Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

High throughput screening and quantitative structure efficacy relationship models for designing displacers for antisense oligonucleotide purification in anion-exchange systems

Nihal Tugcu[a]; C. B. Mazza[a]; C. M. Breneman[b]; Yogesh S. Sanghvi[c]; S. M. Cramer[a]
[a] Chemical Engineering Department, Rensselaer Polytechnic Institute, Troy, NY, U.S.A. [b] Chemistry Department, Rensselaer Polytechnic Institute, Troy, NY, U.S.A. [c] Manufacturing Process Department, 2292 Faraday Avenue, ISIS Pharmaceuticals, Inc., Carlsbad, CA, U.S.A.

Online publication date: 29 May 2002

To cite this Article Tugcu, Nihal , Mazza, C. B. , Breneman, C. M. , Sanghvi, Yogesh S. and Cramer, S. M.(2002) 'High throughput screening and quantitative structure efficacy relationship models for designing displacers for antisense oligonucleotide purification in anion-exchange systems', Separation Science and Technology, 37: 7, 1667 — 1681
To link to this Article: DOI: 10.1081/SS-120002743
URL: http://dx.doi.org/10.1081/SS-120002743

SEPARATION SCIENCE AND TECHNOLOGY, 37(7), 1667–1681 (2002)

HIGH THROUGHPUT SCREENING AND QUANTITATIVE STRUCTURE EFFICACY RELATIONSHIP MODELS FOR DESIGNING DISPLACERS FOR ANTISENSE OLIGONUCLEOTIDE PURIFICATION IN ANION-EXCHANGE SYSTEMS

Nihal Tugcu,[1] C. B. Mazza,[1] C. M. Breneman,[2] Yogesh S. Sanghvi,[3] and S. M. Cramer[1,*]

[1]Chemical Engineering and
[2]Chemistry Department, Rensselaer Polytechnic Institute, Troy, NY 12180
[3]ISIS Pharmaceuticals, Inc., Manufacturing Process Department, 2292 Faraday Avenue, Carlsbad, CA 92008

ABSTRACT

A high throughput screening (HTS) technique was developed for the rapid evaluation of displacers for the purification of antisense oligonucleotides using anion-exchange systems. By employing this technique, a large number of potential displacers with a variety of structural properties were evaluated in parallel, dramatically decreasing the time required for displacer discovery. A database was generated containing molecular descriptors of the screened displacer probes as well as their displacement data

*Corresponding author. E-mail: crames@rpi.edu

obtained from the HTS experiments. A subset of the probe data was used to derive a predictive quantitative structure efficacy relationship (QSER) model using a partial least squares (PLS) approach. The resulting model was well correlated with $r^2 = 0.915$. In addition, the model was shown to predict the molecules not included in the model successfully.

The results presented in this paper demonstrate the utility of the HTS technique for the rapid evaluation of displacers for the purification of antisense oligonucleotides. These results applied in concert with the QSER modeling, can aid in the a priori design of high-affinity displacers for anion-exchange systems.

INTRODUCTION

Significant advances in antisense therapeutics over the last decade have generated substantial interest in the use of antisense oligonucleotides as drug candidates. There are currently over a dozen antisense oligonucleotide drug candidates in clinical trials (1–3). The rapid development of oligonucleotide therapeutics and the projection that demands for oligonucleotides could reach the level of metric tons per year, poses a significant challenge to separation scientists to develop cost effective processes that can deliver high purity and yields (3–5). Several separation methods are currently in use for the purification of oligonucleotides at both the analytical and process scale. These are reversed phase chromatography (6–12), ion-pair reversed phase chromatography (13,14), and anion-exchange chromatography (3,15–17). Separations by ion exchange and reversed phase chromatography have been generally carried out in the gradient mode. Recently, it has been shown that the displacement mode of chromatography can also be employed successfully for the purification of oligonucleotides resulting in high purity and high yields. Sample displacement and displacement using high and low molecular weight displacers were shown to be effective techniques for process scale purifications (3,18–20).

While column based methods development protocols have been developed for the identification of low molecular weight displacers in ion-exchange systems, this technique can be labor intensive. Accordingly, there is an urgent need to develop a more rigid method for the identification of high affinity low molecular weight displacers.

High throughput screening (HTS) has been used widely in drug discovery to identify novel lead compounds from diverse as well as narrow directed libraries (21). Recently, an effective technique for the HTS of potential cation-exchange low molecular weight displacers has been developed (22). In contrast to conventional

column techniques, this HTS technique enables the rapid evaluation of potential low molecular weight displacers using parallel displacement experiments.

Quantitative structure retention relationships (QSRR) models have been reported for small molecules for various modes of chromatography (23–25). Predictive QSRR models based on the partial least squares (PLS) approach have been demonstrated for a relatively large data set in ion-exchange systems (26). Partial least squares with latent variables (LV) has the advantage of deriving predictive models in cases where the number of data points is limited and the number of variables is large. Recently, predictive quantitative structure efficacy relationship (QSER) models were developed successfully for the evaluation of potential low molecular weight displacers using batch cation-exchange experimental data (21). In the present work, the HTS technique is employed to identify potential low molecular weight anion-exchange displacers for the purification of antisense oligonucleotides. Further, a QSER model based on the PLS routine is employed to predict displacer efficacy of molecules not included in the derivation of the model.

EXPERIMENTAL

Materials

Bulk strong anion-exchange, Source 30Q (30 μm) was donated by Amersham Pharmacia Biotech (Uppsala, Sweden). For displacement experiments, Source 30Q stationary phase was slurry packed in 100 × 5 mm I.D. column. A strong anion-exchange, Poros HQ/H (10 μm), prepacked 100 × 4.6 mm I.D. column, was obtained from Perseptive Biosystems (Framingham, MA).

Sodium hydroxide and sodium chloride were purchased from Fischer Scientific (Pittsburgh, PA). Cyclamic acid, 1,2-naphtaquinone-4-sulfonic acid, 1,8-naphthosultam, saccharin (sodium salt, hydrate), sulfanilamide, pantothenic acid, hydroflumethiazide, 2,3-dihydro-3,3-dimethyl-1,2-benzisothiazole-1,1 dioxide, 1,3,6-naphthalenetrisulfonic acid, sunset yellow FCF, tartrazine, orange G, new coccine, acid yellow 34, allura red AC, rhodamine B, fast green FCF, sulfonazo III, amaranth, methyl red, orange II, benzoic acid, 1,5-naphthalenedisulfonic acid, *p*-toluene sulfonic acid, benzenesulfonic acid, 1,2-benzenedisulfonic acid, brilliant black BN, brilliant blue Coomassie R-250, 3-hydroxy-4-(2-hydroxy-4-sulfo-1-naphthylazo)-2-naphtalenecarboxylic acid, 2-(4-sulfophenylazo)-1,8-dihydroxy-3,6-naphtalenedisulfonic acid, chromotrope 2R, pyrenetetrasulfonate, 8-hydroxy-1,3,6-pyrenetrisulfonic acid, caffeine, sodium sulfite, phenyl phosphate, and calmagite were purchased from Aldrich (Milwaukee, WI). Suramin (hexasodium salt) was obtained from Alexis Corporation (San Diego, CA) and sucrose octasulfate was purchased from

Toronto Research Chemicals, Incorporation (Ontario, Canada). Phosphorothioate oligonucleotides were synthesized at ISIS Pharmaceuticals (Carlsbad, CA).

Equipment

Oligonucleotide analysis was carried out using a Waters 600 multisolvent delivery system, a Waters 712 WISP autoinjector, and a Waters 484 UV–VIS absorbance detector controlled by a Millenium chromatography software manager (Waters, Milford, MA). Displacement experiments were carried out using a Waters 590 HPLC pump (Waters, Milford, MA) connected to a chromatography column via a Model C10W port valve (Valco, Houston, TX). Fractions of the column effluent were collected using a LKB 2212 Helirac fraction collector (LKB Bromma, Sweden).

The modeling work was carried using a Micron 300 MHz and 64 Mb RAM computer.

Procedure

High Throughput Screening

The bulk stationary phase (Source 30Q; 1.8 mL) was first washed with deionized water and then the mobile phase buffer, 20 m*M* NaOH + 500 m*M* NaCl and allowed to equilibrate for 2 hr. After gravity settling of the stationary phase, the supernatant was removed and 21 mL of 15 mg/mL oligonucleotide in the mobile phase buffer was then equilibrated in a shaker for 12 hr at 23°C. The supernatant was then analyzed by high temperature (70°C) anion-exchange chromatography to determine the oligonucleotide concentration and the amount adsorbed on the stationary phase was calculated through a mass balance. The supernatant was then removed and 25 μL aliquots of the stationary phase with adsorbed oligonucleotide were added to separate vials. Aliquots (300 μL) of 10 m*M* solutions of each displacer in the mobile phase buffer were then added to each vial, and was allowed to equilibrate for 6 hr. After equilibriation was complete, the stationary phase was allowed to gravity-settle and the supernatants were removed and analyzed to determine the percentage of oligonucleotide displaced by each displacer. These experiments were carried out in duplicate.

Displacement

For column displacement experiments, the column was initially equilibrated with the mobile phase solution 20 m*M* NaOH + 500 m*M* NaCl and

then sequentially perfused with feed, displacer, and regenerant solutions. The oligonucleotides (60 mg) were purified using 5 m*M* amaranth as the displacer. Appropriate fractions (200 μL) of column effluent were collected during the displacement experiments for subsequent analysis of oligonucleotides and displacer. The displacement experiment was carried out at a flow rate of 0.2 mL/min and the effluent was monitored at 254 nm.

Displacement Fraction and High Throughput Screening Supernatant Analysis

Experiments with 45 min linear gradients were carried out using high temperature (70°C) anion-exchange chromatography (Poros HQ/H) (Buffer A: 20 m*M* NaOH, Buffer B: 20 m*M* NaOH + 2.5 *M* NaCl). The column effluent was monitored at 254 nm and the flow rate was 1 mL/min.

THEORY

Modeling

The molecular structures of the displacers were energy minimized and their 2D, 3D, and transfer atomic equivalent descriptors (27) were calculated. The descriptors were employed in concert with the experimental HTS data to generate a QSER model based on the PLS approach (GAPLS, MIT module, MIT, MA) with latent variables to aid in the prediction of displacer efficacy. This model is generated with a training set and the predictive power is analyzed using a set of molecules not used in the generation of the model.

Partial Least Squares

The Genetic Algorithm/Partial Least Squares (GAPLS) application used in this work examines the molecular descriptors of each molecule in a fraction of the data set called the training set and reduces the number of descriptors by using the genetic algorithm approach with a "leave one out" technique (GAPLS, MIT module, MIT, MA). The application generates a QSER model based on the PLS approach with the descriptors that render the highest correlation coefficients for the model. Briefly, the PLS with latent variables (28,29) can be expressed as:

$$y = a_1\mathrm{LV}_1 + a_2\mathrm{LV}_2 + \cdots + a_m\mathrm{LV}_m, \quad (1)$$

where y is the dependent variable (i.e., percent oligonucleotide displaced), LV_i the ith latent variable and a_i the ith regression coefficient corresponding to LV_i. Each latent variable, LV_i, can be expressed as a linear combination of the independent variables x_i:

$$LV_i = b_1x_1 + b_2x_2 + b_3x_3 + \cdots + b_nx_n, \quad (2)$$

where x_i is the ith independent molecular descriptor and $b_1, b_2, \ldots, b_n$ are the descriptors coefficients. The first latent variable accounts for most of the variance, while consecutive latent variables account for relatively smaller amounts of variance. In addition, the latent variables in a model are orthogonal to each other.

Descriptors

In this study 2D, 3D, and transferable atom equivalent (TAE) descriptors were employed (26). The 2D and 3D (i.e., connectivity descriptors, volume, shape, radius, octanol/water partition coefficient, etc.) descriptors are useful descriptors as they generally permit an immediate interpretation of interactions occurring in the systems being examined. On the other hand, molecular electron density-derived descriptors can provide high-quality models for many systems, but require additional interpretation (30). A variety of molecular descriptors can be derived from electron density distributions obtained from ab initio calculations (30). However, these calculations can be lengthy due to the large amount of integrals involved. Breneman and co-workers have developed the TAE method (26) derived from the theory of atoms in molecules pioneered by Bader (31), which can evaluate accurately the electron density of a molecule and generate descriptors with a substantial reduction in computational time (30). The descriptors generated by the TAE method have been employed previously for QSRR in reversed-phase chromatography and ion-exchange chromatography (26,32). In addition, the TAE descriptors have been used in the generation of QSER models for data obtained from the HTS technique for rapid evaluation of displacers in cation-exchange systems (22).

RESULTS AND DISCUSSION

The HTS technique was employed for the rapid evaluation of potential displacers for the purification of phosphorothioate antisense oligonucleotides. In addition, a predictive quantitative structure-based model was developed to aid in the identification of high-affinity low molecular displacers for these anion-exchange systems.

Structures of representative molecules in the data set are shown in Fig. 1. The potential displacer molecules screened in this work were selected in order to cover a set of chemically diverse structures. While doing so, we also included molecules that previously had shown sufficient efficacy for oligonucleotide displacement (19).

The molecules in the data set contain various combinations of benzene rings, azo linkage moieties, sulfonates, sulfates, and sulfonamides. In addition, several families of related compounds were incorporated in the data set. For

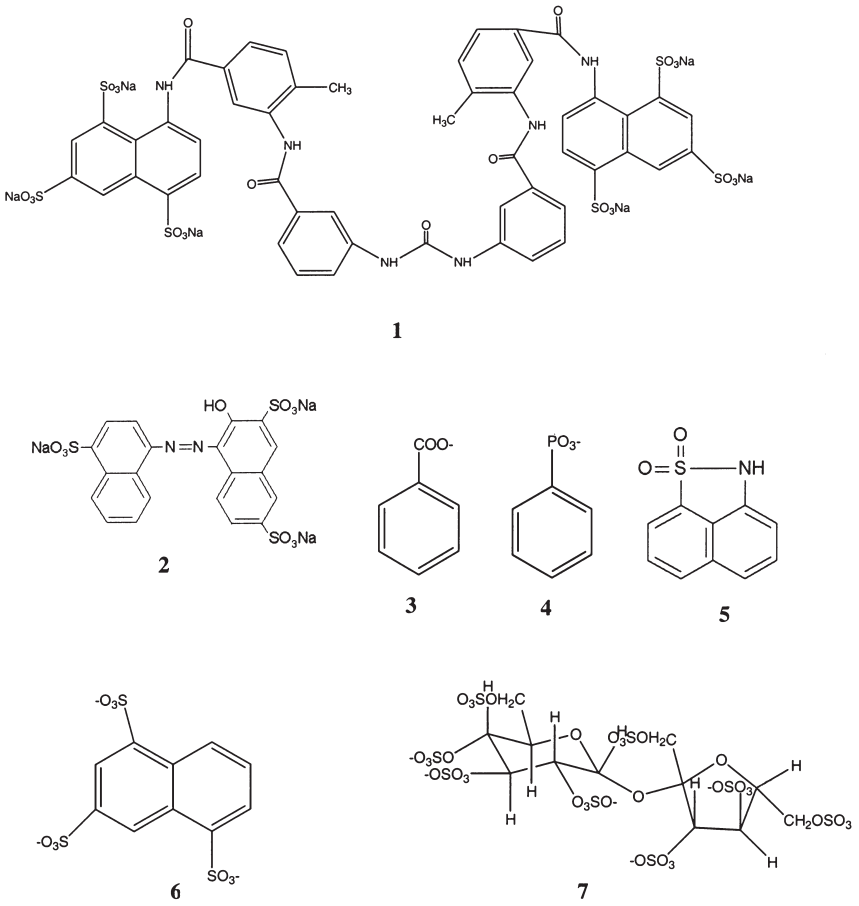

Figure 1. Representative probes employed in this work; suramin (1), amaranth (2), benzoic acid (3), phenyl phosphate (4), 1,8 naphthosultam (5), 1,3,6 naphthalenetrisulfonic acid (6), and sucrose octasulfate (7).

Table 1. Displacers That Were Evaluated Using High Throughput Screening and the Average Percent Displaced

	Percent Oligos Displaced		
Displacer	Average	Vial #1	Vial #2
Buffer control	1.9	2.1	1.7
Sulfonilamide	18.9	18.7	19.1
Panthothenic acid	19.5	18.6	20.4
Cyclamic acid	21.0	21.2	20.8
1,2-Naphtoquinone-4-sulfonic acid	22.9	22.1	23.8
Hydroflumethiazide	23.0	23.1	22.8
p-Toluene sulfonic acid	25.2	25.1	25.2
Methyl red	26.8	26.1	27.5
2,3-Dihydro-3,3-dimethyl-1,2-benzisothiazole-1,1 dioxide	27.0	27.7	26.2
1,5-Naphthalene disulfonic acid	28.4	28.1	28.7
Phenyl phosphate	29.6	31.7	27.5
Rhodamine B	29.6	28.4	30.9
Fast green FCF	29.9	30.5	29.2
1,8-Naphtasultam	30.7	30.0	31.5
1,2-Benzenedisulfonic acid	31.0	30.4	31.6
Sodium sulfite	32.4	32.3	32.5
Benzenesulfonic acid	33.1	33.3	32.9
Caffeine	33.3	34.3	32.4

3-Hydroxy-4-(2-hydroxy-4-sulfo-1-naphtylazo)-2-naphtalene carboxylic acid	33.3	33.2	33.5
Benzoic acid	33.6	34.4	32.8
Tartrazine	34.7	33.9	35.6
Saccharin	35.9	36.0	35.9
1,3,6-Naphthalenetrisulfonic acid	36.0	36.2	35.8
Acid yellow 34	37.4	38.3	36.4
Sucrose octasulfate	37.5	37.1	38.0
Orange II	40.2	39.9	40.5
Allura red AC	43.3	42.1	44.6
Chromotrope 2R	44.7	44.2	45.2
Orange G	45.4	46.5	44.3
Sunset yellow	46.2	46.2	46.2
2-(4-Sulfophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid	47.8	46.4	49.3
Brilliant blue Coomassie R-250	52.8	51.6	54.1
Suramine	54.0	54.8	53.3
Amaranth	54.1	55.1	53.0
Calmagite	55.9	55.2	56.6
New coccine	57.2	58.0	56.4
Sulfonazo III	57.6	54.6	60.6
Pyrenetetrasulfonate	61.8	59.9	63.7
Brillaint black BN	68.1	67.9	68.4
8-Hydroxy-1,3,6-pyrenetrisulfonic acid	73.2	71.5	74.8

example, molecules that belong to the sulfonamide family included 1,2-naphthaquinone-4-sulfonic acid, 1,8-naphthosultam, sulfanilamide, hydroflumethiazide, 2,3-dihydro-3,3-dimethyl-1,2-benzisothiazole-1,1 dioxide, and saccharin. In addition, several compounds related to amaranth were examined (i.e., sunset yellow, fast green FCF, sulfonazo III, etc.).

A total of 39 molecules were employed in this study using the HTS experimental technique (described in the "Experimental" section). The data from duplicate experiments is shown in Table 1 with an increasing order of total oligonucleotides (%) displaced by a given displacer.

The percent oligonucleotides displaced for these potential displacer molecules were in the range of 19–73%. The results indicate (Table 1) that there is a correlation between the number of aromatic rings in the structure of the displacer and the value of percent oligonucleotide displaced. For example, the highest percent displaced values were obtained for pyrene tetrasulfonate and 8-hydroxy-1,3,6-pyrenetrisulfonic acid with four aromatic rings in their structure. Whereas, *p*-toluene sulfonic acid with a single aromatic ring exhibited lower displacement capacity. In addition, several dyes that are structurally similar such as sulfanozo III, new coccine, sunset yellow, orange G, brilliant black BN, calmagite, cooamassie blue, and amaranth performed well in the HTS analysis. All of these molecules had more than one aromatic ring and more than two negative charges. Molecules with lower percent displaced values had less charges and/or less number of aromatic rings. In addition, dyes that also contain a positive charge (e.g., Fast green FCF and Rhodamine B) did not perform as well as the other dyes with multiple aromatic rings. In general, molecules that belong to the sulfonamide family, 1,2-naphthoquinone-4-sulfonic acid, 1,8-naphthosultam, sulfanilamide, hydroflumethiazide, and 2,3-dihydro-3,3-dimethyl-1,2-benzisothiazole-1,1 dioxide had percent displaced values in the range of 19–31%. In this family, saccharin performed the best with a 36% oligonucleotide displaced value.

As described in the "Theory" section, the GAPLS with latent variables approach was used to generate a QSER model for the antisense oligonucleotide. The QSER model was generated with a fraction of the data set called the training set and the model results are presented in Fig. 2. As seen in the figure, the experimental and estimated percent oligonucleotides displaced for the training set are very similar; the model was well correlated with a correlation coefficient of 0.915. Further, the training set consists of different classes of molecules indicating that the GAPLS approach is a powerful technique to create a well-correlated quantitative structure based models using molecules having diverse structures.

The predictive power of the QSER model was then evaluated employing molecules not included in the training set. These test set results are shown in Table 2. The predicted and experimentally observed percent oligonucleotide

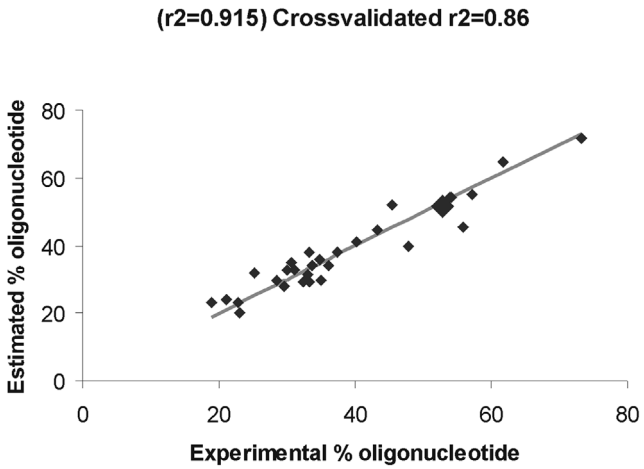

Figure 2. Plot of experimental vs. estimated % oligonucleotide displaced values by the QSER model in the training set.

displaced values are quite similar, with an average difference between experimental and predicted values of 5% oligonucleotide displaced (Table 2). These results indicate that once the model is created, it is then possible to predict the efficacy of untested molecules of potential anion-exchange displacer compounds in a very short time without having to perform initial experiments.

As seen in Fig. 2, amaranth has a relatively high value of percent oligonucleotide displaced. Accordingly, a displacement experiment was carried out using amaranth as the displacer on a Source 30Q column.

Table 2. Comparison of Quantitative Structure Efficacy Relationship Predictions and the Experimentally Observed Values for the Molecules in the Test Set

Displacers	Observed	Predicted
Sulfonazo III	58	45
Sunset yellow	46	43
Brillant black	68	63
Chromotrope 2R	45	48
Methyl red	27	22
2,3-Dihydro-3,3-dimethyl, 1,2-benzisothiazole-1,1-dioxide	27	26

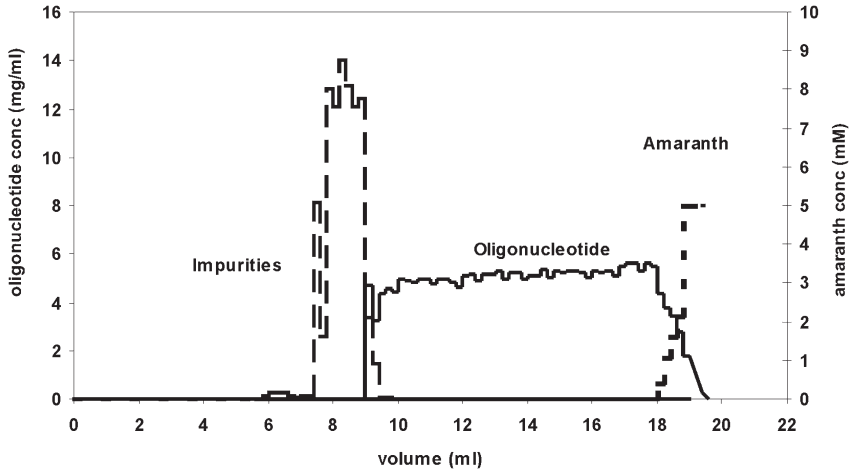

Figure 3. Displacement separation of 60 mg of oligonucleotides using amaranth as a displacer. Column: 100 × 4.6 mm I.D. Source 30Q; Mobile phase: 20 *mM* NaOH + 500 *mM* NaCl; Displacer: 5 *mM* amaranth; Flow rate: 0.2 mL/min.

As seen in Fig. 3, displacement of 60 mg of oligonucleotides was carried out successfully using amaranth as the displacer. The displacement separation resulted in a purity of ~99% full-length product and yield of 87% as determined by anion-exchange analysis. These results are similar to previous results using other stationary phase materials (19,20). If necessary, the low molecular weight displacers (e.g., amaranth) can be separated readily from the oligonucleotide using size-based purification methods (33).

CONCLUSIONS

A HTS technique was employed successfully for the rapid evaluation of a large number of structurally diverse low molecular weight anion-exchange displacers for the purification of antisense oligonucleotides. By using the HTS technique, it was possible to rapidly screen several molecules for their efficacy and to identify new displacer lead compounds. In general, the probes containing more aromatic rings as well as charges showed the highest affinity.

A predictive QSER model of displacer efficacy was derived by using the PLS approach. The power of this QSER model was demonstrated successfully using test molecules not included in the derivation of the QSER model.

We are in the process of evaluating the displacers with the highest percent oligonucleotide displaced values such as pyrene tetrasulfonate and 8-hydroxy-1,3,6-pyrenetrisulfonic acid for their column performance. An important aspect of these studies will involve the ability to remove these displacers from the stationary phase material subsequently. For this purpose, the HTS technique will be adapted to screen for various regeneration methods for these displacers prior to conducting the actual column experiments.

The success of this work now enables the a priori design of a variety of potential displacers for oligonucleotide purification.

ACKNOWLEDGMENTS

The authors acknowledge Prof. Jim Moore (Department of Chemistry, Rensselaer Polytechnic Institute) for his valuable discussions related to this work. The authors acknowledge NIH Grant GM 47372-04A2 and NSF Grant BES-0079436 for funding this research. This work was also partly funded by Isis Pharmaceuticals and Amersham Pharmacia Biotech.

REFERENCES

1. Bennet, C.F.; Cowsert, L.M. Antisense Oligonucleotides as a Tool for Gene Functionalization and Target Validation. Biochim. Biophys. Acta **1999**, *1489*, 19–30.
2. Deshmukh, R.R.; Cole, D.L.; Sanghvi, Y.S. Purification of Antisense Oligonucleotides. Methods Enzymol. **1999**, *313*, 203–226.
3. Deshmukh, R.R.; Leitch, W.E., II; Cole, D.L. Application of Sample Displacement Techniques to the Purification of Synthetic Oligonucleotides and Nucleic Acid: A Mini-Review with Experimental Results. J. Chromatogr. A **1998**, *806*, 77–92.
4. Brown, K.D. Process Specialists See Promise in Oligonucleotide Therapeutics. Gen. Eng. News **1998**, *18*, 1.
5. Sanhgvi, Y.S.; Andrade, M.; Deshmukh, R.R.; Holmberg, L.; Scozzari, A.N.; Cole, D.L. Chemical Synthesis and Purification of Phosphorothioate Antisense Oligonucleotides. In *Manual of Antisense Methodology*; Hartman, G., Endres, S., Eds.; Kluwer Academic Publishers: Norwell, **1999**, 3–23.
6. Hummel, M.; Herbst, H.; Stein, H. Reversed-Phase High-Performance Liquid Chromatography of Very Long Oligodeoxyribonucleotides. J. Chromatogr. **1989**, *477*, 420–426.

7. Hill, T.L.; Mayhew, J.W. Convenient Purification of Tritylated and Detritylated Oligonucleotides up to 100-mer. J. Chromatogr. **1990**, *512*, 415–431.
8. Arghavani, M.B.; Romano, L.J. A Method for the Purification of Oligonucleotides Containing Strong Intra- or Intermolecular Interactions by Reversed-Phase High-Performance Liquid Chromatography. Anal. Biochem. **1995**, *231*, 201–209.
9. Ikuta, S.; Chattopadhyaya, R.; Dickerson, R.E. Reversed-Phase Polystyrene Column for the Purification and Analysis of DNA Oligomers. Anal. Chem. **1984**, *58*, 2256–2257.
10. Maa, Y.; Horvath, C. Rapid Analysis of Proteins and Peptides by Reversed-Phase Chromatography with Polymeric Micropellicular Sorbents. J. Chromatogr. **1988**, *445*, 71–86.
11. Huber, C.G.; Oefner, P.J.; Bonn, G.K. High-Resolution Liquid Chromatography of Oligonucleotides on Nonporous Alkylated Styrene–Divinylbenzene Copolymers. Anal. Biochem. **1993**, *212*, 351–358.
12. Germann, M.W.; Pon, R.T.; Van De Sande, J.H. A General Method for the Purification of Synthetic Oligodeoxyribonucleotides Containing Strong Secondary Structure by Reversed-Phase High-Performance Liquid Chromatography on PRP-1 Resin. Anal. Biochem. **1987**, *165*, 399–405.
13. Jost, W.; Unger, K.; Schill, G. Reversed-Phase Ion-Pair Chromatography of Polyvalent Ions Using Oligonucleotides as Model Substances. Anal. Chem. **1982**, *119*, 214–223.
14. Allinquant, B.; Musenger, C.; Schuller, E. Reversed-Phase High-Performance Liquid Chromatography of Nucleotides and Oligonucleotides. J. Chromatogr. **1985**, *326*, 281–291.
15. Pearson, J.D.; Regnier, F.E. High-Performance Anion-Exchange Chromatography of Oliogonucletides. J. Chromatogr. **1983**, *255*, 137–149.
16. Drager, R.R.; Regnier, F.E. High-Performance Anion-Exchange Chromatography of Oligonculeotides. Anal. Biochem. **1985**, *145*, 47–56.
17. Cubellis, M.V.; Marino, G.; Mayol, L.; Piccialli, G.; Sannia, G. Use of Fast Protein Liquid Chromatography for the Purification of Synthetic Oligonucleotides. J. Chromatogr. **1985**, *329*, 406–414.
18. Gerstner, J.A.; Pedroso, P.; Morris, J.; Bergot, B.J. Gram-Scale Purification of Phosphorothioate Oligonucleotides Using Ion-Exchange Displacement Chromatography. Nucleic Acids Res. **1995**, *23*, 2292–2299.
19. Shukla, A.A.; Deshmukh, R.R.; Moore, J.A.; Cramer, S.M. Purification of Oligonucleotides by High Affinity, Low Molecular Weight Displacers. Biotechnol. Prog. **2000**, *16*, 1064–1071.
20. Tugcu, N.; Deshmukh, R.R.; Sanhgvi, Y.S.; Moore, J.A.; Cramer, S.M. Purification of Oligonucleotides at High Column Loading by High Affinity, Low Molecular Weight Displacers. J. Chromatogr. A **2001**, *923*, 65–73.

21. Gallop, M.A.; Barret, R.W.; Dower, W.J.; Fodor, S.P.A.; Gordon, M. Applications of Combinatorial Technologies to Drug Discovery. 1) Background and Peptide Combinatorial Libraries. J. Med. Chem. **1994**, *37*, 1233–1251.
22. Mazza, C.B.; Rege, K.; Breneman, C.M.; Dordick, J.S.; Cramer, S.M. High Throughput Screening and Quantitative Structure Efficacy Relationships of Displacer Molecules for Ion Exchange Systems. Submitted for publication in Biotechnol. Bioeng., **2001**.
23. Carr, P.W. Solvatochromism, Linear Solvation Energy Relationships and Chromatography. Microchem. J. **1993**, *48*, 4–28.
24. Forgács, E.; Csarthati, T. *Adsorption Phenomena and Molecular Interactions in Chromatography*; Molecular Basis of Chromatographic Separations, 1st Ed.; CRC Press: Boca Raton, 1997; 10–12.
25. Kaliszan, R. Quantitative Structure-Retention Relationships Applied to Reverse-Phase High-Performance Liquid Chromatography. J. Chromatogr. A **1993**, *656*, 417–435.
26. Mazza, C.B.; Breneman, C.M.; Cramer, S.M. Predictive Quantitative Structure Retention Relationship Models for Ion-Exchange Chromatography. **2001**, in preparation.
27. Breneman, C.M. RECON4-10, RPI, Troy, NY.
28. Livingstone, D. Supervised Learning. *Data Analysis for Chemists*; Oxford Science Publications: Oxford, 1995; 138–161.
29. Wold, S. Cross-Validatory Estimation of the Number of Components in Factor and Principal Components Models. Technometrics **1978**, *20*, 397–405.
30. Breneman, C.M.; Thompson, T.R.; Rhem, M.; Dung, M. Electron Density Modeling of Large Systems Using the Transferable Atom Equivalent Method. Comput. Chem. **1995**, *19*, 161–179.
31. Bader, R.F.W.; Carroll, M.T.; Cheeseman, J.R.; Chang, C. Properties of Atoms in Molecules: Atomic Volumes. J. Am. Chem. Soc. **1987**, *109* (26), 7968–7979.
32. Breneman, C.M.; Rhem, M. A QSPR Analysis of HPLC Column Capacity Factors for a Set of High-Energy Materials Using Electronic Van der Waals Surface Property Descriptors Computed by the Transferable Atom Equivalent Method. J. Comp. Chem. **1997**, *18* (2), 182–197.
33. Kundu, A.; Shukla, A.A.; Barnthouse, K.; Moore, J.; Cramer, S.M. Displacement Chromatography of Proteins Using Sucrose Octasulfate. BioPharm **1997**, *5*, 64–68.