β-CD groups onto introduction of 3-glycidoxypropyl groups and hydrolysis of the oxirane ring to diol groups. The MFP materials having hydrophobic-diol phases were prepared from starting silica materials with average pore diameters of 6 nm–10 nm. Serum proteins were completely recovered from them at the first injection by changing the balance of hydrophobic and hydrophilic phases. The obtained MFP materials could tolerate over 500 injections of a 20 μL serum sample with no change of back pressure and column efficiency. The MFP packing materials having β-CD-diol groups had been prepared using an 8 nm pore size silica. Bovine serum albumin BSA was eluted from the material only bonded with β-CD as a tailing peak with protein recovery of 90%. However, from the β-CD-diol material BSA was eluted with a protein recovery of almost 100%. This is due to low surface coverage of the material bonded only with β-CD. The β-CD-diol material can be used for over 400 injections of a 20 μL serum sample. Fig. 3 shows chromatograms of direct injection analysis of chlorpheniramine in human serum on the β-CD-diol packings.

![Fig. 1 Schematic representation of an internal-surface reversed-phase ISRP material](image)

Proteins do not adsorb on the hydrophilic exterior surfaces and do not penetrate into the hydrophobic interior surfaces while analytes can reach the interior surfaces and be separated.

![Fig. 2 Separation of cephalosporins in human serum by direct injection onto the ISRP materials designed by Hagestand and Pinkerton](image)

Peak assignments: 1. cefotaxime 10 μg · mL⁻¹ 2. cefmenoxime 10 μg · mL⁻¹ 3. cefamandole 20 μg · mL⁻¹. HPLC conditions: eluent 100 mmol/L phosphate buffer-acetonitril 12:1, final pH 6.9, flow-rate 0.8 mL/min, detection 254 nm, injection volume 20 μL. Dotted lines indicate serum blank.

It was found that protein-based CSPs worked as MFP materials because a protein especially a glycoprotein has hydrophilic external surface. Ovomucoid-based CSP could be used for direct serum injection assays of chiral drugs.

2 Protein-based chiral stationary phases

A protein and glycoprotein respectively consist of
amino acids and amino acids and sugars both of which are chiral. Thus all proteins have the ability to discriminate a chiral molecule. However only a limited number of proteins have been investigated as HPLC CSPs. Protein-based CSPs are of special interest because of their unique enantioselective properties and because they are suitable for separating a wide range of enantiomeric forms. The advantages of protein-based CSPs generally include the use of an aqueous mobile phase as for reversed-phase HPLC enantioselectivity for a wide range of compounds and direct analysis without derivatization. The disadvantages have included low capacity lack of column ruggedness and limited understanding of the chiral recognition mechanism. A few trials were made to overcome these disadvantages of protein-based CSPs as described below.

2.1 Chiral stationary phases based on serum albumin fragments

The bovine serum albumin BSA fragment columns obtained by other investigators could have less capacity and enantioselectivity and be less stable than the intact BSA column. We isolated the BSA fragment of molecular mass 35 236 estimated by electrospray ionization mass spectrometry. The estimated molecular mass was in good agreement with the predicted molecular mass 35 234 of the half-cystinyl BSA fragment which is ascribable to amino acid sequences 1-307 having eight disulfide bonds and one half-cystinyl bond in the 34th. Next the intact and fragmented BSA were bound to N,N'-dissucinimidyl carbonate DSC-activated aminopropyl-silica gels. The bound amounts of the BSA fragment were 2.2-2.7 times more than that of the intact BSA. Chiral resolution of 2-arylpropionic acid derivatives benzodiazepines warfarin and benzoid was attained with the BSA fragment columns. Fig. 4 shows chromatograms of benzoin on the BSA and BSA-fragment columns. The BSA fragment columns gave higher enantioselectivity for lorazepam and benzoin because of the larger bound amounts and lower enantioselectivity for other compounds tested compared with the BSA column. The lower enantioselectivity might be due to changes in the globular structure of the BSA fragment and/or changes in the local environment around the binding sites.

Similarly a human serum albumin HSA fragment was isolated by size-exclusion chromatography following peptic digestion of HSA. Matrix-assisted laser-desorption ionization time-of-flight MALDI-TOF mass spectra indicated three M + H\(^+\) ion peaks whose molecular masses were estimated to be 34 816, 29 178 and 44 134. The molecular masses estimated were in good agreement with the predicted molecular weights of amino acid sequences 1-308, 49-308 and 1-387. The fragment protein whose molecular mass was about 35 000 was mainly observed. Next the intact and fragmented HSA were bound to DSC-activated aminopropyl-silica gels. Though the retentivity and enantioselectivity of all solutes tested were lower on the HSA fragment column than the HSA column benzodiazepines oxazepam 2-arylpropionic acid derivatives ibuprofen...
ketoprofen, pranoprofen and flurbiprofen and warfarin were still enantioseparated on the HSA fragment column. However no enantioseparation of benzoin was attained. As reported by He and Carter primary binding sites for benzodiazepines and 2-arylpropionic acid derivatives are located in the subdomain IIIA while that of warfarin in the subdomain IIA. It is interesting that benzodiazepine oxazepam and 2-arylpropionic acid derivatives ibuprofen ketoprofen pranoprofen and flurbiprofen participate in enantioselective binding interaction with the N-terminal half of HSA despite the fact that the primary binding sites of these compounds are on the other half of HSA. Fig. 5 shows the enantioseparations of pranoprofen and warfarin on the HSA and HSA fragment columns. Though the HSA column showed higher enantioselectivity than the HSA fragment column the enantioseparations of pranoprofen and warfarin were attained with a shorter analysis time on the HSA fragment column. This could be due to removal of the non-specific binding sites of HSA changes in the globular structure of the HSA fragment and/or changes in the local environment around the binding sites. Further the HSA fragment column was as stable as the intact HSA column for repetitive injection of samples. These results suggest that the HSA fragment column could be useful for the enantioseparation of solutes having strong affinities for HSA.

![HSA column](image)

![HSA fragment column](image)

### Fig. 5 Chromatograms of pranoprofen [a] and warfarin [b] on HSA and HSA-FG75 columns

HPLC conditions: column size 2.0 mm i.d. × 100 mm, eluent 50 mmol/L sodium phosphate buffer pH 7.5, 1-propanol 94:6 v/v, flow rate 0.2 mL/min, UV detection 210 nm, temperature 25 °C, loaded amount 100 ng.

#### 2.2 a1-Acid glycoprotein

Since the molecular mass of a1-acid glycoprotein AGP from human plasma ranges from 38 800 to 48 000 it is generally assumed to be 40 000. Recently we reported that the average molecular mass of AGP could be ca. 33 000 by MALDI-TOF mass spectra and that the sugar content of AGP could be estimated to be 34%. There is no precise information on the chiral recognition sites and mechanism of AGP because of the lack of information on the tertiary structure. Further the role of sugar moieties in enantioselective bindings by AGP has not been investigated precisely. We prepared partially deglycosylated pd-AGP by removal of a sugar moiety of AGP by treatment with N-glycosidase. The average molecular mass of pd-AGP was estimated to be ca. 30 600 by MALDI-TOF mass spectrometry. Fig. 6a and b shows chromatograms of ethotoxin enantiomers on the AGP and pd-AGP columns respectively. The retentivity and enantioselectivity of the
neutral and basic solutes tested on the pd-AGP column were significantly or not significantly larger in most solutes than those on the native AGP column. A possible explanation is that by cleavage of a sugar chain pd-AGP could become more hydrophobic than AGP and or that a solute could be easily accessible to the specific or non-specific binding sites of pd-AGP. Further study is required to clarify the chiral recognition mechanism of AGP and the role of the sugar moiety on chiral recognition.

![Chromatograms of ethoatoin enantiomers on AGP](image)

**Fig. 6** Chromatograms of ethoatoin enantiomers on AGP

- a) and pd-AGP b) columns

HPLC conditions: column size: 2.0 mm i. d. x 100 mm; eluent: 10 mmol/L phosphate buffer pH 5.0; flow-rate: 0.2 ml min⁻¹; detection: 210 nm; temperature: 25 °C; loaded amount: 100 ng.

### 2.3 Ovomucoid and ovoglycoprotein

CSPs based on ovomucoid from chicken egg whites OMCHI were developed by Miwa et al. The OMCHI column can resolve a wide range of acidic and neutral enantiomers.

Various ovomucoids such as ovomucoid from turkey egg whites OMTKY and OMCHI exist as three tandem independent domains. In order to gain information regarding the enantioselective recognition mechanism of ovomucoid proteins it is of interest to know whether chiral recognition is expressed by all three domains by only one domain or by a combination of domains. Each domain and combination domains first and second and third domains were isolated purified and characterized. Further columns were made with purified OMTKY and OMCHI domains to test chiral recognition properties. The third domain of OMTKY and OMCHI consisted of glycosylated OMTKY3S and OMCHI3S and unglycosylated domains OMTKY3 and OMCHI3. The third domains of the OMTKY and OMCHI domains were found to be enantioselective for at least two classes of compounds benzodiazepines and 2-arylpipionic acid derivatives as shown in Tables 1 and 2 respectively. Glycosylation of the third domain did not affect chiral recognition. Further the chiral recognition mechanism of the OMTKY3 was elucidated by using NMR measurements molecular modeling and computational chemistry. Fig. 7 illustrates the binding orientations of the enantiomers of U-80413 which is one of 2-arylpipionic acid derivatives in each of the two surface regions identified on the OMTKY3. The first group of amino acids Val6 Arg21 Pro22 Leu23 Lys34 and Phe53 is on the left while the second group Val41 Val42 Leu48 and Lue50 is on the right. The tubular structure represents the protein backbone and side chains of selected amino acid residues Arg21 Lys34 and Phe53 are shown. The peptide strand is wrapped around an α-helix and held in place by three disulfide bonds. The N-terminal is at the top right and the C-terminal is in the back. The dockings in the second site to the right were of higher energy and without apparent points of interaction that could produce chiral recognition. This site could account for the nonspecific binding site. The selected specific binding model for each of the  R- and S- U-80413 with OMTKY3 is shown in Fig. 8. One can see similarities and differences in orientation and intermolecular interactions between the  R- and

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*HPLC conditions: column size: 2.0 mm i. d. x 100 mm; eluent: 20 mmol/L phosphate buffer pH 6.9; 2-propanol 79:30; flow-rate: 0.1 ml min⁻¹; column temperature; ambient.

OMTKY3 unglycosylated third domain of OMTKY3S glycosylated third domain of OMTKY3OMTKY2 second domain of OMTKY1 first domain of OMTKY1.
phenyl group of $\text{R}^-\text{U-80413}$ and Phex 53. However,$\text{R}^-$ neither the first nor the second domain of OMTKY gave appreciable chiral recognition abilitit.$\text{R}^-$

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<th>Table 2 Retention factors and enantioseparation factors of benzodiazepines and 2-arylpropionic acid derivatives on OGCHI domain columns</th>
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*HPLC conditions: column 2.0 mm i. d. × 150 mm; eluent: 20 mmol/L phosphate buffer pH 6.8; 2-propanol 99:1; flow-rate: 0.1 mL/min; column temperature: ambient.


Recently[6] we isolated and characterized a new protein from chicken egg white[6][27][28]. It was termed ovoglycoprotein from chicken egg white[6] OGCHI[6]. In addition[6] it was found that 10% of OGCHI was included in crude OMCHI preparations[6][28]. Moreover[6] OMCHI and OGCHI columns were made from isolated[6] pure proteins and were compared with regard to chiral recognition abilities. It was found[6][28] that the chiral recognition ability of OMCHI reported previously[6][28] came from OGCHI[6] and that OMCHI had no chiral recognition ability. Further[6] it was found that OGCHI is preferentially bound to DSC-activated aminopropyl-silica gels compared with OMCHI[6] even though the average molecular mass of OGCHI and OMCHI are 30,000 and 27,000[6] respectively[6][29][30]. This is why CSPs based on crude OMCHI[6] which are now commercially available[6] show moderate chiral recognition ability.

The effects of the sugar moieties of OGCHI on the enantioseparations of various solutes were investigated.[6] The retentive and enantioselective properties of the OGCHI[6] asialo OGCHI and asialo-agalacto OGCHI columns were compared. Generally[6] removal of a sialic acid or sialic acid-galactose group of OGCHI resulted in no change or an increase of the retention factors and enantioseparation factors of solutes tested. It was concluded that sialic acid and sialic acid-galactose groups of OGCHI did not participate in chiral discrimination of the solutes tested[6][31]. Moreover[6] partially deglycosylated[6] pEG[6] OGCHI and completely
deglycosylated OGCHI and cd-OGCHI were obtained by treatments of OGCHI with N-glycosidase and a mixture of endoglycosidase and N-glycosidase respectively. The average molecular masses of pd-OGCHI and cd-OGCHI were estimated to be about 28 400 and 21 400 respectively. It was found that the pd-OGCHI column showed excellent chiral recognition abilities comparable to the OGCHI column and that the retention and enantioselectivity of basic solutes tested on the pd-OGCHI column were higher than those on the OGCHI column while those of acidic solutes on the pd-OGCHI column were lower. Further cd-OGCHI still showed chiral recognition abilities for various solutes tested. These results revealed that the chiral recognition site of OGCHI existed on the protein domain of OGCHI.

OGCHI was bound to aminopropyl-silica gels via an amino or carboxyl group of OGCHI and cd-OGCHI were prepared via a carboxyl group of OGCHI and that the amino group of OGCHI is suitable for chiral resolution of acidic solutes and those via an amino group of OGCHI are suitable for chiral resolution of basic solutes. It was suggested that electrostatic interaction between an amino or carboxyl group of OGCHI and a charged solute should play an important role in chiral recognition of the solute. Further chiral recognition properties of OGCHI and a protein from Japanese quail egg white (OGj, PQ) were compared. The average molecular masses of OGCHI and OGj, PQ were estimated to be about 30 000 and 27 400. The OGCHI column is suitable for chiral resolution of basic compounds while the OGj, PQ column is suitable for that of acidic compounds. With regard to chiral resolution of neutral compounds it is dependent on the compound to be resolved which column could be suitable. The results obtained revealed that chiral recognition of various solutes should be efficiently attained by using both columns complementarily.

2.4 Chiral stationary phases based on other proteins

CSPs based on lysozyme and pepsin were developed by us. By using a mixture of phosphate buffer and organic modifier as an eluent basic and uncharged enantiomers were resolved on both CSPs while no resolution of acidic enantiomers was observed. Further the pepsin and OMCHI were mixed-immobilized onto the same porous aminopropyl-silica gels. The retentive and enantioselective properties of the mixed-protein-based CSP were compared with those of pepsin- and OMCHI-based CSPs. The pepsin-OMCHI-based CSP showed similar enantioselectivity with the pepsin-based CSP because OMCHI has no enantioselectivity. In addition the pepsin-OMCHI-based CSP was more stable than the pepsin-based CSP for repetitive injections of samples and continuous flow of an eluent. The pepsin-OMCHI-based CSP was further stabilized by crosslinking with glutaraldehyde.

3 Uniformly sized molecularly imprinted polymers

Since molecular imprinting techniques can afford complementary binding site s for a template molecule Fig. 9 the MIPs are used for chromatographic separations SPE membranes antibody-mimics and sensors for the purpose of specific recognition of the target molecule . Usually non-aqueous bulk polymerization methods have been utilized to obtain MIPs. The disadvantage of the method is that the obtained block polymers had to be crushed ground and sieved to produce packing materials. The MIPs obtained are unsuitable for HPLC packing materials owing to random shape and size distribution. Uniformly sized MIPs have been prepared through a combination of a typical multi-step swelling and polymerization method and molecular imprinting technique . The advantages of the method are as follows it is easy to prepare uniformly sized and monodispersed particles easy to perform situ mod-

![Fig. 9 Schematic diagram of preparation of non-covalent MIPs](image-url)
ification and suitable for preparing HPLC packing materials. We have prepared uniformly sized MIPs for basic and acidic drug enantiomers and applied the obtained MIPs for resolution of drug enantiomers by HPLC.

Recently, selective enrichment and pretreatment of analytes in complex matrixes have been attained with SPE based on MIPs. The SPE based on MIPs has been generally carried out by an off-line mode. We have prepared a RAM-MIP, a uniformly sized MIP selectively modified with a hydrophilic external layer through a combination of molecular imprinting and hydrophilic surface modification techniques. Further, the obtained RAM-MIP for 2-arylp propaneic acid derivatives was applied for the direct serum injection assays of the drug by a column-switching consisting of a RAM-MIP and a conventional C18-silica column.

3.1 Chiral separation using molecularly imprinted polymers

We prepared uniformly sized MIPs for S-4-propanoate and S-chlorphenamine by a two-step swelling and polymerization method using methacrylic acid, MAA and ethylene glycol dimethacrylate as the functional monomer and crosslinker, respectively. The retentive and enantioselective properties of these two drugs and their structurally related compounds on the MIPs were evaluated using an aqueous eluent. Hydrophobic and ionic interactions could mainly be responsible for the retention and enantioseparation of propanolol and chlorphenamine in an aqueous eluent. The MIP showed the highest recognition for the template molecules and slight recognition for their structurally related compounds. Further, the MIPs for 2-arylpropanic acid derivatives and S-naproxen and S-ibuprofen were prepared using 4-vinylpyridine 4-VPy and EDMA as the functional monomer and crosslinker, respectively by a two-step swelling and polymerization method. The obtained MIP for S-naproxen was evaluated in aqueous eluent. The baseline resolution of naproxen enantiomers was attained by optimization of the preparation method of the uniformly sized MIP and separation parameters such as flow-rate and column temperature. Fig. 10[48] shows the effects of eluent pH on the retention properties of S-ibuprofen benzoic acid benzensulfonic acid and benzene on non-imprinted EDMA styrene-EDMA and V-Py-EDMA and S-ibuprofen imprinted V-Py-EDMA materials respectively[49]. Since the first two polymers had no ionizable groups in the polymer backbone solutes were mainly retained with hydrophobic interactions. Benzensulfonic acid was not retained on the EDMA and styrene-EDMA materials among the eluent pHs tested. It is thought that the apparent pK_a value of benzensulfonic acid is < 2.3. On the other hand, benzensulfonic acid was retained on the non-imprinted and S-ibuprofen imprinted V-Py-EDMA materials by decreasing the eluent pH. The retention of benzensulfonic acid was due to ionic interactions of sulfonyl groups of benzensulfonic acid with the positively charged V-Py-EDMA materials. It was reported that the apparent pK_a value of the pyridyl group of the matrix was ~ 4[50]. However, the retention data of benzensulfonic acid suggested the shift of the average apparent pK_a value of 4-VPy-EDMA materials to < 3. Benzoic acid was more retained by hydrogen bonding interaction with a pyridyl group on the non-imprinted and S-ibuprofen imprinted V-Py-EDMA materials than the EDMA and styrene-EDMA materials. The retention factor of S-ibuprofen increased with the
order EDMA\textsuperscript{c} 4-VPy-EDMA\textsuperscript{c} styrene-EDMA\textsuperscript{c} and \textsuperscript{d} S\textsuperscript{d}-ibuprofen imprinted 4-VPy-EDMA materials. S\textsuperscript{d}-Ibuprofen could be retained mainly with hydrophobic interactions with EDMA and styrene-EDMA materials and hydrophobic and hydrogen bonding interactions with 4-VPy-EDMA materials. Further S\textsuperscript{d}-Ibuprofen was more retained by the molecular imprinting effect on the S\textsuperscript{d}-ibuprofen imprinted 4-VPy-EDMA materials than the non-imprinted ones.

3.2 Restricted access media-molecularly imprinted polymers

We have prepared a RAM-MIP material\textsuperscript{a} a uniform-sized MIP for S\textsuperscript{a}-naproxen selectively modified with a hydrophilic external layer\textsuperscript{a} through a combination of molecular imprinting and hydrophilic surface modification technique\textsuperscript{a} \textsuperscript{51,52}. Further\textsuperscript{a} we prepared RAM-MIP materials for S\textsuperscript{a}-naproxen and -ibuprofen\textsuperscript{a} and tried to apply the respective RAM-MIP for direct serum injection assays of the drug by column-switching system\textsuperscript{a} consisting of the RAM-MIP material and a conventional C18-silica column. However\textsuperscript{a} leakage of the imprint molecule prevented accurate and precise assays of the drug. Thus\textsuperscript{a} the RAM-MIP material for S\textsuperscript{a}-naproxen was applied for the assays of ibuprofen in rat plasma\textsuperscript{a} \textsuperscript{52}. Fig. 12\textsuperscript{a} parts a\textsuperscript{a} b and c\textsuperscript{a} shows chromatograms of standard ibuprofen samples\textsuperscript{a} 5.0 \textmu g mL\textsuperscript{-1}\textsuperscript{a} control plasma sample and control plasma sample spiked with 5.0 \textmu g mL\textsuperscript{-1}\textsuperscript{a} of ibuprofen\textsuperscript{a} respectively\textsuperscript{a} using column-switching techniques. Fig. 12 illustrates the fact that ibuprofen is separated from the ordinary components of plasma samples\textsuperscript{a} and that ibuprofen is almost completely recovered from the serum samples. Further\textsuperscript{a} S\textsuperscript{a}-naproxen\textsuperscript{a} imprint species\textsuperscript{a} appeared on a chromatogram. However\textsuperscript{a} it was completely separated from ibuprofen on a C18 column.

The developed method should have wide applicability for the determination of a drug in biological fluids.

4 Conclusions

The author has prepared LC packing materials for direct serum injection assays of drugs and their metabolites\textsuperscript{a} LC packing materials for resolution of enantiomeric drugs\textsuperscript{a} and uniformly sized MIPs for drugs and their metabolites. These materials could be
useful for specific purposes for the assays of drugs and their metabolites and resolution of enantiomeric drugs. However, we require further studies to clarify the chiral recognition sites and mechanism on OGCII. In addition, MIPs for hydrophilic compounds proteins and peptides are the targets to be pursued. In the future, we will need further LC packing materials for high-performance separations as well as for selective separations.

Finally, the author would like to thank many people for their help and encouragement. First of all, he thanks the people who studied and are studying in his laboratory. Dr. H. Matsunaga, Ms. H. Sanbe and Ms. C. Kagawa as colleagues in the laboratory are greatly appreciated for their talented assistance. Also, thanks are due to many coworkers for their help and discussions. Dr. T. Pinkerton, Pharmacia & Kalamazoo, MI USA Prof. J. Markley, University of Wisconsin-Madison, Madison, WI USA Prof. N. Tanaka, Kyoto Institute of Technology, Kyoto, Japan Prof. K. Hosoya, Kyoto Institute of Technology Dr. K. Kimata, Nacalai Tesque Kyoto and Dr. H. Wada, Shinwa Chemical Industries Kyoto. Last but not least, the author thanks Professor T. Uno, Professor Emeritus kyoto University of Kyoto, Japan, and Professor T. Nakagawa, Kyoto University, for a lot of support at the start of my scientific career.

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