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DETERMINATION OF ^{14}C IN ALCOHOLIC BEVERAGES

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ABSTRACT. A simple and quick method for the determination of ^{14}C in ethanol has been developed, using an ultra low-level liquid scintillation counter. I have studied factors influencing the lower limit of detection and have shown that liquor can be measured directly without pretreatment. Results of measurements on Austrian wines are presented and compared with results obtained from tritium measurements. The applicability and limitations of the results to age determination are discussed.

INTRODUCTION

Radiocarbon has been injected into the atmosphere in the course of atmospheric nuclear bomb tests in an amount of ca 6.2MCi, mostly in the late 1950s and early 1960s. The activity concentration of ^{14}C rose from 6.1 to 12.2pCi/gC in the Northern Hemisphere. After the treaty on the ban of atmospheric nuclear bomb tests in 1963, the activity concentration of both ^{14}C and tritium decreased, slowly approaching pre-bomb levels. Enhanced ^{14}C activity concentrations in the atmosphere caused, via assimilation of plants (also via the food chain), equal distribution of excess ^{14}C in all biological material. In assimilation, ^{14}C is incorporated into carbohydrates and, therefore, alcohol which is produced by fermentation of sugar. Sugar reflects the ^{14}C activity concentration of the ambient atmosphere, which in turn can be used to determine the age of the respective alcohol-containing liquid. Similarly the activity concentration of tritium can be used for the same purpose.

OBJECTIVES

An original aim of this study was to monitor the mean ^{14}C activity concentration in the atmosphere via the concentration in ethanol. The discussion of the results will show that there are so many factors influencing the concentration in ethanol that it cannot be taken as an exact indicator for the air concentration. (Therefore, in the future, the concentration of ^{14}C in the air will be measured using carbon dioxide, which will be separated from the air during krypton enrichment for ^{85}Kr determination.)

The age of wine and spirits is of great commercial interest, since monetary value increases with age. Age determination then is important in controlling the age declaration. These measurements can also be used to detect admixtures of synthetic alcohol. Since ^{14}C decays in mineral oil and natural gas, the resulting ^{14}C activity in synthetic alcohol would be extremely low.

Alcoholic beverages are subject to food legislation in Austria and it is important that ^{14}C measurements can be made quickly and easily. Accuracy is not of primary concern in this instance. To meet these requirements, modern equipment is used and measurement conditions are carefully optimized.

INSTRUMENTATION

At our institute, two ultra low-level liquid scintillation "Quantulus" counters from Pharmacia-Wallac are used for a wide range of radionuclide determinations (eg, tritium in precipitation and surface water, ^{226}Ra, ^{222}Rn in mineral and drinking water, ^{90}Sr in dairy products, ^{85}Kr in air, gross α and β counting of waste and drinking water, etc). The extremely low background of these counters makes it very useful not only for low-level measurements, but they also yield accurate results in short measurement times and thus high sample throughput. Control of the routinely recorded pulse-height spectra can detect possible interference (eg, chemiluminescence) and makes development of techniques very easy and time-saving.

DEVELOPMENT OF THE METHOD – OPTIMIZATION

Because of demands for a simple and quick analytical method, procedures such as sample combustion, collection of carbon dioxide in bariumchloride, liberation with phosphoric acid, absorption in amine or benzene synthesis and subsequent measurement (Martin *et al*, 1981) could not be used.

Schönhofer & Henrich (1985) showed that pulse-height spectra can be seen clearly with the Quantulus even when mixing untreated liquor with a scintillation cocktail (Simon, Rauschenbach & Frey, 1986; Resmini & Volonterio, 1974; Guérain & Tourlière, 1975; Fischer *et al*, 1980). I also used direct measurement to investigate the factors influencing the results of liquid scintillation counting, namely, type of vial and cocktail, range of miscibility, mixing ratio and ethanol concentration. Instruments were regarded as constant and no attempt was made to optimize by changing their characteristics.

Only disposable standard 20ml vials were tested. Polyethylene vials were not considered because, although they show low backgrounds, their diffusion properties are highly unfavorable if samples are kept for some time. In glass vials, the measurements are disturbed by interference from the Cerenkov radiation produced by ^{40}K background, which is much higher than in plastic vials (Schönhofer & Henrich, 1985). Wallac teflon vials show ideal properties, but are too expensive for routine work both in cost and cleaning time after analysis. Vials made from polyethyleneterephthalate (PET) (Wheaton) show somewhat higher background levels when other radionuclides such as tritium or ^{222}Rn are determined. However PET vials are transparent and thus the sample can be checked for homogeneity. Furthermore, PET vials showed no diffusion with scintillation cocktails (Schönhofer & Henrich, 1987). Subsequently the latter were chosen for the tests. Only recently, teflon-coated polyethylene vials (Zinsser) became available. They show similar background values to the PET vials, which I prefer because of their transparency.

Only commercially available scintillation cocktails were tested, first, for the range of miscibility. Of all cocktails tested, Quickszint 400 (Zinsser) showed the largest range both in mixing ratio and ethanol concentration. For practicability, ethanol concentrations in the range of 35–45% (for unconcentrated liquor) and 80–95% (a range easily achieved by distillation) were studied. In the lower range, a mixture of 1:1 was possible; in the higher concen-

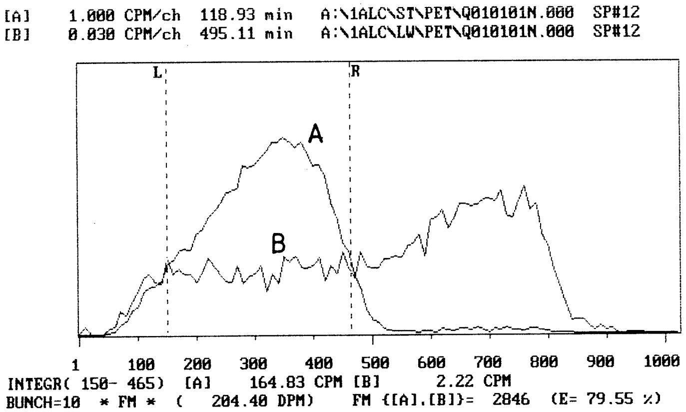


Fig 1. Pulse height spectra of background and internal standard, 5ml 95% ethanol + 15ml Quickszint 400, PET-vial; A = internatl standard, B = background; note the different scales (ratio 1:0.03)

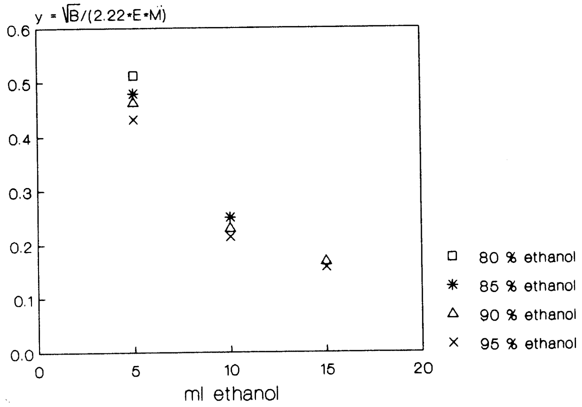


Fig 2. Dependency of y = $\sqrt{B}/(2.22\times E\times M)$ on the amount of ethanol of indicated concentration in a total sample of 20ml, PET-vial, Quickszint 400

tration range, mixing ratios of 5ml ethanol plus 15ml cocktail up to 15ml ethanol plus 5ml cocktail were investigated. An example for pulse height spectra obtained for background and standard is shown in Figure 1, with a background of 2.22cpm and an efficiency of 79.55% in a window optimized to the maximum figure of merit of 2846. The results for the concentration range 80–95% are summarized in Figure 2, with respect to the influence of different concentrations and mixing ratios on the quantity $y = \sqrt{B}/(2.22\times E\times M)$. This is related to the lower limit of detection and used frequently in literature. B is the background, E the efficiency in %, and M the mass of carbon in the sample. The mixing ratio plays an important role, obviously due to the higher amount of incorporated carbon, but with a given mixing ratio, the ethanol concentration has much less influence. The values obtained are compared in Table 1 with values from the literature (Simon, Rauschenbach & Frey, 1968; Resmini & Volonterio, 1974; Guérain & Tourlière, 1975). Val-

TABLE 1

Comparison of obtained results on performance with results from the literature

Author	Vial	Ethanol (ml)	Ethanol (%)	Cocktail	Ml	E%	BG cpm	E^2/B	LLD* 100 min dpm/gC	LLD* 500 min dpm/gC	y**
Simon	Low K glass	10	94–99	toluene	10	63.1	18.7	213	0.53	0.24	0.79
Resmini	Low K glass	12	95	p-xylene	11	70	18.3	268	0.39	0.18	0.59
	Low K glass	16	95	p-xylene	7	61	19.4	192	0.34	0.15	0.51
	Nylon	18	99.5	p-xylene	7	. . .	not reported			. . .	0.39
Guérain	Poly-ethylene	10	96	Instagel	10	65.2	8.7	488	0.34	0.15	0.51
Schönhofer	PET	10	95	Quick.400	10	80.1	2.2	2900	0.142	0.064	0.214
	PET	15	95	Quick.400	5	67.1	1.9	2397	0.105	0.047	0.157

* Lower limit of detection based on 3σ of background, expressed in dpm/g carbon, related to 100min counting time and 500min counting time, respectively

** $y = \sqrt{B}/(2.22 \times E \times M)$

ues for LLD and y for comparable samples and cocktails, are better, by a factor of 2–4. Better values have never been reported.

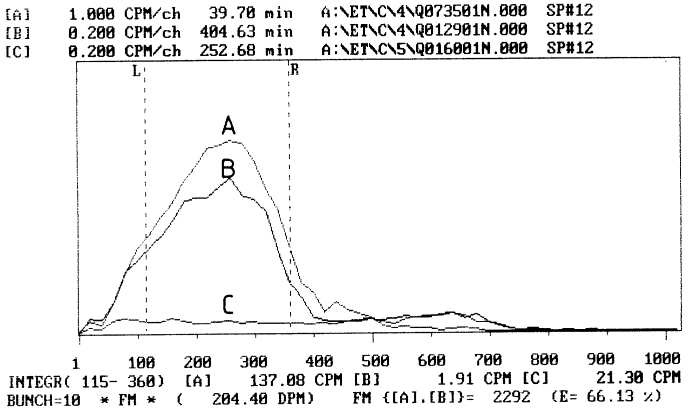
ANALYSIS

There were two types of measurements: 1) spirits not requiring the concentration steps, and 2) of wine, spirits, etc, requiring concentration and/or purification.

Each sample was compared to a background sample and a standard containing ethanol in approximately the same concentration. For background, synthetic alcohol without ^{14}C has to be used. Analytical grade absolute alcohol from Merck was found free of ^{14}C by comparison to a certified synthetic sample. For the standard, an ethanol solution of labeled glycerol tripalmitate supplied in capsules by Pharmacia – Wallac, with an accuracy of ca ±3.5% was used. Both radioactive standards and background samples can be refrigerated and used for several months without any change in properties. Sets of different concentrations were normally made and appropriately chosen for measurement.

For the first type of measurement, spirits and scintillation cocktails were mixed in a volume ratio of 1:1. Uncolored spirits with an ethanol concentration of ca 40% are very suitable. Occasionally very long-lasting chemiluminescence was observed, which can easily be detected because Quantulus allows measurement of delayed coincidence pulses. Appropriate windows must be set to cut off the chemiluminescence contribution. Figure 3 shows the spectra of Russian vodka, standard and background. The vodka was originally submitted for ^{137}Cs analysis resulting from the Chernobyl fallout. Since "radioactivity" was ordered we also analyzed it for ^{14}C. Background was 1.91cpm and efficiency 66.13% for the optimized window. The result of 7.90pCi/gC indicates that it is of natural origin.

For the second analysis of colored spirits and wine, it was necessary to



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[A]    1.000 CPM/ch   39.70 min   A:\ET\C\4\Q073501N.000  SP#12
[B]    0.200 CPM/ch  404.63 min   A:\ET\C\4\Q012901N.000  SP#12
[C]    0.200 CPM/ch  252.68 min   A:\ET\C\5\Q016001N.000  SP#12
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          1     100    200    300    400    500    600    700    800    900   1000
INTEGR( 115- 360)  [A]    137.08 CPM [B]      1.91 CPM [C]     21.30 CPM
BUNCH=10  * FM *  (   204.40 DPM)    FM {[A].[B]}=  2292  (E= 66.13 %)
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Fig 3. Pulse height spectra of Stolichnaya vodka, 40.65 vol % ethanol, 10ml vodka mixed directly with 10ml Quickszint 400; A = internal standard, B = sample, C = background

concentrate and purify to obtain reliable results. From Figure 2, it can be seen that a concentration of at least 85% ethanol is necessary for a mixing ratio of at least 1:1, improving the value of y by a factor of 2. Further improvement is possible by use of 90% ethanol which is easily achieved by distillation. Higher concentrations would improve the results somewhat, but would cause much more work, especially when absolute ethanol should be produced (Kostadinov & Yanev, 1986). With spirits, it is sufficient to apply a one-step distillation over a Vigreux column. With wines, a two-step distillation is used. First a rough and quick distillation is carried out, collecting the fraction between 76° and 79°C. The remaining liquid is kept for tritium analysis. Distillation over a small Vigreux column follows. This two-step method proved to be faster than using large columns to achieve the concentration in one step. The ethanol concentration is determined with a hand-held densitometer (DMA35, Paar, Graz, Austria), which is based on an oscillator measurement and thus allows for using small sample volume (ca 2ml) which, moreover, can be recovered. Operation is simple and results are fast.

From the liquid residue of the first distillation, some pure water is distilled for determining the tritium concentration. The method is described elsewhere (Schönhofer & Henrich, 1985).

In both cases the samples are measured in shorter cycles. Individual spectra are controlled especially for chemiluminescence and summed for each sample with the automatic "CUMU" program of the Quantulus software.

The limiting factor in this kind of research is always the availability of representative samples. Commercial interest is mostly focused on old wines because of their high prices, but it is extremely difficult to obtain reliable reference samples, not to mention the costs. For this study we obtained, at no cost, well-defined annual wine samples spanning the period of 1969 through 1986, as well as two samples from 1952 and 1960, from a vintager of excellent reputation. The samples came from the Langenlois region, some

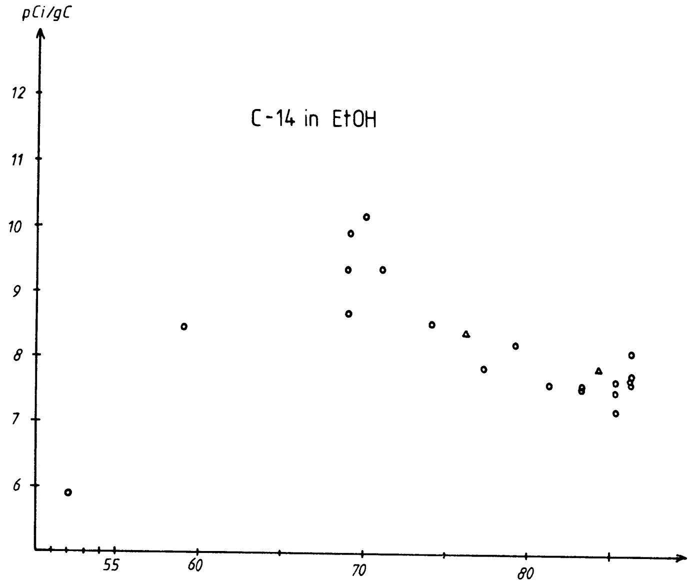

Fig 4. ^{14}C in ethanol distilled from wine; O = Langenlois; Δ = Stainz

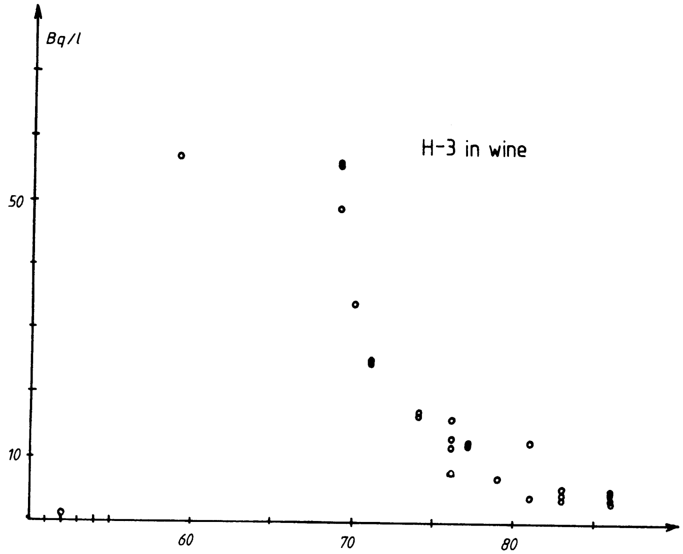

Fig 5. Tritium in wine from the Langenlois area

60km northwest of Vienna, north of the Alps. Two additional samples were chosen from Stainz, ca 20km southwest of Graz, near the Yugoslav border and south of the Alps. The reason for this choice was to measure variation with time within a small well-defined area as well as between different areas. Areas north of the Alps are influenced mostly by air masses from the Atlantic, whereas, south of the Alps, the climate is dominated from the Mediterranean Sea.

RESULTS

^{14}C results are presented in Figure 4. A smooth curve was not obtained and there are marked differences even between samples from the same year. The two samples from Stainz (Δ) are within the variation of the samples from Langenlois.

Most of the wines were also analyzed for tritium (Fig 5). Compared with ^{14}C data a much steeper rise is observed from 1952 (note the extreme low value) to 1959, as well as a much faster decrease towards pre-bomb levels. The variation of the values for the same year are pronounced at low tritium values after 1975.

DISCUSSION

The variations in both ^{14}C and tritium activity concentration in wine are not surprising. These differences originate both from concentration variations in the atmosphere and from varying wine-producing techniques throughout the industry.

Several reasons exist for atmospheric variations. Seasonal variations are known to occur both for ^{14}C (eg, Povinec *et al*, 1986) and tritium (Schönhofer & Weisz, 1987). Before ca 1980, the seasonal variations, especially for tritium, were even more pronounced (Rank & Rajner, 1979). Thus it can be assumed that the exact time of the vegetation period is influential. Regional variations have been observed, especially in connection with the Suess effect (Awsiuk & Pazdur, 1986).

The wine industry and legislation might also influence the activity concentrations. In most countries, the addition of sugar before fermentation is allowed, to a certain extent. This is only allowed for table wine, which is not kept for a long time. Thus, this is of no importance in the case of age determination. In many countries, it is legal to blend wine with a certain percentage of younger wine, therefore correctly labeled wine may contain ^{14}C and tritium different from the concentration typical for the indicated year.

Addition of ethanol, produced synthetically from oil, would change the ^{14}C activity concentration to lower values and could therefore be detected. This can be used to detect the addition to certain wines with high alcohol concentration and to spirits.

As for the applicability of this method to age determination, Figures 4 and 5 clearly show that more data is necessary, particularly for wines of vintage prior to 1969. Due to the observed variations and small yearly changes after ca 1975, the accuracy of this method is rather poor for this period. In any case, measurement of both ^{14}C and tritium seems to be essential for more precise age determination of older wines. Because of the inherent variations of ^{14}C concentrations in wine, extremely accurate determinations are unnecessary and emphasis can be placed on high sample throughput for routine analysis.

CONCLUSIONS

A simple and quick analytical method for determination of ^{14}C in alcohol has been developed by using a commercially available ultra low-level liquid scintillation counter and optimizing counting conditions with respect to vial, cocktail, mixing ratio and ethanol concentration. Work has to continue to obtain a complete curve of the ^{14}C and tritium activity concentration in wine throughout the past four decades before the method can be used for routine determination of age.

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