

## CORRESPONDENCE

some observations of the author, which are felt to be not very rare, on discussion with many persons in the academic field

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## Split second and Olympics

Jha<sup>1</sup> points out an error of magnitude ~20 ms (originating from differential arrival time of starting signal at extreme

lanes), which is twice as large as the resolving time (10 ms) used to determine winners in modern competitive international athletics. This, however, is only the tip of the iceberg. Much larger uncertainties due to athletes' differential response to stimuli used for starting the events should form the basis of a more serious objection to deciding winners with 10 ms (or perhaps better, in future) resolution. The magnitude of such an uncertainty in the case of a sound-based stimulus, such as pistol firing, can be as large as a few hundred milliseconds<sup>2</sup>.

Furthermore, while the error reported by Jha<sup>1</sup> can be rectified by using a light-based starting signal instead, the differential response to visual stimulus, whose magnitude can be of the order of a few tens to hundreds of milliseconds<sup>3</sup>, would still give rise to large uncertainties. Therefore, assuming that the

winner of a 100-metres dash be decided solely by competitors' relative ability to run, new means of correcting for this uncertainty should be devised.

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## SCIENTIFIC CORRESPONDENCE

### DNA-binding monitoring made easy

Binding of molecules on deoxyribonucleic acid (DNA) has been a subject of active interest<sup>1</sup>. Understanding the mechanism of such processes helps in gaining insight into the mutagenic, carcinogenic, tumorigenic and anti-neoplastic properties of different compounds<sup>2</sup>. The modes of binding involve electrostatic, hydrophobic, intercalative and groove-oriented processes.

Various methods are used to locate the molecule on the DNA<sup>1</sup>. Here, we describe a novel procedure to test the binding of fluorophore-bearing molecules on DNA. The compound is incubated with DNA and treated with cetyl trimethyl ammonium bromide (CTAB). DNA forms a 1:1 insoluble complex with CTAB<sup>3</sup>. The precipitated DNA-CTAB complex is insoluble in water and only sparingly soluble in most of the common solvents. But, this can be readily dissolved in sodium dodecyl sulphate (SDS) micelles. If the test compound is capable of binding with DNA, it will be trapped within the DNA structure before precipitation. The DNA-compound-CTAB complex solubilized in SDS will give the characteristic

fluorescence emission spectrum of the compound. *Such a signature of the compound detected in the precipitated complex provides a direct evidence for its interaction with DNA.* To ensure that no unbound material adheres to the solid complex, the precipitate is washed with aqueous buffer.

Many aromatic compounds known to bind on DNA possess an emission spectrum. Change in fluorescence intensity of the compound alone cannot be taken as a conclusive proof for its binding since either increase or decrease in intensity has been observed with different compounds. The DNA-CTAB complex has been shown to retain its structural integrity in the solubilized form in SDS<sup>3</sup>. Thus, the drug or ligand molecule will also be retained in the precipitated complex.

The above method has been demonstrated for the first time by us in the dye ethidium bromide which is known to intercalate efficiently with DNA as shown in several studies<sup>4</sup>. The precipitated complex on centrifugation was visibly coloured red indicating the presence of the dye within DNA. Fluorescence

spectrum of the dye could be detected in SDS solution of the complex. Conversely, a non-binding compound will not produce its spectrum in the solubilized complex. However, shifts in fluorescence maximum and reduction in intensity are generally observed. Furthermore, in principle, any other spectroscopic property of the molecule may be used to identify its presence in the complex.

Here we describe the details of the experimental methodology for the intercalating compound, ethidium bromide. In a typical experiment with ethidium bromide, 100 µg salmon testis DNA (Sigma Chemical Co., St. Louis, MO, USA) was incubated at ambient conditions with 10.0 nMol of ethidium bromide in 1.0 ml TE buffer (0.01 M Tris-HCl, 0.001 M EDTA, pH 8.0). After 10 min incubation, 90 µmol of CTAB (dissolved in 0.3 ml TE) was added<sup>3</sup>. The precipitated DNA-ethidium bromide-CTAB complex was collected and dissolved in 1.0 ml of 0.01 M SDS. The fluorescence emission spectrum of the solution was studied in a Hitachi F4010 fluorescence spectrophotometer at an

excitation wavelength of 506 nm. The spectrum showed an emission maximum at 615 nm, characteristic of ethidium bromide.

This method may not be applicable for a compound which binds to DNA exclusively by electrostatic mechanism since the phosphate sites will not be available to CTAB for subsequent precipitation. The method is ideally suited for intercalating compounds or for compounds strongly binding to DNA by non-electrostatic mechanisms. We

have extended this method to test various compounds including an alkaloid, deoxytubulosine, isolated from the flowers of *Alangium lamarckii*.

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3. Kumjappu, J. T. and Nair, C. K. K., *Indian J. Chem.*, 1992, **A31**, 432-435
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## OPINION

## On the recentness of current researches in earth science in India

*Salil Agrawal and Vinod Agrawal*

The questions pertaining to the output of our community of geo-scientists, in terms of effort and money spent by

them, customarily, and perhaps because it is convenient too, are replied with the 'number' of research papers published

by them. The harder question of the quality of research remains unanswered, mostly getting drowned in the debate

Table 1. Frequency tabulation for references cited

Class	Class limits (both years included)	50 research papers published in India in the year 1991*		50 research papers published from abroad in the year 1991**	
		Frequency	Relative percentage	Frequency	Relative percentage
	In or before 1931	11	01.114	25	01.014
1	1932-36	4	00.405	4	00.162
2	1937-41	7	00.709	10	00.406
3	1942-46	5	00.507	6	00.243
4	1947-51	7	00.709	17	00.690
5	1952-56	19	01.925	23	00.933
6	1957-61	35	03.546	40	01.623
7	1962-66	60	06.079	57	02.312
8	1967-71	119	12.057	153	06.207
9	1972-76	155	15.704	245	09.939
10	1977-81	182	18.440	476	19.310
11	1982-86	227	22.999	742	30.101
12	1987-91	156	15.805	667	27.059
Total		987	100.00	2465	100.00

\*Selected from:

1. *Journal of Geological Society of India*. 2. *Indian Journal of Earth Sciences*. 3. *Indian Journal of Geology*  
4. *Indian Minerals*.

\*\*Selected from:

1. *Contributions to Mineralogy and Petrology*. 2. *Mineral Deposita*. 3. *Geological Magazine*. 4. *Journal of Petrology*.