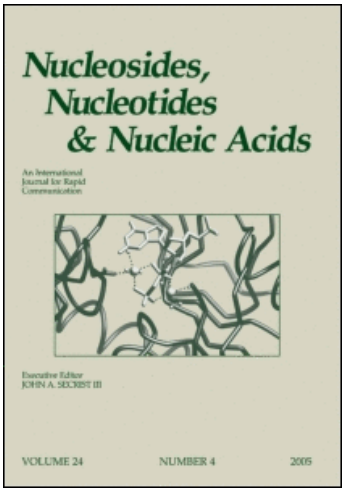

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A Rapid Method for Purification of Oligonucleotides

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To cite this Article Kumar, Ajay , Dawar, Hema and Mathur, Sudhakar(1992) 'A Rapid Method for Purification of Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 11: 9, 1575 — 1578
To link to this Article: DOI: 10.1080/07328319208021350
URL: http://dx.doi.org/10.1080/07328319208021350

NUCLEOSIDES & NUCLEOTIDES, 11(9), 1575-1578 (1992)

A Rapid Method for Purification of Oligonucleotides

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Abstract: A simple, rapid and novel method for purification of the oligonucleotide using silica gel matrix is described.

Synthetic oligonucleotides are widely used in modern molecular biology. Phosphoramidite synthesized oligonucleotides require a final reaction step with concentrated ammonia to effect their complete deprotection. The resulting by products, e.g., benzamide and small amount of truncated oligonucleotide sequences, need to be removed from the oligonucleotides before the latter may be used in various enzymatic processes (1). The removal of the by products had been carried out by time-consuming molecular exclusion chromatography (1) or by ethanol/Mg^{2+} precipitation (2). A rapid method for the purification through extraction with n-butanol has been reported recently (3). However, the assessment of purity of the butanol extracted oligonucleotide carried out by UV spectra was poor. We here describe a simple, rapid, and novel silica based technique for the purification and isolation of oligonucleotides.

An oligonucleotide d(ACTGGCGCCGTTGCCACCGTTGCCG) was synthesized at 0.2 μmole scale using Pharmacia Gene Assembler employing β-cyanoethyl phosphoramidite chemistry (3). Coupling efficiency was exceeded 98% per residue during the synthesis. The protected oligonucleotide was treated with 25% ammonia solution at 55°C for 16 hrs. The ammonia solution was completely evaporated in a speed vac concentrator and the crude oligonucleotide was redissolved in autoclaved water. 100 μl containing 5 OD A_{260} nm of the crude oligonucleotide solution was added to 300 μl of 7M sodium perchlorate solution and this solution was then poured in a microfuge tube containing 200 mg of presoaked column chromatography silica gel. The microfuge tube was left at room temperature for 5 min. and then centrifuged for one min. at 5000 rpm. The supernatant was decanted and the silica gel was washed thrice with one ml of absolute ethanol. Subsequently 1 ml of the autoclaved water was added to the microfuge tube and incubated at 90°C for two min. The aqueous supernatant containing the oligonucleotide was decanted.

TABLE-1. Summary of the Purification Data

S.No.	Method	% Recovery	Purification Time (min.)
1.	Silica Gel Matrix	55	15
2.	Butanol Extraction		
	First Time	83	15
	Second Time	60	
3.	OPC Cartridge	40	90
4.	Sephadex, Gel Exclusion Chromatography	45	60

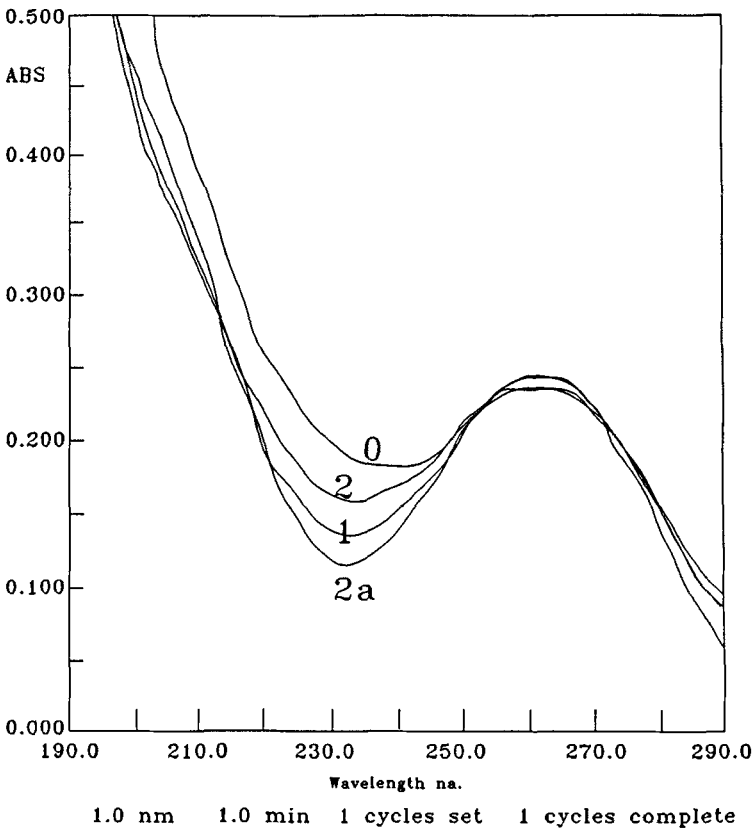

Figure-1. UV spectrum of the oligodeoxynucleotide (ACTGGCGCCGTTGCCACCGTTGCCG) in water. (0) Crude Oligonucleotide; (1) oligonucleotide purified by silica gel matrix; (2) oligonucleotide purified by n-butanol extraction first time; (2a) oligonucleotide purified by n-butanol extraction second time.

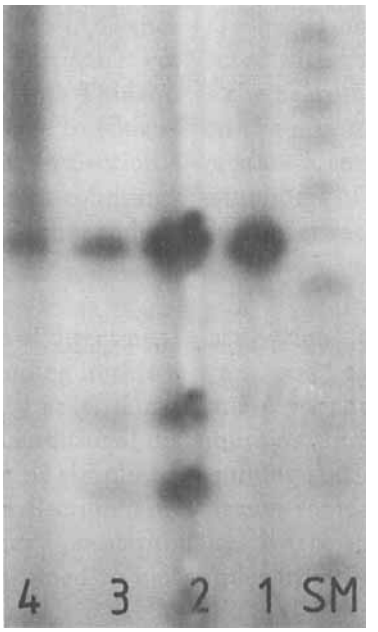

Figure-2. Autoradiogram of the oligonucleotide d(ACTGGCGCCGT TGCCACGTTGCCG). (SM) Size markers (8 to 32 mer); (1) oligonucleotide purified by silica gel matrix; (2) oligonucleotide purified by n-butanol extraction; (3) oligonucleotide purified by Sephadex method; (4) oligonucleotide purified by OPC Cartridge method.

A comparison of the time required for purification and % recoveries by four different methods are summarized in table-1.

Crude oligonucleotide and those purified by n-butanol extraction method and silica gel matrix method were analyzed for organic contaminants and small truncated oligonucleotide sequences by UV spectroscopy and autoradiography. As shown in Fig.-1 the organic contaminant concentration (those absorbing between 200-240 nm) are greatly reduced by silica gel method in the first attempt. However, butanol extraction was repeated twice to get rid of the complete impurities. An autoradiogram of the oligonucleotide purified by all the four methods along with size marker (8-32 mer) is shown in Fig.-2. The autoradiogram was obtained by running an analytical PAGE-7M urea gel. The equal amount of P^{32} labelled oligonucleotide samples were loaded. The autoradiogram clearly shows that the oligonucleotide was best purified by silica gel matrix method as it removed all the truncated oligonucleotides. The truncated sequences were least removed by the butanol extraction method. The amount of truncated oligonucleotides removed by OPC cartridge and Sephadex method was intermediate. The % recoveries and time (15 min.) required for the purification of the oligonucleotides by butanol extraction and silica gel matrix method were comparable (55-60%). However, other methods such as molecular exclusion chromatography and OPC cartridge methods were time-consuming and recoveries were poor.

Conclusion: A comparison of the number of oligonucleotides of varying length purified by four methods suggest that the silica gel matrix method is most reliable as it completely removes all the impurities in a single attempt.

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Received 9/26/91
Accepted 5/28/92