:HROM. 4263

THIN-LAYER CHROMATOGRAPHY OF MALTO-OLIGOSACCHARIDES*

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(Received July 10th, 1969)

SUMMARY

Procedures for the separation, location and elution of malto-oligosaccharides are described. Best separations were obtained on glass plates (20 × 46 × 0.5 cm) coated with Kieselguhr G, serially developed in solvents of varying proportions of 1-butanol, pyridine and water. Major zones were temporarily visualized by exposing the plates to iodine vapors. The located zones were eluted from the thin-layer plates into fiberglass filter pieces with water. Radioactivity of the zone can be determined directly by placing the filter in a toluene-based scintillation fluid and counting in a scintillation counter. After radioactivity determination, the carbohydrates can be eluted from the filter with water and quantitatively measured.

INTRODUCTION

A procedure for the separation, recovery and determination of both carbohydrate content and radioactivity of malto-oligosaccharides was required as part of a study of *in vivo* starch synthesis[1]. Paper chromatographic separations require several days, and generally are only capable of separating malto-oligosaccharides of degree of polymerization (DP) below 10 (*cf.* refs. 2 and 3). Several thin-layer chromatographic procedures have been reported[4–6]; but these procedures have been designed primarily for the separation and qualitative or quantitative measurement of the fractions directly on the thin-layer plate[5]. This communication describes a thin-layer chromatographic system suitable for the separation of malto-oligosaccharides with DP of up to 20–25. A convenient procedure for the location and elution of zones will be described.

* Purdue Agricultural Experiment Station Journal Paper No. 3711.
** This study was begun when one of us (J.C.S.) was a visiting professor of Horticulture at The Pennsylvania State University, University Park, Pa.

MATERIALS AND METHODS

Equipment

The Shandon 500 Chromatank* chambers were obtained from Colab Laboratories, Inc., Chicago Heights, Ill. Carbohydrates were streaked on the thin-layer plates with a Micro Spray Pistol obtained from Brinkmann Instruments, Inc., Westbury, N.Y. A fixed thickness applicator was also obtained from Brinkmann Instruments, Inc.

Materials

Kieselguhr G was a product of E. Merck, Darmstadt, G.F.R. Solvents were of reagent grade and used as received. The malto-oligosaccharide mixture, prepared by partial acid hydrolysis of amylose, was a gift from Dr. H. F. ZOBEL.

Procedures

A Kieselguhr G layer 250 μ thick was spread on glass plates 20 × 46 × 0.5 cm. The plates were allowed to dry at room temperature overnight prior to use. The malto-oligosaccharide mixture was solubilized in 90% dimethylsulfoxide (DMSO) and aliquots (0.5–20 mg) were applied as narrow streaks 2 cm above the lower edge

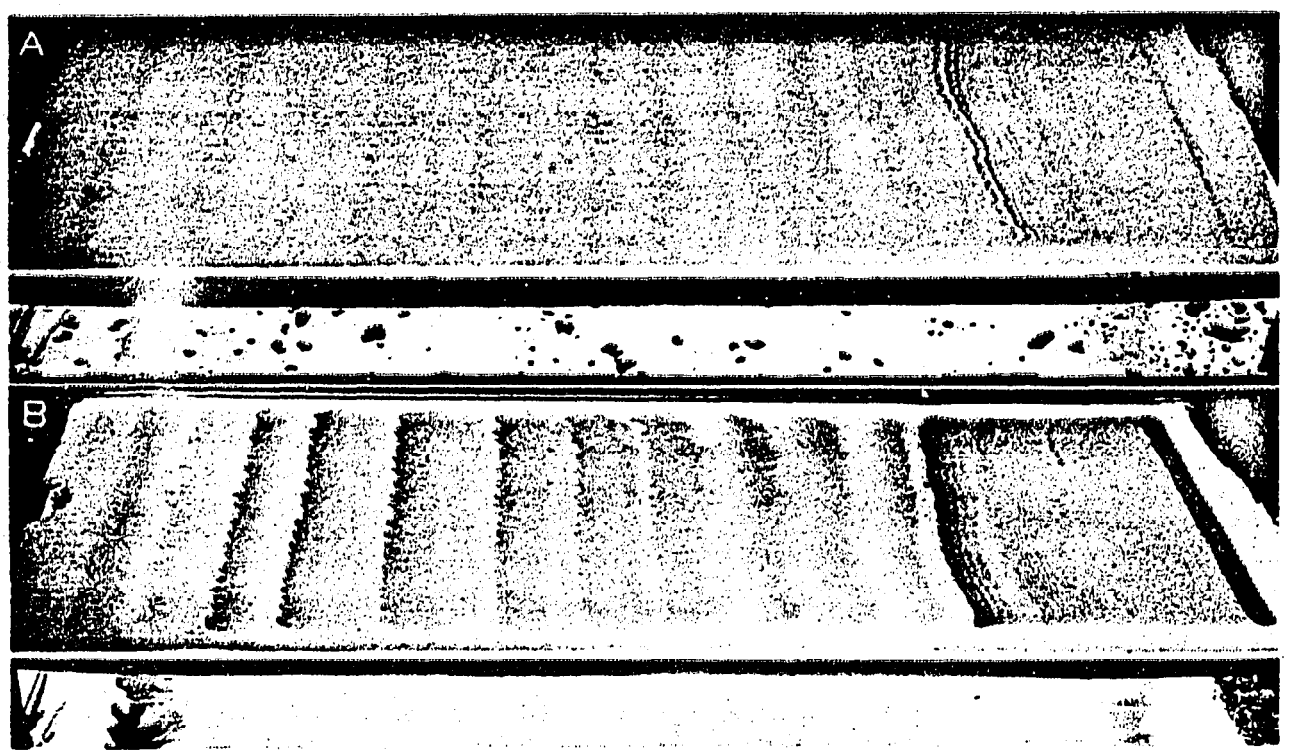

Fig. 1. (A). Developed thin-layer chromatogram exposed to iodine vapors. A 6 mg sample of malto-oligosaccharides was separated as described for the large plates in Table II. (B). The plate pictured in (A) after spraying with 20 ml of detection reagent (18 ml of 95% ethanol, 1.0 ml concentrated H_2SO_4 and 1.0 ml of anisaldehyde) and heating at 100° for 25 min[4].

* Mention of a trademark name, proprietory product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.

of the plate. The plates were developed in solvents containing various proportions of 1-butanol, pyridine and water as will be noted in the results.

The malto-oligosaccharide zones were visualized by spraying with 20 ml of anisaldehyde–H_2SO_4–ethanol (1:1:18) spray reagent and heating at 100° for 15 to 30 min[4]. The malto-oligosaccharides so detected could not be eluted for the determination of radioactivity and carbohydrate contents. In order to elute the carbohydrate zones from the plates the zones were located by placing the plate, adsorbent side down, over a glass tray containing iodine crystals (Fig. 1A). The malto-oligosaccharides complex with the iodine vapors more rapidly than the Kieselguhr, and thus show up as light yellow bands across the plate which can be marked on the back of the glass. The zone should contain at least 100 μg of malto-oligosaccharides in order to show up well. When the plate is removed from the presence of the iodine vapors, the color disappears rapidly and the malto-oligosaccharides are left unchanged.

The malto oligosaccharides can be eluted from the plate by first removing a streak of Kieselguhr 0.5–1.0 mm wide from between each zone. Place the plate in a humid chamber. The malto-oligosaccharides can be chromatographed off the plate with water and into Whatman GF-82 fiberglass paper pieces (1.5 mm × 4 cm) (Fig. 2).

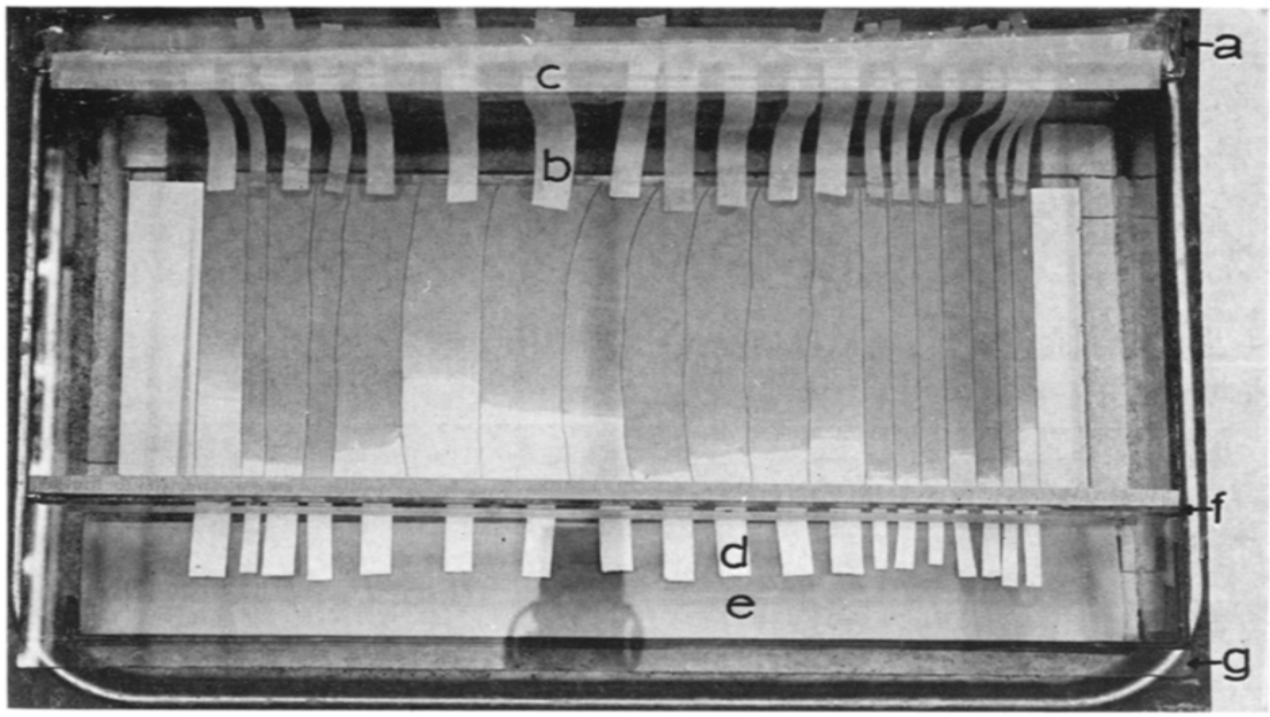

Fig. 2. Elution apparatus (51 cm long × 37 cm wide × 10 cm high) was constructed from 3 mm thick window glass held together with polyethylene tape. Water is carried by capillarity from a chromatograph trough (a) to the zones by wicks (b) cut from Whatman No. 54 chromatograph paper. Strips of teflon are taped to the cover glass (c) and top of the wall (not shown) where they contact the wicks. Fiberglass filter pieces (d) are placed on a glass plate covered with a sheet of teflon (e). The teflon is used to inhibit any transfer of compound from the paper. A movable glass piece (f) with a strip of teflon on the lower edge holds the filters firmly in contact with the chromatograph zones and also makes it possible to maintain high humidity around the chromatogram even if the cover glass (g) above the fiberglass filters is removed to allow drying. To aid in maintaining high humidity a shallow pan of water (not shown) was placed directly below the thin-layer chromatogram. The entire apparatus is kept in a metal tray to facilitate handling.

TABLE I

R_P VALUES OF MALTO-OLIGOSACCHARIDES SEPARATED BY THIN-LAYER CHROMATOGRAPHY

Glass plates 5 × 20 cm and 20 × 45 cm were coated with a 250 μ thick layer of Kieselguhr G. The malto- natively narrow streaks 16 cm long were applied to the large plates. All solvent compositions are given

Solvents	*Degree of polymerization*									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
Small plates										
70:20:10	0.86	0.56	0.25	0.08	0.03	0.01				
60:30:10	0.90	0.76	0.57	0.37	0.18	0.10	0.06	0.04	0.03	
50:40:10	0.92	0.83	0.77	0.53	0.31	0.19	0.12	0.08	0.05	
40:50:10	0.94	0.90	0.81	0.70	0.52	0.36	0.25	0.18	0.12	0.10
65:20:15[d]	0.89	0.78	0.60	0.39	0.20					
50:40:20[d]	0.93	0.89	0.84	0.78	0.70	0.63	0.55	0.44	0.36	0.30
60:20:20[c]	0.90	0.82	0.70	0.54	0.37	0.23	0.16	0.10	0.07[b]	0.03
50:30:20	0.93	0.90	0.86	0.79	0.70	0.61	0.51	0.39	0.29	0.20
45:35:20	0.95	0.91	0.87	0.81	0.74	0.67	0.61	0.53	0.44	0.35
40:40:20	0.96[a]	0.93[a]	0.90[a]	0.85	0.80	0.73	0.65	0.54	0.44	0.34
35:45:20	0.96[a]	0.94[a]	0.89[a]	0.85	0.79	0.73	0.68	0.59	0.50	0.41
30:50:20	0.96[a]	0.92[a]	0.90[a]	0.87	0.83	0.78	0.71	0.63	0.52	0.42
50:28:22	0.93	0.88	0.82	0.75	0.67	0.58	0.50	0.39	0.31	0.23
45:33:22	0.97	0.92	0.86	0.80	0.74	0.69	0.64	0.58	0.50	0.44
40:38:22	0.93[a]	0.89[a]	0.84	0.81	0.77	0.73	0.68	0.62	0.55	0.49
39:39:22	0.95[a]	0.90[a]	0.85	0.82	0.78	0.73	0.68	0.62	0.57	0.50
30:48:22	0.93[a]	0.90[a]	0.85[a]	0.81	0.78	0.73	0.66	0.61	0.53	0.45
42:34:24	0.96[a]	0.91[a]	0.87	0.83	0.78	0.75	0.70	0.65	0.59	0.52
40:36:24	0.92	0.88	0.83	0.80	0.76	0.73	0.68	0.64	0.60	0.55
38:38:24	0.92	0.87	0.82	0.77	0.75	0.67	0.60	0.54	0.49	0.44
50:20:30[c]	0.88	0.81	0.67	0.49	0.31	0.25	0.18	0.14	0.11	0.08
40:30:30	0.94	0.90	0.86	0.82	0.79	0.74	0.68	0.62	0.55	0.49[b]
60:40:30[d]	0.92	0.90	0.84	0.77	0.71	0.64	0.57	0.49	0.39	0.31
Large plates										
45:35:20	0.94	0.90	0.86	0.81	0.77	0.69	0.61	0.51	0.42	0.34
40:38:22	0.93	0.90	0.86	0.81	0.75	0.70	0.62	0.53	0.45	0.37
65:20:15[d]	0.87	0.77	0.61	0.42	0.24	0.14	0.09	0.05	0.03	0.02
60:40:30[d]	0.91	0.87	0.83	0.77	0.69	0.61	0.53	0.42	0.33	0.25
50:40:20[d]	0.94	0.91	0.87	0.81	0.74	0.65	0.55	0.44	0.33	0.25

[a] Denotes detectable zones which were very close together.
[b] Very dark narrow zones composed of several different size malto-oligosaccharides. Thus all zones
[c] These solvents separated into two phases during chromatography.
[d] These solvents were used in the earlier study and are included for comparison.

RESULTS AND DISCUSSION

WEILL AND HANKE[4] reported the separation of malto-oligosaccharides up to DP 9 on thin-layer plates coated with Kielselguhr G. One of their better solvents was composed of 1-butanol–pyridine–water (13:4:3). In our laboratory this solvent separated malto-oligosaccharides of DP 5 or smaller but the larger components remained near the origin. These same workers also reported the separation of malto-oligosaccharides to DP 10 with a 1-butanol–ethanol–water (5:3:2) solvent. We found that this system resolved components to DP 7; but the larger malto-oligosaccharides appeared as a smear up the lower half of the plate. HUBER *et al.*[6] reported the separation of megalosaccharides up to about DP 35 using continuous TLC. In this separation components smaller than DP 19 were chromatographed off the plate. Their optimum

oligosaccharides, dissolved in 90% dimethylsulfoxide, were applied as a narrow band 1 cm long. Alter- in the order 1-butanol–pyridine–water.

11	12	13	14	15	16	17	18	19	20	21	22	23
0.07	0.06	0.04	0.03									
0.22	0.17	0.14	0.09	0.06[a]	0.05[a]	0.03[a]	0.02[a]					
0.02												
0.15	0.10[a]	0.07[a]	0.05[a]	0.04[a]	0.03[a]							
0.28	0.22	0.16	0.13	0.10	0.08	0.07						
0.27	0.19	0.15	0.11[a]	0.08[a]	0.06[a]	0.06[a]						
0.34	0.26	0.20	0.16	0.12	0.09	0.08						
0.34	0.26	0.20	0.15[a]	0.12[a]	0.09[a]							
0.19[b]	0.16	0.14	0.10	0.08	0.05							
0.35	0.29	0.22	0.18	0.15	0.12	0.10[a]	0.09[a]					
0.42	0.36	0.29	0.24	0.20	0.16							
0.41	0.35	0.28	0.22	0.17	0.12	0.10[a]	0.08[a]					
0.37	0.31	0.25	0.20	0.15								
0.44	0.35	0.28	0.22	0.16	0.13[a]	0.11[a]	0.09[a]					
0.48	0.41	0.34	0.27	0.22	0.18	0.15[a]	0.14[a]	0.13[a]				
0.38	0.34	0.29	0.25	0.21								
0.47[a]	0.38[a]	0.33[a]	0.28[a]	0.23[a]	0.19[a]	0.16[a]						
0.23	0.21	0.19	0.15	0.12	0.09	0.07						
0.28	0.23	0.18	0.15	0.12	0.10	0.08	0.04					
0.31	0.26	0.22	0.17	0.12	0.10	0.08	0.06	0.05	0.04	0.04	0.03	0.03
0.01												
0.18	0.17[b]	0.13	0.10	0.07	0.06[a]	0.05[a]	0.04[a]	0.02[a]				
0.19	0.14	0.10	0.08	0.04								

below this are of unknown degrees of polymerization.

separations were obtained on thin-layer plates coated with a mixture of three parts Silica Gel G and one part Kieselguhr G. A solvent system of 1-propanol–nitromethane–water (5:2:3) was considered to be the best. When this system was tested in our laboratory there was incomplete separation and considerable smearing between zones.

Preliminary studies indicated that malto-oligosaccharides larger than DP 12 could be separated with 1–butanol–pyridine–water solvents in ratios of (6:4:3) and (5:4:2). We have reported partial separations of malto-oligosaccharides derived from pullulanase digestion of β-amylase limit dextrins by using long plates (45 × 20 cm) and multiple developments[1]. The plates were first developed 23 cm in 1-butanol–pyridine–water (5:4:2), then to 32–34 cm in (6:4:3) and finally to 43–44 cm in (13:4:3). The plates were completely dried between each development. During the above study[1] we noticed that quite often the components DP 13 to DP 17 were

TABLE II

R_P VALUES OF MALTO-OLIGOSACCHARIDES SEPARATED BY UNIDIMENSIONAL MULTIPLE THIN-LAYER CHROMATOGRAPHY

Small plates prepared as described in Table I were developed their entire length 1, 2 or 4 times in 1-buta- once 25 cm in (39:39:22); once 30 cm in (50:28:22) and finally 40 cm in (70:20:10). All plates were dried

Solvent	*Times developed*	*Degree of polymerization*											
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*[c]	*8*	*9*	*10*	*11*
Small plates													
40:40:20	1	0.92[a]	0.89[a]	0.84	0.80	0.75	0.69	0.61	—	0.53	0.44	0.34	0.27
40:40:20	2	—[b]	—[b]	0.99[a]	0.98[a]	0.96[a]	0.94[a]	0.91	0.86	0.85	0.76	0.65	0.56
40:40:20	4	—[b]	—[b]	—[b]	0.96[a]	0.95[a]	0.94[a]	0.93[a]	0.92	0.90	0.85	0.79	0.71
Large plates													
Plate 1		0.95	0.89	0.83	0.75	0.65	0.58	0.49	—	0.42	0.35	0.29	0.25
Plate 2		0.87	0.84	0.74	0.67	0.59	0.53	0.46	—	0.41	0.35	0.30	0.26

[a] Same as in Table I.
[b] Zones crowded together near the front.
[c] Relative R_P value of a DP 8 standard chromatogramed on the same plate.

concentrated into a very narrow region. In an effort to find a solvent composition which can be used for optimum separation we have tested a number of systems composed of varying proportions of 1-butanol, pyridine and water chosen from points on a tri-linear graph. These solvents were first tested on 5 × 20 cm plates coated with Kieselguhr G. Several mixtures resulted in good separations of malto-oligosaccharides (Table I). Multiple development of the small plates could be used to resolve the larger components (Table II). In an effort to obtain maximum separation on a single chromatogram, large plates were serially developed in several solvent combinations. One of the better separations utilized two developments (18 and 25 cm) with a solvent shown to move the larger malto-oligosaccharides followed by development 2/3 of the distance with a solvent capable of separating the intermediate size components. The final full plate development separated the smallest compounds (Table II and Fig. 1).

If the separated malto-oligosaccharides are to be removed from the Kieselguhr G, a non-destructive method for the location of the zones is required. Iodine vapors

TABLE III

RECOVERY OF MALTOSE FROM THIN-LAYER CHROMATOGRAM PLATES

Maltose (0.5–4.0 mg) was streaked on a 20 × 20 cm thin-layer plate. After drying 2 × 20 cm zones containing the maltose were eluted with water into fiberglass filters. The maltose was then eluted from the filters into 10 ml scored tubes and the quantity recovered measured by the phenol–H_2SO_4 procedure[9].

Maltose added (mg)	*Maltose found (mg)*	*Recovered (%)*
0.0	0.04[a]	
0.5	0.45	90.0
1.0	0.87	87.0
2.0	1.83	91.5
4.0	3.99	99.7

[a] This amount in the blank was substracted from each standard sample.

nol–pyridine–water solvents. The large plates were serially developed once 18 cm in (39:39:22); between developments

12	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*
0.20	0.15	0.12	0.09	0.08											
0.44	0.35	0.27	0.22	0.16	0.13	0.10[a]	0.09[a]	0.07[a]							
0.62	0.50	0.40	0.28	0.22	0.15	0.12[a]	0.10[a]	0.08[a]	0.07[a]	0.06[a]					
0.25	0.24	0.24	0.23	0.22	0.21	0.20	0.19	0.19	0.18	0.18	0.17	0.16	0.16	0.15	0.12
0.25	0.24	0.23	0.23	0.21	0.19	0.17	0.16	0.14	0.13	0.11	0.09	0.07	0.06	0.04	0.02

have been used to visualize temporarily amino acids on paper chromatograms[7] and steroids on thin-layer chromatograms[8]. The malto-oligosaccharide zones complexed with the iodine vapors when sufficient carbohydrate was present (4–20 mg on a 45 × 20 cm plate). The zones appear as faint yellow bands and are best seen when viewed with light from below (Fig. 1A).

Once the zones are located they can be conveniently eluted from the plate into pieces of chromatograph paper or fiberglass filters (Fig. 2). Maltose and malto-oligosaccharides essentially move with the water front and are completely transferred to the filter in one development (Tables III and IV). Thin-layer chromatograms con-

TABLE IV

RECOVERY OF MALTO-OLIGOSACCHARIDES FOLLOWING CHROMATOGRAPHY

Samples of a mixture of malto-oligosaccharides (4–20 mg) were streaked on 20 × 45 cm thin layer plates. The chromatograms were serially developed once to 23 cm in the 1-butanol–pyridine–water (50:40:20) solvent, once to 33 cm in the (60:40:30) solvent and once to 41 cm in the (65:20:15) solvent. The zones were detected with iodine vapor. The Kieselguhr was scored between each zone and the malto-oligosaccharides eluted into fiberglass filters with water until saturated and then removed. New filters were added for the second elution. For the third elution the zones were scraped off the plate and put in 10 ml scored tubes. Water was added and heaten 20 min in a boiling water bath. After centrifugation an aliquot was taken for carbohydrate determination. The total carbohydrate content was measured as described in Table III.

Malto-oligosaccharide added (mg)	*Malto-oligosaccharide recovered in all zones*							
	1st elution		*2nd elution*		*3rd elution*		*Sum*	
	(mg)[a]	*%*	*(mg)*[a]	*%*	*(mg)*[a]	*%*	*(mg)*[a]	*%*
4.0	3.21	80.3	0.02		0	0	3.23	80.7
6.0	5.24	87.3	0.40		0	0	5.64	94.0
10.0	8.34	83.4	1.80	18.0	0	0	10.14	101.0
20.0	16.49	82.4	1.66	8.3	0.27	1.3	18.42	92.1

[a] All samples were corrected for the slight contamination in the Kieselguhr G. A zone above the front was eluted and used as a blank.

taining between 4 and 20 mg of malto-oligosaccharide mixture were developed by a three-development system. The zones were visualized with iodine and eluted with water. Over 80% of the carbohydrate was recovered in the first elution (Table IV). The small amount of carbohydrate not eluted the first time was confined to the origin. With such a system one can measure the radioactivity of a zone by placing the dried filter, plus carbohydrate, in a toluene based scintillation fluid and counting as previously described[1]. After counting, the filter can be removed, and dipped twice in toluene to remove the scintillators. The carbohydrates can be eluted from the filter with water and quantitatively measured[1].

ACKNOWLEDGEMENT

The authors are grateful to Mrs. LOUANNA BATEY for her excellent technical assistance.

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