# Liposomes

## a practical approach

Edited by
**R R C New**

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IRL PRESS
at
OXFORD UNIVERSITY PRESS
Oxford New York Tokyo

Oxford University Press,
Walton Street, Oxford OX2 6DP

Oxford is a trade mark of Oxford University Press

Published in the United States
by Oxford University Press, New York



*British Library Cataloguing in Publication Data*
Liposomes.
1. Organisms. Liposomes.
I. New, R.R.C. II. Series 574.87'34

*Library of Congress Cataloging in Publication Data*
Lipsomes: a practical approach/edited by R.R.C. New.
(The Practical approach series)
Includes bibliographical references.
1. Liposomes. I. New, R. R. C. (Roger R. C.) II. Series.
RS201.L55L55 1989 574.87'4—dc20 89-22802
ISBN 0-19-963076-3.
ISBN 0-19-963077-1 (pbk.)

Previously announced as:
ISBN 1 85221 059 1
ISBN 1 85221 060 5

Typeset and printed by Information Press Ltd, Oxford, England.

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# THE
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# Preface

Over the last twenty years the liposome has changed its status from being a novel plaything for the laboratory worker to a powerful tool for the industrialist—with the gap between the ideal desired characteristics of liposomes and what is technically feasible becoming narrower all the time. The properties of membrane preparations have been researched extensively, and ingenious ways have been found of manipulating them to confer behavioural characteristics which stretch the imagination—sensitivity to heat, light, pH, magnetic field, and chemical structure. Few other areas of study can routinely bring into play such a wide range of phenomena.

Liposomes may be defined simply as lipid vesicles enclosing an aqueous space. They were brought to the attention of the scientific world by A.D. Bangham in 1965, and proposed as useful models for cell membranes. Indeed, using the definition above, even cells and organelles themselves may be considered to be just sophisticated types of liposome. Using these artificial membrane vesicles, great insight was brought to many aspects of cell physiology such as permeability, fusion, membrane-bound enzyme properties etc, and will continue to do so. More recently, the potential of liposomes in the medical field is slowly becoming realized, with several clinical trials in progress examining their use as drug delivery agents. Applications in the areas of diagnosis, immuno-modulation, and genetic engineering have been identified and developments will follow.

In spite of numerous books and papers written on the subject, many people are still unclear about what liposomes are, and how work employing them is carried out. The aim of this book is to dispel some of that mystery. It has been written with two groups of people in mind. Firstly, the laboratory worker, who wishes to have at his/her fingertips detailed, tried-and-tested methods which will be accepted by experienced workers in the field as giving results which are reliable and convincing. The methods presented here are not a comprehensive list of everything which can be, or has been done, but are a careful selection of the most useful and most easily applied methods for the general laboratory. The second category of reader is the graduate student who may have had little exposure to membrane techniques, and who will benefit from an understanding of the theory behind membrane processes. It is hoped that this may act as an introductory textbook for the basic principles of liposomology, before embarking on detailed study of more learned treatises.

*Liverpool School of Tropical Medicine* Roger R.C.New
August 1989

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*The support of the Leverhulme Trust in the preparation of this book is gratefully acknowledged by R.R.C.New.

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# Abbreviations

| | |
|---|---|
| ADC | analogue to digital converter |
| AM | ammonium molybdate |
| ANS | anilinonaphthalene sulphonate |
| APSA | *N*-(*p*-aminophenyl) stearylamide |
| α-T | α-tocopherol |
| α-TS | α-tocopherol succinate |
| BCECF | 2,7-biscarboxyethyl-5(6)-carboxyfluorescein |
| BCIP | 5-bromo, 4-chloro, 3-indolyl phosphate |
| BHT | butylated hydroxytoluene |
| BPS | biotinylated phosphatidyl serine |
| BSA | bovine serum albumin |
| CDI | carbodiimide |
| CF | carboxyfluorescein |
| CHEMS | cholesterol hemisuccinate |
| CL | cardiolipin |
| CMC | critical micelle concentration |
| DCCI | dicyclohexyl carbodiimide |
| DLPC | dilauroyl phosphatidyl choline |
| DMPC | dimyristoyl phosphatidyl choline |
| DMPG | dimyristoyl phosphatidyl glycerol |
| DODAC | dioctadecyl ammonium chloride |
| DOPC | dioleyl phosphatidyl choline |
| DPPA | dipalmitoyl phosphatidic acid |
| DPPC | dipalmitoyl phosphatidyl choline |
| DPPG | dipalmitoyl phosphatidyl glycerol |
| DRV | dried-reconstituted vesicle |
| DSPC | distearoyl phosphatidyl choline |
| DTT | dithiothreitol |
| EDCI | 1-ethyl-3-(dimethyl aminopropyl)-carbodiimide |
| ESR | electron spin resonance |
| F | fluorescence |
| FPL | French pressed liposomes |
| FTS | freeze-thaw sonication |
| G6PDH | glucose-6-phosphate dehydrogenase |
| HDL | high density lipoproteins |
| IUV | intermediate-sized unilamellar vesicle |
| LDL | low density lipoprotein |
| LPC | lyso-phosphatidyl choline |
| LUV | large unilamellar vesicle |
| MEL | micro-emulsification liposomes |
| Mes | morpholino ethane sulphonic acid |
| MLV | multi-lamellar vesicle |
| Mops | morpholino propane sulphonic aicd |
| MPS | monocyte phagocyte system |
| MTT | 3-[4,5-Dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide |
| MVL | multi-vesicular liposome |
| NHSIA | *N*-hydroxysuccinimido-iodoacetate |

| | |
|---|---|
| PA | phosphatidic acid |
| PC | phosphatidyl choline |
| PCS | photon correlation spectroscopy |
| PE | phosphatidyl ethanolamine |
| PG | phosphatidyl glycerol |
| PHA | pulse height analysis |
| PI | phosphatidyl inositol |
| PMSF | phenyl methyl sulphonyl fluoride |
| PS | phosphatidyl serine |
| PTA | phosphotungstic acid |
| PTR | phase transition release |
| PVP | polyvinyl pyrrolidone |
| RES | reticular endothelial system |
| RET | resonance energy transfer |
| REV | reverse-phase evaporation vesicle |
| RSVE | reconstituted Sendai virus envelope |
| SAMSA | 5-acetylmercaptosuccinic anhydride |
| SATA | succinimidyl-5-acetylthioacetate |
| SFFF | sedimentation field flow fractionation |
| SM | sphingomyelin |
| SMPB | *N*-succinimidyl (4-[*p*-maleimidophenyl])butyrate |
| SPDP | *N*-succinimidyl pyridyl dithiopropionate |
| SPLV | stable plurilamellar vesicle |
| SUV | small unilamellar vesicle |
| TAC | time to amplitude converter |
| TBA | thiobarbituric acid |
| TBS | Tris-buffered saline |
| TEA | triethylamine |
| TEP | 1,1,3,3, tetraethoxypropane |
| TMS | trimethyl silane |
| TNBS | trinitrobenzene sulphonic acid |
| TNS | (6-[*p*-toluidinyl]naphthalene-2-sulphonate) |
| TO | triolein |
| WCOT | wall-coated open tubular |

# Contents

CHAPTER 1

# Introduction

ROGER R.C.NEW

## 1. AIM OF BOOK

This book is intended both as a compilation of methods which may act as a useful reference source for workers already in the field of liposomes, and as a simple guide to workers outside the field who are wondering whether liposomes might have some application to their own speciality, and if so, what sort of liposome is best to use. Consequently, for most of this volume the emphasis is not on the applications liposomes can be put to, since it is assumed the reader will already have his/her own uses in mind—uses which may be entirely original, and could not be anticipated by the authors of this book. Instead, we hope to make readers sufficiently well-informed about liposomes that they may choose for themselves the best methods to adopt for their purpose, and in attempting this, we have concentrated heavily on methodology as the means of classifying different areas of the subject.

The first half of the book describes in detail the different ways of making, modifying, purifying, and characterizing liposomes, while the last half discusses ways in which information can be obtained about the behaviour of the finished product in different biological systems. This introductory chapter gives a very simple guide to what liposomes are, and outlines the general principles involved in choosing a given type of liposome for a particular application.

## 2. STRUCTURE OF LIPOSOMES

Liposomes are simply vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules (usually phospholipids). They form spontaneously when these lipids are dispersed in aqueous media, giving rise to a population of vesicles which may range in size from tens of nanometres to tens of microns in diameter. They can be constructed so that they entrap quantities of materials both within their aqueous compartment and within the membrane. The value of liposomes as model membrane systems derives from the fact that liposomes can be constructed of natural constituents such that the liposome membrane forms a bilayer structure which is in principal identical to the lipid portion of natural cell membranes—the 'sea of phospholipids' in the Singer and Nicholson model. The similarity between liposome and natural membranes can be increased by extensive chemical modification of the liposome membrane, and may be exploited in areas such as drug targeting or immune modulation, both *in vivo* and *in vitro*, where the ability to mimic (or to improve upon) the behaviour of natural membranes, and also to be degraded by the same pathways, makes them a very safe and efficacious vehicle for medical applications. Alternatively,

**a** **Chemical structure** **Name**

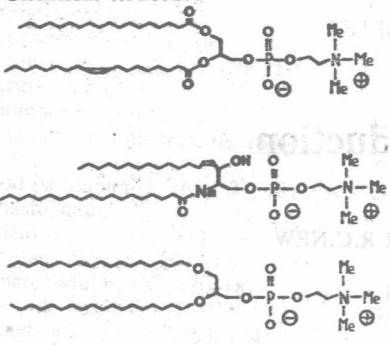
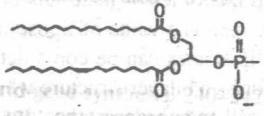
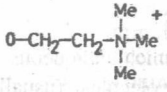
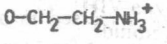
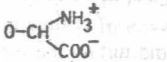
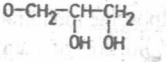
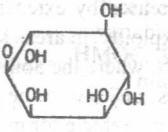



**Figure 1(a) Three main classes of choline-containing phospholipids.** Choline-containing phospholipids are the most abundant phospholipids in nature. In phosphatidyl cholines (lecithin), a three-carbon glycerol bridge links two long chain fatty acids with a phosphoryl choline moiety; by convention, the fatty acids are said to occupy the 1 and 2 positions of the glycerol bridge while the polar headgroup is in position 3. The bridge carbon in position 2 (the middle carbon of the three-carbon glycerol bridge) is asymmetric—i.e., it displays optical activity because each of the four bonds is joined to a different chemical group. Natural phospholipids can have many different fatty acids conjugated in positions 1 and 2, usually with the longer and/or more unsaturated chain in position 2 as shown here (see also *Table 2*). Sphingomyelin consists of a single fatty acyl chain conjugated via an amide linkage to the nitrogen of sphingosine, which is again linked to phosphoryl choline. The lipid portion without the headgroup is known as ceramide. In addition to sphingomyelin, this ceramide residue is found in molecules known as gangliosides and cerebrosides, which contain polysaccharide headgroups in place of phosphoryl choline. Hydrocarbon chains can also be joined to the glycerol bridge via ether linkages as in the third class of phosphocholine lipids, of which a synthetic analogue useful in liposome formulations is shown here. In nature, diether phospholipids usually have a glycerol headgroup, while choline or ethanolamine headgroups are more usually found in molecules containing one each of the acyl and ether linkages (e.g. in plasmalogens). These lipids are considerably less prevalent in nature than the diacyl phosphatidyl cholines.

**b** **Phosphatidyl moiety** **Headgroup** **Common name abbreviation**



| Phosphatidyl | | |
|---|---|---|
|  | choline | PC |
|  | ethanolamine | PE |
|  | serine | PS |
|  | glycerol | PG |
|  | acid | PA |
|  | inositol | PI |