High-performance liquid chromatography on polypyrrole-modified silica

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ABSTRACT

Bare silica and C_{18}-bonded silica were modified with polypyrrole chloride and polypyrrole chloride–dodecyl sulfate. The packing materials were characterised using scanning electron microscopy, energy dispersive X-ray analysis, elemental analysis and surface area analysis. Both reversed-phase and anion-exchange chromatographic behaviour were determined using a selection of test compounds.

INTRODUCTION

Silica is the most popular supporting material used in liquid chromatography at present [1]. It is readily available in appropriate particle sizes, pore sizes, pore volumes and surface areas for high-performance liquid chromatography (HPLC). Furthermore, it can be modified to provide stationary phases with different interaction modes. Functional groups such as alkyl, phenyl, amino and cyano groups have been bonded to silica. Such functional groups attached have included polar compounds such as safrole [2], electron donor–acceptors such as caffeine [3–5], hydrogen bonding ligands such as pyridyl [6], metallic compounds such as copper (II) [7] and zirconium oxide [8], compounds for protein separations such as melittin [9], concanavalin A [10] and N-hydroxysuccinimide ester [11], and compounds for chiral separations such as L-proline [12], acetylquinine [13] and cellulose [14].

More recently, polymer-coated silica gels have become popular since they combine the mechanical properties of silica with the dynamic chemical properties of polymers. For example, poly(alkyl aspartamide) [15], alkyl polysiloxanes [16], polyvinylpyrrolidone [17], poly(2-sulfoethyl aspartamide) [18], polyethyleneimine [19,20], polyamine [21], poly(butadiene-maleic acid) [22] and polyvinylimidazole [23] have been coated on silica gels. More complicated polymer-coated silica stationary phases such as polymer-diol-silica [24] and C_{18}-polymer-silica [25] have also been synthesised. The polymer coating not only improves the selectivity but also the chemical stability of the stationary phases.

Conducting polymers such as polypyrrole are known to posses unique physical and chemical properties [26]. They are conductive, electroactive and stable in aqueous as well as organic solvents. Conducting polymers coated on to reticulated vitreous carbon have previously been utilised as chromatographic stationary phases in these laboratories. Both reversed-phase as well as anion-exchange chromatographic interactions were reported [27–29]. However, to date these polymers have not been coated on silica substrates.

In this work, bare silica (termed silica) and C_{18}-bonded silica (termed C_{18}) have been coated with polypyrrole chloride (PP/Cl) or polypyrrole chloride–dodecylsulfate (PP/Cl/DS) and selectivity differences were considered. Characterisation of the coated phases was carried out using scanning electron microscopy (SEM), energy dispersive X-ray (EDX) analysis, elemental analysis and surface area analysis. Both reversed-phase and anion-exchange chromatography were characterised.

TABLE I

HPLC CHARACTERISATION CONDITIONS

Column	C_{18} based	Silica based
Mobile phase	methanol–water (65:35)	methanol–water (50:50)
Flow-rate-(ml/min)	0.50	0.30
Test compounds	Benzene 50 ppm	
	Toluene 50 ppm	
	Dimethylphthalate (DMP) 10 ppm	
	Diethylphthalate (DEP) 10 ppm	
	Phenol 30 ppm	
	Benzoic acid 20 ppm	
	Aniline 20 ppm	
	N,N-Dimethylaniline (DMA) 20 ppm	
	Theophylline 10 ppm	
	Caffeine 10 ppm	

EXPERIMENTAL

Reagents and materials

Analytical reagent (AR) grade chemicals were used unless otherwise stated. Silica gel (10-μm Spherisorb, PhaseSep) and C_{18}-bonded silica gels (10-μm ODS-Hypersil, Shandon) were used as substrates in separate parts of this work. Pyrrole was distilled before use. Sodium chloride and sodium dodecyl sulfate (SDS) were used as supporting electrolytes, *i.e.* counterion sources and a 1.5 M FeCl$_3$ laboratory reagent (LR) grade solution was used to induce polymerisation. The chromatographic test compounds (Table I) were the same as those used previously [27]. Methanol and purified water were used as solvents. Ammonium acetate and a phosphate buffer (mixture of Na$_2$HPO$_4$ and NaH$_2$PO$_4$) were added to the mobile phase as required.

Instrumentation

SEM and EDX were carried out using a Hitach S-450 scanning electron microscope (Hitachi, Japan), connected to a TN-2000 Microtrace detector (United Science, USA). Elemental analysis results were obtained from the Analytical Laboratory Australian National University. Surface areas of stationary phases were measured on an Area Metre II (Strohlein Instruments, Germany). The HPLC experiments were conducted using a Kortec K350 HPLC Pump (ICI, Australia) an ERC-7210 spectrophotometric detector at 254 nm (ERMA Optical Works, Japan) and a Rheodyne 7512 injector (USA) with a 20-μl sample loop.

Column preparation

The polymer-modified stationary phases were prepared as follows: (1) 1 g of silica or C_{18} was placed in a 100-ml beaker; (2) 1 ml distilled pyrrole was added to the beaker, and stirred for 10 min with a glass rod; (3) 100 ml of distilled water were placed in the beaker, and 0.01 mole of counterions (Cl$^-$ or DS$^-$) were added as required. The mixture was stirred with a magnetic stirrer to form a suspension. (4) 1 ml of 1.5 M FeCl$_3$ solution was added and the solution was stirred vigorously for 30 min; (5) the coated particles were collected using a büchner filter and washed with 200 ml of water; (6) all packings were dried at 62°C in vacuum for 3 h before use.

The packing procedures employed for this column system have been described previously [30]. All columns were washed with methanol for 8 h before use.

RESULTS AND DISCUSSION

Preliminary characterisation of the polypyrrole-

TABLE II

ELEMENTAL ANALYSIS OF VARIOUS PACKINGS

Packing	C%	H%	N%	Cl%	S%	Polymer%[a]
C_{18}	9.45	1.80	0.0	0.0	0.0	0.0
PP/Cl/C_{18}	11.82	1.99	0.69	0.64	0.0	3.9
PP/Cl/DS/C_{18}	12.59	2.10	0.59	0.46	0.31	4.8
Silica	0.0	0.0	0.0	0.0	0.0	0.0
PP/Cl/silica	4.91	0.33	1.16	0.99	0.0	7.4

[a] Polymer% (w/w) was calculated from the weight difference between polypyrrole-modified supports and bare supports.

coated phases was carried out to ensure that the silica and C_{18} were in fact coated using the polymerisation procedure. Polypyrrole chloride-modified silica, polypyrrole chloride-modified C_{18} and polypyrrole chloride–dodecylsulfate-modified C_{18} were termed PP/Cl/silica, PP/Cl/C_{18} and PP/Cl/DS/C_{18}, respectively. Elemental analysis (Table II) indicated that the polypyrrole loadings on C_{18} were relatively low.

It was verified that using C_{18} both DS^- and Cl^- ions were incorporated when the polymer was formed from a SDS–$FeCl_3$ solution.

It was found that the polymer coating changed the specific surface area in all cases (Table III). Surface areas were determined by BET. After placing a sample bottle and an empty reference bottle into a liquid nitrogen bath, a pressure difference between the two bottles was recorded and the specific surface area of the phases was calculated according to a manufacturer provided chart. With the C_{18} material change in surface area was more obvious when the surfactant SDS was present. However, the results indicated that the surface area of the porous silica and C_{18} materials did not change dramatically with the polymer coating. These changes may in fact be due to complications that arise from the use of nitrogen in measuring the surface area of polar phases.

A micrograph of a polypyrrole-coated C_{18} packing is shown in Fig. 1. The other polypyrrole-modified packings have similar micrographs. Although the characteristic black colour of the polypyrrole-coated particles was visible to the naked eye, no significant difference was observed using SEM.

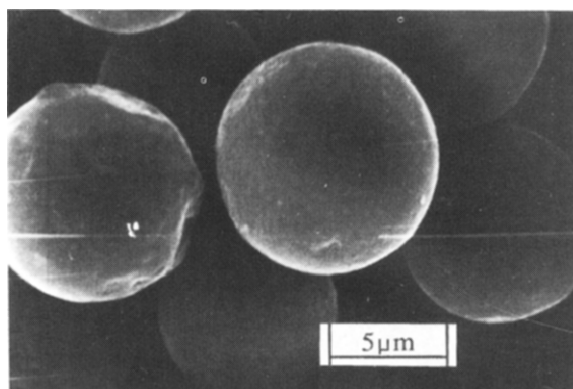Counterions in polypyrrole-coated stationary

Fig. 1. Scanning electron micrograph of silica-based polypyrrole packings. Sample: PP/Cl/DS on 10-μm Hypersil ODS.

phases were detected using EDX analysis. Not only the counterion but also the silica background signals appeared on all spectra (Fig. 2). When poly-
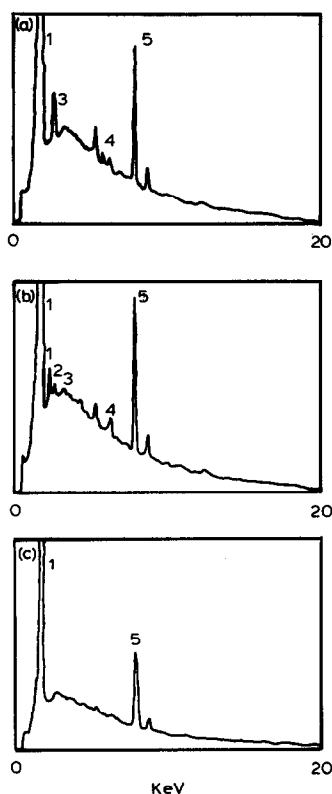

Fig. 2. EDX spectra of silica-based polypyrrole packings. Spectrum on (a) PP/Cl/C_{18}, (b) PP/Cl/DS/C_{18} and (c) C_{18}. Responses due to (1) Si, (2) S, (3) Cl, (4) Fe and (5) Cu. EDX condition: high voltage = 20 kV; accumulation time = 200 s.

TABLE III

SURFACE AREA ANALYSIS OF VARIOUS PACKINGS

Packing	Specific surface area (m²/g)	Estimated[a] polymer thickness (nm)
C_{18}	98.5	–
PP/Cl/C_{18}	96.5	0.26
PP/Cl/DS/C_{18}	83.4	0.33
Silica	149	–
PP/Cl/silica	137	0.33

[a] Assuming polymer density = 1.5 g/cm² [26,31] polymer thickness = polymer%/(polymer density × specific surface area).

TABLE IV

CHROMATOGRAPHIC INTERACTIONS ON POLYPYRROLE CHLORIDE-COATED SILICA

Column: PP/Cl/silica 64 mm × 1.6 mm I.D.

Test compounds	In methanol–water (50:50)		In methanol–water (50:50) with 0.1 M ammonium acetate	
	k'	Relative k' vs. benzene	k'	Relative k' vs. benzene
Benzene	0.41	1.0	0.28	1.0
Toluene	0.67	1.6	0.59	2.1
DMP	0.83	2.0	0.63	2.2
DEP	0.85	2.1	0.64	2.8
Phenol	0.38	0.92	0.31	1.1
Benzoic acid	∞	∞	9.6	34
Aniline	3.3	8.0	0.18	0.63
DMA	9.1	22	0.86	3.0

mers were chemically synthesised using $FeCl_3$, Cl^- as well as $FeCl_4^-$ was incorporated as a counterion which was confirmed by the existence of Fe signals in the spectra. This has also been reported by other workers [32]. The addition of DS^- to the monomer solution resulted in incorporation of DS^-.

Reversed-phase chromatographic characterisation

Chromatographic interactions on polypyrrole-modified silica and C_{18} stationary phases were characterised using the test compounds already listed. A comparison of chromatographic interactions on bare silica and polypyrrole-modified silica is summarized in Table IV and a comparison of chromatographic interactions on C_{18} and polypyrrole-modified C_{18} summarized in Table V. The relative capacity factor (k') vs. benzene was calculated by dividing capacity factors of test compounds by the capacity factor of benzene.

Polymer-coated silica. Using bare silica none of the test compounds investigated were retained except for aniline and N,N-dimethylaniline (DMA). These compounds are known to interact with the silanol groups on silica surfaces [1].

Retention of all compounds on polypyrrole-modified silica was observed (Table IV), which indicated that a reversed-phase chromatographic interaction was generated by PP/Cl. Retention of all compounds except benzoic acid, aniline and DMA was low and the results were comparable with other

TABLE V

CHROMATOGRAPHIC INTERACTIONS ON POLYPYRROLE-COATED C_{18}

Mobile phase: methanol–water (65:35); 0.5 ml/min.

| Test compounds	C_{18}		PP/Cl/C_{18}		PP/Cl/DS/C_{18}	
	k'	Relative k' vs. benzene	k'	Relative k' vs. benzene	k'	Relative k' vs. benzene
Benzene	2.02	1.00	1.86	1.00	1.87	1.00
Toluene	3.66	1.81	3.33	1.79	3.40	1.82
DMP	0.89	0.44	1.25	0.67	0.87	0.47
DEP	2.13	1.05	2.71	1.46	1.90	1.02
Phenol	0.60	0.30	0.70	0.38	0.61	0.33
Benzoic acid	0.12	0.06	∞	∞	∞	∞
Aniline	0.84	0.42	0.63	0.34	2.42	1.29
DMA	4.29	2.12	3.19	1.72	11.3	6.00
Theophylline	0.26	0.13	6.23	3.35	0.70	0.37
Caffeine	0.31	0.15	6.04	3.24	1.14	0.61
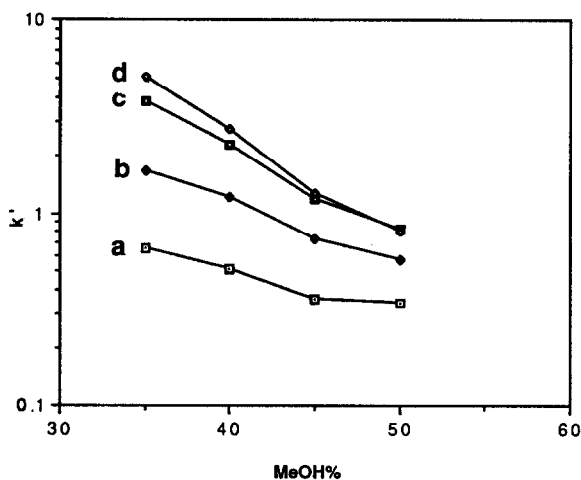
Fig. 3. Capacity factors of benzene, toluene, DMP and DEP vs. methanol (MeOH) concentration in the mobile phase. Column: PP/Cl/silica in a 64 mm × 1.6 mm I.D. column; detector: UV at 254 nm; sample: a = 50 ppm benzene; b = 50 ppm toluene; c = 10 ppm DMP; d = 10 ppm DEP.

polypyrrole columns previously investigated [27]. These results indicated that PP/Cl contributed only minor hydrophobic effects. Retention increased with decreased methanol concentration (Fig. 3) which is typical of reversed-phase behaviour. As expected, PP/Cl also introduced a certain degree of polarity to the silica surface, probably from the polar part of the polymer matrix. The low retention values obtained for phenol confirmed that PP/Cl had very low levels of interaction with unisolated acidic molecules. Both aniline and DMA were retained which verified that polypyrrole was capable of basic interactions. This is due to the fact that electrons are lost during polymerisation and positive sites are formed on the polymer. Amines with unshared electron pairs can then access these sites.

The addition of salt (0.10 M phosphate buffer) to the mobile phase (methanol–water, 50:50) reduced the capacity factors for aniline and DMA. This was due to solvation of the positive sites on the polymer matrix as has been reported previously for other stationary phases [33].

Polymer-coated C_{18} phases. Capacity factors on the polypyrrole-modified C_{18} columns for benzene, toluene, dimethylphthalate (DMP), diethylphthalate (DEP) and phenol compounds, were similar to those obtained using non-coated C_{18} packings.

There were two possible reasons for this. The polypyrrole coating was porous enough to allow molecules to pass through and to interact with the C_{18} coating and/or the C_{18} surface was not fully covered. Although the polypyrrole coating did not alter the selectivity of the C_{18} stationary phase it did contribute to the retention properties.

Capacity factors obtained for benzene and toluene decreased marginally with both polymer coatings. This indicated that polypyrrole-coated C_{18} was less hydrophobic than the C_{18} packing. Capacity factors for DMP and DEP were slightly increased on PP/CL/C_{18} but remained unchanged on PP/Cl/DS/C_{18}. This indicated that the PP/Cl/C_{18} was less hydrophobic than PP/Cl/DS/C_{18}. This was propably due to the contribution of the non-polar end of the surfactant causing an increase in the hydrophobicity of the stationary phase. Phenol was not retained significantly on any of the coated and uncoated C_{18} phases investigated. Aniline and DMA were retained significantly on all stationary phases. Chromatograms obtained on C_{18} phases revealed that both of these compounds gave tailing responses. Polypyrrole coating of the C_{18} material reduced this tailing but did not eliminate it. However, the presence of the PP/Cl/DS coatings on C_{18} resulted in increased levels of interaction with aniline and DMA. This may have been due to the polar end of the surfactant. A similar effect was reported previously by others who have incorporated surfactants into conducting polymers [34].

Anion-exchange chromatographic characterisation

Benzoic acid was permanently retained on polypyrrole-modified phases unless a salt was induced in the eluent but it was eluted in the void volume on C_{18} and bare silica columns. This indicated that the polypyrrole coating had anion-exchange capabilities as reported previously [27–29].

The effect of pH on retention by ion exchange was investigated. It was found that the capacity factor for benzoic acid increased with decreasing pH of the phosphate buffer (Fig. 4).

The effect of ionic strength on ion exchange was also investigated. The capacity factor of benzoic acid decreased with an increasing ionic strength of the buffer (Fig. 5). The retention of aniline and DMA were not altered significantly (Fig. 5).
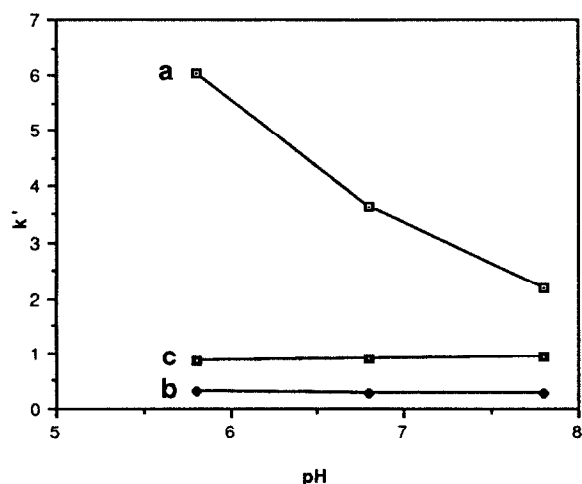
Fig. 4. Capacity factors *vs.* pH of the mobile phase. Column: PP/Cl/silica in a 64 mm × 1.6 mm I.D. column; detector: UV at 254 nm; sample: a = 20 ppm benzoic acid; b = 20 ppm aniline; c = 20 ppm DMA.

Chromatographic performance

Using optimized conditions from Figs. 3, 4 and 5, it was found that the polypyrrole-modified silica column was not efficient enough to separate a group of test compounds. Chromatographic separations were carried out using polypyrrole-modified C_{18} phase (Fig. 6). The polypyrrole-modified C_{18} col-
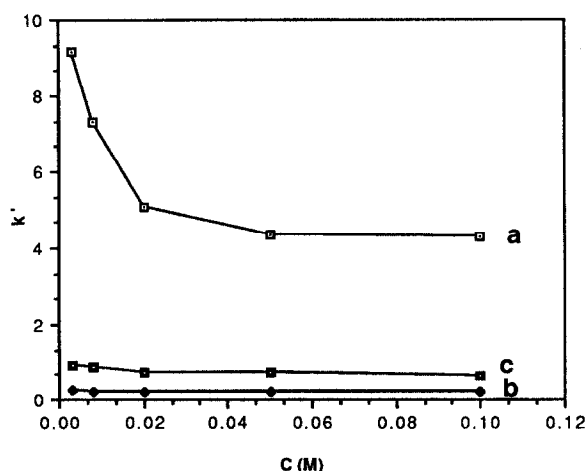

Fig. 6. Separations on a polypyrrole-coated C_{18} stationary phase. Column: PP/Cl/C_{18} in a 64 mm × 3.2 mm I.D. self-compressed column [30]; eluent: methanol–water (65:35) at 0.3 ml/min; detector: UV at 254 nm. Responses due to: (a) 1 = 5 ppm theophylline and 2 = 5 ppm caffeine; (b) 1 = 15 ppm phenol, 2 = 5 ppm aniline, 3 = 5 ppm DMP, 4 = 50 ppm benzene, 5 = 5 ppm DEP, 6 = 50 ppm toluene and 7 = 5 ppm DMA.

Fig. 5. Capacity factors *vs.* ionic strength of the mobile phase. Column: PP/Cl/silica in a 64 mm × 1.6 mm I.D. self-compressed column [30]; detector: UV at 254 nm; sample: a = 20 ppm benzoic acid; b = 20 ppm aniline; c = 20 ppm DMA.
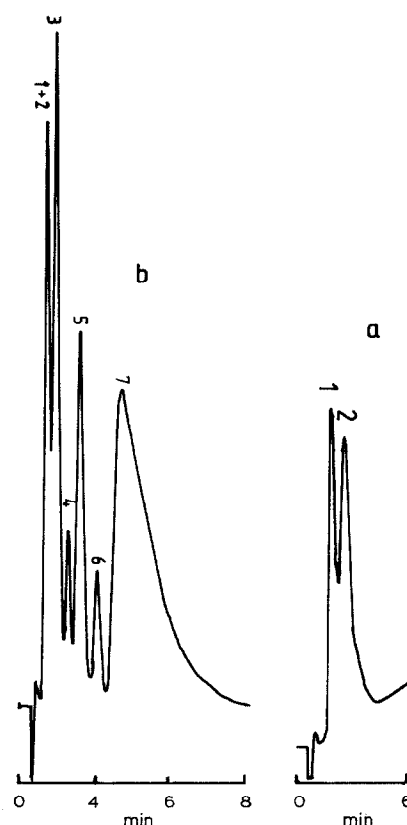
umn displayed different selectivity to the C_{18} column. Retention on C_{18} column was summarized in Table V. For example, although benzoic acid eluted first on the C_{18} column, it did not elute from the PP/Cl/C_{18} column. In addition, phenol and aniline could be separated on the C_{18} column, but not on the PP/Cl/C_{18} column. Furthermore, benzene and DEP as well as aniline and DMP could be separated on the PP/Cl/C_{18} column but not on the C_{18} columns. Basic drugs, caffeine and theophylline, were separated on the PP/Cl/C_{18} column (Fig. 6a) but they were eluted almost in the void volume under the same conditions using a C_{18} column. This verified that the polypyrrole coatings can provide unique selectivities when used in chromatography.

CONCLUSIONS

Using a chemical polymerisation procedure, silica-based packing materials were coated with conducting polymers. Elemental analysis indicated that the polymer loading was low on the C_{18}-based supports but slightly higher on the bare silica packings.

Changes in chromatographic interactions were found when polypyrrole-modified C_{18} columns were examined. Similar behaviour was observed on polypyrrole-modified bare silica. Mixed mode phases capable of anion exchange as well as hydrophobic interactions were obtained.

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