Effect of the phosphorylation state of thymidine derivatives on Sephadex K_d values*

It has been demonstrated[1-3] that gel filtration chromatography on Sephadex G-25, G-50 and G-75 will separate bases, nucleosides, nucleotides, oligonucleotides and polynucleotides from each other. Each variety of Sephadex has its specific range of maximum usefulness, and no one variety is effective over the whole range of molecular sizes.

During studies with the reaction reported by SCHRAMM *et al.*[4] for polymerizing 5'-thymidylic acid, products were isolated[5] containing more than one phosphorus per nucleoside and therefore not belonging to the above series. These products were examined for size by passage through Sephadex and were found to have elution positions corresponding to greater sizes than predicted from their molecular weights.

To explore this observation further in a systematic manner, thymidine and eighteen of its derivatives varying in size and state of phosphorylation have been comparatively chromatographed on Sephadex G-25 and G-50 columns (Table I).

TABLE I

COMPILATION OF COLUMN CHARACTERISTICS

Sephadex type	*Column dimensions (cm)*	*Flow rate (ml/min)*	V_t *(ml)*	V_o *(ml)*
G-25	1.0 × 175	0.70	67.6	60.3
G-50	1.0 × 170	0.30	87.7	53.4
G-75	2.3 × 36	0.70	96.0	50.9

A few of the compounds have also been studied on a G-75 column. The results are reported in Table II using K_d units derived from eqn. (1)[6]:

$$K_d = \frac{V_e - V_o}{V_t} \qquad (1)$$

where V_e, V_o and V_t are the elution volume, outside volume and inside volume, respectively. For G-25 and G-50, V_o was the V_e of S-RNA. With G-75, DNA was used. For all three columns, V_t was determined[9] as the V_e for tritium water minus V_o. Thymine had K_d = 1.13 on G-25 and 1.12 on G-50. The eluant was 0.005 *M* triethylammonium bicarbonate.

The data from the decrease in K_d values with increasing size along each homologous series were fitted successfully to an exponential drop in K_d with linear increase in the number of thymidine units per molecule. From these expressions and from the specific G-25 K_d for $p(tp)_2$, molecular weights were calculated for the K_d = 0.04 member of six of the series (Table II).

It is seen that larger G-numbered Sephadexes can resolve higher molecular weight compounds. In using Sephadex as a chromatographic medium to assay the

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TABLE II

THYMIDINE DERIVATIVES ON SEPHADEX

Terminal phosphorus	*Sephadex type*	*Compounds[a] and K_d values*						*Mol. wt. for K_d = 0.04*
None[b]		t	tpt	$t(pt)_2$	$t(pt)_3$	$t(pt)_5$	$t(pt)_9$	
	G-25	0.99	0.51	0.34	0.12	0.04	0.01	1,800
	G-50	0.98	0.61	0.54	0.30	0.14	0.05	3,000
5′-Phosphate[c]		pt	$(pt)_2$	$(pt)_3$	$(pt)_4$	$(pt)_6$	$(pt)_{10}$	
	G-25	0.31	0.13	0.06	0.02	—	—	1,100
	G-50	0.50	0.30	0.22	0.12	0.06	—	2,150
	G-75	0.80	0.65	0.53	0.44	0.30	0.15	5,200
3′-Phosphate[d]		tp	$(tp)_2$					
	G-25	0.32	0.12					—
	G-50	0.47	0.30					
3′,5′-Diphosphate[d]		ptp	$p(tp)_2$					
	G-25	0.07	0.04					700
	G-50	0.22	0.18					—

[a] Thymidine is represented by t and esterified phosphate by p such that pt is 5′-thymidylic acid, tp is 3′-thymidylic acid, and ptp is thymidine 3′,5′-diphosphate. Degree of linear oligomerization is represented by the subscripts.

[b] The last four compounds were synthesized from 5′-phosphates by cleavage with *E. coli* alkaline phosphatase.

[c] Prepared according to KHORANA AND VIZSOLYI[7].

[d] Prepared according to KHORANA[8].

degree of chemical polymerization of 5′-thymidylic acid, G-25 would be a poor choice since no clear distinction could be made between a tetramer and any higher polymer. Conversely, G-75 should give useful size information up to the eicosamer.

It is also evident from the data that comparison on one type of Sephadex of the different states of terminal phosphorylation yields the order *none* > 5′ (*or* 3′) > 3′,5′ for the molecular weight at any K_d value. An explanation might be that phosphate ester groups are associated with vicinal water molecules which are tightly enough bound to give the effect of a higher molecular weight in solution.

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A simple device for applying solutions to non-bound preparative thin-layer chromatographic plates

MOTTIER AND POTTERAT'S[1] method of covering glass plates with dry adsorbent has been used by MISTRYUKOV[2,3], ČERNÝ *et al.*[4] and VACÍKOVÁ *et al.*[5] to make preparative thin-layer chromatographic plates. The preparation of non-bound plates is less cumbersome and quicker than that of preparative bound plates. No special applicator is required to spread the adsorbent, and no need exists for the somewhat lengthy drying procedure[6] which is required to avoid cracking of bound plates of sufficient thickness for separations on a preparative scale.

The most tedious operation in the use of non-bound plates has been the application of the substrate solution. Micropipettes have been used commonly, but with these it is difficult to apply the solution in a uniform thin line. MORGAN'S[7] device for obtaining a series of spots by a row of capillary tubes was tried in this laboratory, but the tubes tended to become clogged with adsorbent. RITTER AND MEYER[8] have reported an apparatus with which the solution is placed onto the adsorbent from a hypodermic needle as the plate is moved slowly back and forth. This procedure was not satisfactory in our hands. A device consisting essentially of a wedge has been described[9] for the application of microliter quantities of solution to electrophoresis paper. A simpler and readily assembled wedge of greater capacity is described in this note.

Fig. 1. Side view of the applicator.