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Chiral selectors from fruit: application of citrus pectins to enantiomer separations in capillary electrophoresis ☆

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Abstract

Pectins were investigated as chiral selective agents in capillary electrophoresis. Successful enantioresolution of antihistaminic and antimalarial compounds, as well as others, was achieved by utilizing potassium polypectate as the chiral selector. Changes in pH, chiral additive concentration and capillary type were studied in relation to chiral resolution. The effect of degree of esterification of pectin materials on chiral recognition was also evaluated. Published by Elsevier Science B.V.

Keywords: Enantiomer separation; Chiral selectors; Pectins

1. Introduction

Oligo- and polysaccharides have tremendous versatility as chiral selectors in capillary electrophoresis (CE). Although cyclodextrins and their derivatives have been the most commonly used carbohydrate-based chiral selectors [1,2], other neutral and ionic polysaccharides exhibiting enantioselectivity in CE include dextran, dextrin, heparin and chondroitin sulfate, as well as others [3]. The diverse structures and functionality of polysaccharides seem apt to yield additional chiral selectors; however, the chiral recognition of many polysaccharides remains unexplored. Given the importance of polysaccharides in biological systems [4], the investigation stereoselective interactions of naturally occurring polysaccharides seems well advised.

Pectins are a family of heterogeneous polysaccharides found in the cell walls of higher plants where they contribute to the firmness and structure of plant tissue [5]. Pectins are comprised primarily of D-galacturonic acid units joined through α-1,4-linkages. A partial structure is shown in Fig. 1. Much of the interest in pectic substances stems from their ability to form gels under appropriate conditions [6]. Intermolecular interactions of pectins are influenced by molecular mass and by degree of esterification of

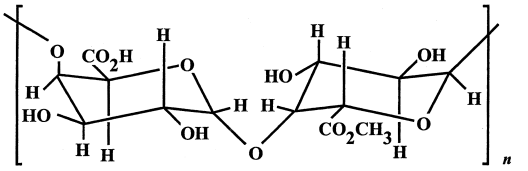

Fig. 1. Partial structure of citrus pectin.

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the galacturonic acid units. The degree of esterification can be modified by either chemical or enzymatic means [7,8]. The interruption of the polygalacturonate backbone by rhamnose units produces kinks in the pectin chain that limit interchain association [9]. Applications of pectins in the food and pharmaceutical industries include use as a gelling agent, fat replacement and drug delivery vehicle [6,10]. The most common commercial sources of pectins are apples and citrus fruits.

Depending on pH, pectins have been shown to form complexes with cationic species such as proteins through electrostatic interactions [11]. Complex formation is believed to involve the carboxylate groups of the pectin and the positively charged amino groups of the protein [12]. Interactions with cations weaken as the number of esterified carboxylate groups on the pectin chain increases [9]. Pectins have also been shown to interact with pharmaceutical compounds. Because pectins are commonly present in fruits and vegetables and as food additives, stereoselective interactions of pectins with chiral drugs might have consequences for drug–food interactions [13,14]. To date, however, the chiral recognition of pectins has not been investigated for cationic drugs. We investigated the chiral recognition of pectins by utilizing these polysaccharides as chiral selectors in CE.

2. Experimental[2]

2.1. Reagents

Polygalacturonic acid sodium salt (sodium polypectate), polygalacturonic acid potassium salt (potassium polypectate) and esterified pectins (all from citrus fruits) were obtained from Sigma (St. Louis, MO, USA). Sodium and potassium polypectates had reported purities of 85–90%. Esterified pectins had degrees of esterification of 22% (DE 22) and 64% (DE 64) according to vendor information. The various racemic analytes were obtained from Aldrich (Milwaukee, WI, USA), Sigma and the United States Pharmacopeia (Rockville, MD, USA). Structures of the analytes are shown in Fig. 2.

2.2. Instrumentation

Experiments were performed on Bio-Rad BioFocus 3000 (Hercules, CA, USA) and Beckman P/ACE 5010 (Fullerton, CA, USA) instruments using a capillary of 50 cm×50 μm I.D. The effective length of the capillary was either 45.4 cm (Bio-Rad) or 43.0 cm (Beckman). The capillary was thermostated at 20°C with a liquid coolant. Separations were performed in the constant voltage mode with an applied voltage of 20 kV. Injections were performed in the hydrodynamic mode. The analytes were detected by measurement of ultraviolet (UV) absorbance at a wavelength of 214 nm. Uncoated capillaries (360 μm O.D.×50 μm I.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Coated capillaries (BioCap XL, 375 μm O.D.×50 μm I.D.) were obtained from Bio-Rad Labs. According to vendor literature, the coated capillaries have a layer of polyacryloylaminoethoxyethanol bonded to the inner surface of the capillary.

2.3. Procedure

Buffers were prepared from concentrated H_3PO_4 or from equimolar portions of sodium phosphate monobasic and dibasic. Sodium or potassium polypectate was added at the desired concentration (w/v), and the solutions were filtered through 0.45-μm nylon filters. Prior to the first injection, uncoated capillaries were purged with 0.1 *M* NaOH for 2 min, water for 3 min, and run buffer for 30 min. Subsequent injections were preceded by a 2-min purge with 0.1 *M* NaOH, and a 3-min rinse with run buffer. Samples were initially dissolved in water at a concentration of 0.5 mg/ml. Samples for injection were prepared by diluting the sample in run buffer or

[2]Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement of the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

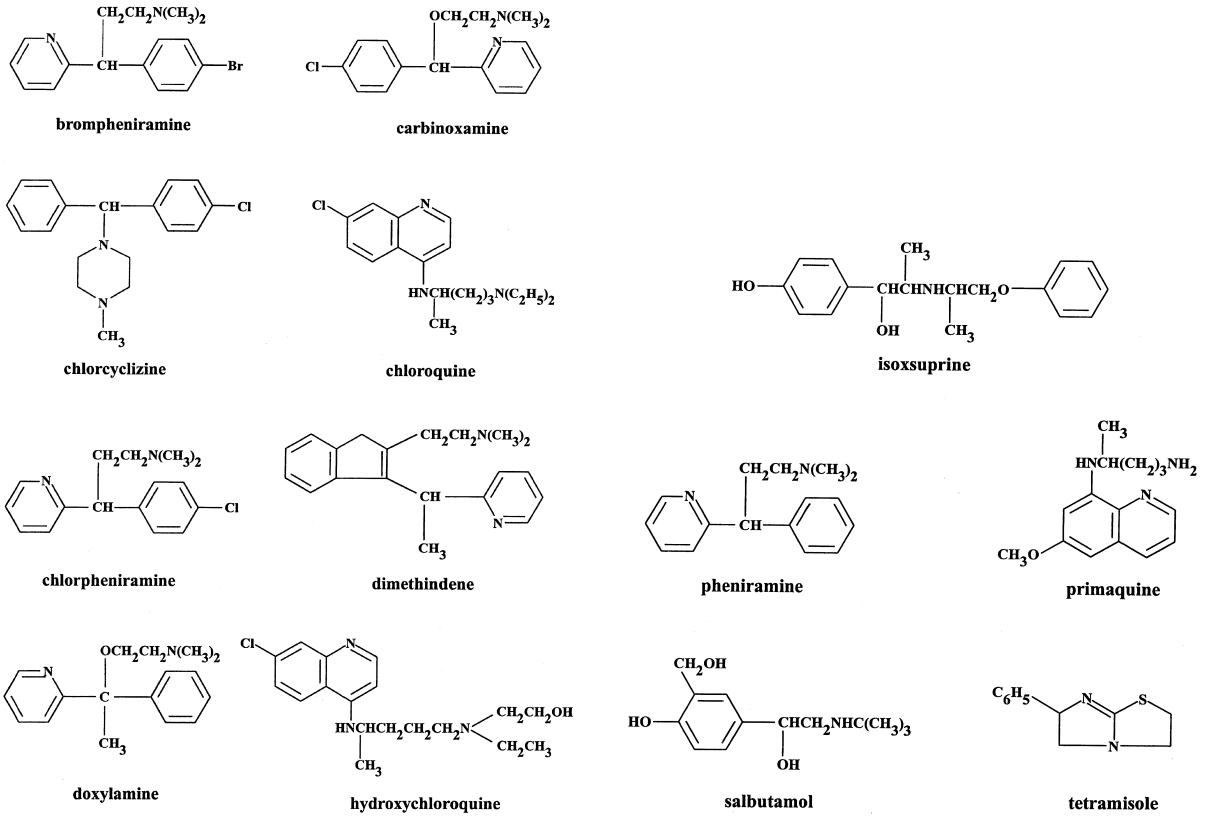

Fig. 2. Structures of chiral compounds studied.

water so that the final concentration was 0.1 mg/ml. All sample injections were performed in duplicate.

3. Results and discussion

Based on studies of pectin–protein interactions, potassium and sodium polypectates were chosen for preliminary study [12]. These non-esterified materials were anticipated to have a high concentration of free carboxyl groups. Electrophoretic experiments were conducted on uncoated fused-silica capillaries. Pectin–protein binding studies have indicated that pectins can bind some of the same proteins as heparin [15]. Therefore, compounds resolved with heparin as a chiral additive in CE were chosen for initial study [16]. Enantioresolution of additional compounds was also explored.

Preliminary investigations focused on the use of sodium polypectate as a chiral additive. However, solutions of this material tended to be turbid and have significant insoluble material. Therefore, potassium polypectate was chosen for further study. Potassium polypectate was readily water soluble up to a concentration of 2.5% (w/v). Running buffer solutions containing potassium polypectate had a slight yellow color, but this color did not present any problems in detection of the analytes. Care was taken to exclude divalent cations such as Ca^{2+} that might promote gelation of the running buffer solutions. Gel formation dramatically changed the viscosity of the run buffer and tended to cause capillary blockage. The pK_a of pectin carboxyl groups can range from 3

to 5, depending on the molecular mass and degree of esterification [17]. Under the experimental conditions used for the majority of separations performed, little deprotonation of the carboxyl groups would be expected, and the polypectate chains should primarily be in the neutral state.

3.1. Electrophoretic results

When potassium polypectate was incorporated as a chiral additive to the run buffer, successful resolution of antihistaminic and antimalarial compounds, as well as others, was achieved. The results are summarized in Table 1. A typical electropherogram is shown in Fig. 3. Migration times of all analytes increased when pectin was added to the run buffer. The addition of the polypectate to the run buffer produced a noticeable increase in viscosity that may be responsible for the delayed migration [18].

The highest resolution was observed for antimalarial compounds such as primaquine. These compounds also had the longest migration times. Each of the antimalarial compounds studied has three potentially protonated nitrogens that could contribute to interaction with the pectin selector. Lower, but significant, enantioselectivity was also observed for several antihistaminic compounds. Higher resolution was obtained for carbinoxamine (R_s=1.8) than for chlorpheniramine (R_s=0.9) under identical experimental conditions, despite longer migration times for chlorpheniramine. At pH 2.0, pheniramine was not resolved, but the structurally similar compound doxylamine was readily resolved (R_s=1.6). The lowest degree of resolution was observed for the broncho- and vasodilating compounds. In general, the degree of enantioselectivity demonstrated with potassium polypectate was lower than that observed with heparin-based selectors for the same analytes [16].

3.2. Separation optimization

Based on the results of protein–pectin interaction studies [11], pH was anticipated to be an important variable in optimizing CE results. The effect of pH on the enantioresolution of several different compounds was studied, and the results are summarized in Fig. 4. In general, a decrease in resolution was observed as pH increased. However, not all compounds were affected to the same extent. Although enantioresolution of chloroquine disappeared at pH 4.0, the resolution of salbutamol remained fairly

Table 1
Summary of electrophoretic data for potassium polypectate chiral selector[a]

	% CS[b]	pH	Migration time (min)	R_s
Antihistamines				
Brompheniramine	2.0	2.0	16.59/16.95	1.1
Carbinoxamine	2.0	2.0	13.96/14.59	1.8
Chlorcyclizine	2.0	2.5	11.59	0.0
Chlorpheniramine	2.0	2.0	16.35/16.66	0.9
Dimethindene	2.0	2.5	13.44/13.70	0.8
Doxylamine	2.0	2.0	11.91/12.10	1.6
Pheniramine	2.0	2.5	11.87/12.07	0.8
Antimalarials				
Chloroquine	2.0	2.0	19.94/20.90	1.9
Hydroxychloroquine	1.5	2.0	16.90/17.62	1.8
Primaquine	2.0	2.0	19.46/20.36	1.6
Broncho- and vasodilators				
Isoxsuprine	1.5	2.5	12.46/12.66	0.6
Salbutamol	2.0	3.0	11.06/11.16	0.7

[a] Separation conditions: uncoated capillary of 50 cm (effective length 45.4 cm)×50 μm I.D.; run buffer, 25 m*M* H_3PO_4 containing potassium polypectate; applied voltage, 20 kV.
[b] % (w/v) chiral selector added to run buffer.

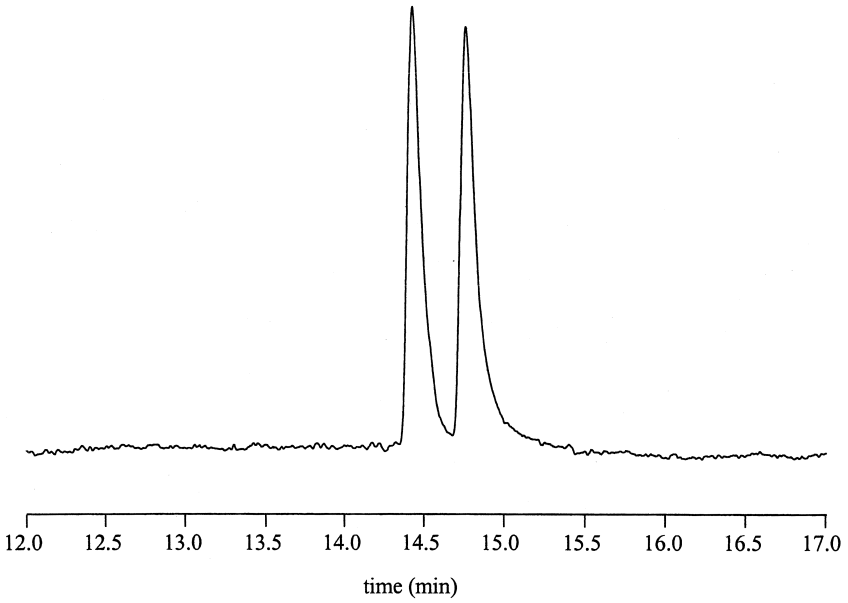

Fig. 3. Separation of primaquine enantiomers. Separation parameters: uncoated capillary of effective length 43.0 cm×50 μm I.D.; run buffer, 25 m*M* H_3PO_4 (pH 2.5) containing 1.5% (w/v) potassium polypectate; applied voltage, 20 kV.

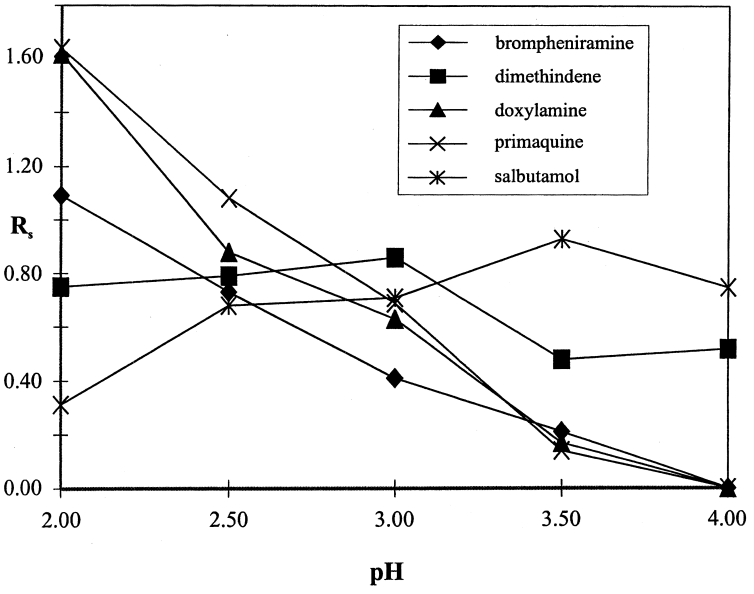

Fig. 4. Effect of pH on resolution of selected compounds. Separation conditions: uncoated capillary of 50 cm (effective length 45.4 cm)×50 μm I.D.; run buffer, 25 m*M* phosphate containing 2.0% (w/v) potassium polypectate; applied voltage, 20 kV.

Table 2
Effect of chiral selector concentration on migration time and resolution[a]

% CS[b]	Carbinoxamine		Chlorpheniramine		Chloroquine	
	t_M[c]	R_s	t_M	R_s	t_M	R_s
0.00	4.28	0.0	4.26	0.0	4.48	0.0
0.50	8.93	0.0	9.89	0.3	9.02	0.5
1.00	11.99	0.6	15.57	0.0	15.38	1.3
1.50	12.05	0.9	15.90	0.5	19.54	1.4
2.00	11.11	0.8	14.32	0.8	16.20	1.2
2.50	9.84	0.4	12.52	0.6	9.75	0.3

[a] Separation conditions: uncoated capillary of 50 cm (effective length 45.4 cm)×50 μm I.D.; run buffer, 25 m*M* H_3PO_4 (pH 2.5) containing potassium polypectate; applied voltage, 20 kV.
[b] Chiral selector concentration (%, w/v).
[c] Migration time in minutes of first enantiomer.

consistent above pH 2.5. A decrease in migration times with increasing pH was observed for all compounds (results not shown). The decrease in migration times can be attributed to increasing electroosmotic flow at higher pH values [18]. Enantioresolution of the target compounds above pH 4.0 was marginal or nonexistent.

The effect of chiral selector concentration on resolution was also investigated. Table 2 summarizes the results. Chiral selector concentrations of 1.5 to 2.0% (w/v) proved to be optimum for most analytes. Chiral selector concentrations greater than 2.5% were not studied because of problems solubilizing the potassium polypectate. Initial increases in chiral selector concentration were accompanied by an increase in migration time (Table 2). An increase in

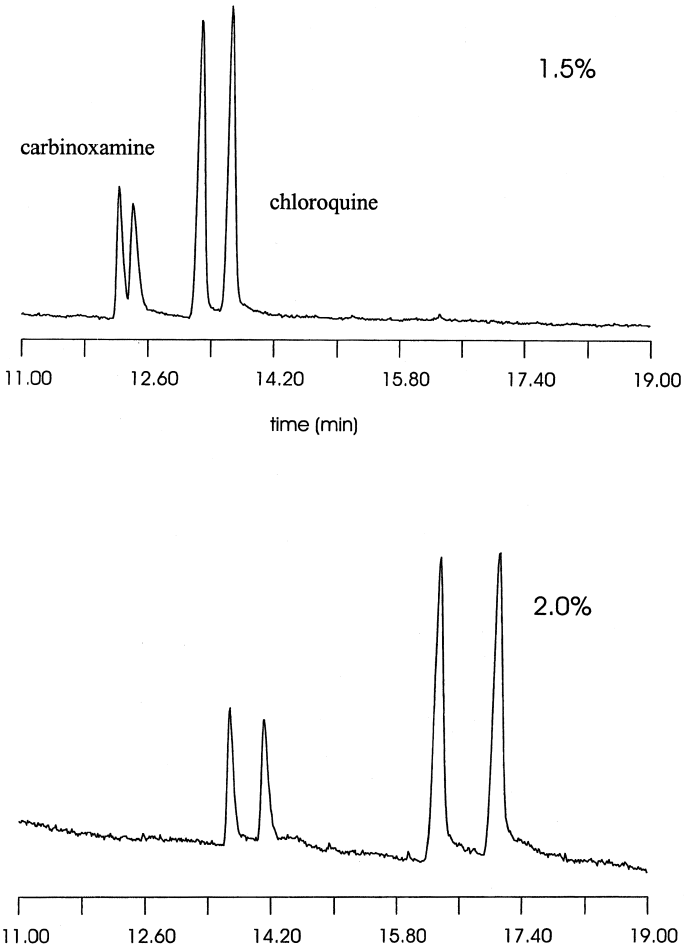

Fig. 5. Enantioseparation of carbinoxamine and chloroquine at different chiral selector concentrations. Parameters: 25 m*M* H_3PO_4 (pH 2.0) containing potassium polypectate.

viscosity of the run buffer is probably the cause of the retarded migration. At pectate concentrations greater than 1.5%, migration times began to decrease. The cause of this decrease is unclear; however, aggregation of the pectin at higher concentrations may alter interactions between the analyte and the chiral selector. Fig. 5 illustrates the dramatic effect of a slight increase in chiral selector concentration for the resolution of carbinoxamine and chloroquine enantiomers. Clearly, both pH and chiral selector concentration should be given consideration for optimizing separations with pectin-based additives.

Some analyte peaks tended to suffer from tailing that might arise from interaction of the cationic analytes with the negatively charged capillary wall [18]. Peak tailing reduced resolution and resulted in separations with low efficiency. A coated capillary was investigated to mitigate this interaction. Fig. 6 shows separations of tetramisole and isoxsuprine on the modified capillary. These compounds had very poor peak shapes on the bare silica capillary. However, other compounds did not exhibit measurable improvement. The coated capillary was not studied at pH below 2.5 because of possible instability of the coating at low pH. However, peak shape improved for most compounds on the uncoated capillary at pH 2.0 as protonation of the surface silanols of the capillary wall occurred and analyte adsorption was minimized.

3.3. Chiral recognition of pectin-based selectors

At low pH, electrostatic interactions would not be expected to play a major role in pectin–analyte interactions because protonation of the pectin chains would occur. Therefore, hydrophobic or hydrogen bonding interactions would be anticipated to dominate selector–selectand associations [6]. Differences in selectivity between carbinoxamine and chlorpheniramine and between pheniramine and doxylamine tend to support the importance of hydrogen bonding in chiral recognition. The oxygen atom adjacent to the chiral center of carbinoxamine and doxylamine appears to contribute to enhanced chiral recognition. Selectivity also seemed to be related in a general way to the number of potential interaction sites on the analyte. The enhanced selectivity observed for the antimalarial compounds might originate from their ability to interact with multiple sites on a single pectin strand or to interaction with multiple pectin chains. Aggregation of pectins in solution has been observed, even at low concentration [19], and low pH ($pH < pK_a$) would promote aggregation of nonesterified pectin by eliminating the repulsive force of

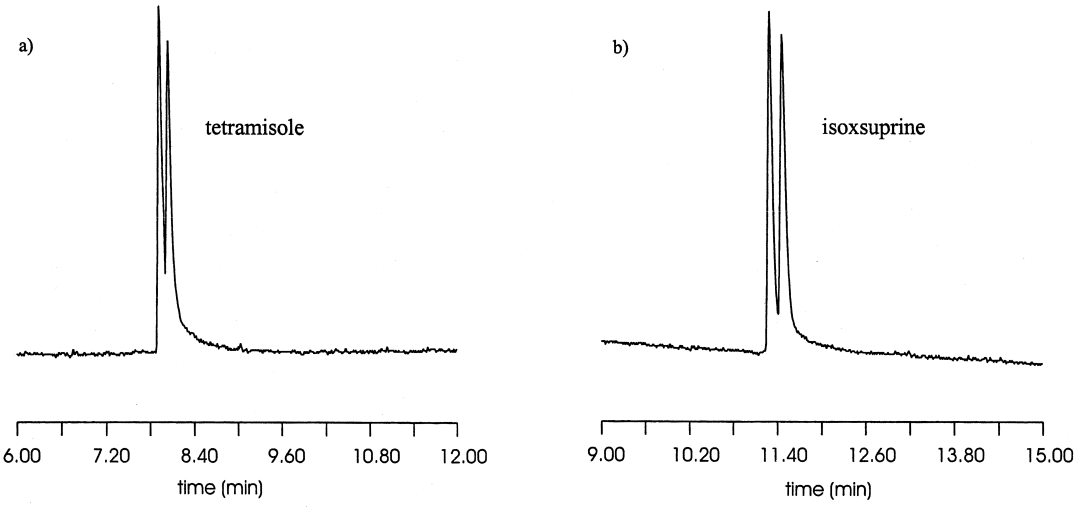

Fig. 6. Separation of tetramisole (a) and isoxsuprine (b) enantiomers on a coated capillary. Separation conditions: coated capillary of 50 cm (effective length 45.4 cm)×50 μm I.D.; run buffer, 25 m*M* H_3PO_4 (pH 4.0) containing 1.5% (w/v) potassium polypectate; applied voltage, 20 kV.

negatively charged carboxylate groups. Aggregation of the polypectate chains might also result in a more ordered polysaccharide system [9] that enhances chiral recognition.

The degree of esterification of the pectin material was also studied in relation to chiral selectivity. Partially esterified pectins (22% and 64%) were utilized as chiral additives to the run buffers and the results were compared to the non-esterified pectin. Although some of the compounds were resolved with the DE 22 material, resolution tended to be lower than that observed with the polypectate additive, and migration times also decreased. A comparison of results is shown in Table 3. The DE 64 material produced a slight resolution of chloroquine enantiomers. Electropherograms comparing the three additives are shown in Fig. 7. Esterification of the pectin chains had a deleterious effect on enantioresolution of all the compounds investigated, and the impact on chiral resolution increased as the degree of esterification of the pectin increased. This further supports the importance of the carboxylic acid moieties of the pectin material in chiral recognition.

Because these polysaccharides were not characterized in terms of molecular mass, the possibility of differences in chiral recognition arising from variations in molecular mass ranges of the three pectin materials should not be overlooked. However, Nishi et al. demonstrated that above a certain molecular mass of dextran, resolution and selectivity were only slightly changed by increases in molecular mass of the polysaccharide [20]. Esterification of the polypectate chains might result in a change in conformation of the chains that induces steric hindrance to pectin–analyte interactions [7]. Methoxylation of the carboxyl groups also alters interchain associations [17] that may participate in the chiral recognition process.

Caution should be used in generalizing the results of this study to other pectins. Pectins have tremendous variability in their composition depending on their source and the method of preparation [6,21].

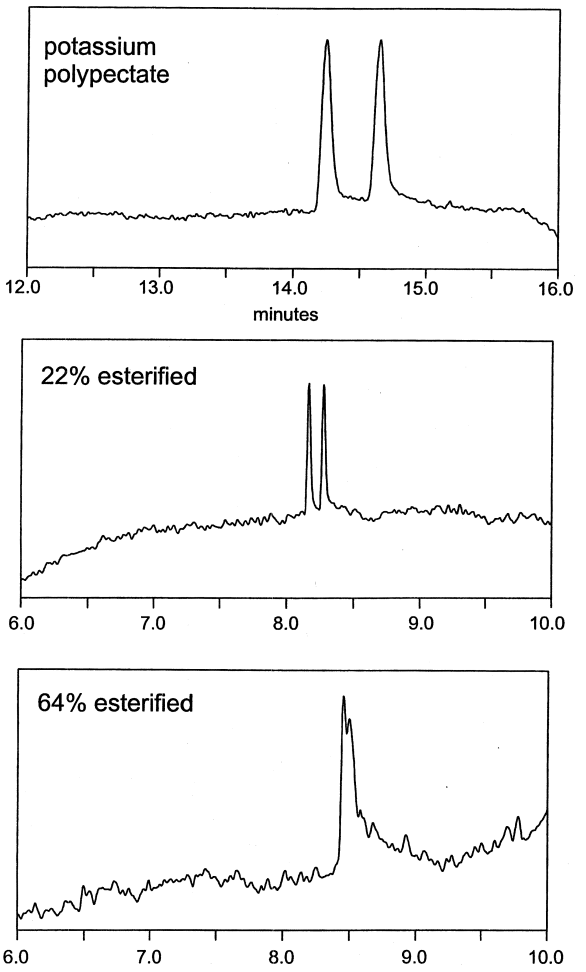

Fig. 7. Effect of degree of esterification on the separation of chloroquine enantiomers. Parameters: 25 m*M* H_3PO_4 (pH 2.5) containing 1.5% (w/v) pectin; applied voltage, 20 kV.

Table 3
Comparison of electrophoretic data for esterified and nonesterified pectins[a]

	Polypectate		DE=22%	
	t_M[b]	R_s	t_M	R_s
Brompheniramine	13.38/13.51	0.7	8.36	0.0
Chloroquine	14.24/14.65	1.6	8.17/8.28	0.7
Chlorpheniramine	13.28/13.40	0.7	8.18	0.0
Dimethindene	14.19/14.36	0.7	8.02	0.0
Doxylamine	11.27/11.43	0.8	7.36/7.40	0.3
Isoxsuprine	11.43/11.54	0.7	8.02	0.0
Pheniramine	13.22/13.40	0.8	8.08	0.0
Primaquine	13.01/13.38	1.5	8.30/8.42	0.9

[a] Separation conditions: uncoated capillary of 50 cm (effective length 45.4 cm)×50 μm I.D.; run buffer, 25 m*M* H_3PO_4 (pH 2.5); applied voltage, 20 kV.

[b] Migration time in minutes.

Therefore, other types of pectin might exhibit enhanced or reduced chiral recognition when compared to the material used in this study.

4. Conclusions

Pectin materials derived from citrus fruits proved to be effective chiral additives for the separation of enantiomers of basic compounds using CE. Both chiral selector concentration and pH were critical parameters in optimizing the separations. Investigations of structurally related compounds and of partially esterified pectins provided insight into possible chiral recognition mechanisms. However, the heterogeneity of pectin-based selectors adds complexity to elucidation of the chiral recognition process associated with these polysaccharides.

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