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MODIFICATION OF A SINGLE-STEP SEPARATION PROCEDURE FOR SEVERAL PROTEIN CONSTITUENTS OF VENOM OF THE INDIAN COBRA (*NAJA NAJA*)

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SNAKE venoms are the complex mixtures of cardio-toxins, neurotoxins, cytotoxins, enzymes and other protein constituents with several or many pharmaco-

logic activities. The single-step procedures for the fractionation of venom proteins are not uncommon¹⁻³.

Achyuthan *et al* have developed a single-step procedure for the separation of protein constituents of *Naja naja* venom⁴. The advantage of changing the pH of second and third buffers (both from 7.5 to 7.0) in the purification of ATPase is shown elsewhere⁵. Now, we report the effect of the increased column length and decreased flow-rate on the separation of protein constituents of *N. naja* venom.

N. naja venom (Batch No. 127) from Haffkine Institute, Bombay, India, and CM-Sephadex C-25 (4.5 meq/g) from Sigma Chemical Company, Missouri, USA were used. *N. naja* venom was fractionated on CM-Sephadex C-25 column (1.2 × 125 cm) by stepwise elution with phosphate buffers of various molarities and pH, as given in figure 1. Protein was estimated by Miller's modification of Lowry's method⁶. The various enzyme activities were screened using standardized methods. Neurotoxin activity was checked by animal experiments. The identification of the protein fractions are also shown (figure 1). The colorimetric measurements were made in Bausch and Lomb, Spectronic-20.

The increased column length (from 2.5 × 74 cm to 1.2 × 125 cm) and the decreased flow-rate (from 120 ml/hr to 40 ml/hr) gives a better separation (more than 20 components instead of 11 components). For instance, Fraction A containing acetylcholinesterase, L-amino acid oxidase and phospholipases has been sub-fractionated into five protein(s) components.

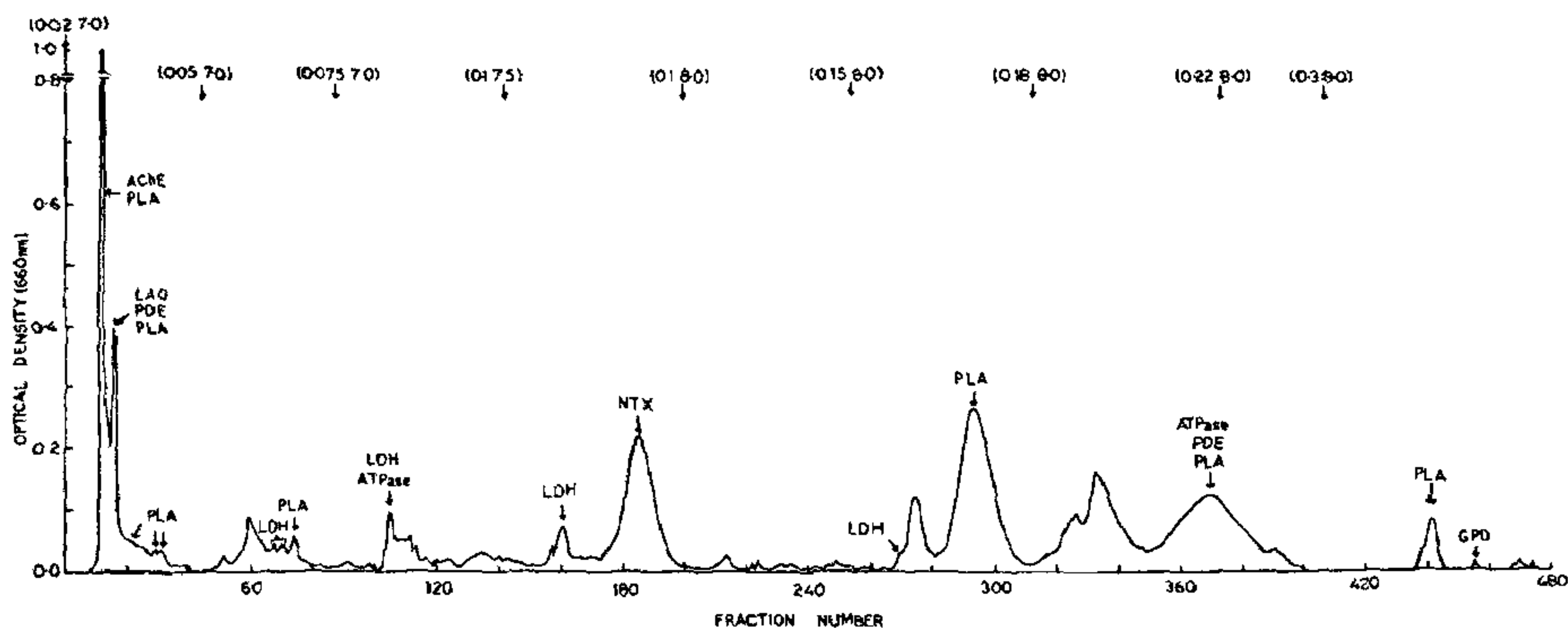


Figure 1. CM-Sephadex column chromatography of *N. naja* venom. Load; 400 mg in 2 ml of 0.02 M phosphate buffer (pH 7); dimensions of column packing, 1.2 × 125 cm; flow-rate, 40 ml/hr; fraction volume, 5 ml; temperature, room temperature (~ 26°C). Elution was carried out stepwise with phosphate buffers of the molarities and pH as indicated. Recovery, 83%. AchE—Acetylcholinesterase, LAO—L-Aminoacid oxidase, ATPase—Adenosine triphosphatase, GPD— α -Glycerophosphate dehydrogenase, LDH—Lactate dehydrogenase, NTX—Neurotoxin, PDE—Phosphodiesterase, PLA—Phospholipase.

Thus the longer columns with slow flow-rate has allowed better separation of variably sized molecules. Therefore the use of ion exchange-cum-molecular filtration is highly advantageous in the fractionation of venoms. Details of these findings are discussed elsewhere.

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SCANNING ELECTRON MICROSCOPIC STUDIES OF ARCHAEOAN LIFE FORMS FROM KARNATAKA, INDIA

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OFTEN, an investigator engaged in the study of Archaeoan life forms, faces the problem of whether a morpho-type preserved in a rock specimen under study in thin sections, is really a structurally preserved organism or is related to other natural inorganic processes. As the scanning electron microscope (SEM) can produce high magnification, high resolution, and depth of field, it aids in identifying the true nature of the morphotype under examination. It also helps in establishing the relationship between the matrix and morpho-type. The purpose of this paper is to present the results of a study, using the SEM, aimed at eluci-

dating the real nature of some suspected organic life forms.

Samples of chert from Dodguni (Long: 76°45' E and Lat. 13° 15' N: Survey of India toposheet No. 57 c/14) situated within the Chitradurga schist belt of Archaeoan age in the Tumkur district of Karnataka, were considered ideal for the study, because of the presence of algal life forms in them reported by Pichamuthu¹ and Gowda², but considered by some to be mineralogic artifacts^{3,4}. The samples were polished and etched with HCl for 5 to 6 hr and then treated with HF for 10 to 12 hr and the etched surfaces were washed thoroughly with distilled water and given a gold coating 200 to 300 Å thick. Photomicrographs were taken under a JEOL scanning electron microscope.

Figures 1,3,5 are thin section photomicrographs of the chert taken under microscope. Figures 1 and 3 are filamentous forms and figure 5 is a globular structure. Figures 2 and 4 are SEM micrographs of filamentous algae taken from etched surface of the chert. Figure 6 is a spheroidal type. SEM micrographs clearly distinguishes between the filaments and the matrix as well as between the globular bodies. The syngenetic nature of the algal filaments are quite obvious from the SEM photomicrographs. These observations negate the possibility of these features being mineralogic artifacts. Had they been mineralogic artifacts, they would have been lost during the strong HF etching and HCl etching and would have appeared as a part of matrix rather than stand out predominantly as filamentous and globular structures amidst the etched out matrix material.

Scanning electron microscopic studies clearly establish, that the algal filaments reported earlier by Pichamuthu¹ and Gowda² in the Archaeoan Dodguni cherts of Karnataka are real microfossils but not mineralogic artifacts as considered by Schopf³ and Prasad⁴.

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