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A LOW ANGLE NEUTRON DATA ACQUISITION SYSTEM FOR MOLECULAR BIOLOGY

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Introduction

The low angle Brookhaven spectrometer system utilizing a 2-dimensional position sensitive counter was designed to accomodate a variety of experiments in molecular biology requiring good low angle resolution. Biological structures requiring low angle analysis techniques fall into two groups:

1) non-ordered systems, i.e. proteins or protein complexes in solution (Engelman and Moore, 1975), and 2) ordered systems with large spacings like muscle, collagen, membranes, etc. (Worcester et al., 1975; Miller et al., 1975; Schoenborn, 1976).

For structural investigations into such systems, data is ideally needed to a low scattering angle of $.2^\circ$ at $4.5 \text{ \AA}$ or a minimum Q of $.005 \text{ \AA}^{-1}$ ($Q = \theta \cdot 2\pi/\lambda$). Depending on the type of structure, data often extends to the high angle region, say 30° . Apart from the low angle requirements, the spectrometer has to have good resolution to resolve diffraction peaks from samples with crystal spacings up to $1000 \text{ \AA}$ or even larger. While it is desirable to build a spectrometer to such scattering conditions, given reactor conditions might not permit this and compromises have to be made between flux, resolution and lowest angle. The low angle spectrometer described here was designed to be used at the HFBR beam pipe working at $\sim 4.2 \text{ \AA}$ or at the H4 satellite station working at $2.4 \text{ \AA}$ with an eventual move of this system to the cold moderator B beam at H9 (Fig. 1).

Spectrometer Parameters

In order to assign general dimension to such a spectrometer, the sample dimension, counter resolution, beam divergence and flux have to be considered. Biological samples are often very small since many cellular subfraction occur in very small amounts which are difficult to extract and purify. For solution scattering, samples of ~ 0.1 cc are typical with protein concentration in the range of 5 to 30 mg/cc. Since the samples are often dissolved in H_2O , D_2O or mixtures thereof, the sample thickness are of the order of a few millimeters for an attenuation of $\sim 1/e$. For solution scattering, a sample diameter of 6 mm was chosen with sample thickness of 2 to 6 mm depending on the sample's absorption (Fig. 2). Such a sample size is also well matched to the counter resolution which is < 3 mm.

The other factors to consider to attain a lowest scattering angle are wavelength, beam collimation and beam stop characteristics. For most practical considerations, the obtainable flux at a given wavelength and collimation will decide what lowest angle data can be measured.

For the Brookhaven High Flux Beam Reactor with a D_2O moderator, the low energy neutron distribution is nearly maxwellian, peaking at ~ 1.1 Å. Due to the rapid decrease in flux with increasing wavelength, experiments with $\lambda > 5$ Å are at present not feasible. The impending installation of the cold moderator will increase the usable wavelength range to at least 10 Å.

At present, the low angle Biology spectrometer is either used at 4.2 Å at a beam pipe (H4B) or at the H4 satellite station as shown in Fig. 3.

At H₄S, monochromatic neutrons are selected from a pyrolytic graphite crystal set for 2.37 Å, a wavelength suitable for good $\lambda/2$ attenuation by a tuned graphite filter. The graphite filter is composed of 8 stacked graphite plates which a combined thickness of 5 cm. The beam flux characteristics for λ and $\lambda/2$ were measured with an analyzer crystal and the absorption curves are shown in Fig. 4. With 8 filters, the flux at $\lambda/2$ is less than 10^{-3} of the flux at $\lambda = 2.37$ Å. The setting of the filter is easily adjusted by rotation of the filter around two orthogonal axes perpendicular to the beam. This adjustment is done with an analyzer crystal set for $\lambda/2$. To test if the filter setting is correct in a configuration where the counter is set for low angles and is not movable to higher angles, the linear absorption coefficient of a set of absorbers is measured and compared to previously determined absorption coefficients for λ and $\lambda/2$. The percentage $\lambda/2$ contamination is then readily calculated.

The desired beam divergence with a beam diameter that is matched to the flat cylindrical solution sample cells (Fig. 2) is achieved by variable length collimators made from a steel pipe with beam defining discs spaced every 5 cm. These beam defining discs have a 6 mm ϕ circular hole and are made from cadmium fronted by a 2 mm thick boron carbide-lead absorber which is beveled toward the beam defining edge of the cadmium disc. These collimators of 0.7 m, 1.4 m and 2.1 m length give a well defined circular beam with divergence of .5°, .25° and .15°.

For most solution scattering experiments, the 1.4 m collimator is used yielding a flux at $2.37 \text{ \AA}$ of $5 \cdot 10^7 \text{ n/cm}^2 \text{ min}$. The beam stops used in such experiments are 2 or 5 cm long aluminum cylinders of various thickness packed with fine grain (200 mesh) boron carbide powder. Tests with boron carbide, boron polyethylene, Li^6 metal, Li^7 fluoride and Li^6 carbonate showed that boron carbide gave less beam stop scattering than the other materials

Two Dimensional Detector

This low angle spectrometer uses a two dimensional position sensitive detector with an active area of $18 \text{ cm} \times 18 \text{ cm}$. This detector is a multiwire proportional counter using the $^3\text{He} (n,p) ^3\text{H}$ reaction which has a reaction energy of 764 keV and a cross section of 5327 barns for thermal neutrons. The counter operates at about 3 kV with a gas filling of 6 atm ^3He , 4 atm Ar, and 0.5 atm CO_2 or CH_4 with an efficiency close to 75% at $2.4 \text{ \AA}$.

The neutron position is determined from the induced charge on two orthogonal grids of resistive wires by a charge division technique. A detailed description of the counter parameters and read out electronics is given by Alberi et al., 1975, and a summary of the characteristics is given in Table 1. The only modification made to this counter over the last three years has been the addition of a getter purifier to reduce contamination of the highly purified gases.

Monitoring the pulse height distribution does indicate some minute changes in the counting gas composition requiring an occasional adjustment of the anode voltage to maintain a reasonable pulse height

spectra and gamma ray discrimination. The main disadvantage of such high pressure counters is caused by the required thick window of 9.5 mm aluminum with a resultant 7% beam attenuation by scattering.

A revised version of the chamber (20 x 20 cm) has recently been developed. It simplifies the construction, and improves the efficiency, spatial resolution and gas stability. The two dimensions are readout from one cathode and the anode. The anode also serves as a pulse height monitor to discriminate against gamma rays. The anode to cathode gaps are 7.5 mm.

One cathode consists of thin metal strips spaced about 5 mm on a glass plate. The strips are interconnected by resistors. Similarly the anode wires (25 μ m dia., 2.5 mm spacing) are also interconnected by resistors. See Fig. 5.

The position of the interaction is read out from the ends of each resistor chain by a charge division method.

The gas, 6 atm ^3He , 4 atm Argon and .5 atm CH_4 is circulated with a small pump through a purifier removing harmful outgassing and decomposition products.

The position read out accuracy is better than 0.3% of full scale for x and y and is completely linear as measured by a uniformly spaced (1 cm) raster scan. For routine linearity tests a calibration plate with neutron transparent concentric circular rings implanted into the absorber material of boron carbide mixed with epoxy is mounted on the counter window and the counter is then exposed to incoherently scattered

neutrons by a polyethylene sample. The spacings of the observed ring pattern is then used to calculate the grid parameters and test for linearity.

The counting linearity of the detector was measured by a grid scan with a small highly collimated beam. The counter showed a periodic counting rate variation of 3% in the vertical direction. This gain variation is caused by a small fluctuation in the anode field due to the wire spacing of 2.54 mm. No correction is made for this variation since in all cases data is integrated over a few cells averaging this gain change.

The computer system for the present low angle spectrometer is shown in Fig. 6, and uses two SDS 920 computers, one to control the sample positioning and exposure duration based on a flux monitor, the other computer is used to accumulate the 2D data array and provide some on line display of the diffraction patterns. The active counter window is decoded into a 128 x 64 matrix with a data processing rate capacity $>10^5$ counts/sec. A dead time counting unit is incorporated but seldom used with the low scattering intensity from biological samples. The data is read into the memory of the SDS 920 via a 256 word double buffer. At high counting rates, refreshment of the on-line display is diminished due to the priority interrupt of the counter. Both computers are programmed in machine language. There is no provision for direct data analysis in the 920 system apart from a dump of the data array on a printer or magnetic tape unit. All data processing is done on the central CDC 7600 computing system with the data transferred via magnetic tape. A series of data processing programs have been developed to provide

for data manipulation, various integration and graphic representation. A new spectrometer control system developed for the cold moderator station uses a PDP 11-34 computer for the spectrometer and counter control functions as well as an on-line display. The 2D counter data is decoded into an external direct access memory with a derandomizer input as described by Dimmler and Hardy, 1976. This fortran programmable computer system will permit on-line data analysis like strip or radial integration which are directly displayed. Final data processing will still be done on the CDC 7600 computing system with the data again transferred via magnetic tape.

Monochromators

In both presently used spectrometer configurations pyrolytic graphite monochromators (Riste and Otnes, 1969) are used with a graphite filter for $\lambda/2$ removal at $\lambda = 2.37 \text{ \AA}$ (H4S) or a cooled Be filter at $\lambda = 4.2 \text{ \AA}$ (H4B); pyrolytic graphite monochromators at these wavelengths are efficient (80% or better) but do have a large intrinsic beam divergence ($\sim .4^\circ$).

For operation at the "cold moderator" beam, multilayer monochromators (Schoenborn et al., 1974) will be used at the end of a guide tube, Fig. 7. These devices are made by alternately depositing thin films of two materials with different scattering factors. Such a system is periodic in the direction normal to the plane of the multilayer with a Bragg spacing equal to the combined thickness of two films. If the two materials chosen have opposite scattering factors, like manganese and Germanium,

then multilayers with a small number of bilayers give rise to high reflectivities, small beam divergence and no high order contamination (Saxena and Schoenborn, 1977). If one of the repeating bilayers is a magnetic material like iron, then such multilayers can be used as efficient polarizers (Lynn et al., 1962; Mezzi, 1976). In practice, the Bragg spacing of such monochromators can be anywhere between 40 Å and 200 Å (or longer) with a selectable $\Delta\lambda/\lambda$ lying between .05 and .25 and an efficiency close to 100%. Multilayer monochromators offer, therefore, a number of advantages for low angle work, particularly for experiments with long wavelength neutrons.

Data Collection

In a typical solution scattering experiment like in the determination of radii of gyration of a protein complex, (Moore et al., 1974) the scattering from a number of samples has to be measured. Since the scattering from the minute amount of proteins is very small, the scattering from the solvent, the sample cell and sample independent background have to be measured, scaled and subtracted from the protein data. In a typical case, data from eight different samples are collected: 3 protein samples in buffers with different D₂O concentration, samples of the 3 different buffers, a blocked beam and an empty sample cell. These samples are mounted in a temperature controlled sample changer and exposed to neutrons under computer control. Data from the samples is collected for an appropriate monitor count in the order of one hour per sample but less for the background data. To accumulate statistical significant

data, the samples are recycled until the appropriate exposure time has been achieved. This sample cycling procedure averages long-term background and beam variations and possible sample deteriorations.

Sample to sample scaling is done with the help of direct beam attenuation measurement, while protein concentration is determined by spectroscopic or other techniques. The protein's activity is often measured before and after exposure to neutrons to monitor possible sample denaturation. The data for the individual sample runs are added, scaled to unit monitor flux and radially integrated before background and solvent subtractions.

A set of radially integrated data sets are given in Fig. 8 for empty cell, blocked beam, H_2O and D_2O solutions without protein. Fig. 9 shows a typical radius of gyration data set from acetylcholine receptors. The analysis of such low angle data is not the subject of this paper and is treated by a number of authors, notably Stuhrmann (1973).

The "protein pair" measurements on ribosomes by Engelman and Moore (1974, 1975) has to date been the most successful application of low angle neutron techniques to biological problems. The very weak signal of the deuterated protein pair within the density matched solvent-ribosome entity demands special care in data collection and processing techniques. Fig. 10 shows a data set from recent measurements of the S7 - S9 protein separation in the 30S ribosomal subunit (Langer et al., 1977). In this case, the interference function caused by the separation of the two deuterated subunits is obtained from two samples: 1) a mixture of equal amounts of 30S particles containing the two deuterated proteins and

the same particle with no deuterated proteins, 2) a sample containing an equal amount of 30S particles with one or the other of the proteins deuterated. Hoppe (1973) has shown that in the above described case, simple subtraction of the two data sets will yield directly the interference function caused by the separation of the two deuterated particles.

For lamellar and paracrystalline preparations, sample cell constructions vary widely depending on the preparation techniques used to achieve good orientation. For diffraction experiments from membranes like lecithin bilayers, where orientation is achieved by spreading the lipid on a thin quartz slide, the sample is mounted on a goniometer within a temperature and humidity controlled aluminum chamber. Data is then collected by rocking these oriented specimens through the Bragg angles (Fig. 11). For correct integration of the reflections, the spectrometer characteristics, like beam divergence and sample mosaic have to be known in order to determine the size of the reflection as a function of Bragg angle. The accurate determination of the vertical extent of reflections is particularly important for the weak higher orders (Saxena and Schoenborn, 1977b). Background can then be determined from areas close to the reflections or can be integrated over the same counter region (2 θ) but at a scattering angle before and after the respective Bragg angle. The latter background determination is more accurate but can however only be used for samples with small mosaic width.

For structural analysis of ordered systems with a large mosaic like retinal rods (Yeager, 1975) or oriented purple membranes (King et al., 1977), the use of a 2D counter is particularly advantageous since many reflections occur simultaneously. Data analysis in such cases where reflections often overlap is, however, more difficult. In the analysis of intact retinal rods, Yeager (1975) gave a detailed analysis of this problem. He obtained the background from the equatorial scattering by rotating the retina by 90° from the Bragg reflecting position. To fit a smooth curve to this background data he used polynomial functions with a multiple linear regression analysis. Peak integration was then achieved by fitting gaussians to the background subtracted data.

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Figure Legends

Fig. 1. Shows the beam and spectrometer layout of the Brookhaven high flux reactor with the biology low angle instrument positioned at H4BP.

Fig. 2. Shows a disassembled and assembled solution scattering cell; the assembled cell fits into an automatic sample changer.

Fig. 3. Biology low angle spectrometer layout at either H4BP or H4S.

Fig. 4. Shows the log of the beam intensity as a function of absorber thickness for λ and $\lambda/2$ to determine the wavelength dependent linear absorption coefficients of the absorber material (polyethylene).

Fig. 5. Depicts a cut away drawing of the new 2D position sensitive counter showing the anode and cathode plane.

Fig. 6. Schematic diagram of the spectrometer and counter system using two 920 SDS computers.

Fig. 7. Photodepicting the construction of the guide tube at the cold moderator. The aluminum mounting tube houses the glass guide tube (manufactured by Steichele) and guide tube adjustment and serve as a mounting unit for the boron carbide neutron shield and the outer lead shielding, the base lead plate and support columns are not shown.

Fig. 8. Log intensity data for a typical set of solution, background and buffer samples. The data is radially integrated and expressed as counts/mm² for a unit monitor count averaged from 12 repeat sample exposures. The counting errors are indicated for data at one angle only.

Fig. 9. A typical final data set for radii of gyration measurements; this data set is taken from an unpublished study of acetylcholine receptors by White, Karlin and Schoenborn.

Fig. 10 a. Shows the processed radially integrated interference data from the pair measurements of deuterated proteins S7 and S9. The data trace for the beam independent background (blocked beam) is also given to illustrate the high statistical accuracy required for such data. Data collection for each sample took 17 hrs. in intervals of ~ 40 min. The averaged data is scaled to counts/mm² with a monitor count of $8 \cdot 10^7$ (~ 40 min). The beam flux was $3.5 \cdot 10^7$ n/cm²/min, with a sample to detector distance of 150 cm, a boron carbide beam stop of 2.3 cm ϕ was used in a He flight path.

Fig. 10 b. Data for the 30S sample containing both the S7 and S9 deuterated protein and a data trace for the buffer alone. The low data point at 3.2° is an artifact and is due to an improper set counter window constant in the final computer data processing step. The differential nature of the final interference data set eliminates this artifact as long as the same counter window constants are used for both the signal and noise data (after Langer, Engelman and Moore, 1977).

Fig. 11. Intensity contour map of a dipalmitoyl lecithin sample showing the first 4 orders. This sample has a lamellar spacing of 60 Å and a mosaic of $.2^\circ$.

Table 1

Two-dimensional position-sensitive detector characteristics

1. Neutron detection efficiency at 2.4 Å wavelength	75%
2. Position resolution at $V_a - V_c = 3000$ V	2.7 mm
3. Positional uniformity of detection efficiency	3%
4. Integral linearity	1%
5. Differential linearity	0.1%/ % of full scale
6. Event resolving time	1 μ s
7. Event processing time	3 μ s
8. Gamma-ray sensitivity (^{60}Co) 2.7×10^{-6} detected/incident - γ	
9. Sensitive area	18 cm x 18 cm 6 atm ^3He
10. Gas-filling composition (absolute pressure)	4 atm Ar 0.5 atm CO_2

TWO DIMENSIONAL THERMAL NEUTRON DETECTOR

COMPUTER SYSTEM FOR BIOLOGY LOW ANGLE STATION

RADIAL INTEGRATED NEUTRON SCATTERING DATA FROM
ACETYLCHOLINE RECEPTORS IN 0.2% TRITON X100 IN 80% D₂O

RADIAL INTEGRATED NEUTRON SCATTERING DATA
FROM A 1% SOLUTION OF TRITON X100 IN 80% D₂O

RIBOSOME DATA PAIR INTERFERENCE
DEUTERATED PROTEINS S7-S9

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