

LIQUID SCINTILLATION COUNTING
RECENT APPLICATIONS AND DEVELOPMENT
VOLUME II. SAMPLE PREPARATION AND APPLICATIONS

IN SITU ANALYSIS OF ^3H IN POLYACRYLAMIDE
GEL SLICES AFTER ELECTROPHORESIS

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Radioactive metabolites are often separated by electrophoresis on polyacrylamide gels. The subsequent liquid scintillation analysis of the radioactivity is usually performed after treatment with some quaternary ammonium base solubilizer.

The solubilizer makes the gel swell and at least partly dissolves the radioactive substance. This technique usually produces reliable results with good counting efficiencies. The substance, however, is lost for further analysis.

Another way of attack is to carry out the liquid scintillation analysis with the substance *in situ*. With such a method the radioactive substance is retained in the gel, is easy to handle and is not lost for further analysis.

Such methods have been developed by several authors, usually, however, with low counting efficiencies. Some years ago I developed a highly efficient *in situ* method for dilute mixed gels (Gezelius, 1977). This technique, however, could not be used with more concentrated gels. The method presented here is a modification of the earlier technique and suitable for the more compact gels.

MATERIALS AND METHODS

Electrophoresis was performed on 7% or 15% polyacrylamide gel columns. The columns were then cut into 1 mm discs.

Soluene 350 (Packard Instrument Company Inc.), a quaternary ammonium chloride solubilizer, was used in the controls.

Counting efficiencies were based on determinations of n - ^3H Hexadecane (standard batch nr 20, The Radiochemical Centre,

Amersham, England) as internal standard.

A dialyzed ^3H -labeled sea urchin protein mixture was gel-filtrated on Sepharose 6B (Pharmacia Fine Chemicals Inc. Sweden) and the two most active fractions (I and II) were used as protein standards.

A known amount of standard sea urchin protein was thoroughly mixed with the gel constituents prior to the casting. A uniform distribution of radioactivity in the gel column was obtained in this way for determination of the counting efficiency in the gels.

After Soluene 350 treatment, Permablend III (Packard Instrument Company Inc.) 5.1 g/ 1000 ml toluene was used as scintillation fluid. This fluid was also used in some of the *in situ* experiments. However, in this case butyl-PBD (Ciba-Geigy Inc.) 4 g/ 1000 ml toluene was the preferred scintillation fluid.

The standard (^3H)protein gels and the electrophoresed gels were sliced and each slice was transferred to a glass scintillation vial. They were then handled in four different ways.

A. The slices were treated overnight with 0.2 ml Soluene 350 and 0.02 ml bidistilled water. 5 ml of Permablend III scintillation fluid was then added and the vial was stirred. After standing overnight, to reduce chemoluminescence, the radioactivity was analysed.

B. The slices were soaked in 3 ml ethylene glycol overnight with occasional stirring. To remove the ethylene glycol the slices were washed twice with 3 ml alcohol (99.5%) and then soaked overnight in 3 ml of this medium. The alcohol was then replaced by 3 ml toluene. About 3 hours later, when the slices were completely transparent, the toluene was exchanged for either (1) 5 ml butyl-PBD scintillation fluid, or (2) 5 ml Permablend III scintillation fluid. One hour later the radioactivity was analysed.

C. The slices were soaked overnight in 15% (w/v) trichloroacetic acid (TCA) and then overnight in 3 ml ethylene glycol with occasional stirring. The slices were further treated as in B, with finally either (1) 5 ml butyl-PBD scintillation fluid or (2) 5 ml Permablend III scintillation fluid.

D. The gel slices were treated according to B (1 or 2) or C (1 or 2). After the radioactive analysis the slices prepared according to B2 or C2 were treated overnight with 0.2 ml Soluene 350 and 0.05 ml bidistilled water. After addition of the original sampels of scintillation fluid the vials were treated as in A. (The Soluene 350 and the water can also be added to the Permablend III Scintillation fluid, but the subsequent swelling of the gel slices will then take much more time.) The B1 and C1 slices had to be rinsed twice in 3 ml toluene and soaked for at least 3 hours in this medium prior to Soluene 350 treatment. This was to ensure that only traces of butyl-PBD would remain. (Butyl-PBD reacts with Soluene 350 and produces

quenching substances.) After Soluene 350 treatment overnight the slices were treated as in A.

In some experiments the gel slices were cut in halves and the halves treated in parallel according to A, B or C.

Several temperatures were tried for the dehydration process.

In some experiments treatment with Cellosolve (2-ethoxyethanol; ethylene glycol monoethyl ether) was performed prior to soaking in alcohol (99.5%).

The analysis of the radioactivity was performed in a Nuclear Chicago Mark II liquid scintillation spectrometer.

RESULTS

About 35 different variations of the *in situ* method was originally tried, with about the same number of chemicals. Of all the various chemicals tested as first soaking medium in the dehydration process, however, ethylene glycol was the only one that did not cause shrinkage of the gel slices. Ethylene glycol is a rather viscous medium and thus occasional stirring had to be done.

The effect of soaking in ethylene glycol was very slow at low temperature (6°C). The process speeded up at elevated temperatures; about 40°C was found to be convenient. A positive effect of the elevated temperatures was a slight swelling of the gel slices, especially those of less concentrated gels.

Ethylene glycol had to be replaced by some agent, miscible with toluene and not interfering with the state of the slices. One of the best media tried was Cellosolve. However, gel slices treated in this way were not as transparent in toluene as those where Cellosolve was replaced by alcohol (99.5%) prior to toluene. Soaking in Cellosolve could even be omitted, if the dehydration in ethylene glycol was completed, if not, a severe shrinking of the gel slices took place. If on the other hand ethylene glycol still remained in the slices when they were transferred to toluene, they would not be totally transparent and shrank.

The presence of toluene-insoluble buffer components in the gel slices will also reduce their transparency. However, such components did not seem to affect the counting efficiency notably in small amounts in these compact gels. The buffer components will diffuse out of the gel during soaking in TCA. This also promotes UV-analysis of the banding in the gel.

In contrast to the situation with mixed dilute gels (Gezelius, 1977), soaking in TCA was equally good when performed with gel slices as with whole gel columns. Neither was the TCA soaking found to be an indispensable step in the dehydration process. The soaking in TCA could even be omitted. However,

prolonged soaking in ethylene glycol at 40°C then resulted in a notable loss of activity.

The counting efficiencies are summarized in Table 1.

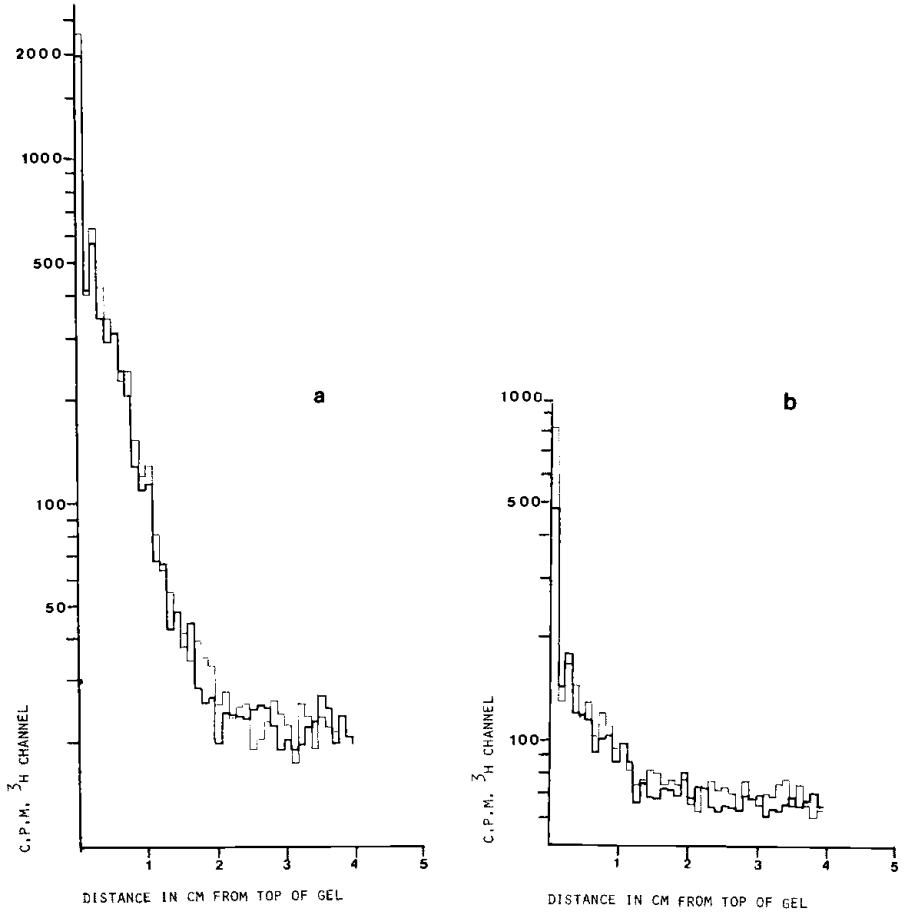


FIGURE 1. (a) Fraction I electrophoresed on a 7% polyacrylamide gel. (b) Fraction I electrophoresed on a 15% polyacrylamide gel. The thicker line indicates treatment according to in situ method C1. The thinner line indicates Soluene 350 treatment according to method D.

TABLE I. Counting efficiencies in percent \pm SD

	Scintillation fluid (5 ml) with		
	Butyl-PBD	Permablend III	Permablend III + Soluene 350 + water
n-(^3H)Hexadecane	54.7 \pm 0.9	50.5 \pm 0.7	48.2 \pm 1.0
Standard (^3H)protein in untreated gel			46.7 \pm 2.8
Standard (^3H)protein in situ			
in TCA-soaked gel 7%	42.0 \pm 2.3	38.1 \pm 1.7	45.0 \pm 3.5
in TCA-soaked gel 15%	39.2 \pm 0.9	35.5 \pm 1.2	40.5 \pm 1.1

The counting efficiencies obtained with the *in situ* technique was found to depend on the concentration of the gel (Table 1). This is also illustrated in Figure 1 and 2, where the good efficiency and reliability of the *in situ* method moreover is demonstrated.

DISCUSSION

The counting efficiencies obtained with *in situ* methods depend on various concurrent factors. The metabolites separated on the gel column must be fixed in a spaced way to minimize self-absorption. The spaced fixation must not be disturbed during dehydration and the following procedures. However, the counting efficiencies of the *in situ* method depend on the concentration of the gel. This is even more obvious when compared with those obtained with the *in situ* method described earlier (Gezelius, 1977) for a mixed gel of 2% polyacrylamide and 0.5% (Table 2). The reduction of the counting efficiencies in more concentrated gels is probably due to increased self-absorption.

Soluene 350 (cf Paus, 1971) was chosen as solubilizer as before (Gezelius, 1977). In the present experiments a highly quenching yellow discoloration only rarely appeared after Soluene 350 treatment.

The mobility and solubility and thus the diffusion rate of the (^3H)proteins in the gels and media was unexpectedly slow, and treatment according to method B gave good results (Fig. 2).

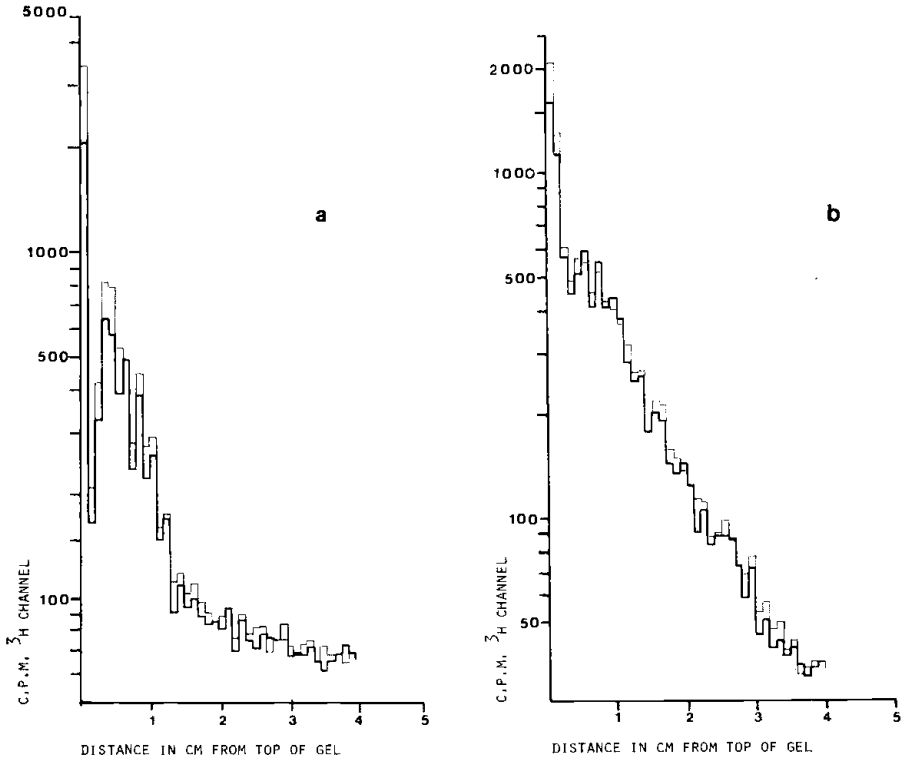


FIGURE 2. (a) Fraction II electrophoresed on a 7% polyacrylamide gel. (b) Fraction II electrophoresed on a 15% polyacrylamide gel. The thicker line indicates treatment according to in situ method B1. The thinner line indicates Soluene 350 treatment according to method D.

TABLE II. Counting efficiencies in percent \pm SD

	Scintillation fluid (5 ml) with		
	Butyl-PBD	Permablend III	Permablend III + Soluene 350 + water
n-(³ H)Hexadecane Standard (³ H)RNA in untreated gel	63.2 \pm 1.8	59.4 \pm 1.2	57.3 \pm 1.2
Standard (³ H)RNA in soaked gel 0.5% agarose + 2% acrylamide			54.7 \pm 3.8
	58.3 \pm 3.7	55.2 \pm 2.2	53.1 \pm 4.8

Boyd and Mitchell (1966) used acetic acid as first step in their process, however, the counting efficiency of the method was only about 23% for ³H. Soaking in 10% acetic acid (or TCA) was also an indispensable step in the *in situ* technique described earlier (Gezelius, 1977). Why this method did not work with more compact gels and why soaking in TCA (or acetic acid) was not indispensable in the present technique is not clear. Neither is it quite understood why ethylene glycol was the only substance found to function in the method proposed here.

Ethylene glycol has to be completely replaced by some toluene miscible substance, otherwise the gel slices start to shrink. The closely related Cellosolve, did not alone give gel slices as transparent as those obtained after additional treatment with alcohol (99.5%). This might be due to slower diffusion rates with Cellosolve.

Soaking in Cellosolve could also be omitted and was so in the final method. However, in some experiments it seemed as if soaking in Cellosolve in some way promoted good results, perhaps by making the *in situ* method less rude. It might well be, that with some gel compositions or buffer systems such a treatment will be an indispensable step to obtain good counting efficiencies.

The *in situ* technique presented here is more laborious than the one described earlier (Gezelius, 1977). This is mainly due to low diffusion rates in the gels especially for the viscous ethylene glycol.

The main advantages of the *in situ* technique are easy handling, little risk of contamination, low quenching, high counting efficiency and high reliability. It is also easy to cut out a special band after electrophoresis and to analyse it for radioactivity and the substance is not lost for further analysis.

ACKNOWLEDGMENTS

The technical assistance of Mr. Bo Molin is greatly acknowledged.

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