

SECTION

B

SUMMARY

Title: Quantitative estimation of active ingredients in some Sri Lankan medicinal plants using ion induced desorption techniques.

Research Institutes:

- a. Department of Physics, University of Colombo and
- b. Natural products development group, ITI (former CISIR)

Chief Scientific Investigators:

- (i) Dr. D.D.N.B. Daya (University of Colombo) ao5
- (ii) Mr. M.S.B. Mohottalage (ITI- former CISIR) (Followed by Dr. G.A.S. Premakumara, ITI and Mr. K.R.D. Dayananada, ITI)

Period of Contract: From 15-05-1998 to 31-10-2001

Scientific Background and Scope/Objectives of Project

✓ Being a tropical country, in Sri Lanka, plants naturally contain many active compounds. Hence, isolating active compounds from plants grown in Sri Lanka has

become a very important research area in Sri Lanka. Mass spectrometry plays a vital role in confirming the structure of new compounds isolated from natural resources. It would be interesting and time-saving if known or suspected active ingredients in a plant can be detected quickly from the crude extract of plant material before an extensive analysis is carried out. It will also be a foundation for an attempt of quantitative estimation of active ingredients directly from crude extracts without purification. This is not possible with conventional mass spectrometry such as GCMS since they are usually not applicable to run crude extracts without purification.

One major objective of this project is to try out a novel "soft ionization" mass spectrometry technique namely plasma desorption mass spectrometry (PDMS) to detect active ingredients of medicinal plants directly from their respective crude extracts. This idea has been favourable as it incorporates only solid samples in the form of thin layers. This was an important requirement for the aim of direct quantitative estimation without purification. Other objectives were comparison of PDMS mass spectra with those from other mass spectrometric techniques and initiation of a data base of plasma desorption mass spectra of medicinal plants

Experimental Methods (major steps carried out):

- ✓ (i) Improvement of the existing mass spectrometer in order to enhance mass resolution and molecular ion yield required for the direct analysis of crude extract.
- ✓ (ii) Mass analysis of a variety of crude extracts of medicinal plants including those containing nerve stimulants using plasma desorption mass spectrometer built at the Dept. of Physics, University of Colombo.

- ✓(iii) Investigation of the possibility of quantitative analysis of active ingredients of crude extracts using PDMS; reproducibility tests and drawing calibration curves.
- ✓(iv) Modification of the plasma desorption mass spectrometer to introduce bombarding the sample by primary ions from its front side and trying out different sample substrates.
- ✓(v) Comparison of the PDMS spectra with EI and MALDI mass spectra.
- ✓(vi) Development of software for a new database of PDMS of medicinal plants.

Results and Discussion

✓ Mass resolution of the home-built plasma desorption mass spectrometer at the Department of Physics of the University of Colombo, was increased significantly (i.e. 3.5 times for Berbarine) by elongating the flight path of secondary ions (i.e. from 34cm to 94cm) thus improving the resolution of the mass spectra from crude extracts. An electrostatic wire guide (kept at -300V) stretched axially along the flight path of secondary ions, as a remedy for the low ion transmission experienced in a long flight path, helped increase the secondary ion yield typically by two orders of magnitude. This is an important achievement in our strategy towards the quantitative estimation of active ingredients from their crude extracts because it helps produce significant mass spectra directly from crude extracts.

✓ It has been successfully demonstrated that the plasma desorption mass spectrometry (PDMS) is capable of producing clean mass spectra directly from their crude extracts of medicinal plants including those containing nerve stimulants without purification (see the full report for the details of crude extracts used).

✓ A new front side bombarding mechanism introduced as a modification to the existing mass spectrometer yielded good mass spectra comparable to back side bombardment thus enhancing the flexibility of the sample preparation for PDMS using different thick metal substrates. This also enhanced the performance of the mass spectrometer for the analysis of crude extracts.

✓ In the attempt of quantitative estimation, applicability of PDMS for constructing a calibration plot was demonstrated during this study. A nearly linear variation of ion yield (peak area) with the log value of the concentration (e.g, for berberine) can be seen. Emerging of a definite pattern or behavior of the calibration curve is an encouraging indication for a quantitative estimation. Difficulty has been encountered with some samples in making homogenous sample layers. This difficulty imposed problems in producing reliable calibration curves for such samples and thus restricting quantitative estimation for such samples.

✓ The initial results in the comparison of EI and PDMS spectra indicated that PDMS has more capability to produce clean mass spectra depending on the compound used especially with respect to the natural product compounds (crude extracts) used in this study. A new software for a new data base of PDMS of medicinal plants has been developed. Once a list of peak of an unknown compound is entered, a priority list of probable compounds is displayed. There is an option to see the stored mass spectra of all the probable compounds in the list. This has the possibility for expansion by adding more mass spectra of known compounds in future.

Conclusions and recommendations

✓ The performance of the home-built plasma desorption mass spectrometer at the Department of Physics of the University of Colombo has been improved to make it more suitable to produce significantly clear mass spectra from crude extracts without purification. The finding that PDMS can successfully be employed to produce clean mass spectra of active compounds directly from crude extracts of medicinal plants has been identified as a significant advantage over the conventional mass spectrometry such as GCMS and it will be greatly helpful in natural product research in Sri Lanka in future. In the attempt of quantitative estimation, reproducible calibration curves have been produced but the difficulty in preparing uniform sample surfaces has imposed restrictions in this context. In the final stage of the project, it has been shown that the modification to introduce front bombarding mechanism has been successful and it facilitates the flexibility of sample preparation. Hence, finding an alternative sample preparation technique is recommended as a future work for a better quantitative study. A software for a new data base of PDMS of medicinal plants has been developed. ✓

Publications on the work done under the contract:

Already one international and 05 local communications have been made (pl. see the list below). One full paper is being written for submission to an international journal of mass spectrometry

List of publications on the work done under the contract

- [1] Plasma desorption mass spectrometry (PDMS) of crude extracts of medicinal plants S.A. Dahanayaka, D.D.N. Barlo Daya, T.R. Ariyaratne and M.S.B. Mohottalage, proc. 55th Annual Session, Sri Lanka Assoc. Advmt. Sci. **E2-16** (1999) 224.

- [2] Plasma desorption mass spectrometry (PDMS) of crude extracts of selected medicinal plants,
✓ S.A. Dahanayaka, D.D.N. Barlo Daya, G.A.S. Premakumara and T.R. Ariyaratne,
Proceedings of the 56th Annual Session of SLAAS (Part 1-Abstracts) E2 (2000) 238 : 259
- [3] Plasma desorption mass spectrometry (PDMS) of plant extracts and its potential applications in quantitative estimations
✓ S.A. Dahanayaka, D. D.N.Barlo Daya, M.S.B. Mohottalage, G.A.S. Premakumara and T.R. Ariyaratna,
Abstracts, Annual Session, Faculty of Science, University of Colombo, Vol.1(2000)
- [4] Quick detection of active compounds in crude extracts of selected plants using plasma desorption mass spectrometry (PDMS),
S.A. Dahanayake, D.D.N. Barlo Daya, M.S.B. Mohottalage,
G.A.S. Premakumara and T.R. Ariyaratne,
Proceedings (Abstracts), Desorption -2000, St.malo,
France (2000)-P46
- [5] Improved performance of a linear mode plasma desorption time-of-flight mass spectrometer with an electrostatic wire guide
S.A. Dahanayake, D.D.N.B. Daya, T.R. Ariyaratne and M.S.B. Mohottalage,
Proceedings of the Technical Sessions, 18 (2002)17-24, Institute of Physics, Sri Lanka