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ON THE APPLICATION OF THE LIQUID SCINTILLATION COUNTING OF ^{14}C-LABELLED PHYTOPLANKTON.

by

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Summary

The paper describes two methods concerning the use of liquid scintillation technique in assaying weak beta emitters incorporated in phytoplankton material with special reference to the ^{14}C-method in primary productivity measurements. The problem of β-absorption and quench correction is discussed, and a line of procedure is pointed out. Results from using two commercially available scintillation cocktails/tissue solubilizers are presented. Furthermore, the preparation and calibration of the NaH$^{14}CO_3$-solutions for primary productivity measurements is described.

"On the Application of the Liquid Scintillation Counting of ^{14}C-labelled Phytoplankton".

INTRODUCTION

Measurements of the radioactivity of labelled phytoplankton samples as well as standardization of the sodiumcarbonate solution used for primary productivity measurements has for many years been carried out by using the Geiger-Müller technique. This method is still used, but now to a smaller extent The method has the advantage that it is relatively inexpensive compared to other methods and that the samples after measurements can be stored for later recounting.

It has, however, many disadvantages. The efficiency of an ordinary thin endwindow Geiger-Müller detector does never exceed 10%. The most serious problem when counting G -M is to compare the samples activity with that of the applied ^{14}C working solution. This is mainly due to hard controllable differences in the β-absorption phenomenon both from sample to sample and between phytoplankton and the precipitated working solution.

The reliability of the selfabsorption curve, which is necessary for calculation of the absolute activity of the tracer solution, when using the bariumcarbonate precipitation method, has been much discussed. It was experimentally found,that the extrapolation to zero thickness activities of the curve,does not represent the real conditions of radiation of labelled plankton on the filters.

In 1965 Steemann Nielsen therefore developed a new technique for G-M measurements of the absolute activity in the ^{14}C-solution, the "Biological Method". The advantage of this method is,that the total radioactivity of the working solution and of the radioactivity of the filtered planktonmaterial are made in an identical way.

Since the publications by Shindler (1966), Wolfe and Schelske (1967) and others the use of the liquid scintillation (LS) technique for measuring radioactivity of ^{14}C-labelled phytoplankton samples, and of the ^{14}C solution, has increased rapidly.

During refined sample preparation methodology it is possible with this technique to calibrate the countings to a constant efficiency f.ex. in absolute units. This can be done to almost any kind of ^{14}C-labelled material.

But neither is this technique without pitfalls when applied to labelled phytoplankton material.

It is a well known fact, that the counting efficiencies for liquid scintillation samples varies with the composition of the liquid.

There are especially two phenomenons bothering the counting efficiencies:

1) Bad contact between the scintillation fluors and the week β-particles from ^{14}C incorporated in planktonic algae (or other particles of a size comparable to the β-range).
2) Impurities from solubilizers, plankton material, filters and alike.

Both phenomenons quench the available energy, reducing both the pulseheights and the total number of detectable nuclear events. Counting efficiencies as measured in a pulseheight channel are therefore uncertain, particularly when assaying weak-energy radionuclides as ^{14}C.

During sample preparation, phenomenon no 1 must be completely removed (and checked). But no 2 can be handled through proper quantitation and correction.

Besides being a very accurate technique the liquid scintillation technique has been developed to handle larger amounts of samples without much human effort.

So it appears simple and time saving compared to the G -M technique.

- To see if or how the β-absorption problem can be avoided or removed the ^{14}C Agency carries out a data-analysis of old and new data concerning phytoplankton samples counted both by the G -M and the LS technique.

This is done as many users still want to employ the much cheaper G -M equipment. In the following the methods applied at the ^{14}C Agency for preparation of the working solution and for measurements of the radioactivity by the liquid scintillation technique are described.

THE WORKING SOLUTION

Preparation of the carbon-14 labelled sodium hydrogen carbonate.

At our Institute, carbon-14 labelled bariumcarbonate ($Ba^{14}CO_3$ from the Radiochemical Centre, Amersham) constitutes the basic carbon-14 source. This is mixed with inactive barium carbonate ($Ba^{12}CO_3$) of high chemical quality.

The final concentration finishup with 2.2 mmol dm^{-3}, except for the 1 µCi solution, where the concentration is 1.8 mmol dm^{-3}.

With these concentrations ampoules are produced in four standard versions with a total activity of 1, 4, 10, and 20 µCi per cm^3, corresponding to the specific activities 0.56, 1.8, 4.5, and 9.1 mCi per mmol respectively.

Line of procedure.

Preparation of the working solution at the Carbon-14 Agency is mainly carried out according to Steemann Nielsen (1952) and is as follows:

STEP 1 The ^{14}C-labelled $BaCO_3$ plus the calculated amount of inactive $BaCO_3$ is placed inside the testtube (P). This tube (P) is placed in a 1 litre suctionflask (S), in which previously has been added 15 cm^3 0.5 M sodium hydroxide (NaOH).

The suctionflask is evacuated and 0.5 M hydrochloric acid is slowly run into the testtube through the funnel (T).

This releases carbondioxide, which diffuses down to the sodium hydroxide-solution, with which it reacts, and is thus absorbed in the liquid phase.

Allthough experiments have shown that more than 99% of the CO_2 from the $BaCO_3$ is absorbed in the NaOH-solution within 15 minutes, the apparatus is left to stand for at least 60 minutes (usually over night).

After this period the vacuum is released and the testtube (now containing $BaCl_2$ and HCl) is removed.

STEP 2 The content of the suctionflask is diluted by freshly prepared double distilled water, the pH is adjusted to a value between 9.5 and 10 and made up to a final volume of quite 1 litre.

The working solution thus made up is then transferred into ampoules by means of an automatic dispenser. Finally the ampoules are flame sealed, autoclaved, and leakage tested.

Standardization of the working solution.

STEP 3 During the dispensing operation 4 ampoules out of 1000 are selected for calibration and standardization. A special procedure of selection is undertaken to control, amongst others, that no radioactivity has been lost during the dispensing and sealing operation. Serial no. 1, 333, 666 and 1000 is systematically selected.

The content of the 4 ampoules are transferred quantitatively to 4 measuringflasks (500 cm^3) and diluted with 0.05 M sodiumhydroxide. The pH of these solutions is kept high - between 11 and 12 - to prevent loss of radioactive carbondioxide during the preparation of the samples.

From each of the measuringflasks, aliquots of 1.0 cm^3 are transferred to 6 LS-glass vials containing 10 cm^3 of the scintillation cocktail Ready-Solv HP (Beckmann). 3 blanks are also prepared by adding precisely 1 cm^3 of the inactive 0.05 M sodiumhydroxide solution to 3 glass vials containing 10 cm^3 of the same scintillation cocktail.

STEP 4 All 27 samples are kept over night in the dark at room temperature for decay of chemiluminescence. Next day the 3 blanks are counted in the liquid scintillation counter.

After having secured that no significant contamination or chemiluminescence is present, a known, carefully weighed amount of a ^{14}C-n-hexadecane-standard is added to the 3 blanks. The ^{14}C-standard used is delivered from the National Bureau of Standards (NBS), Washington D.C.

The radioactivity of the 27 samples are then measured after normal light and temperature equilibration in the

liquid scintillation counter.

Statistics and certification.

STEP 5. Each radioactive sample is counted 3 times and 3×10^5 counts are recorded from each as a minimum. The uncertainty due to the radioactive decay mechanisms per se, is about 0.4% at the 95% confidence interval. Thus playing an insignificant role in the overall uncertainty on the final measure of radioactivity per ampoule.

A computerprogramme gives us:

1. mean values of the cpm Net counting rate for the 3 repeated measurements of each subsample,
2. mean values of cpm for the 6 subsamples from each ampoule,
3. mean values of cpm for the internal standard,
4. mean value of cpm for all the subsamples from the 4 ampoules,
5. the coefficient of variation (CV)"within"and"between" ampoules,
6. the counting efficiency (by internal standards), and
7. the dpm for the mean value for all 4 ampoules.

Each time a batch of ampoules has been produced the absolute radioactivity is measured and a certificate for the batch is worked out. This shows all technical batch data and the coefficient of variation within the batch with respect to disintegration rate from spot test samples as described above.

The certificate is always delivered together with ampoules supplied from the Agency.

The accuracy with respect to the disintegration rate given in the certificate is expressed as an estimate on the overall uncertainty, which we have found is less than $\pm 2.5\%$

Such a figure can be achieved in many ways. We have chosen to calculate this estimate of uncertainty in accordance with the recommendation of the International Commission on Radiation Units and Measurements (ICRU Report 12). The limits of uncertainty were taken as the arithmetic sum of the uncertainty due to random variations, calculated at the 95.0% confidence interval, plus the estimated systematic uncertainties.

Occasionally 3 ampoules out of a batch are tested for non-volatile radioactivity after acidification. I.e. 1-2 drops of concentrated HCl are added to the recently opened ampoules and after about 20 minutes the content of the ampoule is counted in the scintillation cocktail Ready Solv HP.

Hitherto no radioactivity above background level has been detected.

This test is particularly important when measuring the production of dissolved organic carbon.

FILTERED PHYTOPLANKTON

Filter treatment.

It is assumed that the phytoplankton is on membrane filterdiscs, and that they have been treated with formaldehyde or another histological fixative.

The filters are placed in a desiccator for 5 minutes over the fumes from concentrated hydrochloric acid to remove inorganic carbonate.

The filters are then dried in a desiccator containing both silica-gel and sodalime. The role of the sodalime is to remove excess hydrochloric acid.

After these treatments the rims of the filters are punched off and the area with the filtered phytoplankton is placed in the glass scintillation vial. The filter is then ready for the final treatment by either of the two following scintillation cocktails before the counting procedure.

Determination of the radioactivity according to Method 1. Soluene-350/PCS (S/PCS).

Since 1974 and up to 1978 the routine procedure for sample preparation and counting system used at the Carbon 14 Agency has been as follows:

If the filter is completely dry, it should be wetted by adding 0.1 cm^3 distilled water.

The phytoplankton and the filter is dissolved by adding 1 cm^3 of the tissue solubilizer Soluene-350. Complete dissolution takes 2-4 hours at room temperature.

When using membrane filters of cellulosenitrate the solution becomes yellow-brownish.

To avoid colour-quenching it is necessary to bleach the sample by adding a few drops of 30% hydrogen peroxide. After storage at room temperature for at least 3 hours the sample is decolourized and 10 cm^3 of the scintillation cocktail PCS, diluted with distilled water to 90%, is added. After having tightly capped and gently shaken, the sample is stored in darkness at room temperature for at least 3 days for the decay of chemiluminescence.

The radioactivity is then measured in the scintillation counter after temperature equilibration.

Determination of radioactivity according to Method 2. Ready Solv HP (RSHP).

At the ^{14}C Agency we now run a second method as daily routine. Both methods are run parallel as two alternative obtional methods.

This second method involves only one operation as follows:

The central part of the filter, punched out and treated as previously described, is placed in the glass scintillation vial. 10 cm^3 of the scintillation cocktail RSHP is added, the vial is immediately tightly capped, shaken and stored in darkness for about 24 hours, whereafter radioactivity measurement can take place after temperature equilibration in the scintillation counter as above.

As no significant chemiluminescence has ever been found in the samples treated by this scintillation cocktail, and according to test of the sample stability from 0 sec. to 6 days, it was found that the radioactivity could be measured already after storage of about 10 hours. It is, however, recommended to wait 24 hours until the radioactivity is measured to ensure that all the planktonmaterial has been completely solubilized. This is particularly important when greater amounts of algae are filtered.

Quench correction.

To ease international cooperation in the field of phytoplankton primary production, our aim is to measure all radioactive samples in absolute units. I.e. the liquid scintillation counter should be calibrated in true disintegrations per unit time: dpm.

It is a well known fact that filtered phytoplankton samples give severe and very varying quench effect, when counted in the liquid scintillation counter. So a reliable calibration curve (or function) is a very important tool in order to have the output converted to true dpm.

Regularly, i.e. once or twice a year, a quench correction curve is constructed as follows:

In each of 5 vials is placed a filter with inactive algae prepared in the same way as previously described. Scintillation cocktail/tissue solubilizer is added and the samples are treated as described before, according to one of the two methods of radioactivity determination.

It should be emphasized that each scintillation cocktail demands its own quenchcorrection function.

A known, carefully measured amount of the NBS ^{14}C-n-hexadecane-standard is pipetted to all 5 vials, and the radioactivity of the 5 vials is measured 3 times each.

About 200 mm^3 of carbon tetrachloride is pipetted to five evaporating devices, which fit at the top of each vial, where they are placed.

These devices are the so called "Hat-trick" from LKB-Instrument (cf.: Reunanen & Soini (1974)).

All 5 vials are replaced in the scintillation counter and are incessantly recounted each for a period of 30 seconds, while the concentration of the quenching agent in the scintillation fluid increases due to diffusion from the "Hat-trick" device. The measurements continue until a suitable quenching level has been reached.

In this way the entire range of actual counting efficiencies can be covered with relatively few calibrated samples.

Figure 1 shows a typical quench correction curve constructed as just described with inactive plankton-material on nitratefilters in the combined scintillation cocktail/tissue solubilizer Ready Solv HP.

Along the ordinate are the calculated counting efficiencies and along the abscissa the internal sample channels ratios (ISCR).

Curve fitting and calculation of parameters.

In many liquid scintillation counters with more than one channel it is feasible to set the gain and discriminators in such a way that a quenchcurve is very close to a straight line.

This was formerly a very common way of setting the instrument, in order to get easily hand-processable data. But the method could be rather timeconsuming and tedious, and worse: you very seldom hit the optimal setting of the instrument.

We have chosen to fit the curve to a polynomial of the third degree, which for this curve,has the following form:

$$E = A_3X^3 + A_2X^2 + A_1X + A_0,$$

example 1:covering the range from 69% to 89% counting efficiencies (E), the parameters to the curve shown in figure 1 have been calculated to:

$$E = 2.61X^3 - 17.0X^2 + 40.2X + 52.6$$

with the upper (UL) and lower (LL) limits at the 95% confidence interval as follows:

$$E = \begin{matrix} UL \\ LL \end{matrix} \begin{Bmatrix} 3.03 \\ 2.19 \end{Bmatrix} X^3 + \begin{Bmatrix} -14.9 \\ -19.0 \end{Bmatrix} X^2 + \begin{Bmatrix} 43.4 \\ 37.0 \end{Bmatrix} X + \begin{Bmatrix} 54.1 \\ 51.1 \end{Bmatrix}$$

The output data from the LKB liquid scintillation counter is as an option logged on punched papertape and are thus very easily processed by means of suitable EDP-programming.

We run the curve fitting on an IBM-360/370 computer using the Gauss-Newton non-linear iterative method.

Calculation of the parameters is also feasible on small desk-calculators, but the amount of data that can be processed are often very limited.

The shown quench curve has been constructed from 339 observations based upon 10 independent samples. Statistical independence of these samples is considered very important. The data were pooled from two data sets.

When counting unknown samples the calculated parameters are fed to a built-in microcomputer in our liquid scintillation counter.

This computer automatically corrects the raw cpm in accordance with the polynomial of third degree and the calculated parameters. But these calculations can as well be done on a (programmable) desk calculator.

The daily output from countings of ^{14}C-labelled phytoplankton is thus given in dpm, which should be directly comparable with the dpm of the applied working solution.

DISCUSSION

The importance of the basic source of ^{14}C-labelled carbonate.

There seems to be evidence that primary production measured by using $NaH^{14}CO_3$ solutions produced by different institutes may give considerable inconsistency in comparative experiments, although within each institute the reproduceability is satisfactory, when using batches produced in the same way. These discrepancies should be due to:

1) Toxic, non-labelled impurities originating from metal-, rubber-, and plasticparts used during processing.
2) The presence of radioisotopes other than ^{14}C, originating from the industrially prepared ^{14}C-material.

Solutions of $NaH^{14}CO_3$ for measurement of the phytoplankton primary production have been prepared using two methods:

a) By trapping $^{14}CO_2$ gas (released by strong acid from $Ba^{14}CO_3$) in sodium hydroxide solution in an all-glass device.
b) By direct dilution of the industrially produced $NaH^{14}CO_3$ solution of high specific activity. (According to Strickland and Parsons (1960)).

Measurements of photosynthetic capacities of plankton algae have indicated presence of toxic substances in solutions prepared according to method b). Both Nair (1974) and Steemann Nielsen (1977) (the latter partly quoting Nair and partly referring to own unpublished results) report that unexpected low productivity rates (about 50%) have been recorded by using $NaH^{14}CO_3$ solutions prepared according to method b), compared to results (from the same waterbody) obtained with solutions made according to method a). Both authors conclude the

discrepancies being due to toxic substances in the bicarbonate solutions prepared according to method b).

Steemann Nielsen (1977) mentions that certain commercially prepared solutions of $NaH^{14}CO_3$ may contain small amounts of other radioactive material. Williams et al. (1973) reports that up to 150 dpm/μCi may be present in commercial radioactive bicarbonate as nonvolatile radioactivity remaining in solution after removal of inorganic carbonate by acidification. This could be of great importance when determining the rate of release of dissolved organic matter from phytoplankton.

The ^{14}C Agency has always as described before made the working solutions from $Ba^{14}CO_3$ by trapping the $^{14}CO_2$-gas using an all-glass device. Furthermore, the applied sodiumhydroxide and the hydrochloric acid have been of the purest analytical grade.

Standardization of the working solution.

As the ^{14}C-method is prevailing for measuring planktonic primary production and as the only added ingredience is the radioactive working solution we find it very important that these solutions per se have the same effect, if any, on the photosynthetic capacity of the phytoplankton.

When using liquid scintillation counting of weak energy beta emitters as Carbon-14 incorporated in algal material, it is necessary to make sure that the ^{14}C-labelled phytoplankton samples on membranefilters are counted with well determined efficiency, so that the converted measure of radioactivity from each sample can be directly compared with the dpm of the working solution.

As mentioned before, the difficulties, which may arise, are mainly due to a severe and non-uniform quench effect and to the varying degree of homogeneity of the counting solution, plus due to a bad contact between weak energetic β-particles and scintillation fluors.

Despite of a very reliable and accurate quench correction it is necessary to ensure that the plankton material has been completely dissolved and that the scintillation cocktail/tissue solubilizer has the ability to

do so, no matter the composition of the phytoplankton populations.

The importance of a complete dissolution of the ^{14}C-labelled phytoplankton in the scintillation fluid is best illustrated when considering that more than 20% of the ^{14}C β-particles are emitted with a mean energy of about 10 KeV. This means that maximal range in histological fixed biological tissue is less than 0.9 µm. The maximal range for the most energetic ^{14}C -particles (155 µm) is only reached by less than 1% of the emitted particles. The two scintillation cocktails/solubilizers mentioned have been thoroughly tested both in a short term batch experiment and in a long term experiment during the ice free season of a whole year.

This test (Ursin et al. (1979)) has only been published in: "C14C Internal Report No. 1a/79", available on request free of charge.

It was shown that both methods were capable of counting ^{14}C-labelled natural phytoplankton. The "short term esperiment" did not reveal any significant differences between the two methods. The results are seen in table 1.

Method 1 (S/PCS) seems to give higher mean values but this is not statistically significant at the 95% confidence interval.

The "long term experiment" on the contrary showed that the above mentioned tendency in fact was significant when compiling paired results from the most of a growth season.

The results are seen in table 2, 3, and on figure 2.

Differences in mean values are observed, method 1 (S/PCS) giving the highest values, and it is obvious that the ^{14}C-n-hexadecane-standard is counted with a different efficiency than the ^{14}C-labelled phytoplankton. This indicates that the solubilizing capacity of RSHP is insufficient for some types of natural phytoplankton. Thus for investigations requiring a high level of accuracy, S/PCS or dry combustion should be preferred. However, the extremely good reproduceability (table 1 and 3). obtained with RSHP makes method 2 superior, when the aim of the study is comparability, and whereas systematic error of about 6% could be tolerated.

Besides,method 2 is very quick and does not demand much skill for getting a good reproduceability, which is a very important quality. The investigation emphasizes the importance of using natural populations and especially compilation of results through a whole year, when testing such procedures. Otherwise the observed lack of accuracy would not have been revealed.

Our investigation of the two scintillation cocktails/ tissue solubilizers does not guarantee that e.g. oceanic phytoplankton would have given similar results.

The combustion method in an induction furnace (Leco Incorp.) was a modified version of Burniso & Perez (1974).

This combustion method is considered as the most reliable reference method.

Unfortunately it is rather timeconsuming and not suited for handling large amounts of samples.

Curve fitting, precision and accuracy.

How accurate can a quench correction bo done using the described procedure?

When calculating the parameters for the third degree polynomial model we check the differences between the observed values and the computed values according to the applied parameters in the model.

These differences,which are called the residual values,are tested for their mean value and normality.

The mean value is most frequently very close to zero (typical $< 10^{-8}$).

This is of course not surprising as the Gauss-Newton's iterative non-linear regression method uses this criterion when calculating the parameters.

The relative standard deviation on the residual values should be as small as possible. If the relative standard deviation exceeds 1%, we confine the efficiency range thus calculating a new set of parameters including only a limited part of the observations closer to the area, where the unknown phytoplankton samples are expected to "fall".

In this way it should be possible to use only parameters for which the relative standard deviation on the variable R (R = residual value) is below 1% (and the relative standard error of the mean is below 0.1%).

When counting samples with a known amount of the NBS n-hexadecane ^{14}C-standard the relative standard deviation after quench correction has never exceeded 0.25% each time. When pooling results compiled through a period of 8 months the relative standard deviation on the actual quench correction has been calculated to 0.20%. So the contribution from the quench correction process per se to the overall uncertainty at the 95% confidence interval is less than 0.5% (cf.: ICRU Report 12).

How do we quantitatively detect the pulseheight shift?

When counting natural phytoplankton on membrane-filter discs, it would be expected that the sample solution was non-homogeneous.

The homogeneity of the counting solutions was tested using the double ratio technique of Bush (1968).

The results of this qualitative test are seen on figure 3A and B.

The ordinates represent the automatic external standard channels ratio (AESCR), and along the abscissae is the internal sample channels ratio (ISCR).

A homogeneous counting system with the active molecules in perfect solution should give an almost straight line through origo. Deviations from a straight line should indicate heterogeneity (to varying degrees).

One of the basic assumptions for using the AESCR is that the sample system is homogeneous so the fluors or scintillators will be radiated with the same geometry, both from active atoms inside the sample, as from the radiation caused by the external gamma source.

This test is not sensitive and the results are not easily interpreted, but we think that the AESCR is not seriously bothered by the presence of a filter or other particles inside the vial.

However, we concluded that the safest quench correction is done by using ISCR. This choice, consistant with theory, was made because it could not be told, wether another composition of the phytoplankton, or "water" would give a different picture.

Unfortunately this implies that low-activity samples should be counted for a rather long period to get a statistically reliable channels ratio.

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TABLE 1.

1	2	3	4	5	6	7
Group	Filter No.	Type	dpm Mean $\bar{X}_{20}$	SD {s+ / s-}	CV {CV+ / CV-}	Scint. cocktail
I	1-20	A	27241	526 {877 / 417}	1.9% {3.2% / 1.5%}	RSHP
II II	21-40 21-40	A	28315	599 {999 / 476}	2.1% {3.5% / 1.7%}	S/PCS
III	51-70	N	28065	849 {1415 / 675}	3.0% {5.0% / 2.4%}	S/PCS
IV	71-90	N	27000	971 {1619 / 772}	3.6% {6.0% / 2.9%}	RSHP

Results from the Batch experiment, dpm(mean), SD and CV.

1	2	3	4	5
Compared groups	t'	df'	P	Remarks
I/II	6.03	37	P>99.95%	Significant
I/III	3.69	32	P>99.95%	Significant
I/IV	0.976	29	80%<P<90%	Non significant
II/III	1.08	34	80%<P<90%	Non significant

Significance test between means, Batch experiment.
"Modified t'-test" (cf. Bailey 1959).
Meanvalues from table 2, column 4.

TABLE 2

Treatment	Serial no.	cpm "raw"	dpm $\frac{(\text{cpm-blind})}{E}$	Counting efficiency	dpm on the filter
Combustion	1	2018.2	2781		
	2	2056.8	2836		
	3	2208.8	3051		
	4	2096.2	2891		
	5	2299.1	3178		
	6	2350.6	3251	(Combustion	
	7	2214.2	3058	efficiency	
	8	2289.7	3165	~ 98%)	
	9	2175.2	3003		
	10	2134.0	2945		
	11	2117.5	2922		
	12	2184.2	3015		
	$\bar{X}_{12}$	2179±4.6%	3008	0.7084	3069
S/PCS	1	2706.8	3191		
	2	2739.3	3229		
	3	2526.7	2976		
	4	2504.8	2950		
	5	2604.3	3068		
	6	2416.4	2844		
	7	2541.4	2993		
	8	2688.6	3170		
	9	2657.1	3131		
	10	2565.1	3022		
	$\bar{X}_{10}$	2592±4.0%	3057	0.8373	3096
RSHP	1	2796.7	3143		
	2	2614.2	2932		
	3	2519.0	2823		
	4	2861.2	3217		
	5	2542.7	2851		
	6	2666.8	2993		
	7	2646.3	2969		
	8	2688.2	3017		
	$\bar{X}_{8}$	2667±4.4%	2993	0.8686	2993

Dry combustion experiment.

TABLE 3

Scintillation cocktail	CV_w	CV+ CV-
S/PCS	13%	18% 11%
RSHP	6.0%	8.0% 4.8%

Coefficient of variation and corresponding control limits. Long term experiment.

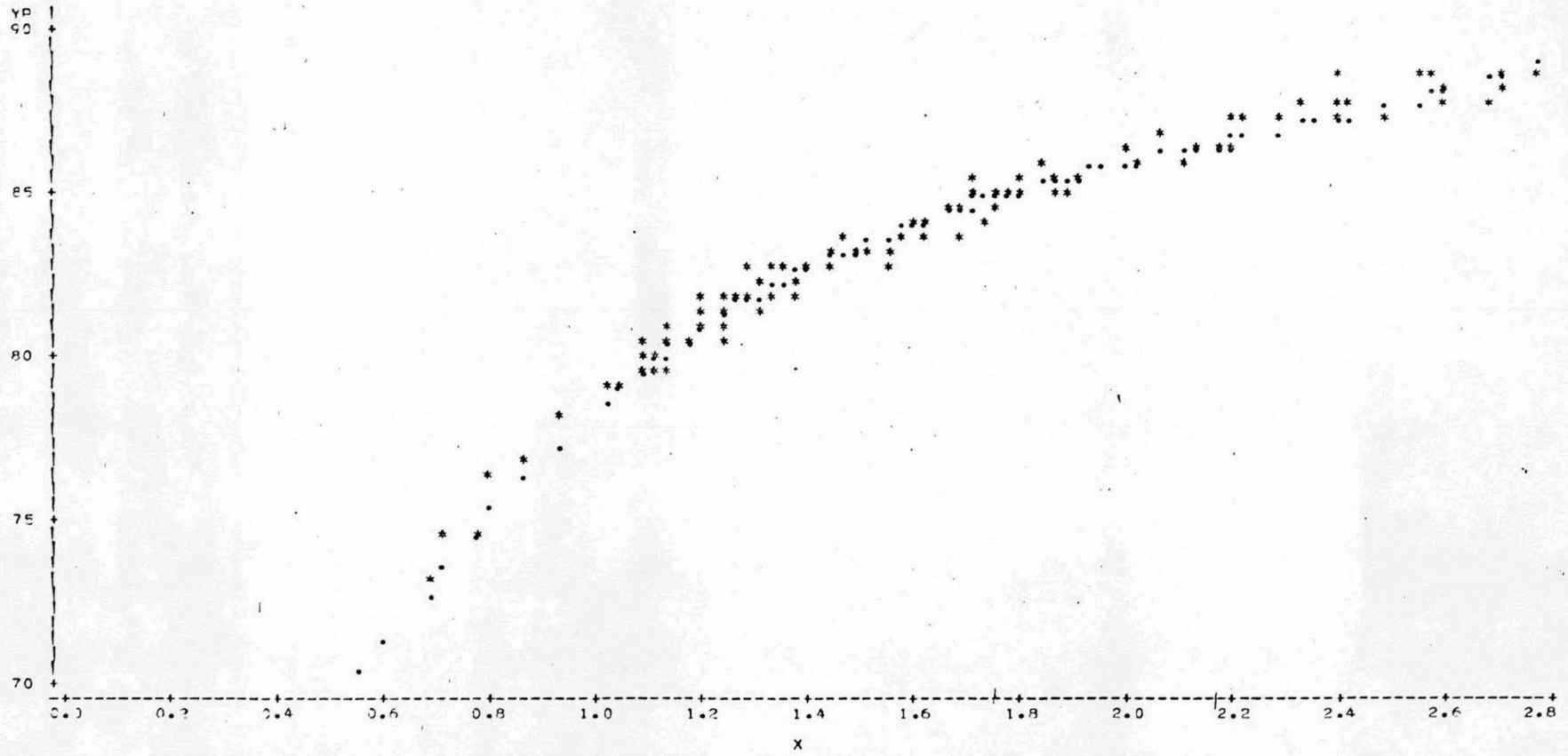

Fig. 1. A typical quench correction curve, RSHP.

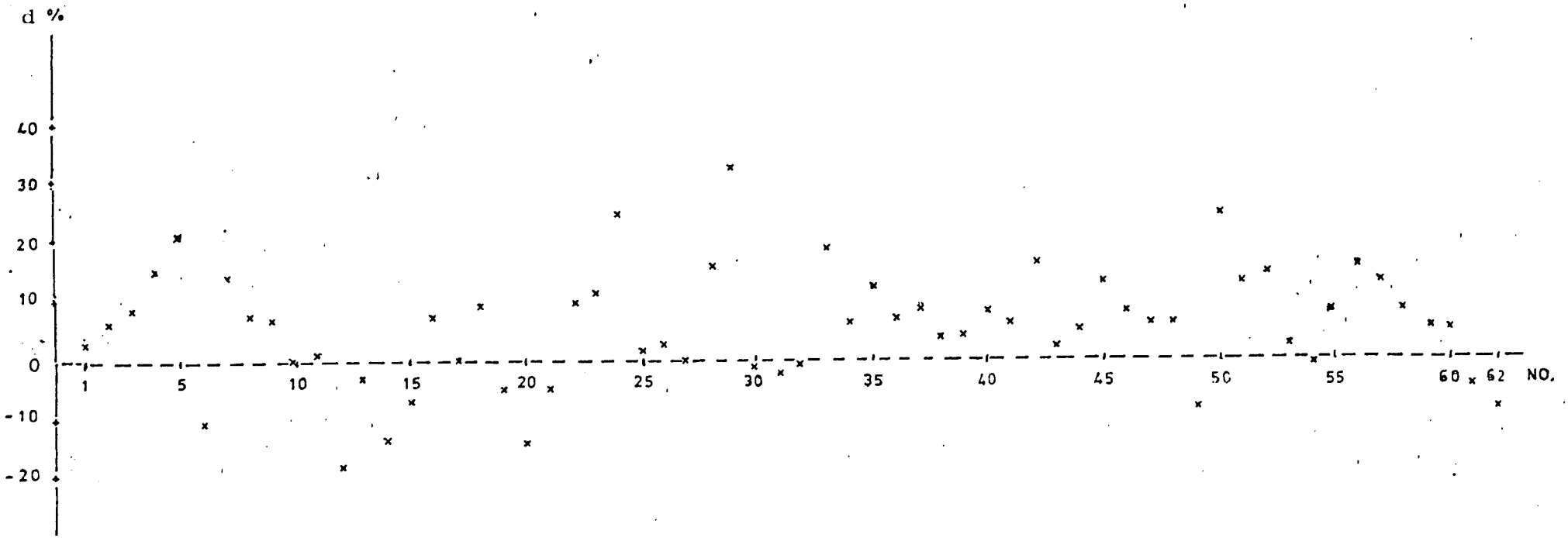

Fig. 2. The relative difference between paired observations (versus serial number), Long term experiment.

$$(d\% = \frac{(\text{dpm S/PCS} - \text{dpm RSHP})}{\frac{1}{2}x(\text{dpm S/PCS} + \text{dpm RSHP})} \times 100)$$

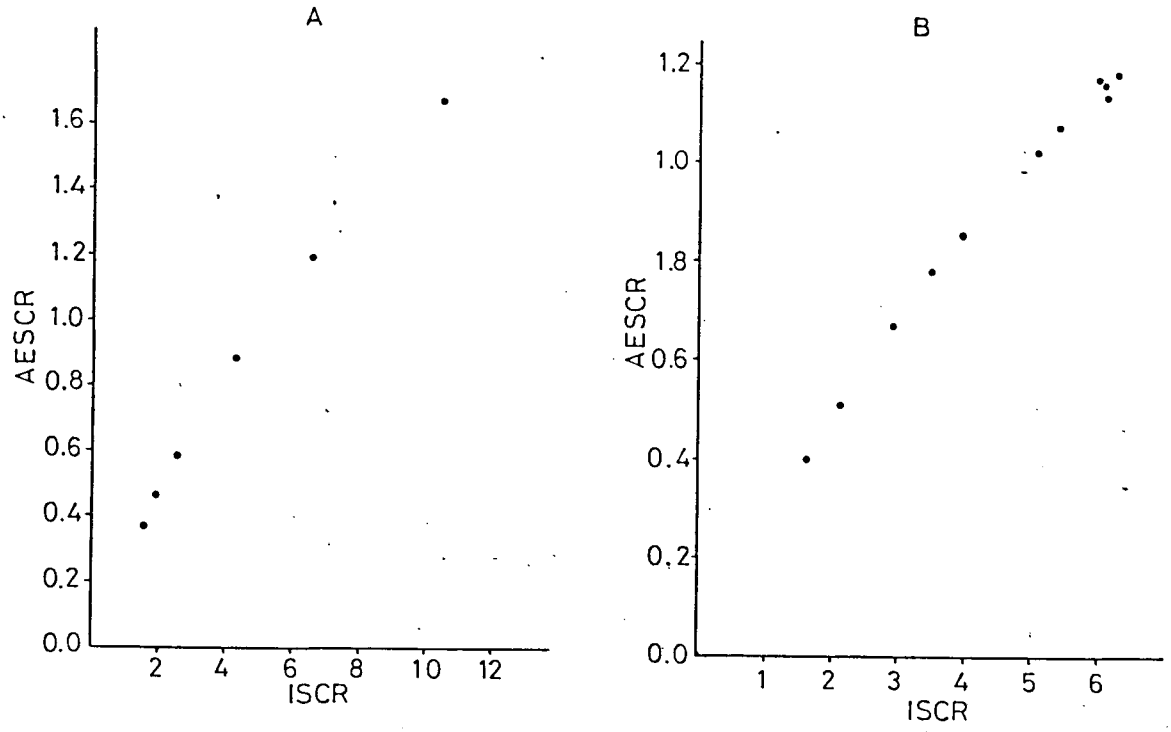

Fig. 3. Qualitative test for sample homogeneity by the "double ratio technique". Bush (1968).

A. LKB standard quench-set in a toluen-based scintillation cocktail inclusive ^{14}C-labelled toluen as the radioactive standard. The LKB quench-set should represent samples of perfect homogeneity.

B. Quench-set with the samples under test. The quenching material is nonradioactive plankton from Lake Lyngby in the scintillation cocktail RSHP. The added radioactive standard is ^{14}C-labelled n-hexadecane.