

**techniques of sample preparation
for liquid scintillation counting**

BRIAN W FOX

TECHNIQUES OF SAMPLE PREPARATION FOR LIQUID SCINTILLATION COUNTING

Brian W. Fox

*Paterson Laboratories,
Christie Hospital & Holt Radium Institute,
Manchester, England*



1976

NORTH-HOLLAND PUBLISHING COMPANY - AMSTERDAM · OXFORD
AMERICAN ELSEVIER PUBLISHING CO., INC. - NEW YORK

© 1976 North-Holland Publishing Company

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the copyright owner.

ISBN North-Holland - series: 0 7204 4200 1

- part 5.I: 0 7204 4216 8

ISBN American Elsevier: 0 444 11056 9

Published by:

NORTH-HOLLAND PUBLISHING COMPANY - AMSTERDAM

Sole distributors for the U.S.A. and Canada:

AMERICAN ELSEVIER PUBLISHING COMPANY, INC.

52 VANDERBILT AVENUE, NEW YORK, N.Y. 10017

This book is the pocket-edition of Volume 5, Part I, of the series 'Laboratory Techniques in Biochemistry and Molecular Biology'.

Volume 5 of the series contains the following parts:

Part I Techniques of sample preparation for liquid scintillation counting, by Brian W. Fox

Part II Isoelectric focusing, by P.G. Righetti and J.W. Drysdale

Printed in The Netherlands

LABORATORY TECHNIQUES
IN BIOCHEMISTRY AND
MOLECULAR BIOLOGY

*Techniques of sample preparation
for liquid scintillation counting*

Edited by

E. S. WORK, W. J. R. SALLI HILL, London
E. WORK - Imperial College, London

Library bound

£ 10.00

2 BIRCHWOOD, Stockton



AMERICAN ELSEVIER PUBLISHING CO. INC. AMSTERDAM, HOLLAND
NORTH-HOLLAND PUBLISHING COMPANY, AMSTERDAM, HOLLAND

LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY

Edited by

T.S. WORK – *N.I.M.R., Mill Hill, London*

E. WORK – *Imperial College, London*

Advisory board

G. POPJAK – *U.C.L.A.*

S. BERGSTROM – *Stockholm*

K. BLOCH – *Harvard University*

P. SIEKEVITZ – *Rockefeller University*

E. SMITH – *U.C.L.A.*

E.C. SLATER – *Amsterdam*



NORTH-HOLLAND PUBLISHING COMPANY – AMSTERDAM · OXFORD
AMERICAN ELSEVIER PUBLISHING CO., INC. – NEW YORK

Contents

| | |
|---|-----------|
| <i>Preface</i> | 7 |
| <i>List of abbreviations</i> | 10 |
| <i>Introduction</i> | 11 |
| Chapter 1. General principles of liquid scintillation spectrometry | 13 |
| 1.1. Scintillation mechanism | 13 |
| 1.2. Quenching | 18 |
| 1.2.1. Impurity or chemical quenching | 20 |
| 1.2.2. Colour quenching | 23 |
| 1.3. Chemiluminescence | 24 |
| 1.4. The counting vial | 27 |
| 1.5. Choice of the scintillation spectrometer | 31 |
| 1.6. Methods of dispensing and cleaning | 35 |
| Chapter 2. Counting systems | 41 |
| 2.1. Homogenous counting systems | 41 |
| 2.1.1. Toluene-based scintillant mixtures | 42 |
| 2.1.2. Dioxane-based scintillant mixtures | 45 |
| 2.1.3. Miscellaneous counting systems | 46 |
| 2.2. Heterogenous counting systems | 48 |
| 2.2.1. Disc counting | 50 |
| 2.2.2. Suspensions and gels | 60 |
| 2.2.3. Colloid counting | 64 |
| 2.3. Cerenkov light | 76 |
| 2.4. Luciferin luciferase assay | 82 |

| | |
|---|---------|
| <i>Chapter 3. Preprocessing techniques: general aims and criteria</i> | 86 |
| 3.1. Combustion techniques | 87 |
| 3.2. Organic solubilizers | 95 |
| 3.3. Degradative methods | 98 |
| 3.3.1. Degradation without volatilization of the products | 99 |
| 3.3.2. Degradation with volatilization of radioisotope | 101 |
| 3.3.3. Enzymatic degradation | 104 |
| 3.4. Precipitation methods | 105 |
| <i>Chapter 4. Animal tissue processing</i> | 197 |
| 4.1. Hard tissue | 109 |
| 4.1.1. Bone, teeth etc. | 109 |
| 4.1.2. Hair, lens, skin, etc. | 114 |
| 4.2. Soft tissues | 117 |
| 4.2.1. Readily homogenised tissues e.g. liver, spleen, etc. | 118 |
| 4.3. Semi-solid or liquid | 121 |
| 4.3.1. Faeces | 121 |
| 4.3.2. Whole blood, plasma etc. | 123 |
| 4.3.3. Radioimmunoassay | 131 |
| 4.3.4. Urine and body water | 134 |
| 4.3.5. Milk and food, etc. | 138 |
| 4.4. Respired gases | 140 |
| <i>Chapter 5. Botanical aspects</i> | 142 |
| 5.1. Higher plant tissue processing | 143 |
| 5.2. Algae, yeasts | 146 |
| 5.3. Soils and nutritional studies | 147 |
| <i>Chapter 6. Cell cultures</i> | 149 |
| 6.1. Virus particles and bacteria | 150 |
| 6.2. Mammalian cells | 151 |
| 6.3. Subcellular organelles (nucleii, ribosomes, etc.) | 152 |
| 6.4. Media and broths | 154 |
| 6.5. Liberated gases | 156 |

| | |
|--|-----|
| <i>Chapter 7. Extracts and chromatographic eluates</i> | 161 |
| 7.1. Aqueous extracts | 162 |
| 7.2. Acid solutions | 164 |
| 7.3. Alkaline solutions | 166 |
| 7.4. Salt solutions | 168 |
| 7.5. Organic solvents | 169 |
| 7.6. Gradient correction procedures | 171 |
| <i>Chapter 8. Macromolecules</i> | 173 |
| 8.1. Proteins and amino acids | 174 |
| 8.2. Nucleic acids, nucleotides, etc. | 177 |
| 8.3. Lipids and steroids | 179 |
| 8.4. Sugars and polysaccharides | 181 |
| <i>Chapter 9. Electrophoresis, centrifugation and chromatography on solid supports</i> | 184 |
| 9.1. Electrophoresis | 184 |
| 9.1.1. Polyacrylamide gel electrophoresis | 184 |
| 9.1.2. Electrophoresis on agarose and starch gels | 188 |
| 9.2. Centrifugation | 189 |
| 9.2.1. Pellets | 189 |
| 9.2.2. Caesium chloride gradients | 191 |
| 9.2.3. Sucrose gradients | 192 |
| 9.3. Chromatography on solid supports | 194 |
| 9.3.1. Thin-layer chromatography | 194 |
| 9.3.2. Paper chromatography | 196 |
| <i>Chapter 10. Inorganic applications</i> | 199 |
| 10.1. Solvent extraction methods | 200 |
| 10.2. Precipitation and complex salt formation | 203 |
| 10.3. Lipophyllic salts | 205 |
| 10.4. Noble gases | 206 |

| | |
|---|------------|
| <i>Chapter 11. Quench correction methods, multiple isotope counting and data evaluation</i> | <i>207</i> |
| 11.1. Quench correction methods | 207 |
| 11.1.1. Internal standard method | 209 |
| 11.1.2. Sample channels ratio techniques | 212 |
| 11.1.3. External standardization | 220 |
| 11.2. Multiple isotope counting | 222 |
| 11.2.1. Two beta-emitters (e.g. ^{14}C and ^3H) | 223 |
| 11.2.2. Gamma- and beta-emitters together | 232 |
| 11.2.3. Automatic quench correction (AQC) | 233 |
| 11.3. Data evaluation | 234 |
| 11.3.1. Some statistical considerations | 234 |
| 11.3.2. Computer assisted data handling | 242 |
| <i>Chapter 12. Geophysics and archaeology</i> | <i>244</i> |
| 12.1. Hydrology applications and tritium dating | 246 |
| 12.2. Fall-out and meteorology | 249 |
| 12.3. Radiocarbon dating | 250 |
| <i>Chapter 13. Miscellaneous applications and future prospects</i> | <i>254</i> |
| 13.1. Flow cells | 255 |
| 13.2. Gas chromatography | 258 |
| 13.3. Analytical applications | 260 |
| <i>Appendix I</i> | <i>264</i> |
| Isotope tables and decay charts | 264 |
| <i>Appendix II</i> | <i>271</i> |
| Properties of solutes, solvents and scintillation mixtures | 271 |
| <i>Appendix III</i> | <i>280</i> |
| Analytical key to sample preparation | 280 |
| <i>Appendix IV</i> | <i>284</i> |
| Glossary | 284 |
| <i>Appendix V, Instruments and chemicals (suppliers)</i> | <i>289</i> |
| <i>References</i> | <i>291</i> |
| <i>Subject index</i> | <i>309</i> |

(内部交流)

TECHNIQUES OF SAMPLE PREPARATION FOR LIQUID SCINTILLATION COUNTING

Brian W. Fox

*Paterson Laboratories,
Christie Hospital & Holt Radium Institute,
Manchester, England*



1976

NORTH-HOLLAND PUBLISHING COMPANY - AMSTERDAM · OXFORD
AMERICAN ELSEVIER PUBLISHING CO., INC. - NEW YORK

© 1976 North-Holland Publishing Company

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the copyright owner.

ISBN North-Holland – series: 0 7204 4200 1

– part 5.I: 0 7204 4216 8

ISBN American Elsevier: 0 444 11056 9

Published by:

NORTH-HOLLAND PUBLISHING COMPANY – AMSTERDAM

Sole distributors for the U.S.A. and Canada:

AMERICAN ELSEVIER PUBLISHING COMPANY, INC.

52 VANDERBILT AVENUE, NEW YORK, N.Y. 10017

This book is the pocket-edition of Volume 5, Part I, of the series 'Laboratory Techniques in Biochemistry and Molecular Biology'.

Volume 5 of the series contains the following parts:

Part I Techniques of sample preparation for liquid scintillation counting, by Brian W. Fox

Part II Isoelectric focusing, by P.G. Righetti and J.W. Drysdale

Printed in The Netherlands

Contents

| | |
|---|-----------|
| <i>Preface</i> | 7 |
| <i>List of abbreviations</i> | 10 |
| <i>Introduction</i> | 11 |
| Chapter 1. General principles of liquid scintillation spectrometry | 13 |
| 1.1. Scintillation mechanism | 13 |
| 1.2. Quenching | 18 |
| 1.2.1. Impurity or chemical quenching | 20 |
| 1.2.2. Colour quenching | 23 |
| 1.3. Chemiluminescence | 24 |
| 1.4. The counting vial | 27 |
| 1.5. Choice of the scintillation spectrometer | 31 |
| 1.6. Methods of dispensing and cleaning | 35 |
| Chapter 2. Counting systems | 41 |
| 2.1. Homogenous counting systems | 41 |
| 2.1.1. Toluene-based scintillant mixtures | 42 |
| 2.1.2. Dioxane-based scintillant mixtures | 45 |
| 2.1.3. Miscellaneous counting systems | 46 |
| 2.2. Heterogenous counting systems | 48 |
| 2.2.1. Disc counting | 50 |
| 2.2.2. Suspensions and gels | 60 |
| 2.2.3. Colloid counting | 64 |
| 2.3. Cerenkov light | 76 |
| 2.4. Luciferin luciferase assay | 82 |

| | |
|---|---------|
| <i>Chapter 3. Preprocessing techniques: general aims and criteria</i> | 86 |
| 3.1. Combustion techniques | 87 |
| 3.2. Organic solubilizers | 95 |
| 3.3. Degradative methods | 98 |
| 3.3.1. Degradation without volatilization of the products | 99 |
| 3.3.2. Degradation with volatilization of radioisotope | 101 |
| 3.3.3. Enzymatic degradation | 104 |
| 3.4. Precipitation methods | 105 |
| <i>Chapter 4. Animal tissue processing</i> | 197 |
| 4.1. Hard tissue | 109 |
| 4.1.1. Bone, teeth etc. | 109 |
| 4.1.2. Hair, lens, skin, etc. | 114 |
| 4.2. Soft tissues | 117 |
| 4.2.1. Readily homogenised tissues e.g. liver, spleen, etc. | 118 |
| 4.3. Semi-solid or liquid | 121 |
| 4.3.1. Faeces | 121 |
| 4.3.2. Whole blood, plasma etc. | 123 |
| 4.3.3. Radioimmunoassay | 131 |
| 4.3.4. Urine and body water | 134 |
| 4.3.5. Milk and food, etc. | 138 |
| 4.4. Respired gases | 140 |
| <i>Chapter 5. Botanical aspects</i> | 142 |
| 5.1. Higher plant tissue processing | 143 |
| 5.2. Algae, yeasts | 146 |
| 5.3. Soils and nutritional studies | 147 |
| <i>Chapter 6. Cell cultures</i> | 149 |
| 6.1. Virus particles and bacteria | 150 |
| 6.2. Mammalian cells | 151 |
| 6.3. Subcellular organelles (nucleii, ribosomes, etc.) | 152 |
| 6.4. Media and broths | 154 |
| 6.5. Liberated gases | 156 |

| | |
|--|-----|
| <i>Chapter 7. Extracts and chromatographic eluates</i> | 161 |
| 7.1. Aqueous extracts | 162 |
| 7.2. Acid solutions | 164 |
| 7.3. Alkaline solutions | 166 |
| 7.4. Salt solutions | 168 |
| 7.5. Organic solvents | 169 |
| 7.6. Gradient correction procedures | 171 |
| <i>Chapter 8. Macromolecules</i> | 173 |
| 8.1. Proteins and amino acids | 174 |
| 8.2. Nucleic acids, nucleotides, etc. | 177 |
| 8.3. Lipids and steroids | 179 |
| 8.4. Sugars and polysaccharides | 181 |
| <i>Chapter 9. Electrophoresis, centrifugation and chromatography on solid supports</i> | 184 |
| 9.1. Electrophoresis | 184 |
| 9.1.1. Polyacrylamide gel electrophoresis | 184 |
| 9.1.2. Electrophoresis on agarose and starch gels | 188 |
| 9.2. Centrifugation | 189 |
| 9.2.1. Pellets | 189 |
| 9.2.2. Caesium chloride gradients | 191 |
| 9.2.3. Sucrose gradients | 192 |
| 9.3. Chromatography on solid supports | 194 |
| 9.3.1. Thin-layer chromatography | 194 |
| 9.3.2. Paper chromatography | 196 |
| <i>Chapter 10. Inorganic applications</i> | 199 |
| 10.1. Solvent extraction methods | 200 |
| 10.2. Precipitation and complex salt formation | 203 |
| 10.3. Lipophyllic salts | 205 |
| 10.4. Noble gases | 206 |

| | |
|---|-----|
| <i>Chapter 11. Quench correction methods, multiple isotope counting and data evaluation</i> | 207 |
| 11.1. Quench correction methods | 207 |
| 11.1.1. Internal standard method | 209 |
| 11.1.2. Sample channels ratio techniques | 212 |
| 11.1.3. External standardization | 220 |
| 11.2. Multiple isotope counting | 222 |
| 11.2.1. Two beta-emitters (e.g. ^{14}C and ^3H) | 223 |
| 11.2.2. Gamma- and beta-emitters together | 232 |
| 11.2.3. Automatic quench correction (AQC) | 233 |
| 11.3. Data evaluation | 234 |
| 11.3.1. Some statistical considerations | 234 |
| 11.3.2. Computer assisted data handling | 242 |
| <i>Chapter 12. Geophysics and archaeology</i> | 244 |
| 12.1. Hydrology applications and tritium dating | 246 |
| 12.2. Fall-out and meteorology | 249 |
| 12.3. Radiocarbon dating | 250 |
| <i>Chapter 13. Miscellaneous applications and future prospects</i> | 254 |
| 13.1. Flow cells | 255 |
| 13.2. Gas chromatography | 258 |
| 13.3. Analytical applications | 260 |
| <i>Appendix I</i> | 264 |
| Isotope tables and decay charts | 264 |
| <i>Appendix II</i> | 271 |
| Properties of solutes, solvents and scintillation mixtures | 271 |
| <i>Appendix III</i> | 280 |
| Analytical key to sample preparation | 280 |
| <i>Appendix IV</i> | 284 |
| Glossary | 284 |
| <i>Appendix V, Instruments and chemicals (suppliers)</i> | 289 |
| <i>References</i> | 291 |
| <i>Subject index</i> | 309 |

Preface

The use of weak beta-emitting radioisotopes in an increasing variety of investigative procedures, particularly in the biochemical field, has stimulated the demand for better methods of assaying those isotopes which are more conveniently incorporated into organic molecules employed as precursors.

The prime object of the present work is to bring together the many different ways that have been designed to prepare the samples in a form suitable for liquid scintillation counting. Although the prime object is to bring the weak beta-emitter into close molecular contact with the primary solvent in the system, the method of doing this is highly dependent on the nature of the sample being measured. The initial sample for assay can exist in any state and associated with many other components which exceed the concentration of the actual beta emitter by many orders of magnitude. Where the level is low, careful thought should be given to the possible means whereby the labelled material itself may be isolated or alternatively the contaminating materials removed from the labelled substance. Occasionally, either one of these processes may be effected by solvent extraction, but before doing so, the sample multiplicity must also be considered. The number of samples may be too great to allow even a simple process of this kind to be conducted within a reasonable time. A decision as to whether or not such a tedious extraction procedure should be contemplated will depend on the level of accuracy needed by the experiment.

Such decisions are often overlooked before assay and many users of liquid scintillation instruments rely solely on the claims of the many commercial scintillation cocktails available, hence incurring unnecessary expense. Often in addition, incorrect application of such cocktails may produce anomalies which are thought to be associated with the experiment itself rather than with the counting conditions employed.

In every counting technique used, some form of 'quenching' (see § 1.2) will be experienced. This reduction of counting efficiency can usually be accurately assessed and allowed for in a number of different ways. However, there are certain situations where these correction procedures cannot be used. It is in these circumstances that anomalous results usually appear and are often unrecognised by the experimenter.

In many, and probably in most biochemical applications, it may not be essential to obtain an accurate assessment of the absolute level of isotope present. Reproducibility of sample counts by careful control of the sample preparation is often the goal to be achieved. The ability to conduct several hundred measurements with a reproducibility of one and often up to 5% between samples is often all that is necessary. The correct initial choice of counting method at the outset will be repaid by the avoidance of many hours of tedious and unnecessary sample preparation, which may otherwise be costly not only in time consumed but also in the preprocessing and scintillation materials used.

Most of the confusion arising from apparently conflicting data in the literature on sample preparation methods, appears to arise from the fact that many authors consistently refrain from stating the reference efficiency of the machine being used. It is thus not possible to determine from the work, whether the increased efficiencies apparently obtained by his method is indeed an improvement or just a demonstration of the acquirement of a scintillation counter with improved engineering. Also since a judicious choice of window settings can appear to confer increased efficiency, open window data should be quoted in making such comparisons for single isotopes. This would avoid giving artifactual increases by quench shifts into lower windows.