

This article was downloaded by:

On: 24 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

### Selection of Adsorbents for the Separation of Chlorophylls

Keiji Iriyama<sup>a</sup>; Masahiko Yoshiura<sup>a</sup>

<sup>a</sup> Division of Biochemistry, The Jikei University School of Medicine, Minato-ku, Tokyo, Japan

**To cite this Article** Iriyama, Keiji and Yoshiura, Masahiko(1982) 'Selection of Adsorbents for the Separation of Chlorophylls', *Journal of Liquid Chromatography & Related Technologies*, 5: 11, 2211 – 2216

**To link to this Article:** DOI: 10.1080/01483918208067629

**URL:** <http://dx.doi.org/10.1080/01483918208067629>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

SELECTION OF ADSORBENTS  
FOR THE SEPARATION OF CHLOROPHYLLS

Keiji Iriyama and Masahiko Yoshiura

*Division of Biochemistry, The Jikei University School of Medicine,  
3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105, Japan*

ABSTRACT

Selection of adsorbents for the separation of chlorophylls has been conventionally attempted. It has been confirmed that Sepharose CL-6B is the best adsorbent for separation of chlorophylls among the adsorbents tested in the present study.

INTRODUCTION

Much attention has been devoted to the selection of adsorbents for the separation of chlorophyll (Chl), as reviewed by Svec (1). Recently, we have developed a method for precleaning Chl in acetone extract from plant materials prior to column chromatographic separation (2,3). Subsequent separation of Chl and yellow leaf pigments from each other was attained by column chromatography on powdered sugar (4); powdered sugar is the most widely used adsorbent for separating Chl. However, preparation of a powdered

sugar column of good quality with good reproducibility requires great care. For this reason, we have developed procedures for the separation of photosynthetic pigments from each other by column chromatography on Sephadex LH-20 (5) or Sephasorb HP Ultrafine (6,7). Omata and Murata (8) have also developed procedures for the isolation of Chl by combining column chromatography on DEAE-Sepharose CL-6B and on Sepharose CL-6B. For preparative purposes, the adsorbent used should have a high capacity to adsorb Chl as well as other photosynthetic pigments.

The maximum amount (mg) of total photosynthetic pigments adsorbed on the top of a column and then separated without overlapping of pigment peaks in a column chromatogram per  $\text{cm}^3$  of adsorbent has been obtained as an index for each adsorbent to select the best adsorbent for the separation of Chl. In a preliminary study, we estimated the maximum amount ( $W_{\text{max}}$ ) for some adsorbents recently developed for the separation of Chl for preparative purposes. By employing the  $W_{\text{max}}$  in selecting the best adsorbent for the separation of Chl, it has been suggested that the values for  $W_{\text{max}}$  tabulated here are practically very useful indices to select the best adsorbent for the separation of Chl.

### EXPERIMENTAL

Höxterman (9) has confirmed, after comparative preinvestigations, that application of the dioxane method (2) is effective for extraction and precleaning of Chl. Indeed, the dioxane method has been used by several investigators (8, 10, 11). The partially purified Chl (Ppt II) prepared from fresh spinach leaves according to the dioxane method (2,4) contained chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), xanthophylls, and nonsorbed carotenes. In this study, the freshly prepared Ppt II was used as test material in obtaining the  $W_{\text{max}}$  of adsorbents to separate Chl for preparative purpose.

A powdered sugar column (2.5 x 35 cm) was prepared according to the procedures described elsewhere (4). Sephadex LH-20,

Sephasorb HP Ultrafine, DEAE-Sepharose CL-6B, and Sepharose CL-6B were purchased from Seikagaku Kogyo Co.. The bed volumes ( $\text{cm}^3/\text{1g}$  of dry adsorbent) in hexane were 1.4 and 1.3 for Sephadex LH-20 and Sephasorb HP Ultrafine, respectively. The bed volumes of DEAE-Sepharose CL-6B and Sepharose CL-6B were not determined because these adsorbents were purchased in suspensions to avoid their chemical degradation. The columns (2.5 x 25 cm) of Sephadex LH-20 and Sephasorb HP Ultrafine were prepared according to the procedures of Iriyama and Yoshiura (5) and Yoshiura *et al.* (7), respectively. The columns (2.5 x 20 cm) of DEAE-Sepharose CL-6B and Sepharose CL-6B were prepared according to the procedures of Omata and Murata (8). All the columns thus prepared were connected via a UV monitor to a fraction collector.

The values for  $W_{\text{max}}$  of Ppt II were determined in the first developing solvent system used for the separation of Chl. When the  $W_{\text{max}}$  was charged on the top of a column and the pigments charged were adsorbed completely on the column bed by washing with the first developing solvent, the complete separation of the Chl-a and Chl-b peaks in a chromatogram, as well as that of the Chl-a and Chl-b bands in the column was always performed. For this reason, the development of the column in the first developing solvent systems was continued until the complete elution of Chl-b from the columns was achieved for the respective case examined.

## RESULTS AND DISCUSSION

The values for  $W_{\text{max}}$  of the adsorbents tested are listed in Table I. The  $W_{\text{max}}$  of powdered sugar was the same as that of Sephasorb HP Ultrafine. The  $W_{\text{max}}$  of Sephadex LH-20 was smaller than that of powdered sugar. Preparation of the columns of Sephadex LH-20 and Sephasorb HP Ultrafine was much easier than the powdered sugar column. In addition, Sephadex LH-20 and Sephasorb HP Ultrafine were more uniform in size and more reproducible in

TABLE I.  
The values for  $W_{\max}$ ★  
for the four adsorbents tested

Adsorbent	Developing solvent system used	Value for the $W_{\max}$ ★ (mg of Ppt II/1 cm <sup>3</sup> of adsorbent)
Powdered sugar	10 % (v/v) diethyl ether in hexane	0.30
Sephasorb HP Ultrafine	10 % (v/v) diethyl ether in hexane	0.29
Sephadex LH-20	0.1 % (v/v) diethyl ether in hexane	0.14
Sepharose CL-6B	0.47 % (v/v) diethyl ether in hexane	1.20

★ For the explanation, see the text.

quality than powdered sugar prepared in the laboratory as an adsorbent. The  $W_{max}$  for Sepharose CL-6B was the largest among the adsorbents tested in this study. Indeed, when the separation of Chl-a and Chl-b in Ppt II was carried out according to the procedures of Omata and Murata (8), their isolation was much easier and more rapid and the yield of Chl-a and Chl-b preparations with one time chromatography were the best. When Chl-a and Chl-b were separated according to the method of Omata and Murata (8) at room temperature, it was found that Chl-a and Chl-b were gradually converted to Chl-a' and Chl-b', respectively, during the course of the column chromatographic separation on the Sepharose CL-6B column developed in the solvent system (hexane/2-propanol = 20 : 1, v/v) as they also recognized. However, when the separation was performed at 4°C or lower, thin-layer chromatographic tests according to the method of Iriyama *et al.* (12) revealed that the degradation of Chl was almost completely inhibited.

It has been found that DEAE-Sepharose CL-6B has an extremely large capacity to adsorb Ppt II (2.46 mg of Ppt II/1 g of adsorbent) and also that separation between the Chl-a and Chl-b bands on the DEAE-Sepharose CL-6B column developed in acetone cannot be attained under the present chromatographic conditions. Omata and Murata (8) used the DEAE-Sepharose CL-6B column to eliminate yellow leaf pigments and degradation products of Chl in Ppt II. We have already developed a procedure for the elimination of xanthophylls in Ppt II by washing Ppt II with 80 % (v/v) aqueous methanol (3). When the further purified Ppt II (Ppt III) thus treated was separated by the column chromatographic procedures developed by Omata and Murata (8), the yields of Chl-a and Chl-b were increased. In addition, good separation of Chl-a and Chl-b in Ppt III was achieved by column chromatography with Sepharose CL-6B (13), but the first developing solvent used was less polar than the solvent system used by Omata and Murata (8).

We have determined the  $W_{max}$  of each adsorbent and confirmed that measurements of the values for  $W_{max}$ , as proposed in this study,

are practically useful to evaluate the separability of photosynthetic pigments for preparative purposes.

#### REFERENCES

- 1) W.A. Svec in "The Porphyrins", edited by D. Dolphin, Vol. V, p. 341, Academic Press, New York, San Francisco, and London, 1978.
- 2) K. Iriyama, N. Ogura, and A. Takamiya, *J. Biochem.*, 76, 901, 1974.
- 3) K. Iriyama, M. Shiraki, and M. Yoshiura, *Chem. Lett.*, 1977, 787.
- 4) K. Iriyama, M. Yoshiura, and M. Shiraki, *J. Liq. Chromatogr.*, 2, 255, 1977.
- 5) K. Iriyama and M. Yoshiura, *J. Chromatogr.*, 177, 154, 1979.
- 6) K. Iriyama, M. Yoshiura, and M. Shiraki, *J.C.S. Chem. Commun.*, 1979, 406.
- 7) M. Yoshiura, K. Iriyama, M. Shiraki, and A. Okada, *Bull. Chem. Soc. Jpn.*, 52, 2383, 1979.
- 8) T. Omata and N. Murata, *Photochem. Photobiol.*, 31, 183, 1980.
- 9) H. Höxtermann, *Studia Biophys. Berlin*, 3, 203, 1978.
- 10) T. Miyasaka, A. Watanabe, A. Fujishima, and K. Honda, *J. Amer. Chem. Soc.*, 100, 6657, 1978.
- 11) A.F. Janzen, J.R. Bolton, and M. Stiliman, *J. Amer. Chem. Soc.*, 101, 6337, 1979.
- 12) K. Iriyama, M. Yoshiura, M. Shiraki, S. Yano, and S. Saito, *Anal. Biochem.*, 106, 322, 1980.
- 13) K. Iriyama, M. Yoshiura, T. Ishii, and M. Shiraki, *J. Liq. Chromatogr.*, 4, 533, 1981.