

## X-ray structural parameters of some Indian coals

Sudip Maity\* and Pinaki Mukherjee

Central Fuel Research Institute, PO FRI, Dhanbad 828 108, India

**Five Indian coals of different ranks (C% ~ 73.6–92.8) have been demineralized by chemical method. Very slow scan X-ray scattering studies have been performed and coal structural parameters determined by a semi-quantitative method. The present study reveals that coal consists of a semi-crystalline turbostratic structure. Also, the high background of the diffraction profiles clearly shows that coal has amorphous carbon in its structure.  $d_{002}$  value decreases and  $f_a$  increases with increase in coal rank. There is a strong linear relationship between C% and volatile matter content with  $d_{002}$  values of coal.**

**Keywords:** Coal, molecular structure, turbostratic structure, XRD.

THE compositional heterogeneity and complexity of coal organic materials require a complex approach for studying their molecular structure. Coal consists of primary macromolecules of polyaromatic–polynuclear structure with some heteroatom groups and their secondary network. The secondary network is made up of aromatic ring-stacking, aliphatic side-chain entanglement, hydrogen bonds, cation bridges, and charge–transfer interactions through oxygen functional groups<sup>1–3</sup>. Many structural models have been proposed for coal considering the presence of aliphatic and aromatic species, amount of oxygen containing functional groups, and several other factors. Instrumental techniques like TEM<sup>4,5</sup>, FT-IR<sup>6</sup>, Raman spectroscopy<sup>7</sup> and NMR<sup>8</sup> have been used for coal molecular structure determination. The role of X-ray diffraction (XRD) study in coal science is enormous. It was initially directed towards measuring the minerals and/or the low- and high-temperature ash in coal. XRD analysis is a fundamental method for evaluating carbon-stacking structure. The degree of ordering, interlayer spacing ( $d_{002}$ ) and crystallite size ( $L_a$ ,  $L_c$ ) have been established as the structural parameters for evaluating the stacking structure of highly crystalline carbon materials<sup>9</sup>. The structure of coal has also been characterized by XRD, and the existence of crystallites in coal structure has been proven by the appearances of the peaks corresponding to the 002, 100, and 110 reflections of graphite<sup>10</sup>. It has an intermediate structure between graphitic and amorphous state, so-called turbostratic structure or random layer lattice structure. Coal also contains significant amount of highly disordered material, amorphous carbon, which is responsible for the background intensity

of the diffractogram. However, the vagueness of the XRD profile of coal with low crystallinity often makes quantitative evaluation of the stacking structure difficult. Fifty years ago, Hirsch<sup>11</sup> and Diamond<sup>12</sup> proposed the statistical interpretation of XRD profiles for carbon materials with low crystallinity, but the recent development of computer techniques has enabled more accurate calculations and detailed analysis of XRD profiles. In the last two decades, extensive X-ray scattering studies<sup>13,14</sup> have been performed on coal for its structure determination.

Slow step scan XRD<sup>15</sup> analysis has been used to give higher resolution of the diffractograms, classifying the carbon-related peaks around 20–26° basically into two categories: one derived from aromatic ring stacking around 26° (so-called  $\pi$ -band or 002 band) and the other around 20° named  $\gamma$ -band, which is believed to be derived from aliphatic chains. The intensity ratio ( $I_{26}/I_{20}$ ) of the two peaks appears to reflect the coal rank<sup>16</sup>.

In this context, structural parameters of Indian coals using XRD have not been attempted. In the present study, a semi-quantitative approach has been used to determine  $d_{002}$ , aromaticity and coal rank ( $I_{26}/I_{20}$ ) of five Indian coals ranging from sub-bituminous to high volatile bituminous. Very slow step scan X-ray scattering studies have been performed on the demineralized coals and deconvolution has been made on the broad hump in the 2 $\theta$  range of 15–32°. This broad hump is fitted into two Gaussian peaks around 20 and 26° and the  $d$ -spacings are taken as the positions of the  $\gamma$ -band and  $d_{002}$  ( $\pi$ -band) respectively. Heights and areas under these two peaks have been used to determine their relative coal ranks and aromaticity ( $f_a$ ). These parameters have been compared with the elemental carbon and volatile matter content of the coals.

Five coal samples covering the range sub-bituminous to high volatile bituminous, collected from different coalfields, namely Muraidih, Mohuda, Sounda, Sirina and Kamptee of India are coded as MH, MO, SO, SI, and KA. Elemental constituent and volatile matter (VM) content were estimated for the coals on dry ash free (daf) basis (Table 1). All coal samples were chemically demineralized to avoid the effect of mineral matter in the diffractogram. Effect of very low content of mineral matter in the demineralized coal is neglected and the effect of heteroatom is insignificant<sup>17</sup>.

Ten grams of each powdered (72 mesh) coal was demineralized by dispersing in 60 ml of conc. HCl solution

**Table 1.** Compositional analysis of coals

	Ultimate analysis (dry ash free basis, %)				
	C	H	N	O by diff	S
MH	92.8	3.5	1.8	1.4	0.5
MO	87.8	5.1	2.4	4.3	0.4
SO	79.2	5.1	2.2	12.7	0.8
SI	75.0	4.4	1.7	16.5	2.4
KA	73.6	4.1	1.8	20.0	0.5

\*For correspondence. (e-mail: sudip\_maity@yahoo.com)

(36.5 wt%) and stirred for 3 h at 60°C in a water bath. Then the coal was filtered and washed with distilled water. The HCl-treated coal samples were then mixed with 60 ml of conc. HF (48 wt%) and stirred for 3 h at the same temperature. Finally, the treated coals were washed with hot distilled water to remove HF and dried in an air-oven at 100°C. Weight loss was then checked to see that demineralization had occurred to at least less than 1% effective mineral matter.

A D-8 ADVANCE (Bruker AXS, Germany) X-ray diffractometer was used to collect X-ray intensities of the demineralized coals in the  $2\theta$  range of 10–115° with Bragg–Brentano geometry using parallel beam  $\text{CuK}\alpha$  (40 kV, 40 mA) radiation. Zero background sample holder was used for coal sample loading and the scan was made in locked couple, step scan mode (0.1°/step) with 6 s at each step.

A Windows NT<sup>TM</sup>-based Diffrac Plus Profile fitting software (M/s Bruker AXS, Germany) was used for deconvolution of the diffractograms in the  $2\theta$  region of 15–32°. This software is used to obtain line positions, intensities, widths and shapes from both resolved and unresolved XRD spectra using least square refinement technique. To get rid of the systematic error related to the least square fitting for the peak positions and intensities, alignment of the X-ray diffractometer was checked before every scan by running standard quartz specimen. The  $2\theta$  scan was refined with  $K_{\alpha 1}$  and  $K_{\alpha 2}$  doublet and the  $K_{\alpha 2}/K_{\alpha 1}$  relative intensity. The least square algorithm refines the background intensities and peak intensities, positions and widths. Two symmetrical Gaussian peaks were fitted to corroborate the  $\pi$ -band and  $\gamma$ -band. Initial guess values for the peak positions and half width were chosen and the profile was fitted. Goodness-of-fit was measured by minimizing four statistical parameters, namely reliability (R), reliability index (RI), Weighted reliability (WR) and theoretical reliability (TR) by several trials. They are expressed as

$$R = 100\% \sqrt{\frac{\sum w(l_o - l_c)^2}{\sum w l_o^2}}, \quad RI = 100\% \frac{\sum |l_o - l_c|}{\sum l_o},$$

$$RW = 100\% \frac{\sum \sqrt{w} |l_o - l_c|}{\sum \sqrt{w} l_o}, \quad TR = 100\% \sqrt{\frac{\sum w l_o}{\sum w l_o^2}},$$

where  $l_o$  and  $l_c$  are observed and calculated intensities and  $w$  is the weighting factor.

A good profile fit is achieved when error values are less than 5% for crystalline materials. In the present study, profile fitting was performed on diffused humps for 170 intensity points and error value could be minimized up to 6.5%, except in one case where error is 4.4%. Considering the nature of the diffractograms, this is the best possible Gaussian fit in the region of study (Figure 1). The minimum error value of 4.4% is achieved for the MH coal, as it has relatively sharper hump in the  $2\theta \sim 15\text{--}32^\circ$  than the other four coals.

Theoretically, the areas under the  $\pi$ - and  $\gamma$ -peaks should be equal to the number of aromatic atoms ( $C_{ar}$ ) and saturated carbon atoms ( $C_{al}$ ), respectively<sup>18</sup>. Therefore, the aromaticity ( $f_a$ ) of coal, the ratio of carbon atoms in aliphatic chains vs aromatic rings can be defined as

$$f_a = \frac{C_{ar}}{C_{ar} + C_{al}} = \frac{A_{002}}{A_{002} + A_{\gamma}},$$

where  $A$  is the integrated area under the corresponding peak, and  $C_{al}$  and  $C_{ar}$  are the number of aliphatic and aromatic carbon atoms per structure unit respectively. Using this relation,  $f_a$  values for all examined coals have been calculated; however, relative intensity ratio ( $I_{26}/I_{20}$ ) for these two peaks is taken as coal rank. The results have been summarized in Table 2.

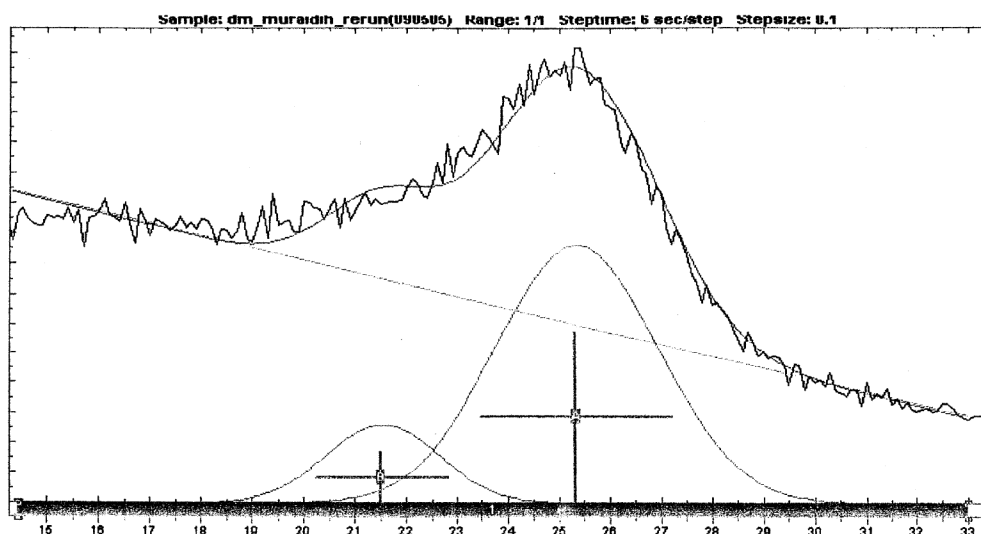


Figure 1. Fitting of two Gaussian peaks for the Muraidih demineralized coal in  $2\theta \sim 15\text{--}32^\circ$ .

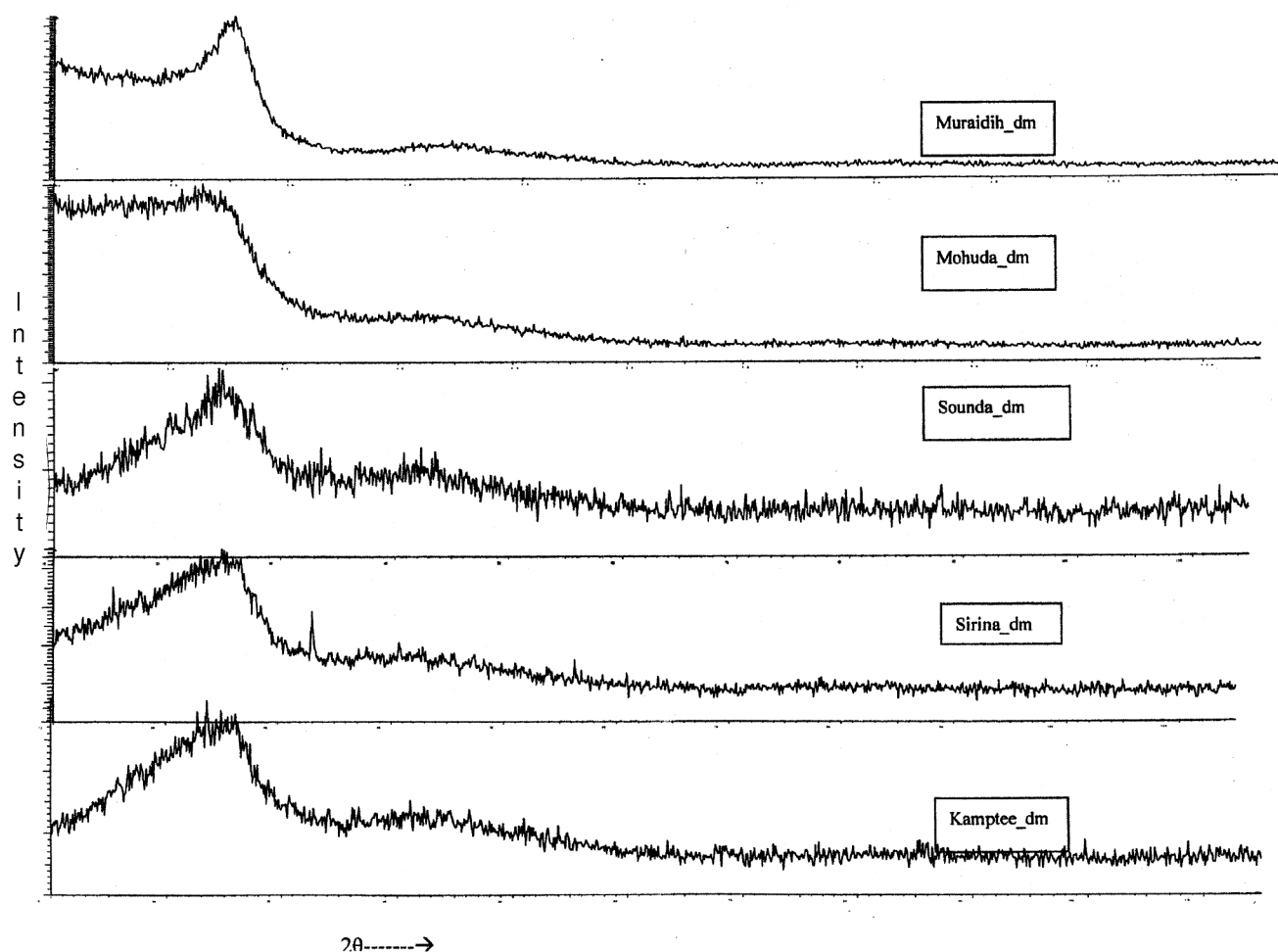


Figure 2. X-ray diffractograms of demineralized coals.

Table 2. Structural parameters extracted from the profile fitting of XRD spectra

Coal type	VM (%)	$d_{002}$ (Å)	$d_{10}$ (Å)	$f_a$	$I_{26}/I_{20}$	$R$ (%)
MH	16.5	3.51	4.13	0.83	3.91	4.4
MO	31.9	3.52	4.17	0.80	3.07	6.0
SO	34.4	3.54	4.45	0.77	2.85	7.9
SI	32.7	3.55	4.20	0.75	2.42	6.5
KA	32.8	3.56	4.29	0.70	2.14	6.5

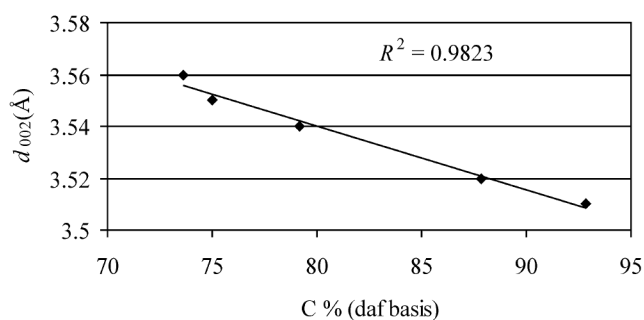


Figure 3. Variation of  $d_{002}$  vs elemental carbon of coals.

A careful study of the diffraction profiles of coals shows the presence of high background intensity (Figure 2) in all the profiles. This is due to the fact that not all carbon atoms are in aromatic structures and a significant amount exists in the form of amorphous carbon. Moreover, all coals contain a clear (002) band and two two-dimensional reflections, (10) and (11). Neither higher order reflections of (00 $l$ ) band nor ( $hkl$ ) reflections are found.

Quantitative comparison has been made for the XRD profile results for the five coals. The  $d_{002}$  has been plotted versus elemental carbon (daf basis) for the coals (Figure 3). It is observed that  $d_{002}$  which ranges from 3.51 to 3.56 Å, decreases with increase in elemental carbon content of the coals and maintains a linear relationship with high correlation coefficient ( $R^2$ : 0.98). It is known that  $d_{002}$  value decreases with increase in coal maturity *vis-à-vis* coal rank<sup>19</sup>. VM values of the demineralized coals have been compared with the  $f_a$ ,  $d_{002}$  and  $I_{26}/I_{20}$  ratio (Table 2). Increase in  $f_a$  is not so prominent for four of the coals with high VM content, but overall it is found to increase with decrease in VM content. This relation is obvious as volatile matter of coals comes from the saturated hydrocar-

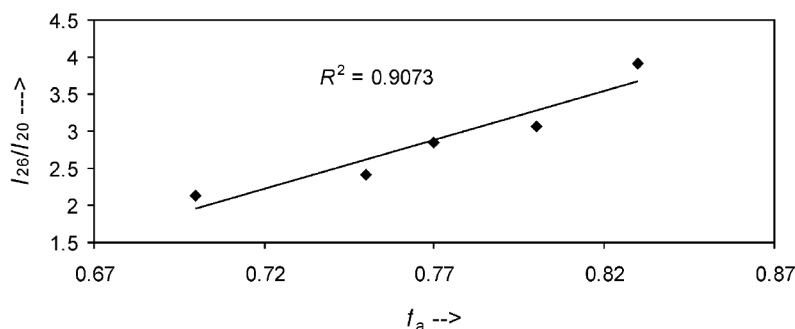


Figure 4. Relation between  $f_a$  and  $I_{26}/I_{20}$ .

bons and hence the decrease of aliphatic side chains increases the aromaticity of coal. A similar relationship exists between VM content and  $d_{002}$  and  $f_a$  values. It is clear from Figure 4 that aromaticity increases with increase in relative ratio of  $I_{26}/I_{20}$  and there is strong linear relationship between  $f_a$  and  $I_{26}/I_{20}$  values ( $R^2$ : 0.91).

Position of the  $\gamma$ -band varies between 4.13 and 4.45 Å and  $d_a$  does not show any correlation to any of the other parameters in this study. Moreover, no earlier researcher has reported any details about the  $\gamma$ -band and established any correlation with other structural parameters of coal.

The present study, aimed towards the determination of X-ray structural parameters of some Indian coals by a semi-quantitative approach, reveals that coal consists of a semi-crystalline turbostratic structure. High background of the diffraction profiles clearly proves that coal has amorphous carbon in its structure. The study reveals that  $d_{002}$  value decreases and  $f_a$  increases with the increase in coal rank, and hence the degree of ordering also increases. This is an initial step towards application of XRD technique to determine X-ray structural parameters of Indian coals. Further studies are in progress on several other Indian coals of varied rank and geological occurrences to determine X-ray structural parameters *vis-à-vis* molecular level understanding of Indian coals.

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## Prevention of radiation-induced damages by aqueous extract of *Ganoderma lucidum* occurring in southern parts of India

Thulasi G. Pillai<sup>1</sup>, Veena P. Salvi<sup>2</sup>,  
Dharmendra Kumar Maurya<sup>2</sup>,  
Cherupally Krishnan Krishnan Nair<sup>2</sup> and  
K. K. Janardhanan<sup>1,\*</sup>

<sup>1</sup>Amala Cancer Research Centre, Thrissur 680 555, India

<sup>2</sup>Radiation Biology and Health Sciences Division,

Bhabha Atomic Research Centre, Mumbai 400 085, India

**Our previous studies have demonstrated that aqueous extract of *Ganoderma lucidum* occurring in South India possessed significant antioxidant activity. The present study was aimed at evaluating the radioprotective properties of the aqueous extract of this mushroom. Single-cell gel electrophoresis (comet assay), protection of radiation-induced plasmid pBR322 DNA strand breaks and inhibitions of lipid peroxidation (TBARS assay) were employed to determine the level of protection offered by the extract. The results indicate that aqueous extract of *G. lucidum* possessed significant radioprotective activity. The findings suggest the potential use of this mushroom extract for the prevention of radiation-induced cellular damages.**

**Keywords:** Comet assay, *Ganoderma lucidum*, lipid peroxidation, medicinal mushroom, radioprotection.

RADIATION protection has significant importance in radiotherapy of cancer, nuclear accidents and even in nuclear warfare. Radiation-induced cell damage results from either damage to cell membrane or DNA<sup>1,2</sup>. Lesions in DNA can be induced either by direct ionization of DNA or indirectly through the reaction of aqueous free radicals leading to base damage, intra- or inter-strand cross-linking and single- or double-strand breaks<sup>1,3</sup>. Unrepaired or misrepaired DNA damage leads to genetic instability, mutations and chromosomal aberrations<sup>1,3,4</sup>. This may lead to the death of progeny after several mitotic cycles; this type of cell death called as 'mitotic or clonogenic death' or 'mitotic catastrophe' is the most common in solid tumours exposed to radiation<sup>3,5-7</sup>. Protection of normal tissues against this cellular damage is important in radiotherapy. The major problem associated with cancer radiotherapy is the severe side effects and damage to normal tissues.

Ionizing radiation is one of the well established and widely used therapeutic modalities either for curative or palliative treatment of tumours in man. The cellular responses include arrest in cell-cycle progression at cell-cycle check points and induction of DNA repair. But the

balance of survival and death signals determines the homeostasis of normal cell systems. In radiotherapy of cancer, normal tissues need to be protected while cancers are exposed to high doses of radiation. A large number of compounds, natural and synthetic, have been evaluated for this purpose<sup>8</sup>. However, most of them failed clinically because of toxicity and side effects. Hence search for an ideal radioprotector is a compelling urgency.

*Ganoderma*, commonly known as reishi, is highly ranked in Oriental folklore. In Chinese medicine, reishi has been considered as a panacea for all types of diseases. Reishi has attracted significant attention in recent years due to its large number of pharmacological properties<sup>9</sup>. The fruiting bodies of this mushroom contain a variety of chemical substances. However, it has been reported that the physiological effects and distinguishing properties of *Ganoderma* are strain-dependent<sup>10</sup>. Recent investigations carried out in our laboratory have shown that *G. lucidum* occurring in the southern part of India possessed significant antioxidant, antitumor and anti-inflammatory activities<sup>11</sup>. We have examined the radioprotective effect of the aqueous extract of this medicinal mushroom and the findings are reported in this communication.

Tris base, high melting agarose, low melting point agarose, Na<sub>2</sub>-EDTA, TritonX-100, sodium sarcosinate, DMSO and propidium iodide were obtained from Sigma Chemicals (St. Louis, Missouri). Plasmid pBR322 DNA was obtained from Bangalore Genei (Bangalore, India). All other chemicals used were of analytical grade and procured locally.

The extract of *G. lucidum* was obtained from mushrooms collected from the outskirts of Thrissur, Kerala, India. The type specimen was deposited in the herbarium of Centre for Advanced Studies in Botany, University of Madras, Chennai, India (HERB. MUBL.3175).

Male Swiss albino mice, 8–10 weeks old and weighing 20–25 g, were selected from an inbred group maintained under standard conditions of temperature (25 ± 2°C) and humidity. All the experiments were conducted strictly according to the guidelines prescribed by the Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted by the Animal Welfare Board, Government of India.

<sup>60</sup>Co-gamma rays in a Gamma Cell 220 (AECL, Canada) at a dose rate of 5.3 Gy/min and Junoir Theratron unit (AECL, Ottawa, Canada) with a dose rate of approximately 0.35 Gy/min at 38 cm were used for irradiation purposes depending on the type of dose given for the experiments.

Sporocarps of *G. lucidum* were dried at 40 to 50°C and several batches of 100 g powder were extracted with distilled water at 80°C for 8–10 h. The extracts were combined, filtered, concentrated and evaporated at low temperature using rotary vacuum evaporator. The residue thus obtained was used for the experiments. The yield was 8%.

The plasmid pBR322 DNA (250 ng in 10 µl in 0.01 M sodium phosphate buffer) was exposed to various doses

\*For correspondence. (e-mail: kkjanardhanan@yahoo.com)