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Some aspects of variability in the examination of biological materials by X-ray spectroscopy

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Abstract

Results are reported of tests to examine aspects of variability when an X-ray spectrometer, with a 25 mCi Americium 241 source of radiation, is used in an air atmosphere with a 40 sample automatic changer. Samples containing theoretically identical components of variability were used, 40 of which were mounted on one sample wheel and 10 on a second. We found significant differences between the first 10 samples from wheel 1 and the last 10 samples from wheel 1. There was an approximate 60-hour period, during which the spectrometer continued to run, between the last sample of the first set and the first sample of the last set. Variability is apparently attributable to electronic effects of unknown and unpredictable origin. In a second test we compared the first 10 samples from wheel 1 with the 10 samples from wheel 2. In this case, over 90 hours intervened between the running of the last sample in the first set and the first sample in the last set, and adjustments of a mechanical nature had to be made to the equipment. Gross differences were apparent between these two sets of samples. The degree of variability demonstrated between the first and the last set of samples on wheel 1 was not unexpected and should not adversely affect experimental work if samples are randomized, and if sub-samples (if required for any purpose) are taken from the total series at random. The variability demonstrated between results from the two sample wheels was great enough to be of major concern in experimental work. The deliberate elimination of extraneous variability is clearly indicated and some suggestions are advanced.

Résumé

Les auteurs donnent les résultats d'essais visant à déterminer les variations d'analyses effectuées à l'aide d'un spectromètre à rayons X en atmosphère normale, muni d'un dispositif automatique de changement pouvant contenir 40 échantillons et dont la source radioactive se compose de 25 mCi d'américium 241. Ils ont utilisé des échantillons théoriquement identiques quant aux variations étudiées; 40 ont été placés sur un des plateaux du dispositif et 10 sur un autre. Une différence significative a été établie entre les 10 premiers et les 10 derniers échantillons du premier plateau. Entre le dernier échantillon du premier groupe et le premier échantillon du dernier groupe, le spectromètre a fonctionné de façon continue pendant environ 60 heures. Les variations sont, semble-t-il, attribuables à des effets électroniques d'origine inconnue et imprévisible. Au cours d'un second essai, les auteurs ont comparé les 10 premiers échantillons du premier plateau avec les 10 échantillons du deuxième. Dans ce cas, une période de 90 heures s'est écoulée entre le passage du dernier échantillon du premier groupe et celui du premier échantillon du dernier groupe; de plus, au cours de cette période, on a dû faire des ajustements mécaniques. Des différences importantes ont été enregistrées entre ces deux groupes d'échantillons. Le degré de variabilité entre le premier et le dernier groupe d'échantillons du premier plateau était prévu et ne devrait pas nuire au travail expérimental si les échantillons ont une répartition aléatoire et si les sous-échantillons (le cas échéant) sont tirés au hasard de la série complète. La variabilité enregistrée entre les résultats des échantillons de chaque plateau était assez significative pour poser un problème sérieux au cours du travail expérimental. Les auteurs ont délibérément éliminé les variations dues à des effets extérieurs et ont énoncé quelques propositions.

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Introduction

In 1968 the Canadian Wildlife Service (CWS) commenced a project designed to attempt the diagnosis of the origins of waterfowl through the chemical elements found in their primary flight feathers. Prior work (Hanson and Jones 1968, Devine and Peterle 1968) indicated that this should be possible. We assume that, because flight feathers are shed once annually and simultaneously, and because the birds are grounded until they grow new feathers, the feathers must in each case uniquely reflect the chemistry of the area in which they were grown.

We discard lead as a potential diagnostic element. Much of our sample material was from shot birds and we assumed that lead shotgun pellets passing through the wings would contaminate feathers. Nonetheless, we examined lead contamination, since there was reason to believe that zinc, copper, and perhaps other elements, which are known to be sometimes incorporated with lead, might also be involved. Tests to that end are reported elsewhere (Kelsall and Burton 1974).

We used the same tests to examine some aspects of variability inherent in our technique and instruments and those results are reported here.

Materials and methods

Pieces of plain, unbleached cotton cloth measuring 0.8 m^2 were mounted as targets on sheets of corrugated cardboard backing. Cotton cloth simulates fairly closely the primary feathers of waterfowl and provides a more uniform matrix for the assessment of results. Eight targets were prepared; two were set aside as controls, and two each were fired at with a 12 gauge shot gun at ranges of 11.4, 22.9 and 45.7 m. Imperial Long Range shells (Canadian Industries Ltd.) containing No. 2 shot were used in all cases.

In the laboratory the targets were divided into pieces approximately 58 cm^2. Five sample squares were cut at random from each of the two controls and five squares containing shot holes were taken at random from each of the squares that had been fired on. In addition, five squares from within the shot pattern, but which did not contain

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shot holes, were selected from each of the two targets that had been fired at from 22.9 m.

Samples were mounted on a 40-sample automatic changer of our own fabrication. They were irradiated by an X-ray source consisting of 25 mCi Americium 241 in an air atmosphere. Chemical spectra were accumulated to 1,000,000 particle counts (a process requiring about 3 h per sample), in 512 channels of energy emission between 0 and 40 keV. Our pulse height analyser (Northern Scientific NS-880) provided a visual display of each spectrum as it was being accumulated and sequential storage of complete spectra on magnetic tape. Output from our magnetic tape was via a conventional teletype on to punch tape.

Forty samples were mounted on one holder (large circular aluminum wheels), and 10 on a second. All samples containing shot holes were mounted so that a single hole and the area around it would be presented to the detector. Because we could not then conveniently process completely randomized data, samples were mounted in the following repeated sequence: 45.7 m with shot hole; 22.9 m with shot hole; 22.9 m without shot hole; 11.4 m with shot hole; control. When the samples on the first wheel had all been examined, a liquid nitrogen Dewar flask on the detector was filled and the second wheel, with the final 10 samples, was put in place.

Data were processed and analysed on an IBM 1130 computer at the Computing Science Centre, Pacific Biological Station, Fisheries Research Board of Canada, Nanaimo, B.C.

Student's "t" test (Simpson *et al.* 1960) was used to present results in graphical form. In Figures 1 and 2 mean values for the first 10 samples from wheel 1 provide the baseline, and deviations from that are expressed in terms of "t" values (above or below the mean) for each of the 512 channels in which alpha, beta and gamma rays were counted.

This presentation has a number of advantages, the chief of which is that background effects are eliminated since they are theoretically constant in all spectra and therefore do not contribute to variability. In addition, it can be readily judged where chemical elements are involved because of the clustering of channels to provide broad peaks in specific areas of emission energy. Variability which might have sufficient significance to affect experimental results is shown also as broad peaks, but in areas where chemical elements are not thought to be involved. Statistical significance is readily judged because, with 18 degrees of freedom (n=2 in all cases), when a peak reaches a height of 2.1 on the Student's t scale then $p = 0.05$, and when a peak reaches 3.9 on the t scale then $p = 0.001$ or less. Cases where single channels reach heights of statistical significance are due either to chance alone, or perhaps in a few cases to the occurrence of error in the data. It is believed that error peaks are minimal.

Results

We used this experiment in two ways to test the sensitivity and value of our equipment and of our electronic and mechanical techniques. First, from experience, we suspected that some of our variability might simply be due to electronic effects over a period of time. In this experiment it took over 150 hours to examine 50 samples, including shut-downs due to power failure and to changing the sample wheels.

Our first test of the effects of variability, in time, compared the first 10 samples from the start of sample wheel 1 with the final 10 samples on the same wheel (Fig. 1). Each set theoretically contained identical components of variability. The only difference in their treatment was that a period of over 60 hours intervened (during which the spectrometer continued to run) between the completion of the last sample in the first set and the first sample in the last set. With 512 channels of emission energy one would expect that approximately 25 would exceed a t value of 2.1, and that 10 might exceed a t value of 2.6, through chance alone. In fact, as Figure 1 shows, about 43 channels exceed 2.1 and about 20 exceed 2.6. This demonstrates that variability is introduced into a sample run over a prolonged period. This variability is apparently attributable to electronic effects which are common in such equipment as ours, but unknown and unpredictable.

Our second test of extraneous variability compared the first 10 samples on wheel 1 with the 10 samples run on wheel 2 (Fig. 2). Again, variability within the two sets of samples should theoretically be the same. However, in this case, more than 90 hours intervened between the running of the last sample in the first set and the first sample in the last set. In addition, adjustments of a mechanical nature had to be made in the equipment. First, the liquid nitrogen Dewar-flask, which supports the spectrometer, was filled. Theoretically, that should have had no effect of a lasting nature on experimental results unless the flask was filled when a sample was being examined by the spectrometer. Care was taken to ensure that that was not the case. Second, one sample wheel had to be substituted for another. Again, that should have made no difference since our wheels were carefully machined to be identical. However, both processes did result in small movements in alignment between the sample and the spectrometer.

Gross differences are apparent between the two sets of samples, with many values, including many adjacent channels, exceeding the t value (3.9) required for a probability of 0.001. Where one might expect one such value due to chance alone (when 512 variables are being dealt with), there are 42 such values (Fig. 2). It is noteworthy that all but a few of these high values lie between 8 and 27 keV, the area where our equipment was most plagued by backscatter peaks. Only the two peaks in the area of 8 to 10 keV seem explicable in terms of chemical elements. They correspond to the positioning of backscatter peaks invariably developed from the tungsten shielding in our radioactive source.

Summary and discussion

The demonstration of some variability over a period of time, beyond that attributable to chance alone, on one sample wheel was not unexpected. The degree of variability shown should not adversely affect experimental work if samples are randomized on the wheel, and if sub-samples (if required for any purpose) were taken from the total series at random.

The variability demonstrated between results from the two sample wheels, however, is large enough to be of great concern in experimental work. The minimum compensation when two or more wheels are being used is to ensure that all samples are randomly distributed on the wheels and that sub-samples, if required, are taken at random.

The deliberate elimination of extraneous variability is clearly indicated. Much of it is probably due to slight differences in both vertical and horizontal alignment as a result of changing sample wheels and of filling the spectrometer's Dewar flask with liquid nitrogen. In our work to date, the positioning of the spectrometer has not been rigid. We have relied on its weight and on great care in handling to prevent movement. This is obviously inadequate and mechanical means must be developed for positioning it in relation to the sample wheel.

Acknowledgements

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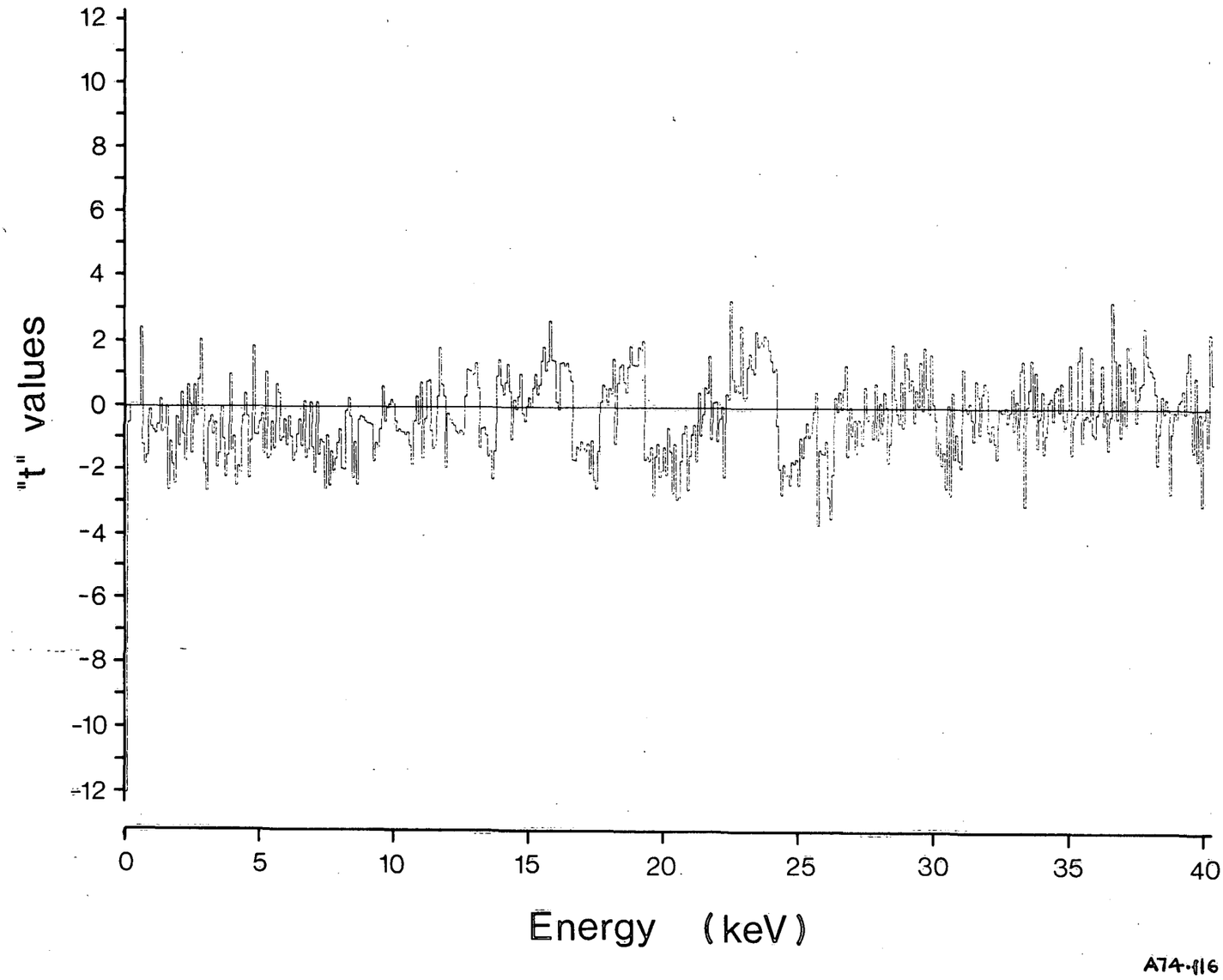

Figure 1
Comparison of the first 10 samples on wheel 1 with the last 10 samples on wheel 1. Calibration is in values of Student's "t" for a 512 channel analysis within the energy range shown

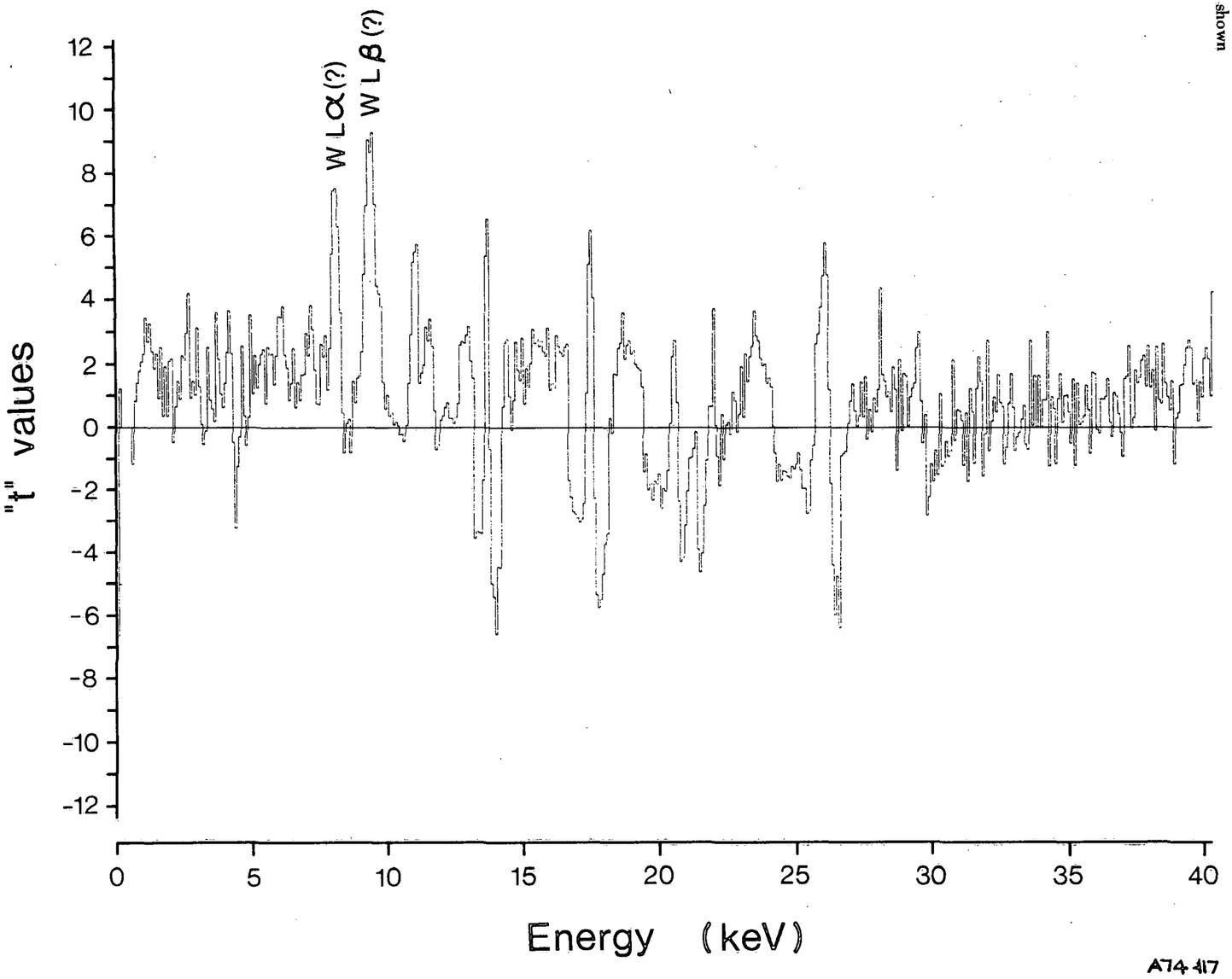

Figure 2
Comparison of the first 10 samples on wheel 1 with the 10 samples on wheel 2. Calibration is in values of Student's "t" for a 512 channel analysis within the energy range shown