

A rapid method for the purification of deprotected oligodeoxynucleotides

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Submitted December 27, 1990

Oligodeoxynucleotides are among the most useful tools in modern molecular biology. Phosphoramidite-synthesized oligonucleotides require a final reaction step with concentrated ammonia to effect their complete deprotection. The resulting organic by-products, e.g. benzamides, need to be removed from the oligonucleotides before the latter may be used in various enzymatic processes (1). Typically, this is achieved by a lyophilization of volatiles (e.g. NH_3), followed by either size exclusion chromatography or precipitation of oligonucleotides in the presence of Mg^{2+} and ethanol (2). Here we describe a rapid method of oligonucleotide purification through extractions with n-butanol. This method obviates the need for prior NH_3 removal, and results in a high yield of oligonucleotides suitable for use in DNA sequencing, PCR amplification, and gel shift analysis of protein-DNA interactions.

Procedure. 100 µl of a deprotected oligonucleotide solution in 30% NH_4OH are vortexed vigorously in a 1.5 ml eppendorf tube with 1000 µl n-butanol (ACS reagent grade) for 15 sec, then centrifuged for 1 min at 12,000 rpm. The single H_2O-containing n-butanol phase is removed and discarded. For some oligonucleotide preparations it may be necessary to repeat the n-butanol extraction to achieve complete removal of contaminants. In these cases, the oligonucleotide pellet is redissolved in 100 µl H_2O and extracted with n-butanol as described above. Following n-butanol extraction, the pellet is dried under vacuum and resuspended in H_2O.

Crude oligonucleotides, and those purified by either the Mg^{2+}/ethanol or n-butanol methods, were analyzed for organic and ionic contaminants by UV/vis spectroscopy and conductivity, respectively. As shown in fig. 1, organic contaminant concentrations (those absorbing between 200–240 nm) are greatly reduced following either Mg^{2+}/ethanol precipitation or n-butanol extraction. Similarly, ionic species are reduced to similar extents by both methods (Table I). Overall recoveries are comparable for both purification methods (typically 90–95% as measured by absorbance at 260 nm). The primary difference between the two methods is the amount of time involved. Lyophilization (3 hrs), freezing prior to centrifugation (20 min), and centrifugation (15 min) all greatly lengthen the time required for oligonucleotide purification by the Mg^{2+}/ethanol method.

We have used n-butanol extracted oligonucleotides for DNA sequencing with Sequenase (US Biochemical Corp., Cleveland, OH) and dideoxy chain termination, PCR amplification of cDNAs, and a gel shift analysis of USF binding to DNA (3). In each case, oligonucleotides purified by the n-butanol method performed as well or better than those purified by other methods. This suggests that n-butanol extracted oligonucleotides should be of sufficient purity for many molecular biological applications.

ACKNOWLEDGEMENTS

This work was supported by The Robert A. Welch Foundation grants G-1195 (M.S) and G-1199 (M.V.D) and by grant GM-38212 (M.S.) from the National Institutes of Health.

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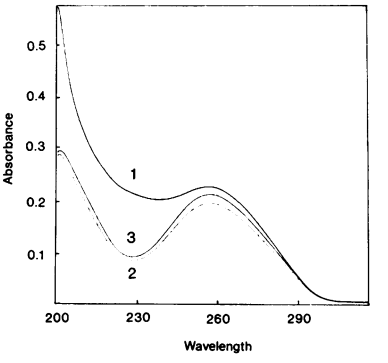

Figure 1. UV spectrogram of the oligodeoxynucleotide GATCTCCGGTCA-CGTGACCGGA. For this analysis oligonucleotides were diluted in a 50 mM potassium phosphate buffer [pH 7]. (1) Impure oligonucleotide; (2) oligonucleotide purified by Mg^{2+}/ethanol precipitation; (3) oligonucleotide purified by n-butanol extraction.

TABLE I. Characteristics of different oligonucleotide preparations

	conductivity (10^{-6}ohm^{-1}cm^{-1})	yield (A_{260})	preparation time
lyophilized	980	100%	3 hr
Mg^{2+}/ethanol precipitated	280	85%	4 hr
n-butanol extracted	470	92%	< 15 min

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