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Large-scale purification of antisense oligonucleotides by high-performance membrane adsorber chromatography

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Abstract

Very high flux ion-exchange membranes were utilized for a novel purification of antisense oligonucleotides (20-mer). Strong anion-exchange membranes were produced by attaching polymeric ligands onto a microporous cellulosic matrix. The oligonucleotides purified were therapeutic single-stranded phosphorothioates deoxyribonucleotides. Although small-scale membrane devices (15 cm^2) had similar resolution to traditional chromatographic columns; their throughputs were superior. Greater than a 1300-fold scale-up produced very similar purity and yields of the phosphorothionate product. Scale-up experiments were conducted with a 2 m^2 surface area membrane module. These modules were easily capable of very high throughputs of 0.5 to 2 l/min. High purity and yields were achieved by both step and linear gradient elution.

Keywords: Membranes; Oligonucleotides; DNA

1. Introduction

Antisense oligonucleotides are an innovative group of biomolecules. They are single-stranded (ss) molecules with 20 to 30 nucleotides in length. Recent clinical successes of these therapeutic molecules has revitalized the interest in manufacturing of these compounds at large-scales. To establish antisense oligonucleotides as a competitive and viable therapeutic option, we are developing cost-effective and scaleable processes to purify these compounds. These molecules are synthesized by automated solid-phase synthesizers and generally purified by chromatography. Both small and large-scale reversed-phase (RP) chromatography has provided adequate GMP purification. Recently, anion-exchange (AX) chromatography has shown potential in purifying these molecules. The AX has shown high purity and good overall process yields. Thus far, we have used traditional bead-based chromatographic media. Here, we will focus upon purification of oligonucleotides by large-scale anion-exchange membrane chromatography.

1.1. Chromatographic membrane adsorbers

Chromatographic membranes are wide pore (5

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μm) cellulosic membranes with grafted polymers containing functional quaternary ammonium groups. These membranes are capable of very high resolution, efficiency, and dynamic capacity. The mass transfer may be an order of magnitude faster than conventional bead-based chromatographic media. The high mass transfer rates are due primarily to the shortened path of molecular diffusion (i.e. the proximity of functional binding groups). The technology and its various aspects have been reviewed recently [1–6]. Several practical applications at various scales have been published recently using membrane adsorber chromatography, especially for DNA and viral reduction [7,8], vaccine protein purification [9] and other protein purification [10–14]. We have evaluated for the first time the use of large-scale membrane adsorbers for high-resolution purification of antisense oligonucleotides.

A variety of high-performance membrane devices have been fabricated. Some are thin diskettes of continuous media or monoliths with very short separation lengths, typically in the 3 to 20 mm length. Others are similar to traditional membranes used for filtration applications. However, the chemistry and porosity has been optimized for chromatographic applications. We have utilized these membranes in our experiments, since they currently offer the best scale-up solution. Examples of diskette type high-performance membrane chromatography (HPMC) devices have been described by Svec and co-workers [15–20], Josic, Strancar and their co-workers [4,21–26] and Freitag and Breier [12].

There are several limitations to the bead-based chromatographic media. Many of these chromatographic limitations can be overcome by membrane-based systems. There is significant mass transfer resistance in chromatographic beads. This becomes the rate-limiting factor during large-scale purification. Generally traditional chromatographic supports are limited to linear velocities of 100–500 cm/h. Column packing at larger scales is often a challenging technology in itself. However, due to the tremendous research efforts over the past 30 years there is a large variety of bead sizes and chemistries; as well as ligand chemistries are currently available. On the other hand, chromatographic membranes can give much higher volumetric flow-rates and have very low mass transfer resistance. This translates into exceptionally high throughput without a large loss of dynamic capacity. Here, there is no column packing technology necessary. Membrane modules may be simply connected together (in series or parallel) in order to achieve the desired capacity. There are relatively few chemistries available at this time. However, the lack of chromatographic variety is likely market-driven, rather than technology-limited.

1.2. Challenges of antisense oligonucleotides purification

Pharmaceutical oligonucleotides are rapidly expanding into a wide variety of medical applications. Now large quantities of oligonucleotides must be synthesized under GMP conditions to support the plethora of clinical trials. The first antisense drug has recently been approved by the US Food and Drug Administration (FDA) for treatment of cytomegalovirus (CMV) retinitis (fomivirsen, Isis Pharmaceuticals). Manufacturing technologies are currently being developed to purify kilogram quantities of such oligonucleotides [27–31]. Although RP and traditional AX methods work well for the purification of oligonucleotides; Q type membrane adsorbers were analyzed for their manufacturing-scale potential. This application also demonstrates the use of very large-scale modules, 2 to 4 m^2, for high-resolution purifications. A purification process may appear suitable at the laboratory scale; however the resolution and yields may decay as the process is scaled to the manufacturing level.

Details of the traditional RP and AX based oligonucleotide purification are given elsewhere [28,30]. There are a number of oligonucleotides in clinical trials, these are summarized in recent reviews [31,32]. The first generation of modified oligonucleotides in the clinic, as well as the ones considered within our text are phosphorothioate deoxyribonucleotides. The diester backbone of the DNA is modified by replacing a non-bridging oxygen atom (at each diester linkage) with a sulfur atom.

The crude oligonucleotide solution of a 20-mer (*n*-mer) phosphorothioate product obtained after automated synthesis has 19-mers as the most significant impurity. They are denoted as the $(n-1)$ impurity,

where *n* denotes the full-length compound. These are multiple species as the deletion could be at any random position of the 20-mer sequence. There are also smaller quantities of the $(n-2)$, $(n-3)$, etc. deletion sequences. In addition to these impurities, there are phosphodiesters generated as a result of the synthetic production process. These are mainly mono-phosphodiesters or $(P{=}O)_1$ and smaller amounts of $(P{=}O)_2$, $(P{=}O)_3$ molecules. The migration of these components on AX analytical column is shown in Fig. 1. Note, that the length based impurities ($n-1$, $n-2$ etc.) are not readily evident in the AX analytical trace and are therefore not marked. Also, there are other impurities present in much smaller quantities which are currently being detailed and characterized. For simplicity we have combined all impurities that migrate later than the *n*-mer as $(n+x)$ components. Similarly we have used the notation Sum $(n-x)$ to denote all deletions, Sum $(P{=}O)_x$ to denote all phosphodiester components, and (P=S) to denote the all-phosphorothioate product molecules. Additional descriptions of these various components of crudes have been presented earlier [27–31].

1.3. Purification of oligonucleotides on membrane adsorbers or other thin supports

There are very few published reports of purifying oligonucleotides on membrane-like materials. There is report of good resolution of 12–16-mer mixed sequence phosphodiesters on small membrane diskettes by Podgornik et al. [26,33]. Excellent separation was obtained with isocratic and gradient elutions even with disks as thin as 3 mm. However, the amount purified was in the micro-gram range and phosphorothioates were not demonstrated. Due to the sulfur modification in phosphorothioates it is extremely difficult to get complete (baseline) separation of 19- and 20-mers all-phosphorothioates on commercially available ion-exchange media. In comparison, it is relatively easy to separate 19- and 20-mers of full-phosphodiester sequences. This apparent loss of resolution in phosphorothioate purifi-

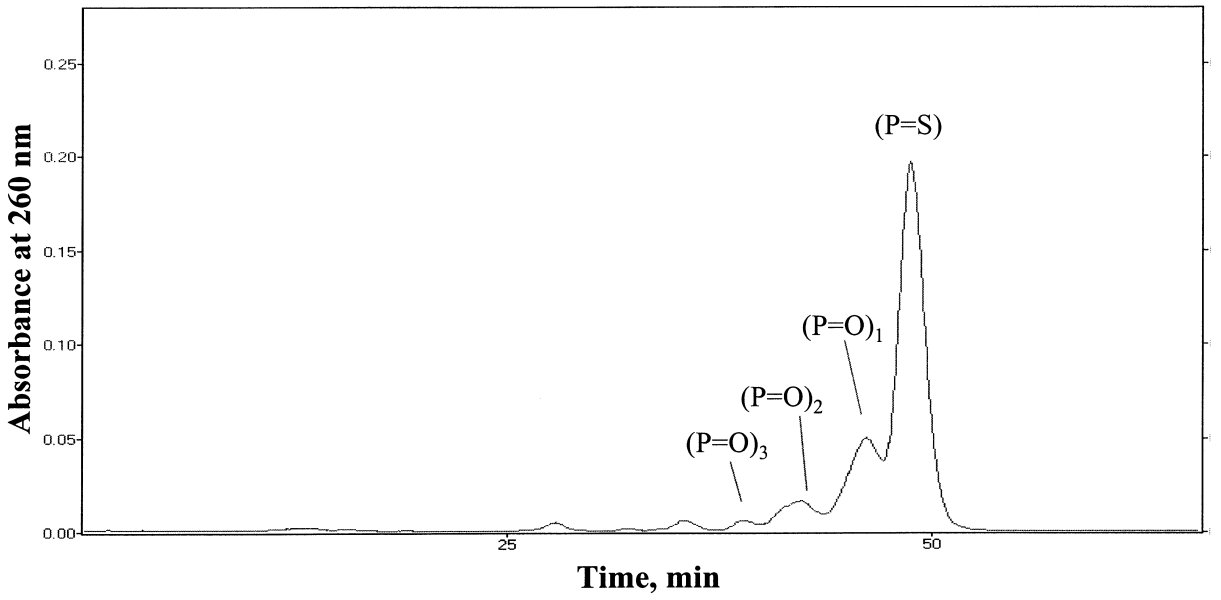

Fig. 1. Analytical purification of ISIS 2302 on conventional beaded chromatographic media. Column: Resource Q 1 ml (3×6.5 mm I.D.), buffer system: A – 20 m*M* NaOH, B – 20 m*M* NaOH+2.5 *M* NaCl. Gradient 0–100% B in 60 CVs. Flow rate 1.0 ml/min. Sample: 200 μg ISIS 2302 crude. Chromatograph: BioCad 60 workstation with UV detection at 260 nm. (Adapted from Ref. [36] with permission, 2000 Am. Chem. Soc.).

cation is probably due to the stereogenicity of the phosphorothioate backbone causing partial separation of multiple stereoisomers of phosphorothioates and band broadening [34]. This work shows phosphorothioate data at gram scale purifications.

2. Methods and materials

2.1. Oligonucleotides and reagents

The oligonucleotides described here are 20-mer phosphorothioates, manufactured at Isis Pharmaceuticals by automated solid-phase synthesis. An antisense inhibitor of cell adhesion molecule ICAM-1, ISIS 2302 (sequence: 5′ GCC CAA GCT GGC ATC CGT CA, all phosphorothioate linkages), has been developed to treat chronic inflammatory diseases). ISIS 2105 is another similar 20-mer but with a different nucleotide sequence (sequence: 5′ TTG CTT CCA TCT TCC TCG TC, all phosphothionate linkages). All crude preparations of the phosphorothioates had the acid labile hydrophobic group 4, 4′-dimethoxytrityl (DMT) removed from the 5′ end (DMT-off) either on the synthesizer or post-synthesis. Thus, the oligonucleotide crudes used for purification had no protecting groups or end-groups on them.

Reagent grade NaCl and HCl were obtained from Sigma (St. Louis, MO, USA), NaOH from Mallinckrodt (Paris, KY, USA). Deionized water (DI) used in these experiments was obtained from a Milli-Q system (Millipore, Newton, MA, USA). Electrophoresis grade Tris–Borate/7 *M* urea was obtained from Beckman Coulter (Fullerton, CA, USA).

2.2. Chromatographic membrane adsorbers and columns

All membrane adsorbers used in this study were obtained from Sartorius (Edgewood, NY, USA). Small-scale experiments were conducted on membranes in syringe–filter-type configuration, Sartobind Q15X, with 15 cm^2 total surface area. Large-scale experiments were performed with the Sartobind 20 Kb (91-Q-20K-60-12) module with 20 000 cm^2 (2 m^2) of membrane area. The membrane module was placed onto a Sartorius polyoxymethylene (POM) core, 90-CR-P060-12, and placed into a special POM housing (90-HS-PO-12). The details of the membrane modules are listed in Table 1. The conventional chromatographic media column used was the same as the analytical column described below.

2.3. Large-scale preparative apparatus

Membrane adsorber (MA) experiments with syringe filters were conducted on the BioCad 60 workstation of PE BioSystems (Framingham, MA, USA). The large-scale MA modules in the filter housing (90-HS-PO-12) were connected to a pumping unit. The pump utilized was either a 200/250 R rotary pump (Flowtech, Atlanta, GA, USA) or a Masterflex 7024-21 peristaltic pump (Cole-Palmer, Chicago, IL, USA). Elution buffer isocratic gradients were performed by manually moving the pump's inlet tubing from one eluent reservoir to the next. Two equal sized reservoirs were connected together to generate linear gradients. The eluent liquid was then drawn from the lowest ionic strength buffer reservoir, while being magnetically-stirred. The volume of the eluents in the two reservoirs controlled the gradient's duration. A ''T'' union at the outlet

Table 1
Details of membrane modules used in the study

Module	Type	No. of layers	Total surface area (cm^2)	Length (cm)	Volume (ml)
Sartobind Q15X	Syringe	3	15	NA[a]	1.0
Sartobind Q20Ka	Radial	30	20 000	30	550
Sartobind Q20Kb	Radial	60	20 000	12	550
Sartobind Q40K	Radial	60	40 000	30	550

[a] NA: Not applicable.

allowed a small sidestream to be diverted to the BioCad workstation. This stream was used for online monitoring of UV (260 and 290 nm), pH, and conductivity.

2.4. Analytical conditions and apparatus

All analytical anion-exchange analysis of fractions were carried out on a Waters (Milford, MA, USA) chromatographic system, with Waters 717 auto sampler, 600E system controller, 991 photodiode array detector and Millennium 2.10 operating software. Capillary gel electrophoresis (CGE) was performed on the Beckman P/ACE 5000 system (Fullerton, CA, USA).

The phosphodiester content of the fractions was measured by analytical AX chromatography, using a 30×6.4 mm I.D. Resource Q (15 μm) column. The analytical separation was performed at 70°C with the same buffer system used in the preparative run. The length-based purity was determined by CGE. The fractions were dialyzed with DI water over ultrafiltration membranes (Millipore, Beverly, MA). The samples were then analyzed on a 47 cm (40 cm to

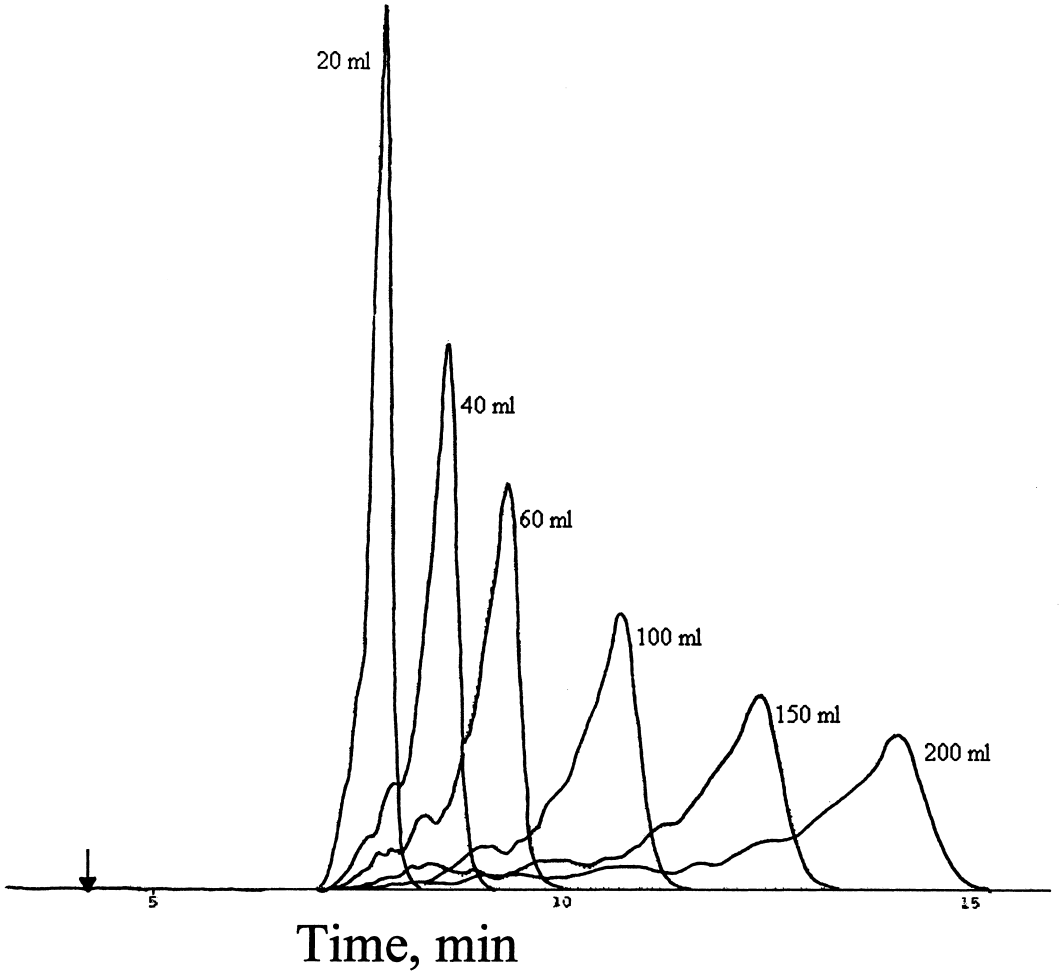

Fig. 2. Purification of ISIS 2302 on syringe membrane adsorbers (MA). MA module: Sartorius Q-15 (three layers, 15 cm^2 membrane area, buffer system: A – 20 m*M* NaOH, B – 20 m*M* NaOH+2.5 *M* NaCl. Gradient 0–100% B with varius elution volumes (20 to 200 ml) as indicated. Flow rate 10 ml/min. Chromatograph: BioCad 60 workstation with UV detection at 260 nm.

detector) Beckman eCAP ssDNA 100 gel filled capillary at a temperature of 40°C, with Tris–Borate/7 *M* urea as the carrier electrolyte [35].

3. Results

3.1. Small scale experiments on syringe filters

The analytical separation of ISIS 2302 crude on small-scale 15 cm^2 syringe filters is shown in Fig. 2. The gradient was from 0 to 100% B. The volume of eluent solution A (20 m*M* NaOH), and B (20 m*M* NaOH with 2.5 *M* NaCl) were varied from 10 to 200 ml. The resolution is comparable to the separation seen on normal high-resolution packed bed columns (Fig. 1) [36]. The operation was conveniently performed on a medium pressure chromatographic workstation.

The separation procedure could be applied to different oligonucleotides with minor variations in gradient conditions. This is similar to results obtained for beaded chromatography supports. Fig. 3A shows the suitability of membrane chromatography for ISIS 2302 with linear gradient elution. Separation using step gradients for ISIS 2105 is shown in the bottom panel of Fig. 3B.

3.2. Large-scale MA experiments

Experiments were conducted on the large-scale membrane adsorber module with surface areas of 2 or 4 m^2 with typical volumetric flow-rates of 0.6 and 1.5 l/min, respectively. Initially, a small load of sample (300 mg of crude unpurified ISIS 2302, DMT-off) was separated on 2 m^2 membrane module. A linear gradient was used to simulate the conditions used for the syringe filters. The gradient was varied from 0% (20 m*M* NaOH) to 100% B in 12 l, where B was 2.5 *M* NaCl solution in 20 m*M* NaOH. The

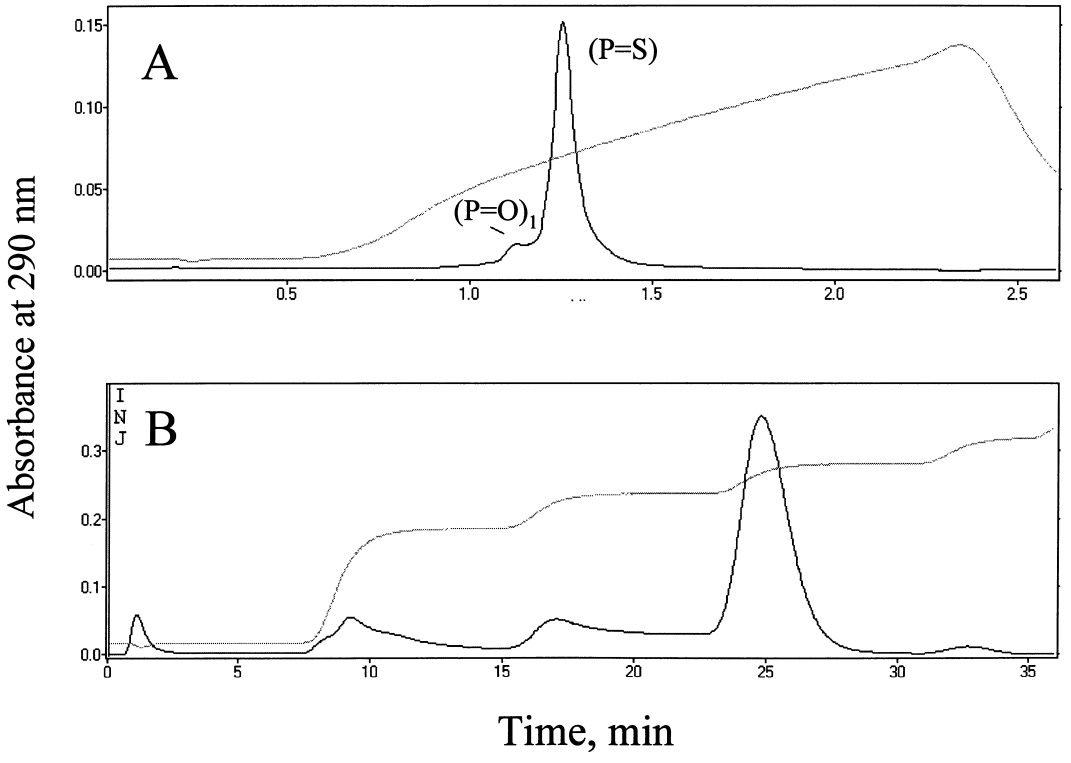

Fig. 3. Purification of ISIS 2302 and ISIS 2105 on small-scale MA with linear and step gradients. (A) ISIS 2302 (100 μl impurity rich sample) separation under linear gradient elution (0–100% B in 20 CVs) and (B) separation of ISIS 2105 with step gradients (30% B, 50% B, 65% B, 80% B, 100% B all for 30 ml). Buffer system same as in Fig. 1. Flow rate 10 ml/min top, 5 ml/min bottom.

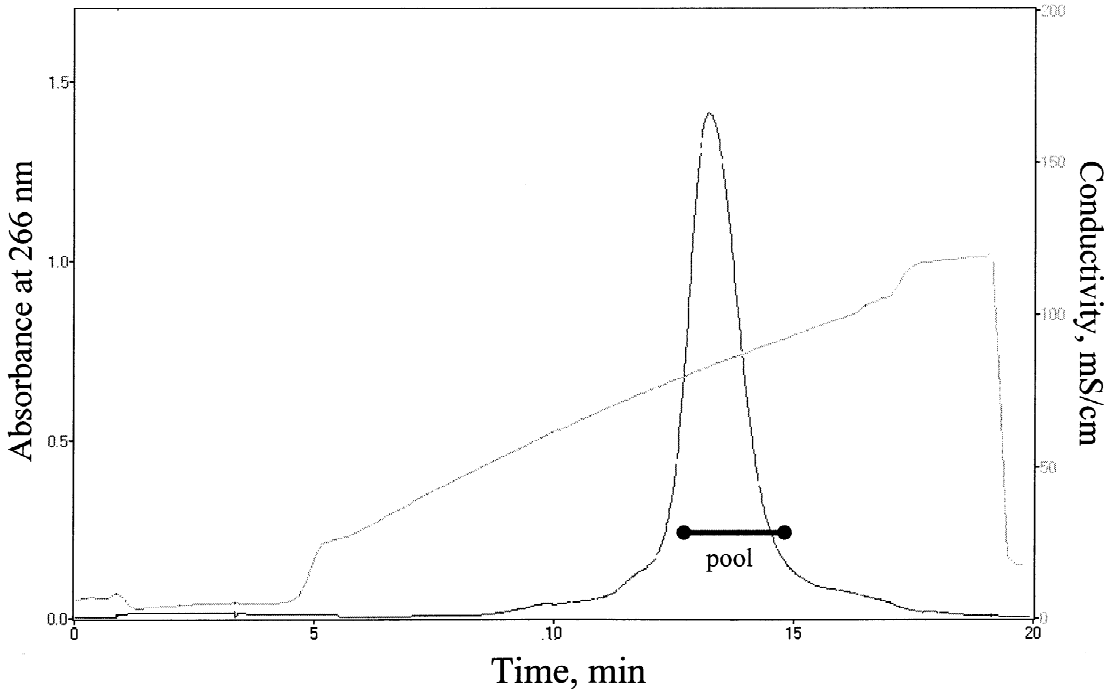

Fig. 4. Purification of ISIS 2302 on large-scale MA module. MA module: Sartobind Q-20K-60-12 (quaternary ammonium functionality, 2 m^2 membrane area, 60 layers, 12 cm length of module). Sample: 300 mg ISIS 2302 DMT-off crude injected in 1 l buffer A. Buffers A: 20 m*M* NaOH, buffer B: 20 m*M* NaOH+2.5 *M* NaCl, gradient 0–100% B in 12 l. Flow rate 620 ml/min, side stream into the BioCad at 40 ml/min.

chromatogram is shown in Fig. 4. The monodiester $(P=O)_1$ species are adequately separated from the main product peak even at this large scale indicating an excellent flow distribution and a low dead volume design. The surface area has been scaled-up more than 1300-fold and the volumetric flow-rate about 100-fold. This chromatogram compares very well to the profile obtained in Fig. 3A. Individual fractions were analyzed for purity by analytical AX. The amount of oligonucleotide was quantitated by UV spectroscopy. The results are plotted graphically in Fig. 5. Pooling the fractions indicated in Fig. 5 gives product pool purity of 91% by AX at a yield of 81% of the starting P=S target product in the feedstream.

Step gradients are often preferred for larger scale processing on ion-exchange chromatography. Therefore, experiments with step gradients and a combination of step and linear gradient runs were also conducted. Fig. 6 shows the purification of 1 g of ISIS 2302 crude at 700 ml/min. The elution consisted of four steps of increasing salt concentration from 15, 32.5, 50, 65 and 100% buffer B. The main product peak elutes at the beginning of the 65% B step. The impurity distribution is shown in Fig. 7. Pooling fractions as indicated in Fig. 6, produces a purity of 95.4% (as measured by analytical AX) with yields of 65% PS. The (P=O) content was only 3.8%. The purity of the full-length product as analyzed by CGE is 90%.

A larger load of 3 g of crude is shown in Fig. 8. The main peak elutes between 50 and 65% NaCl steps and at higher loading it elutes earlier as expected. Pooling fractions 8–18 as indicated in the Fig. 9 gives product purity of 92% (as analyzed by the AX assay) with yields of 62% of the (P=S). The (P=O) content of the pool was 4.8%. The results are summarized later in Table 2.

There is some flow-through of the crude material at the 3-g level of loading. Two strategies were examined to increase the initial capture of the target oligonucleotide. One strategy was to reduce the loading flow-rate; while the second was to recirculate

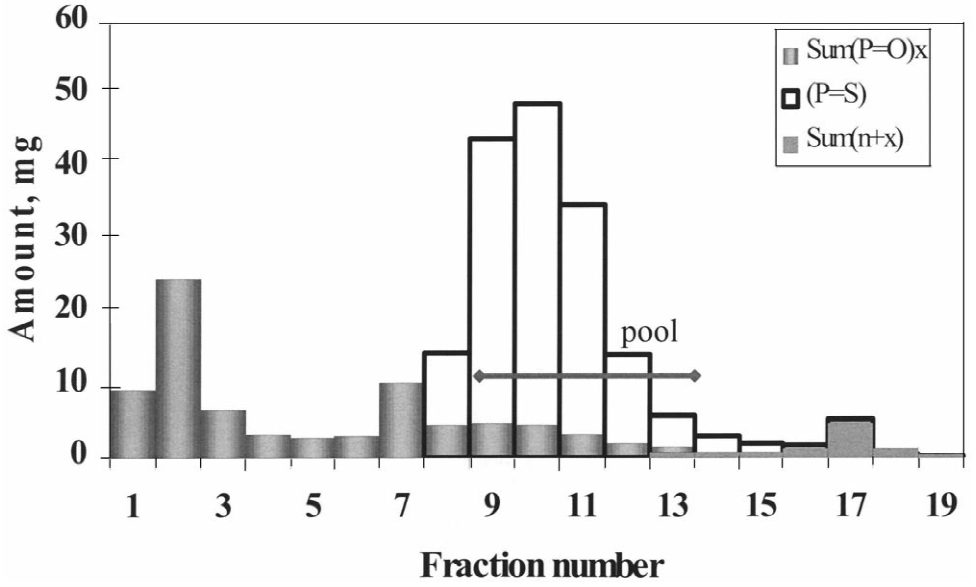

Fig. 5. Impurity distribution for purification in Fig. 4.

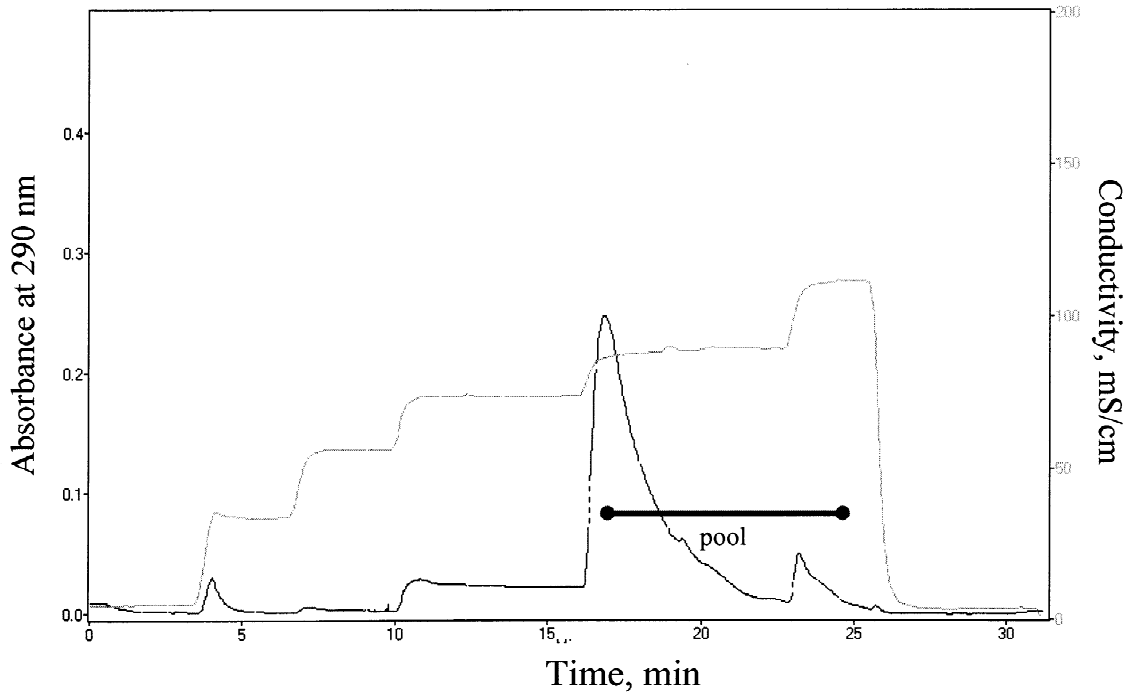

Fig. 6. Purification of ISIS 2302 on large-scale MA module with a step gradient. MA module: Sartobind Q-20K-60. Sample: 1 g ISIS 2302 DMT-off crude injected in 1 l buffer A. Elution: gradient 15% (2 l), 32.5% (2 l), 50% (5 l), 65% (5 l), 100% (2 l), where (v/v%) are from 2.5 *M* NaCl stock in 20 m*M* NaOH. Other conditions same as in Fig. 4.

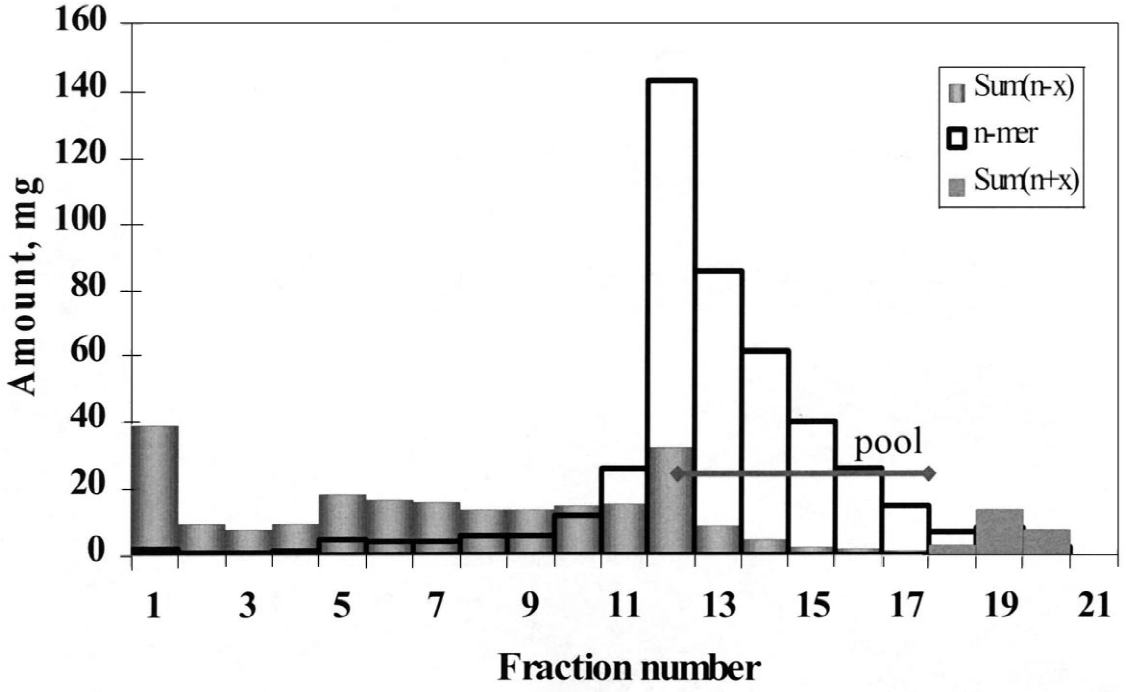

Fig. 7. Impurity distribution for the purification in Fig. 6.

the flow-through. The later strategy appears to increase the binding efficiency of the membrane. The feed was recirculated three times onto the membrane column. The chromatographic elution pattern during the final injection showed a marked reduction in the UV absorbance. The overall product yield was improved by 5%. Although the purification is excellent, at this high flux the binding capacity can be improved by recirculation. Nonetheless, this does reduce the expected throughput; the purification time remains quite fast. Even with the feed recirculation, the entire purification process requires less than 30 min. Fig. 10 shows the experiment where a gradient elution was used in conjunction with an initial ''wash'' step elution. Again the same load of 3 g was separated with gradient NaCl steps of 15 and 32.5% followed by a linear gradient from 32.5 to 100% B in 10 l. The pool had a 90.7% product purity and the yield was 60%. The (P=O) content is relatively higher at 7.1%. The impurity distribution is shown in Fig. 11. Quantitative results for purifications in Figs. 4–11 are summarized in Table 2.

4. Discussion

Experiments demonstrate that the membrane modules have very good selectivity. The resolution between mono-phosphodiesters and all phosphorothioate species was excellent. The purification and reduction of $(n-1)$ species is comparable to that achieved by chromatographic beads. Results shown in Fig. 6 can be compared against our prior published results on conventional chromatographic beads shows that similar purity can be achieved at a lower yield but higher volumetric flow-rates [30]. The full length purity of 90.5% was achieved at yield of 83% (Table 3 in Ref. [30]) on beaded chromatographic media compared to 90% purity at 65% yield for MA purification (Fig. 6). While the yield is lower the purity achieved is very similar and the volumetric flow was 600 versus 35 ml/min. Further optimization can yield higher full-length material. Another key advantage of the MA process was that many optimal process parameters for conventional media could be applied directly. For example, the optimal

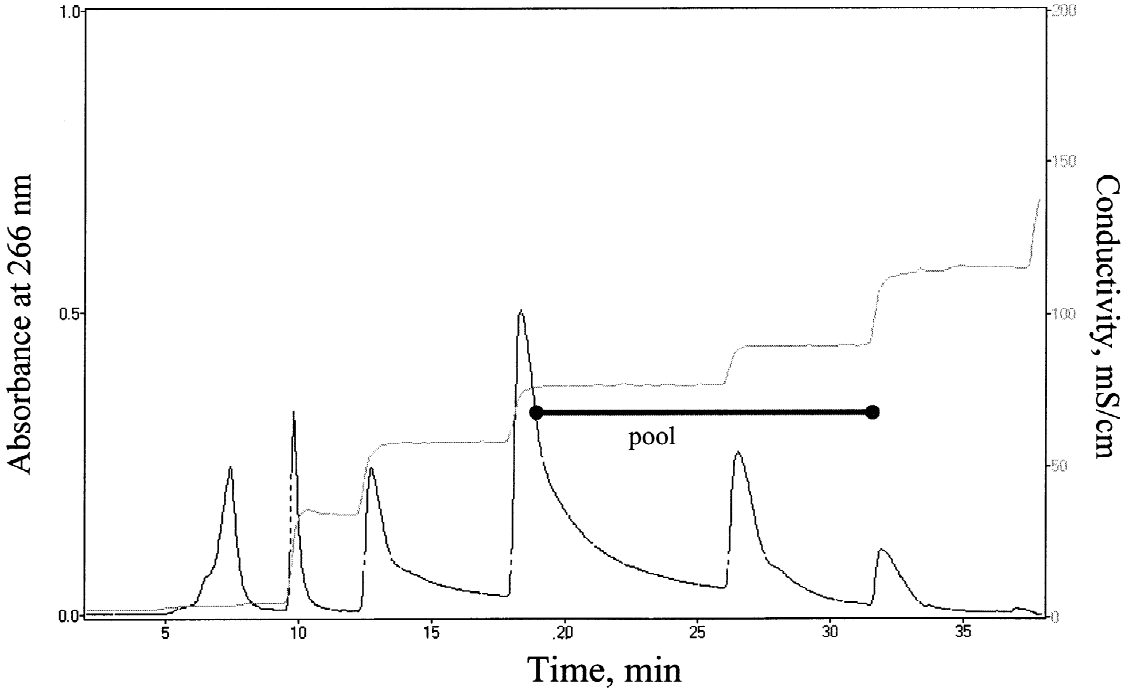

Fig. 8. Purification of 3 g ISIS 2302 on large-scale MA module. Sample: 3 g ISIS 2302 DMT-off crude injected in 1.5 l buffer A. Elution: gradient 15% (2 l), 32.5% (5 l), 50% (8 l), 65% (5 l), 100% (2 l), where (v/v%) are from 2.5 *M* NaCl stock in 20 m*M* NaOH. Other conditions same as in Fig. 4.

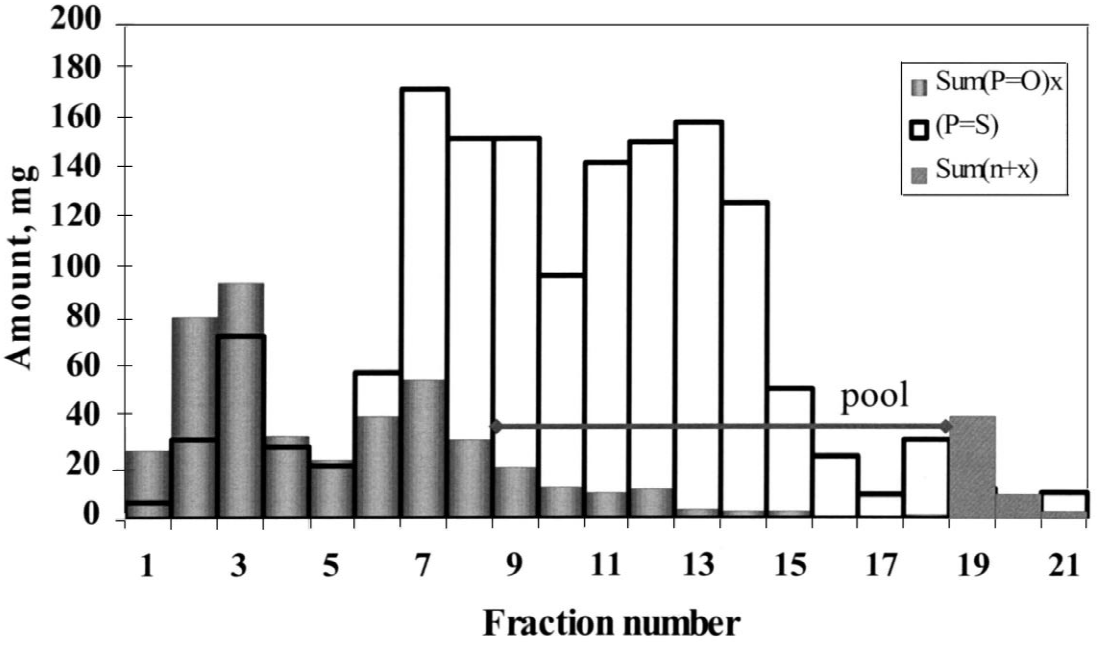

Fig. 9. Impurity distribution for purification in Fig. 8.

Table 2
Analytical summary for purifications in Figs. 4–11

	SAX analysis			CGE analysis		
	Sum $(P{=}O)_x$	(P=S)	Yield	Sum $(n-x)$	n-mer	Yield
Feed	34%	66%		31%	69%	
Product (Figs. 4 and 5)	9%	91%	81%			
Product (Figs. 6 and 7)	4%	96%	77%	10%	90%	65%
Product (Figs. 8 and 9)	8%	92%	72%			
Product (Figs. 10 and 11)	10%	90%	61%	11%	89%	60%

buffer system, pH etc. on beads is the same as that used for MA, so that only the load, flow-rate and gradient conditions needed to be optimized.

The throughput is currently 4-fold faster than traditional chromatography. The membrane modules may be capable of much higher fluxes. However, we did not examine higher flow-rates in these experiments. The total capacity (of this module on a volumetric basis) is possibly lower than conventional media. This may be explained by the fact that the surface area contained within the module is considerably less than the internal surface area for conventional media. However, it should be pointed out that the dynamic capacity would be greater than conventional media at the experimental flow-rates. The dynamic binding capacity at 5% breakthrough with frontal loading was measured to be 10 g for the module at this flow-rate. However, as experiment in Fig. 10 indicates there was some breakthrough even at 3 g. While we were able to empirically solve the

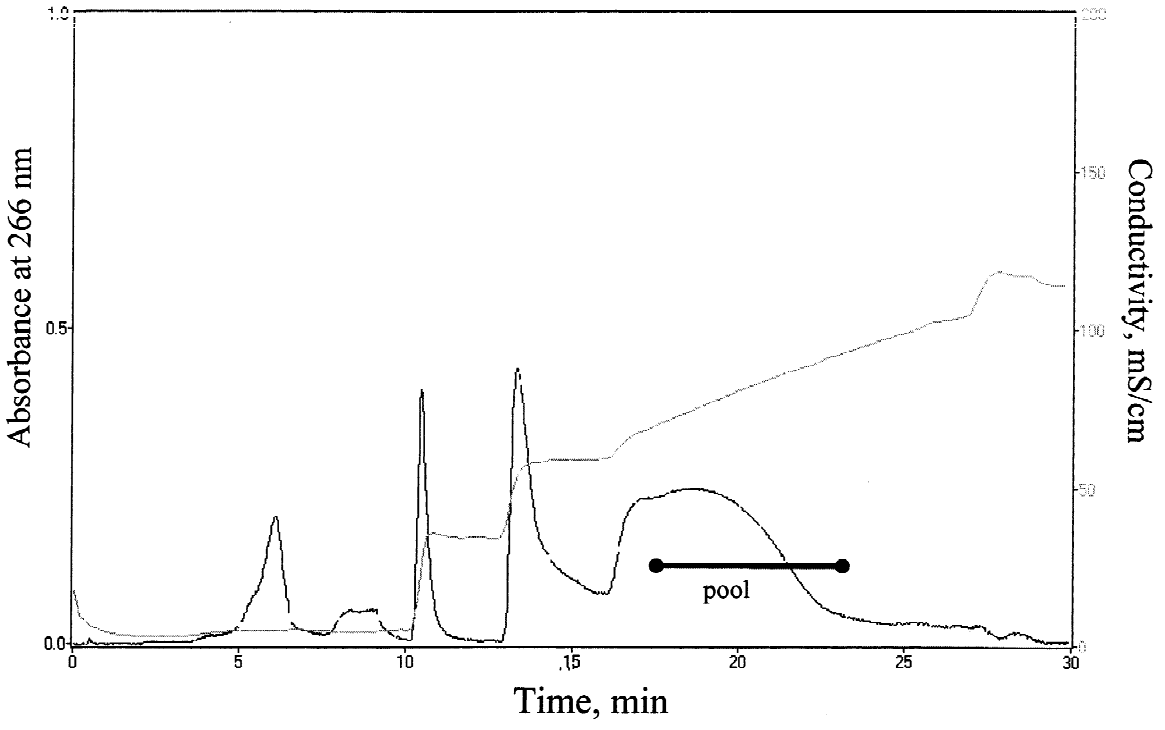

Fig. 10. Purification of ISIS 2302 on MA with step and linear gradients. Sample: 3 g of ISIS 2302 in 2 l. Elution: 15% (2 l), 32.5% (2 l), 32.5 to 100% B in 10 l. Sample was recirculated back into the column. Other details are as Fig. 4.

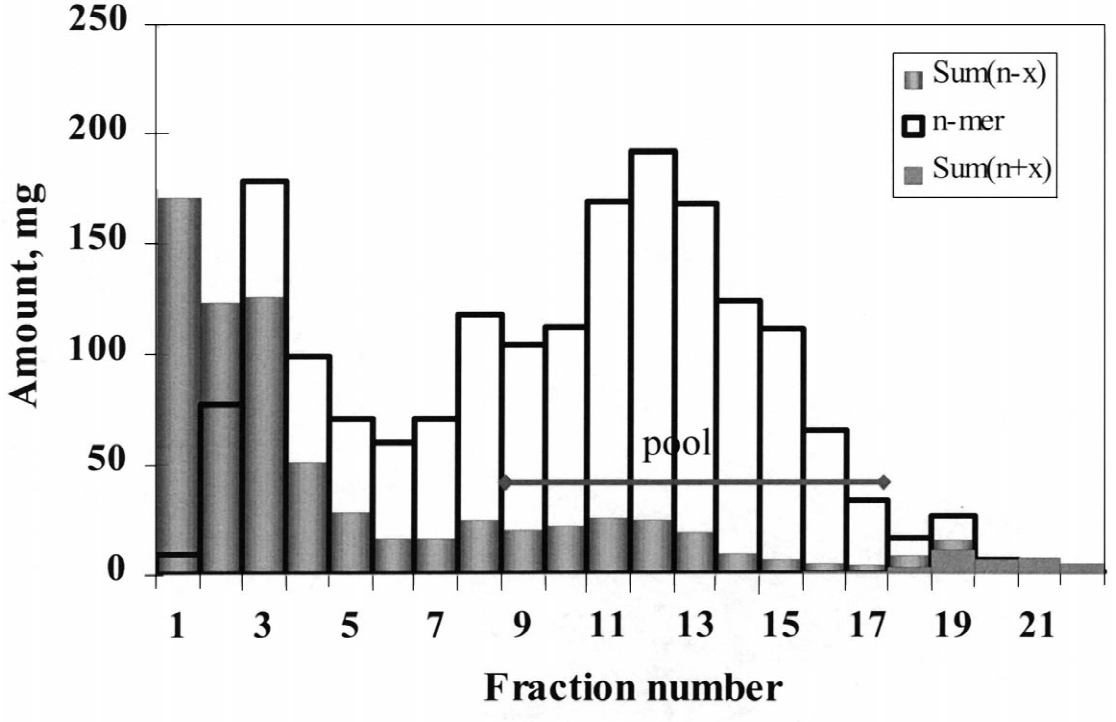

Fig. 11. Impurity distribution for experiment in Fig. 10.

problem by recirculation, this aspect needs to be studied further.

Overall, the membrane modules give an excellent high-resolution purification of the oligonucleotides at a large scale. While, the scale-up was not identical, it was very close to our theoretical prediction. Scaling from 15 cm^2 to 2 m^2 membrane area (a scale-up factor of over 1300) produced surprisingly similar results. Other published data have shown scalability of this technology to 21 m^2 surface area with flow-rates of approximately 10 l/min [37]. A system of this size would be able to purify between 30 and 100 g of crude oligonucleotide within a 15 min cycle (including cleaning and regeneration). Therefore, it can be assumed that an automated chromatography controller could perform approximately 96 cycles per day (assuming a 24 processing period.). Thus, this system could process in the range of 2.9 to 9.6 kg of crude material within a single day.

The membrane adsorbers were reused multiple times for these experiments presented here. The membranes did not need extensive regeneration or cleaning in between runs and a high salt wash (2.5 *M* NaCl) was sufficient to proceed with the subsequent runs. The membranes were cleaned in place for overnight storage with a rinse of 0.5 *M* NaOH solution and storage in 0.1 *M* NaOH. Prior to use the membrane was reequilibrated in buffer A (20 m*M* NaOH). No perceptible performance loss was observed with this procedure and indicates that the membrane adsorbers could be reused just like conventional polymeric chromatographic media.

The hallmark of this technology is that the separations are feasible at very high throughputs. This has a great advantage when dilute feedstreams must be used. The time reduction during the loading step alone could be a great benefit. A hypothetical example of loading 2 l of feed containing 3 g of material is presented for comparison in Table 3. Using load equivalency a 2 m^2 is considered equal to a 100 ml (100×25 mm I.D.) column with conventional beaded media. Assuming a linear gradient processing with 20 column volumes (CVs) as the required volumetric eluent. The flow-rate for axial flow column is 20 ml/min or (0.2 CV/min). Flow rate for cylindrical MA unit is 700 ml/min (~1.3

Table 3
Hypothetical comparison of beaded chromatography media and MA with same dynamic binding capacity

	Conventional column	MA module
Load	3 g	3 g
Column	100 ml (25×100 mm)	2 m^2
Flow-rate	20 ml/min (0.2 CV/min)	700 ml/min (1.3 CV/min)
Sample volume	300 ml	300 ml
Loading time	15 min	0.6 min (1.8 with 3×feed recirculation)
Elution 20 CVs	100 min	15 min
Run time (Load+elution)	115 min	17 min (with recirculation)

CV/min). This shows that the MA system would be able to purify in 1/7th the time of the conventional column. Although we found that there may be loading flux limitations; it is entirely possible that the elution portion of our separation could be accomplished at a greatly accelerated flow-rate. Since the majority of the purification cycle (20 min) is spent on gradient elution, the time-savings could be significant (i.e. 4-fold). Future experiments will examine the effect of increasing the gradient elution rate upon purity and yields. As the sample feed becomes more dilute the throughput difference becomes even more remarkable. It may be expeditious to use membrane adsorbers to capture a dilute target molecule in leu of ultrafiltration (prior to chromatography).

5. Conclusions

Membrane adsorber chromatography is an innovative technology at the early stages of commercialization. This is a first attempt to optimize the separation of a complex oligonucleotide mixture on this novel chromatographic membrane. We have shown that high-resolution purification of antisense oligonucleotides is feasible, as well as practical on this media producing high purity and yields. This technology is easily scaleable from laboratory devices to multigram loading levels required for GMP manufacturing. There is also the promise that the membranes may be scaled-up beyond 21 m^2, permitting commercial manufacturing scale [37]. Nonetheless, it must be stressed that we have only performed a preliminary evaluation of this nascent technology. As the antisense product matures and improves, so shall the membrane purifications. Membrane adsorbers will soon match the optimized results obtained on conventional beaded media. In addition to the high resolution application presented here, many other suitable applications for this technology will emerge. In the future, other applications will be quickly embraced when the membrane's throughput is compared to that of conventional chromatographic media.

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