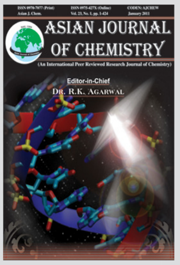
Asian Journal of Chemistry; Vol. 23, No. 6 (2011), 2597-2599

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Determination of Inorganic Anions in *Eucheuma cottonii* and Carrageenan by Suppressed Ion Chromatography

REFILDA SUHAILI[1], EDISON MUNAF[1,*], RAHMIANA ZEIN[1], ANANG SEDYOHUTOMO[2], LEE WAH LIM[2] and TOYOHIDE TAKEUCHI[2,*]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Andalas University, Padang 25163, Indonesia
[2]Department of Chemistry, Faculty of Engineering, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

*Corresponding authors: E-mail: emunaf@fmipa.unand.ac.id; take-t@gifu-u.ac.jp

(*Received*: 28 July 2010; *Accepted*: 14 February 2011) AJC-9618

Inorganic anions in *Eucheuma cottonii* (a species of red algae) and carrageenan were determined by ion chromatography with a packed-column suppressor system. Two packed-column suppressors were connected in series *via* a 10-port switching valve for continuous chromatographic runs. Sodium carbonate-bicarbonate solution was used as the mobile phase and its background conductivity was reduced significantly by the suppressor, leading to achievement of lower detection limits. F^-, Cl^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-} and I^- contained in *Eucheuma cottonii* and carrageenan were determined within 15 min. The detection limits obtained by the present method were 2.7-12 nM for these anions.

Key Words: *Eucheuma cottonii*, Carrageenan, Common inorganic anions, Ion chromatography, Packed column suppressor.

INTRODUCTION

The oceans cover 71 % of the earth's surface and include an abundance of fauna and flora. The chemical composition of seawater is quite constant, but the nutrient levels for the plants in sea water are extremely variable.

Eucheuma cottonii are harvested throughout the world as food sources as well as an export commodity for the production of agar and carrageenan products. F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-} and I^- are minor essential elements in food. Deficiencies of these essential elements can cause poor health. These ions also work as an indicator for the food quality[1].

Since its introduction by Small *et al.*[2], ion chromatography has been developed as a mature tool to determine ionic species not only in environmental and drinking waters, but also in food and other complex samples. In ion chromatography, a suppressor system is commonly used for a sensitive detection of ions on the basis of their electrical conductance. Its function is to chemically reduce the background conductivity of the electrolytes of the eluent before it enters the conductivity cell. Suppressors are used in conjunction with conductivity detectors to improve detection sensitivity, especially for anion analysis. Various types of suppressor systems have been used for anion determinations, involving packed-bed[2], hollow-fiber[3], micro-membrane[4] and electrodialytic suppressors[5].

Packed-column suppressors suffer from a number of disadvantages, involving the need for off-line regeneration, band-broadening occurring in the suppressor resulting in loss of chromatographic efficiency and variable retention in the suppressor column due to ion-exclusion effects. In spite of the above disadvantages, packed-bed suppressors are more rugged and reliable than other suppressors. We have developed a simple packed-column suppressor unit using two monovalent cation exchange guard columns, which have sulfonate functional groups and relatively high ion exchange capacity of *ca.* 2.0 ± 0.2 eq./L. With an aid of a ten port-switching valve, regeneration of the suppressors could be carried out on-line by passing an appropriate acidic solution to replace the accumulated eluent cations and thus allowing continuous suppression[6]. This easy-setup suppressor unit achieved sub-ppb to ppb level limits of detection (S/N = 3) and is comparable to the commercially available electrical anion-self-regenerating suppressors.

The purpose of the present research is to simultaneously determine inorganic anions such as F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-} and I^- in *Eucheuma cottonii* and carrageenan by using two packed-column suppressors connected *via* a 10-port switching valve to allow continuous determination. The proposed suppressed-IC system achieved higher selectivity, shorter analysis time, lower quantitation and detection limits *via* optimization of the chromatographic parameters for routine analysis.

EXPERIMENTAL

Analytical reagent grade chemicals were purchased from Nacalai Tesque (Kyoto, Japan), unless otherwise noted. Standard solutions (0.3 mM for each anion) were prepared by dissolving

NaF, $NaHPO_4$, $NaNO_2$, NaCl, NaBr, NaI, $NaNO_3$ and Na_2SO_4 by using deionized water. All standard solutions were stored in polyethylene containers and kept under refrigeration at *ca.* 4 ºC. The deionized water used throughout this research was prepared in the laboratory using a GS-590 water distillation system (Advantec, Tokyo, Japan). Na_2CO_3 and $NaHCO_3$ were obtained from Wako (Osaka, Japan) and used as the eluent. The eluent was prepared daily before use. The pH of the carbonate/bicarbonate eluent was measured with an IM-20E ion meter (Toa Electronics, Tokyo, Japan). Membrane filters (0.45 μm) for IC were obtained from GL Sciences (Tokyo, Japan). Samples were filtered with the membrane filter before injection to the chromatographic system.

Samples: A 40-day old *Eucheuma cottonii* was harvested from the sub-province of Nangro Aceh Darussalam, Indonesia. The alga was extensively washed with distilled water to remove the particulate material from their surface and was dried under the sunlight. The dried biomass of red alga was cut, ground and then screened to particle sizes of 150-425 μm. Carrageenan was extracted from red algae by alkaline solution. Dried red algae and carrageenan were digested with nitric acid and hydrogen peroxide, followed by filtration with a 0.45-μm membrane filter. The solutions were then stored in polyethylene containers under refrigeration at 4 ºC.

Ion chromatography system: The ion chromatograph consisted of PU 980 and PU-2080i plus HPLC pumps (Jasco, Tokyo, Japan), a Rheodyne Model 5095 injector equipped with a 20 μL sample loop (Cotati, CA, USA), a CM-8020 conductivity detector (Tosoh, Tokyo, Japan), a Model 7610-600 10-port switching valve (Rheodyne) and a Computer-Aided-Chromatography data processor (Nippon Filcon, Tokyo, Japan). The separation column employed was a TSKgel IC-Anion-PW_{XL} column (Tosoh; 50 mm × 4.6 mm i.d.) and two suppressor columns, *i.e.*, TSK guard columns (50 mm × 4.6 mm i.d.), obtained from Tosoh (Tokyo, Japan). The cation-exchange resins of the guard columns possess sulfonate functional groups and relatively high ion exchange capacity of *ca.* 2.0 ± 0.2 eq./L. While one suppressor was being used for chromatographic run, the other suppressor was being regenerated with 0.1 M sulfuric acid (Wako) at a flow rate of 2.0 mL/min by using the PU-2080i plus HPLC pump. The regenerated suppressor was then rinsed with deionized water for future chromatographic run.

Operating conditions: The mixture of Na_2CO_3 and $NaHCO_3$ was used as the eluent for the determination of anions, as commonly employed in ion chromatography. The system was operated under the isocratic mode. To find the optimum conditions for the anions of interest, parameters such as eluent flow-rate and eluent concentration were optimized. The optimum condition was then applied to the determination of inorganic anions in *Eucheuma cottonii* and carrageenan.

RESULTS AND DISCUSSION

Effect of sodium carbonate and sodium bicarbonate concentration on anions separation: The concentration ratio of sodium bicarbonate and sodium carbonate solutions was evaluated for the separation of anions on the TSKgel IC-Anion column. The retention times and resolutions of the analyte ions were strongly dependent on the concentration of the eluent. Sodium carbonate and sodium bicarbonate concentrations investigated were 1.0-1.75 mM. When the concentrations of carbonate and bicarbonate were 1.0 and 1.0 mM, respectively, the resolution was not good. On the other hand, when the concentration s of carbonate and bicarbonate were 1.5 and 1.75 mM, respectively, the resolution of anions was good, but the inlet pressure at 0.7 mL/min was higher than 8 MPa (recommended maximum pressure) and the first eluted anion peak (F^-) was interfered with the system peak (water dip). Operations at higher inlet pressures would shorten the lifetime of the columns. Considering these situations, the optimum eluent concentrations were 1.2 and 1.4 mM for carbonate and bicarbonate, respectively.

Fig. 1 shows the separation of eight anions under the three different conditions. It can be seen that the resolution of the chromatogram obtained under the optimum condition (B in Fig. 1) is better than others and that resolutions of the analyte anions are not satisfactory under the conditions in Figs. 1A and 1C. The retention order under the optimum condition was F^-, Cl^-, NO_2^-, Br^-, NO_3^-, HPO_4^{2-}, SO_4^{2-} and I^-.

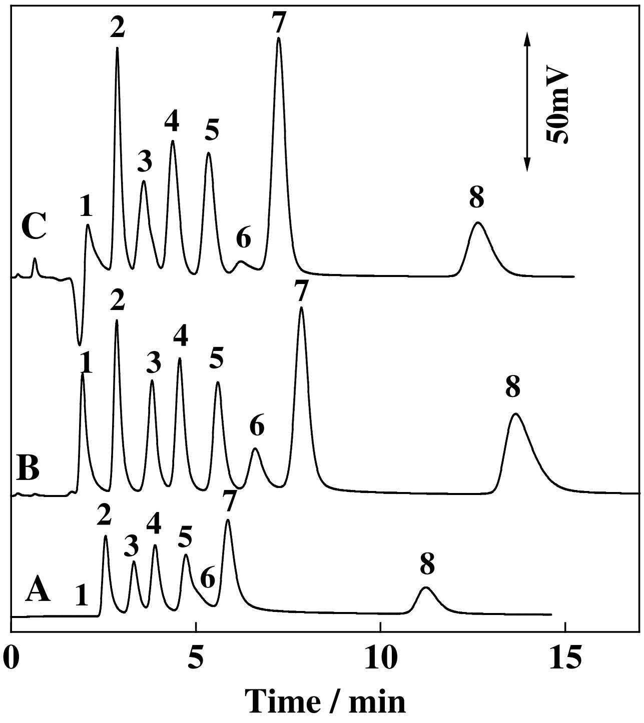


Fig. 1. Effect of Na_2CO_3-$NaHCO_3$ concentration on anion separation. Flow rate, 0.7 mL/min. Concentrations of Na_2CO_3-$NaHCO_3$, A = 1.0-1.0; B = 1.2-1.4; C = 1.5-1.75 mM. Column, TSKgel IC-Anion PW_{XL} (50 × 4.6 mm i.d.). Column temperature, room temperature. Injection volume, 20 μL. Peak, 1 = F^-; 2 = Cl^-; 3 = NO_2^-; 4 = Br^-; 5 = NO_3^-; 6 = HPO_4^{2-}; 7 = SO_4^{2-}; 8 = I^-

Repeatability: Table-1 shows the repeatability of the signal for five successive measurements under the optimum conditions. All of the relative standard deviation (RSD) values for retention time, peak area and peak height were smaller than 1 %. As demonstrated in Table-1, the present system can provide reproducible data.

Calibration curves and detection limits: The linearity and detection limits data are summarized in Table-2. The

TABLE-3
CONCENTRATIONS OF ANIONS CONTAINED IN PURE CARRAGEENAN, EXTRACTED CARRAGEENAN and *Eucheuma cottonii*. OPERATING CONDITIONS AS IN Fig. 2

Samples	Anion contained (mg/100 g)							
	F^-	Cl^-	NO_2^-	Br^-	NO_3^-	HPO_4^{2-}	SO_4^{2-}	I^-
Pure carrageenan	1.75	16.6	1.28	5.22	(445)	8.64	19.2	11.7
Extracted carrageenan	1.80	17.8	0.89	5.10	(442)	8.02	25.1	10.1
Eucheuma cottonii	2.33	10.0	1.71	7.77	(449)	7.99	22.1	15.1

TABLE-1
RELATIVE STANDARD DEVIATIONS (RSDS) OF THE RETENTION TIME AND THE PEAK SIGNALS OF THE INORGANIC ANIONS UNDER THE OPTIMUM CHROMATOGRAPHIC CONDITIONS. EXPERIMENT CONDITIONS WERE SAME AS FIG. 1b

Anion	RSDs (%), n = 5		
	t_r*	Peak height	Peak area
F^-	0.00	0.12	0.38
Cl^-	0.21	0.17	0.32
NO_2^-	0.16	0.28	0.34
Br^-	0.13	0.22	0.39
NO_3^-	0.17	0.25	0.24
HPO_4^{2-}	0.13	0.15	0.44
SO_4^{2-}	0.20	0.28	0.14
I^-	0.10	0.27	0.31

*Retention time.

TABLE-2
SUMMARIZED DATA FOR THE STANDARD CALIBRATION GRAPHS, COEFFICIENT CORRELATION (r^2), RETENTION TIME AND DETECTION LIMITS AT S/N = 3 OF THE ANIONS OBTAINED UNDER THE OPTIMUM OPERATING CONDITIONS. EXPERIMENT CONDITIONS WERE SAME AS FIG. 1b

Anion	[Anion] (mM)	r^2	t_r* (min)	LOD (nM)
F^-	0.1-0.5	0.9958	0.84	39
Cl^-	0.1-0.5	0.9999	2.80	28
NO_2^-	0.1-0.5	0.9987	3.80	44
Br^-	0.1-0.5	0.9995	4.60	37
NO_3^-	0.1-0.5	0.9995	5.61	45
HPO_4^{2-}	0.1-0.5	0.9997	6.70	119
SO_4^{2-}	0.1-0.5	0.9997	8.00	27
I^-	0.1-0.5	1.0000	13.7	61

*Retention time.

calibration curves between the anion peak height and the sample concentration ranged from 0.1-0.5 mM showed good linear relationships with the correlation coefficients (r^2) varied between 0.9958 and 1.0000. Good linearity of the present method can be seen from the table. The separation was completed in 15 min.

Determination of inorganic anions in *Eucheuma cottonii* and carrageenan: The present suppressed ion chromatography system was applied to the determination of inorganic anions in *Eucheuma cottonii* as well as in pure and extracted carrageenan samples. Each sample was injected after filtration with a 0.45 μm membrane filter.

Good separations of inorganic anions were also achieved for the real samples (Fig. 2). The amounts of anions contained in the samples were determined and summarized in Table-3. Table-3 shows the concentrations of anions contained in pure carrageenan, extracted carrageenan and *Eucheuma cottonii*. All of the examined anions except for NO_3^- contained in these samples were determined to be 0.9-25 mg/100 g of the samples.

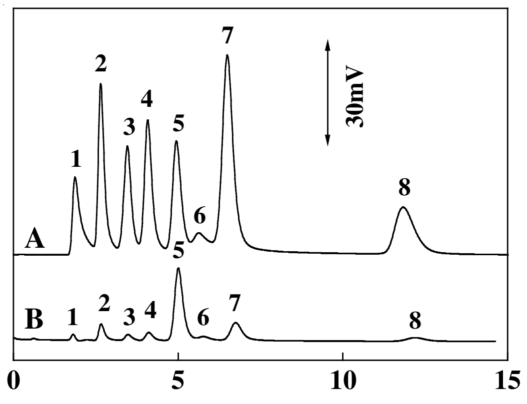


Fig. 2. Separation of inorganic anions in (A) *Eucheuma cottonii* and (B) standard solution. 0.3 mM. Eluent, mixture of 1.2 mM Na_2CO_3 and 1.4 mM $NaHCO_3$. Concentration of standard anions, 0.3 mM each. Other operating conditions are same as in Fig. 1

It is found that the concentration of NO_3^- is much higher than other anions. This is because the samples were treated with nitric acid.

Conclusion

A packed-bed suppression system using a 10-port switching valve was designed for easy and rapid determination of inorganic anions in ion chromatography. By connecting two packed-bed suppressors in series *via* a 10-port switching valve, low background conductivity could be maintained during the operation and it allowed continuous determination by column switching. Seven common inorganic anions (F^-, Cl^-, NO_2^-, Br^-, HPO_4^{2-}, SO_4^{2-}, I^-) could be determined by the present method. The mixture of 1.4 mM sodium bicarbonate and 1.2 mM sodium carbonate was suitable for the determination of the above inorganic anions. The relative standard deviations for the retention time, peak area and peak height of the eight common inorganic anions were acceptable. The present system was satisfactorily applied to the determination of common anions in *Eucheuma cottonii* and carrageenan within 15 min.

REFERENCES

1. A. Csikkel-Szolnoki, M. Báthori and G. Blunden, *Microchem. J.*, **67**, 39 (2000).
2. H. Small, T.S. Stevens and W.C. Bauman, *Anal. Chem.*, **47**, 1801 (1975).
3. T.S. Stevens, J.C. Davis and H. Small, *Anal. Chem.*, **53**, 1488 (1981).
4. J. Stillian, *LC Magazine*, **3**, 802, 806, 808, 812 (1985).
5. D.L. Strong, P.K. Dasgupta, K. Friedman and J.R. Stillian, *Anal. Chem.*, **63**, 480 (1991).
6. L.W. Lim, S. Suenaga, T. Hino and T. Takeuchi, Development of a Simple Packed-Column Suppressor Unit for Conventional ion Chromatography System and its Application to the Determination of Major Anions in Environmental Waters, Presented at the the 3rd China-Japan-Korea Joint Ion Analysis Symposium, Jeju Pacific Hotel, June 10-12 (2009).