Table 1 *Effect of leaf extract of V. rosea on sperm parameters of male albino mice*

Parameter	Control	Experimental
Sperm count ($\times 10^6$/ml)	77.25 ± 9.09 (6)	52.13 ± 3.96** (6)
Percentage of motile sperm	71.67 ± 5.32 (6)	57.17 ± 8.80** (6)
Duration of motility (min)	92.83 ± 11.46 (6)	61.67 ± 5.96** (6)
Normal sperm (%)	85.33 ± 5.92 (6)	69.50 ± 5.96* (6)
Abnormal sperm (%)	14.67 ± 5.92 (6)	30.50 ± 5.96** (6)
Live sperm (%)	75.67 ± 5.57 (6)	61.00 ± 9.53* (6)
Dead sperm (%)	24.33 ± 5.57 (6)	39.00 ± 9.53* (6)

Values are mean ± SD.
$^*P < 0.05$, $^{**}P < 0.01$.

Duration of motility of the sperm of treated mice was 44% of that of controls. The relative percentage of abnormal sperm increased considerably. Among the abnormal sperm, categories like double-tailed, detached head, detached tail, mid-piece bending, irregular head and mid-piece loop formation were predominant. The relative percentage of live sperm decreased significantly (table 1).

Sperm count is considered to be one of the important parameters that affect fertility. Decrease in sperm count of *V. rosea* extract-treated mice opens up a promising avenue for further studies in antifertility methods.

Sperm have two principal attributes, namely motility and fertilizing ability[6]. Motility is an important prerequisite for fertilization in the case of internally fertilizing organisms. Any negative impact on motility would seriously affect fertilizing ability. In increasing the relative percentage of abnormal sperms and decreasing the relative percentage of live sperm, *V. rosea* appears to be deleterious at the level of cauda epididymis.

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QUANTITATIVE ANALYSIS OF THIOCYANATE IN URINE BY HEAD-SPACE GAS CHROMATOGRAPHY

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A widely used method for the determination of thiocyanate in biological fluids is the measurement of red colour of thiocyanate formed by adding ferric ion in acid medium. But it has its limitations because of interfering colours from phenols and keto acids, precipitation of some of the thiocyanate with protein by trichloroacetic acid[1,2], conversion of thiocyanate to cyanide[3], and interferences due to various coloured complexes formed with ferric nitrate reagent in the urine samples of persons under treatment with medicines such as desprin and certain antibiotics.

Other prevailing methods also have limitations, such as, in the case of Aldridge's[4] method of pyridine–benzidine reagent[5], interference by amino acids such as tryptophan and by proteins. Monitoring of thiocyanate in altered thiocyanate metabolism is of great significance, such as during therapy in certain cases of poisoning. Therefore an attempt was made to overcome such interferences in the determination of urine thiocyanate using gas chromatography.

Gas chromatographic (GC) assay of thiocyanate in terms of cyanide was made by taking 10 ml of diluted or undiluted urine in a closed vial (15 ml)

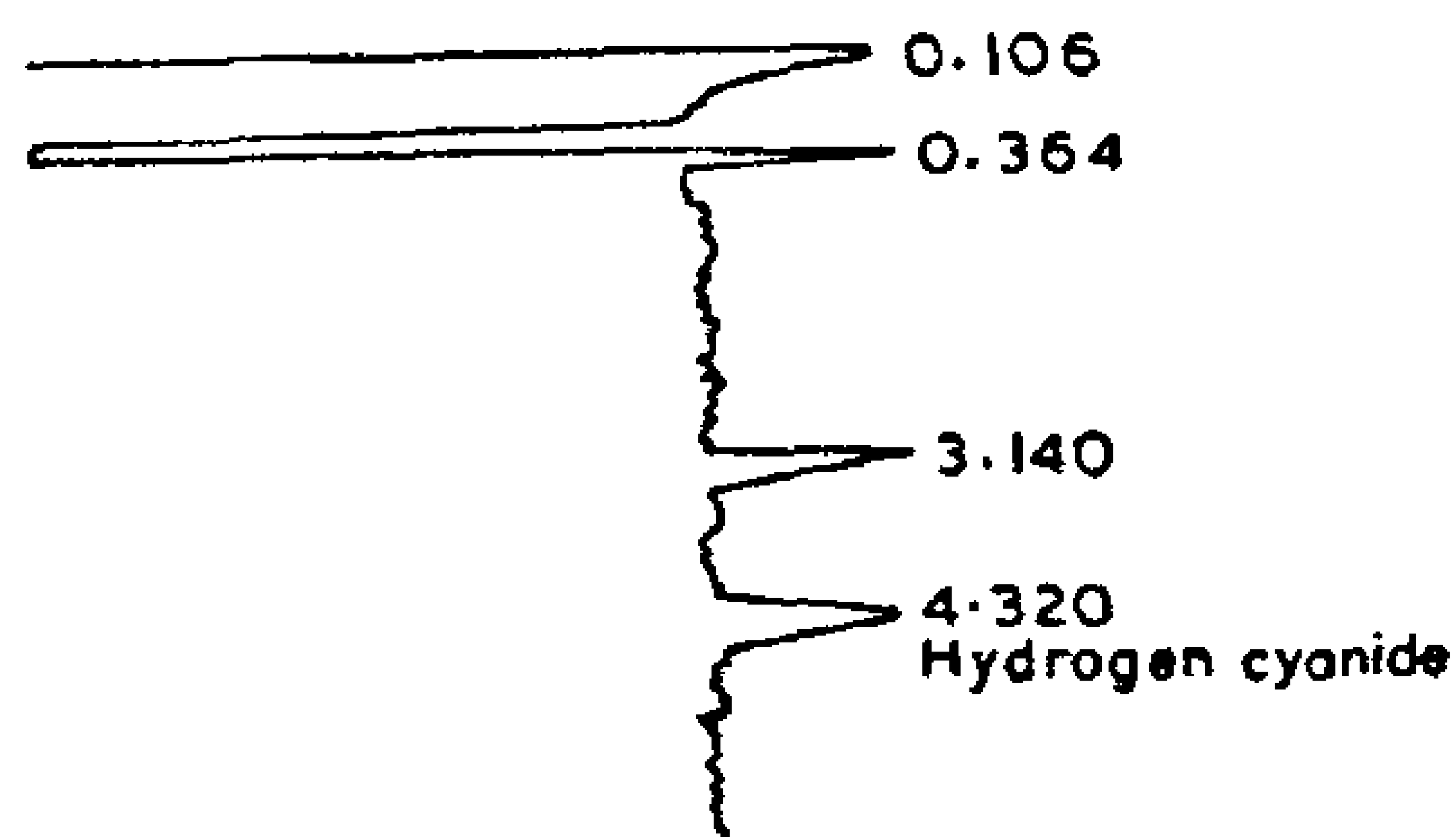

Figure 1. Chromatogram of hydrogen cyanide generated from thiocyanate in urine (head-space analysis, GC/FID).

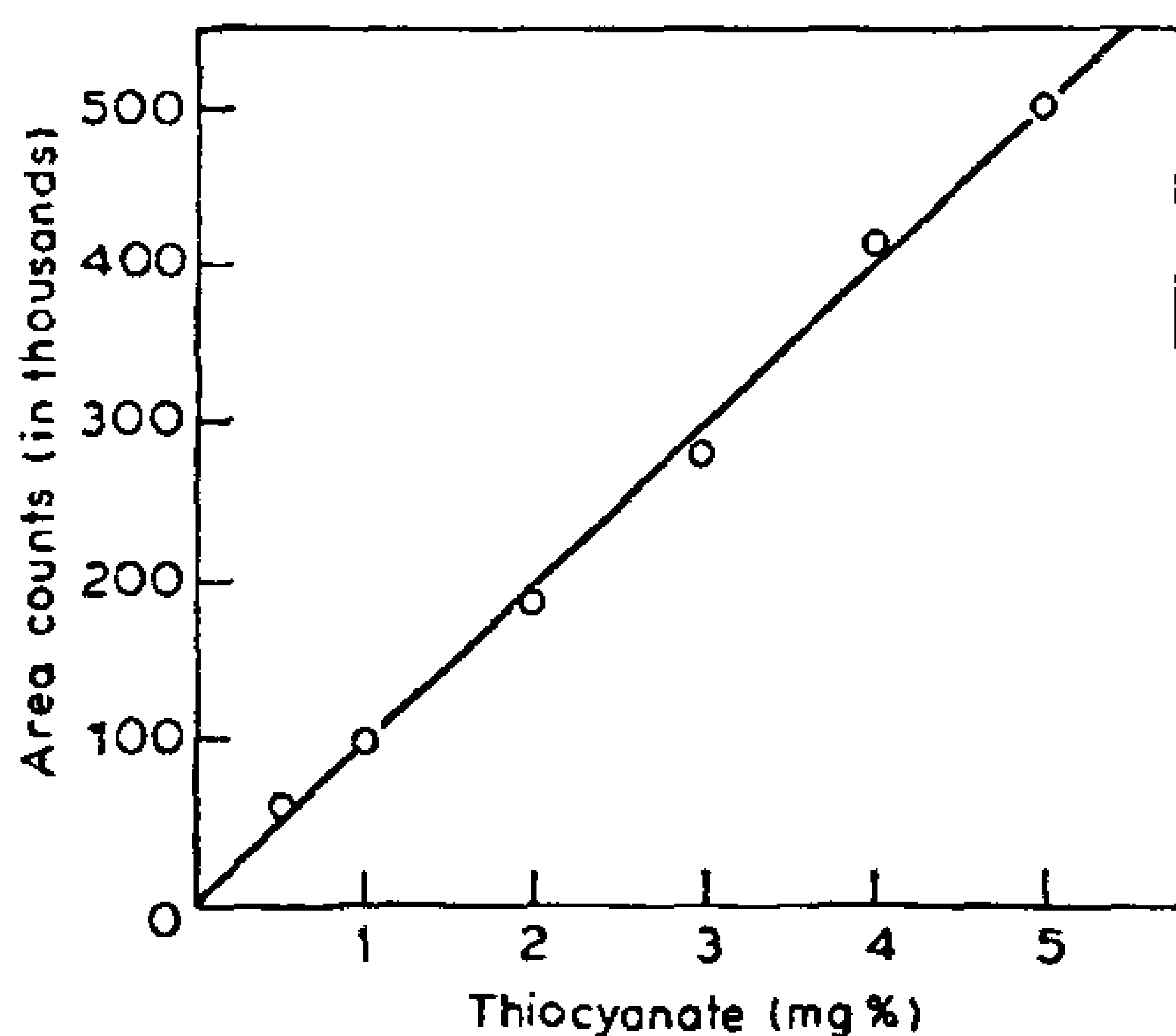

Figure 2. Correlation between Bowler's and GC methods for estimation of thiocyanate in urine.

sealed with Teflon tape and adding 1 ml of 0.1 M potassium dichromate and 2 ml of concentrated sulphuric acid, which converts thiocyanate to cyanide and releases it as hydrogen cyanide. Two ml of head-space gas was injected, using a pressure lock syringe (Dynatech Precision Sampling Corporation, USA) into an isothermal gas chromatograph after a 30 min incubation at 40°C, following the method of McAuley and Reive[6]. A Perkin-Elmer Sigma-300 gas chromatograph equipped with a flame ionization detector and a Teflon column (12′ × 1/8″ OD) packed with Porapak-Q 80/100 mesh was used for the analysis. Nitrogen was used as carrier gas at 45 ml/min. Column temperature was 125°C, detector temperature 230°C, and injector temperature 210°C. Recording and quantification of the peaks were

Table 1 *Recovery of thiocyanate from urine in the GC method*

Sample no.	Amount added (mg%)	Amount found* (mg%)	Recovery (%)
1	0.1	0.090	90 0
2	0.2	0.181	90.5
3	0 5	0.460	92.0
4	1.0	0.940	94 0
5	2.0	1 860	93.0
6	4.0	3.820	95.5

*Means of three replicates.

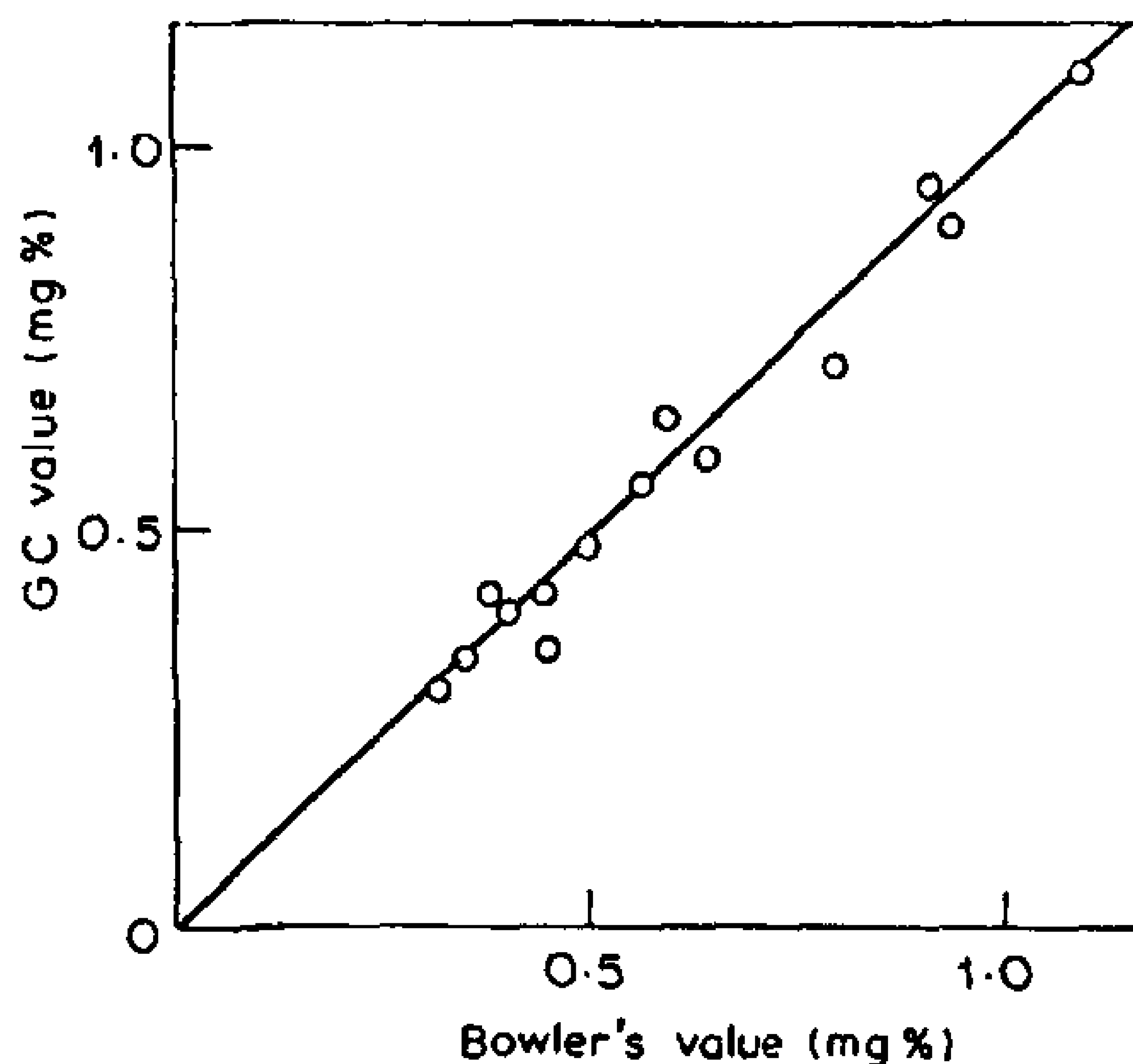

Figure 3. Standard curve for hydrogen cyanide generated from thiocyanate in urine.

done using a Perkin-Elmer LCI-100 laboratory computing integrator.

The retention time for hydrogen cyanide generated

Table 2 *Comparison of thiocyanate values of abnormal urine samples analysed by Bowler's and GC methods*

Sample	Bowler's value (mg%)	GC value (mg%)
1	2.82	0.62
2	4.50	0.14
3	2.50	0.20
4	*	0.33
5	*	0.85
6	2.80	0.50
7	1.55	0.50
8	*	1.25
9	1.65	0.52
10	1.30	0.68

*, Could not be detected.

from thiocyanate was 4.32 min and the resulting peak was uniform and symmetrical with no tailing (figure 1). Several concentrations of thiocyanate, ranging from 0.05 to 10 mg%, were used and a standard graph of area counts vs concentration was plotted; this is linear, as seen in figure 2. As the concentration of thiocyanate in the reaction mixture increased from 0.05 to 5 mg%, the variation coefficient decreased from 6.57 to 1.30% ($n = 10$ for each of the 5 test concentrations). The detector response was checked for every set of analyses by testing one or two known concentrations, allowing a tolerance limit of ±10%. This response factor was used in analysis of samples of urine for thiocyanate levels.

Recovery experiments were carried out by adding known amounts of standard thiocyanate to urine, or cyanide to urine made alkaline. Recovery was 90–95% (table 1) and reproducibility 95–100%. Background cyanide levels in urine would not have any significant effect on the results, being in negligible amount (0.005 mg%)[7]. Phenols, keto acids, sodium thiosulphate, vitamin B_{12} and desprin did not interfere with the assay. In the case of normal urine samples no remarkable difference was noted in thiocyanate values (SD of differences was 0.041 and t was 0.91) determined spectrophotometrically using Bowler's method followed by Denson *et al.*[8] and by the present GC method (figure 3). However, abnormal urine samples, characterized either by the presence of turbidity or by a colour other than the specific red colour of ferric thiocyanate with ferric nitrate reagent, gave lower values by the present method. Urine samples in which thiocyanate could not be estimated by Bowler's method could be analysed by the GC method (table 2).

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ANNOUNCEMENTS

Seminar on Academy–Industry Interaction in Agriculture Biotechnology

Place: Indian Institute of Science, Bangalore
Date: 1 December 1989
Contact: The Secretary
Centre for Advancement of Biotechnology
c/o Vittal Mallya Scientific Research Foundation
K. R. Road
P.O. Box 406
Bangalore 560 004

Solid State Physics Symposium

Place: IIT, Madras
Date: 19–22 December 1989
Contact: Prof. R. Srinivasan
Convener, SSP Symposium
Dept of Physics
Indian Institute of Technology
Madras 600 036

National Seminar on Chemical Physics

Place: Pondicherry University, Pondicherry
Date: 9–11 February 1990
Contact: Prof. A. Srinivasa Rao
Chairman, NSCP
Raman School of Physics
Pondicherry University (JIPMER Campus)
Pondicherry 605 006
Abstracts by 15 December, full papers by 15 January 1990.