THE UNIVERSITY OF HULL

Novel applications of surface-modified sporopollenin exine capsules

being a Thesis submitted for the Degree of

Doctor of Philosophy

in the University of Hull

by

Youkui Huang

B.S (Nanjing University)

December 2013

DECLARATION

The work described in this thesis was carried out in the Department of Chemistry, The University of Hull under the supervision of Dr Andrew Boa and Dr Grahame Mackenzie between September 2010 and August 2013. Except where indicated by references, this work is original and has not been submitted for any other degree.

Acknowledgements

I would like to thank the following people who have assisted me in the course of my research:

Dr. A. N. Boa, my supervisor, for all his valuable help and guidance.

Dr. G. Mackenzie, my co-supervisor, for his ideas and help throughout this study.

Professor B. P. Binks, for his guidance and assistance.

My colleagues Dr. A. D. Taboada, F. Almutairi and A. Tyuwoa, for their assistance in my work.

Dr. K. Welham, for the analysis of products by GC-MS.

Mrs. C. Kennedy, for the analysis of products by elemental analysis.

Mr. B. Knight, for the analysis of products by ICP-MS

Mr. T. Sinclair, for production of the SEM images (Hull).

My friends and family, for their enormous support and encouragement during my study time at university.

And Chinese Scholarship Council, for their provision of a maintenance grant.

Abstract

The external shell (exine) of plant spores and pollen grains is composed of sporopollenin, which is an organic polymer renowned for its exceptional resistance to physical and chemical attack. The resilience of sporopollenin to high temperature, pressures, acidic and basic corrosion is proved by its survival in some sedimentary rocks, which are 500 million years old.

Solid phase organic synthesis is a process where molecules or reagents are used whilst attached to an insoluble and filterable solid support. Sporopollenin could have a series of advantages over commercial resins if it can be applied to solid phase synthesis as it has a constant chemical structure, constant pore size, and in particular chemical and physical stability.

In this study sporopollenin was first extracted from fresh pollen and spores, and then modified to make a basic form by attachment of alkyldiamines, and an acidic form by treatment with chlorosulfonic or sulfuric acid. Detailed studies were undertaken regarding the use of the base form of sporopollenin in Schotten-Baumann type acylations and Knoevenagel condensations, and the acidic form of sporopollenin in the isopropylidene protection of mannose and preparation of cyclic acetal. As a result, aminated sporopollenin particles were shown to be effective scavengers in Schotten-Baumann acylation and they catalyse Knoevenagel condensation successfully.

The sulfonated sporopollenin particles also reveal their ability in catalysing relative reactions.

Raw spores and simply extracted sporopollenin has been investigated for the stabilization of Pickering emulsion by Binks *et al*. In this work, sporopollenin was aminated and their behaviour as emulsifiers has been studied. The amino groups on the surface of sporopollenin will change the hydrophilicity of the particles and affect their behaviour in the emulsions.

Abbreviations

4-MMNO.H_2O	4-methylmorpholine-*N*-oxide monohydrate
CPG	Controlled pore glass
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DVB	Divinylbenzene
EOF	Electro osmotic flow
GC-MS	Gas chromatography–mass spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
IPM	Isopropyl myristate
IR	Infrared
LC-MS	Liquid chromatography–mass spectrometry
MCMs	Mobil Crystalline Materials
NMR	Nuclear magnetic resonance
PAMAM	Polystyrene-supported poly (amidoamine)
PEG	Poly (ethylene glycol)
PEGA	Acrylamidopropyl-PEG-*N*,*N*-dimethylacrylamide
Pepsyn	Poly (dimethylacrylamide resin)
PS	Poly (styrene)
SECs	Sporopollenin Exine Capsules
SFC-MS	Supercritical Fluid Chromatography Mass Spectrometry

SPOS	Solid phase organic synthesis
SPPS	Solid phase peptide synthesis
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TG	TentaGel
UV-VIS	Ultraviolet–visible spectroscopy
XPS	X-Ray photoelectron spectroscopy

CONTENTS

CHAPTER 1 Sporopollenin

1.1 Introduction

Sporopollenin is the word given by Zetzsche *et al*[1] to describe the resistant exine material from both spores and pollen grains. Spores are produced by non-seed bearing plants, for example ferns and mosses, as part of the process of reproduction.[2] Pollen grains are the male reproductive structure of angiosperms (flowering plants) and plants classed as gymnosperms (for example, conifers).[2,3] The inner layer, or intine, consists mainly of cellulose and some polysaccharide material. The outer layer, or exine, is composed largely of a substance known as sporopollenin.[3-6] The study of spores and pollen grains is called palynology and has been used in a wide range of fields including fossil and pollen analysis, allergy studies, and forensic science.[2]

Sporopollenin used in this research is from the spores of *Lycopodium clavatum*, the most widespread species in the genus *Lycopodium* of the clubmoss family *Lycopodiacece*. The spores are monodisperse, with size about 20 μm in diameter, and are almost spherical in shape.

The earliest recorded observation of the exine was from John and Baraconnot,[4,6] in 1814 and 1829 respectively. Both researchers commented on the inertness of the exine compared with the rest of the pollen wall. To describe the exine, Baracannot introduced the term "pollenin", and in 1928, Zetzsche and Huggler introduced the term "sporonin" to describe the resistant exine material from *Lycopodium clavatum* spores.[7] Finally the

collective name of "sporopollenin" was used to describe the resistant exine material of pollen grains and spores by Zetzsche *et al*[1] since they have the same chemical character.

A method of preparation of a morphologically intact pollen or spore wall devoid of any cytoplasmic contents e.g. nitrogen, fats genetic material and proteins were reported by Zetzsche *et al*.[1,3,6] By treating with organic solvents and boiling sodium hydroxide solution, the content could be removed. After the treatment, nitrogen free "membranes" which retained their original shape and consisted of two layers were obtained. The inner layer was stained blue with iodine in H_2SO_4 which is characteristic of cellulose when treated in this condition. Zetzsche and Huggler[6] also discovered that the inner layer could be removed by treatment with hot 85% *ortho*-phosphoric acid over five days. The resistant material left over by these treatments is what is considered to be sporopollenin.

Zetzsche[8] represented all sporopollenins by a general empirical formula based on a C_{90} unit after work on several different pollen 'membranes', Table 1.1.

Material	Empirical formula
Lycopodium clavatum	$C_{90}H_{144}O_{27}$
Equisetum arvense	$C_{90}H_{144}O_{31}$
Ceratozamia mexicana	$C_{90}H_{148}O_{31}$
Picea exelsa	$C_{90}H_{144}O_{26}$
Picae orientalis	$C_{90}H_{144}O_{25}$
Taxus baccata	$C_{90}H_{138}O_{26}$

Table 1.1 Empirical formula of different sporopollenins[9]

1.2 Isolation of sporopollenin from modern pollen and spores

In the literature, there are a series of methods describe how to extract sporopollenin from fresh spores and pollen grains. Some involve harsh treatments with strong acids and bases at elevated temperatures and also more gentle conditions. It has been suggested that harsh conditions may bring changes to the structure of the sporopollenin slightly, for example, the hydrolysis of ester groups.[10,11] This must be taken into consideration when comparing sporopollenin samples prepared with different methods. The main methods are detailed below.

1.2.1 Treatment with alkali and phosphoric acid

Zetzsche *et al* in 1928[3,4,7] described a method by treatment the spores or pollen grains with organic solvents followed by a hot alkali and then finally with hot 80 % *ortho*-phosphoric acid for 6 days. And more recently, Shaw *et al*[11] found that *ortho*-phosphoric acid alone did not completely remove the intine and an extra treatment with 80 % H_2SO_4 was necessary to remove all traces of cellulose.

1.2.2 Acetolysis

Erdtman introduced a method of extraction using acetolysis.[12] This involved treatment of the spores or pollen grains with a 9:1 mixture of acetic anhydride and concentrated H_2SO_4.

1.2.3 Anhydrous HF

A method was developed by Dominguez et al in 1993[13] which involved using anhydrous HF (Hydrofluoric acid) in pyridine for extraction. The reaction took place at 40 °C for 5 hours.

1.2.4 4-Methylmorpholine-N-oxide

Another effective method to isolate the sporopollenin exine from the spore is to use 4-methylmorpholine-N-oxide monohydrate (4-MMNO.H_2O).[14-16] Spores or pollen grains were suspended in molten 4-MMNO.H_2O at 75 °C to release sporoplasts (pollen contents which have been stripped of their exines). Both sporoplasts and sporopollenin can be recovered by using this treatment.

1.2.5 Enzymes

A number of workers have used enzymes to obtain sporopollenin, or have incorporated an enzymatic hydrolysis step into their sporopollenin extraction procedure.[17-20]

1.3 Structure of sporopollenin

Because of the extreme resistance of sporopollenin to chemical attack, the structure of sporopollenin remains unknown. However, suggested mechanisms have been put forward to explain how sporopollenin is produced biochemically and what its structural components are.

1.3.1 Work by Zetzsche et al (1928-1937)[3,4,7,21,22]

In 1928, Zetzsche and co-workers started to study the membranes of spores and pollen and in particular the structure of sporopollenin.[7] The sporopollenin was isolated from various pollen grains, spores and fossil material in order to identify their composition. A summary of their work is shown in Figure 1.1.

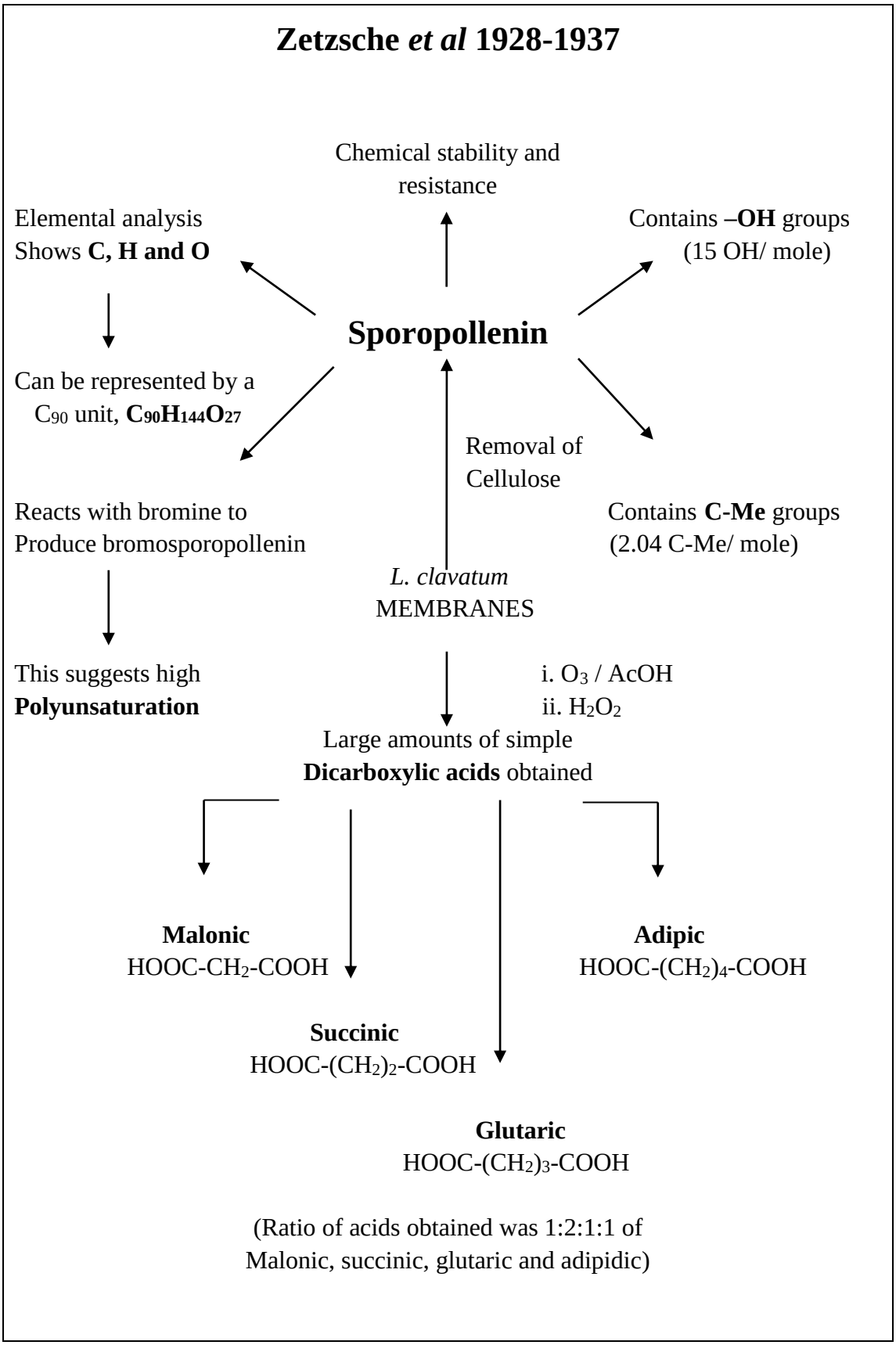


Figure 1.1 A summary of the work carried out by Zetzsche *et al* [6,8,11,22]

1.3.1.1 Elemental Analysis

Results of elemental analyses showed that sporopollenin exines (inner layer removed) are made up of C, H and O. The elemental analysis of a series of sporopollenins is shown in Table 1.2.[3,7,22]

1.3.1.2 Functional Group Identification

a) Kuhn-Roth oxidations with chromic acid indicate the presence of C-Me groups. The amount varies from around 1.70 C-Me/mol (0.90 mmol/g) for Pinus sylvestris to 4.50 C-Me/mol (2.6 mmol/g) for Lange sporonin. And the value for *Lycopodium clavatum* was found to be 2.04 C-Me/mol (1.2 mmol/g).[6,22]

b) That sporopollenin could be acetylated with hot acetic anhydride indicated the presence of hydroxyl groups. By saponification with alkali of the acetyl sporopollenin gave values for the number of hydroxyl groups per C_{90} unit. The number for sporopollenin from Lycopodium clavatum is 15 OH/mol (9 mmol/g).[22]

SPOROPOLLENIN SOURCE	EMPIRICAL FORMULA	C-Me / Mole	OH / Mole
Lycopodium clavatum (spores)	$C_{90}H_{144}O_{27}$	2.04	15
Pinus sylvestris (pollen)	$C_{90}H_{144}O_{24}$	1.70	13
Phoenix dactylifora (pollen)	$C_{90}H_{150}O_{33}$	3.45	/
Tasmanian fossil (pollen)	$C_{90}H_{136}O_{17}$	3.00	/

Table 1.2 Results from analytical experiments carried out by Zetzsche *et al* (1937).[3,22]

1.3.1.3 Reaction with Bromine

Zetzsche *et al* found that sporopollenin contains substantial unsaturation as it readily absorbed large amounts of bromine in CCl_4 to give 'bromosporopollenin' with approximately 50 % bromine by weight difference.[6]

1.3.1.4 Oxidative Degradation

Sporopollenin can be degraded into simple soluble substances by oxidative chemical attack,[6,23] which again demonstrates the unsaturated nature of the material. Zetzsche *et al* exposed *Lycopodium clavatum* membranes to ozone in acetic acid followed by treatment with hydrogen peroxide. In these conditions, much of the exine dissolved and large amounts of a mixture containing simple dicarboxylic acids, especially succinic, glutaric, malonic and adipic acids (ratio of 2:1:1:1), were obtained.

The results from Zetzsche's work leads to the conclusion that spores or pollen grain walls consist of a mainly cellulose inner layer and a very resistant outer layer, which is a polyunsaturated compound with methyl and hydroxyl groups. The only reagents to be able to degrade the polymer were strong oxidizing agents. However, the results from Zetzsche's work did not give any insight into the actual monomers present in the sporopollenin structure as all of the products formed from the degradation experiments were produced under harsh conditions which could have involved modification of the structural units.

1.3.2 Early Work by Shaw and Yeadon (1964, 1966)[3,4,9,24]

After the initial work by Zetzsche *et al*, Shaw and co-workers reinvestigated the experiments and also carried out additional degradative studies in an attempt to determine the composition of sporopollenin. Most of the studies were carried out on sporopollenin from *L. clavatum* and *P. sylvestris*, and the results confirmed Zetzsche's conclusion.

Various methods of degradation were used to degrade sporopollenin into a useful manner in order to gain an insight into its structure and the only effective technique was oxidation. A wide range of reagents including sodium hypochlorite, chromic acid, strong nitric acid, treatment with ozone and KOH fusion were investigated and the most useful technique was found to be the use of ozone and KOH fusion. Results obtained by treatment with ozone are shown in Figure 1.2.

A conclusion given by Shaw and Yeadon from their work is that membranes of *L. clavatum* and *Pinus silvestris* pollen were very similar and contain the following: [23,25]

1) Cellulose.
2) Some polysaccharide material that is more readily hydrolysed than cellulose.
3) A lipid fraction that is resistant to chemical treatments.
4) A lignin-like fraction.

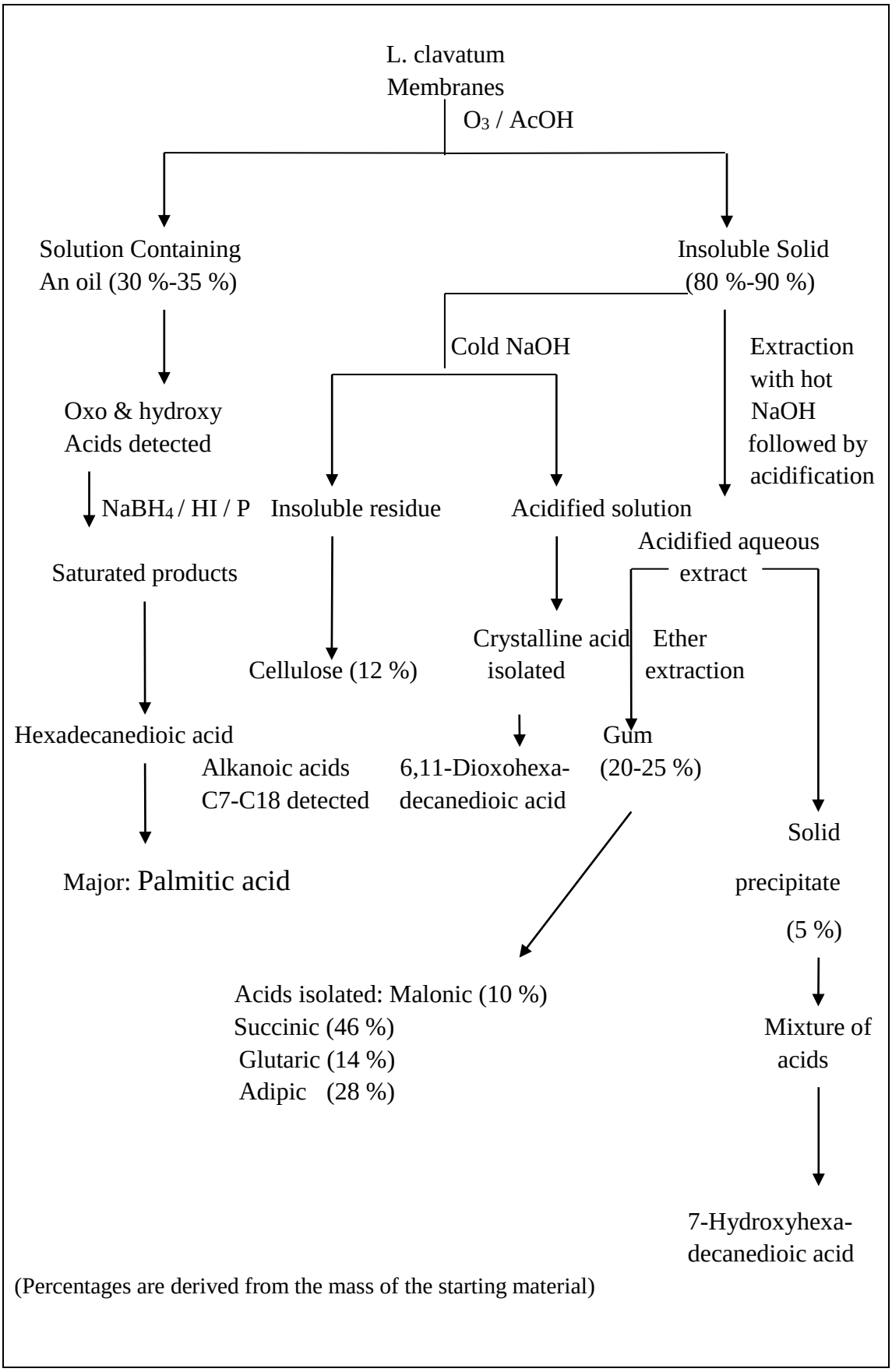


Figure 1.2 Summary of the work with O_3 carried out by Shaw and Yeadon[23,25]

1.3.2.1 Cellulose

Treatment of the membranes of *Lycopodium clavatum* spores with a small amount of ozone[2,24,25] resulted in the formation of an insoluble solid (80-90 % by weight of the original membrane), and a solution from which an oil (30-35 % by weight of the original membrane) was isolated was obtainable. The solid mainly dissolved in sodium hydroxide at room temperature to leave an insoluble residue of cellulose (12 % by weight of original membrane) which was confirmed by hydrolysis with H_2SO_4 to be glucose and by IR comparison with a pure cellulose sample.

1.3.2.2 Additional polysaccharide material

When Shaw *et al* [24] repeated Zetzsche's ozone degradation, they found that most of the membrane dissolved leaving a mixture of simple dicarboxylic acids and a small amount of an insoluble residue. The residue was hydrolysed by acid and then produced cellulose and a solution which contained a mixture of sugars including xylose as the main component, glucose and galactose. Shaw[3,11] suggested that this polysaccharide material could be a hemicellulose or xylan-like material.

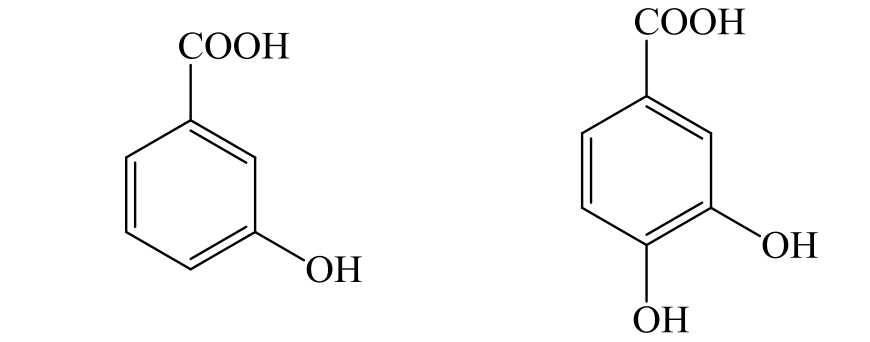
1.3.2.3 Lipid fraction

Non-branched mono- and di- carboxylic acids were obtained from oxidative degradation.[3,24] After treatment with ozone (method of Shaw),[24] an oil resulted which gave positive tests for oxo- and hydroxy-acids. After reduction with $NaBH_4$ the mixture was shown to contain hexadecanedioic acid along with a mixture of alkanoic acids, the major contributor being methyl palmitate.

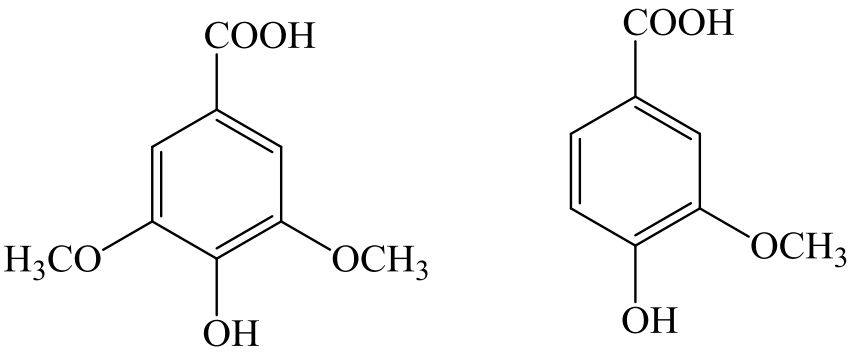
The solution obtained from the treatment of the insoluble solid with NaOH at room temperature was acidified to give crystalline, 6,11-dioxohexadecanedioic acids. And the acidification of the insoluble solid after extraction with hot sodium hydroxide gave a solid precipitate which contained a mixture of acids, one of which was identified as 7-hydroxyhexadecanedioic acid. Ether extraction of the acidified extract gave a gum, which after methylation and examination by gas chromatography yielded a mixture of malonic (10 %), succinic (46 %), glutaric (14 %) and adipic acids (28 %). Shaw *et al*[3,24] suggested that these acids could have been possible oxidative degradation products of the C_{16} acids identified earlier or oxidation products of similar like material of lipid origin.

1.3.2.4 Lignin material

By fusion of the membranes of *L. clavatum* spores with KOH, a mixture of alkanoic acids (C_5-C_{11}) and the phenolic acids *m*-hydroxybenzoic acid, protocatechuic acid and traces of syringic and vanillic acids was obtained, Figure 1.3. Shaw *et al*[24] suggested that a lignin-type material was present from this result. Infrared studies of the membranes showed absorptions around 1500-1600 cm^{-1} which are characteristic of aromatic structures and similar bands have been found in lignins.[24,25] Lignin-type materials usually produce methoxy aromatic groups after methylation with diazomethane followed by KOH fusion. However, when this was carried out on spores from *Locopodium clavatum*, this was not found as expected. Shaw[24] suggested that it was because it was masked by the cellulose and lipid fractions. Shaw *et al* proposed that the sporopollenin found by Zetzcshe was composed of lipid and lignin type fractions.



m-hydroxybenzoic acid Protocatechuic acid



Syringic acid Vanillic acids

Figure 1.3 Phenolic acids obtained by fusion of the membranes of *L. clavatum* spores with KOH

1.3.3 Carotenoids as possible precursors of sporopollenin

When sporopollenin is treated with ozone it produces several different compounds, suggesting that sporopollenin might be made up from different products. However, Shaw[4] proposed that sporopollenins from different plant sources were similar in character, which indicates that their synthesis is directed by the DNA of the plants. Brooks and Shaw[4,26,27] found a correlation of development of the pollen exine from *Lilium henryii* plants and the development chemical substances in anthers. These results suggest that sporopollenin is formed by an oxidative polymerisation of carotenoids and carotenoid esters.[26,27] The development of the pollen grains was accompanied by parallel formation of carotenoids and carotenoid esters in a ratio of 2.2:1.[4]

It is possible to envisage that these molecules could provide the features required of a monomer precursor that could lead to a macromolecular structure analogous to sporopollenin by examining the structures of carotenoids and carotenoid esters.[3] A selection of carotenoids and carotenoid esters is shown in Figure 1.4.

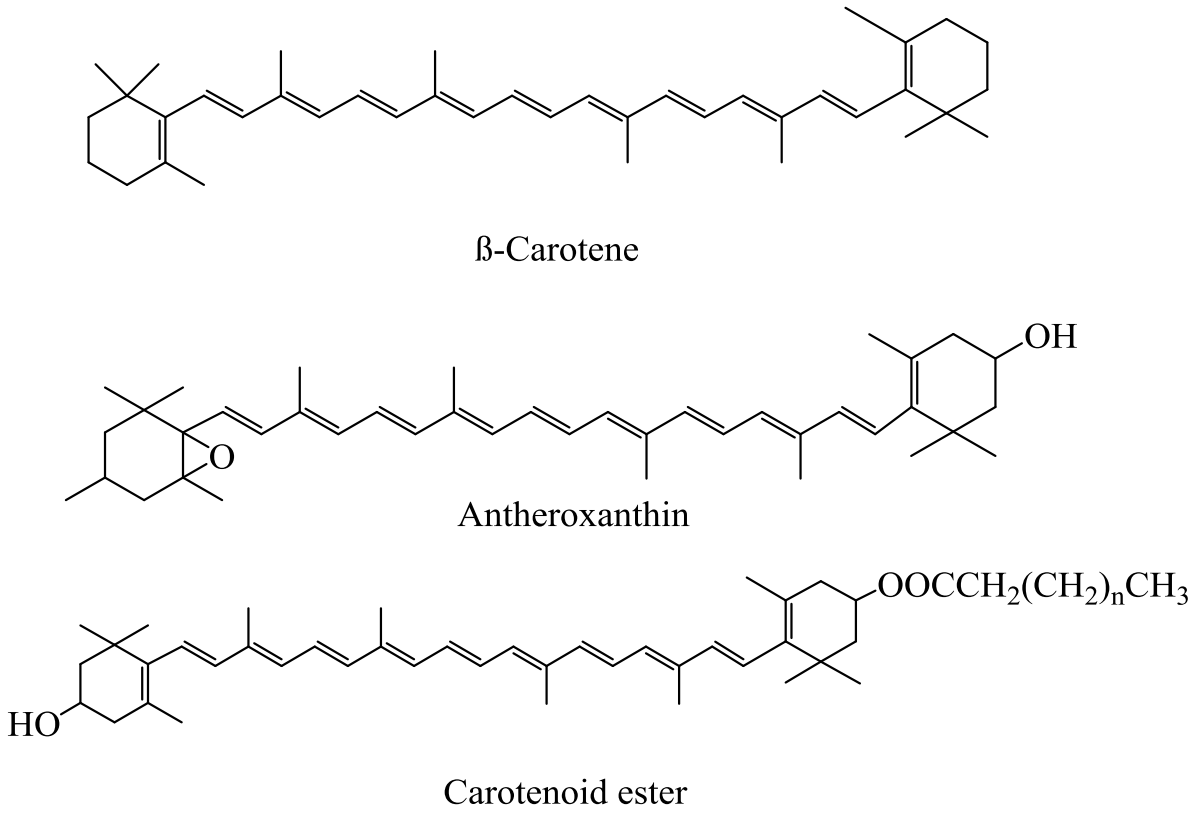


Figure 1.4 Examples of carotenoids and carotenoid esters.

Polymerisation of a number of carotenoids, including β-carotene, carotenoid and carotenoid esters of *L. henryi*, with a trace of an ionic catalyst (BF_3, Boron trifluoride) in the presence of oxygen, gave an insoluble oxygen containing unsaturated polymer virtually chemically and physically identical to sporopollenin.[26] Comparisons between the natural bio-polymer and the synthesised polymer were examined. Similar features exist in:

(i) resistance to acetolysis, (ii) elemental analysis, (iii) infrared spectra, (iv) oxidation products and (v) pyrolysis gas-liquid chromatograms.[27] *p*-Hydroxybenzoic acid was produced as the major component in potassium hydroxide fusion of both polymers. This result led Shaw and Yeadon to revoke their original suggestion that sporopollein is of

lignin origin, and later Shaw proposed that sporopollenin is produced by oxidative polymerisation of carotenoid esters.[23,26]

This proposal was further backed up by examining the pyrolysis products of sporopollenin from various sources such as modern pollen, coals and river shale. The comparison of the product and the pyrolysis products of β-carotene and its oxidative polymer showed that sporopollenin produced typical degradation products comparable with those from such carotenoids.[28] β-Carotene, the β-carotene oxidative polymer and the sporopollenin material produced from ionene and the most of the samples produced naphthalene, 2,6-dimethylnaphthalene and 1-methyl- and 2-methyl-naphthalene.[28] The structures are shown in Figure 1.5.

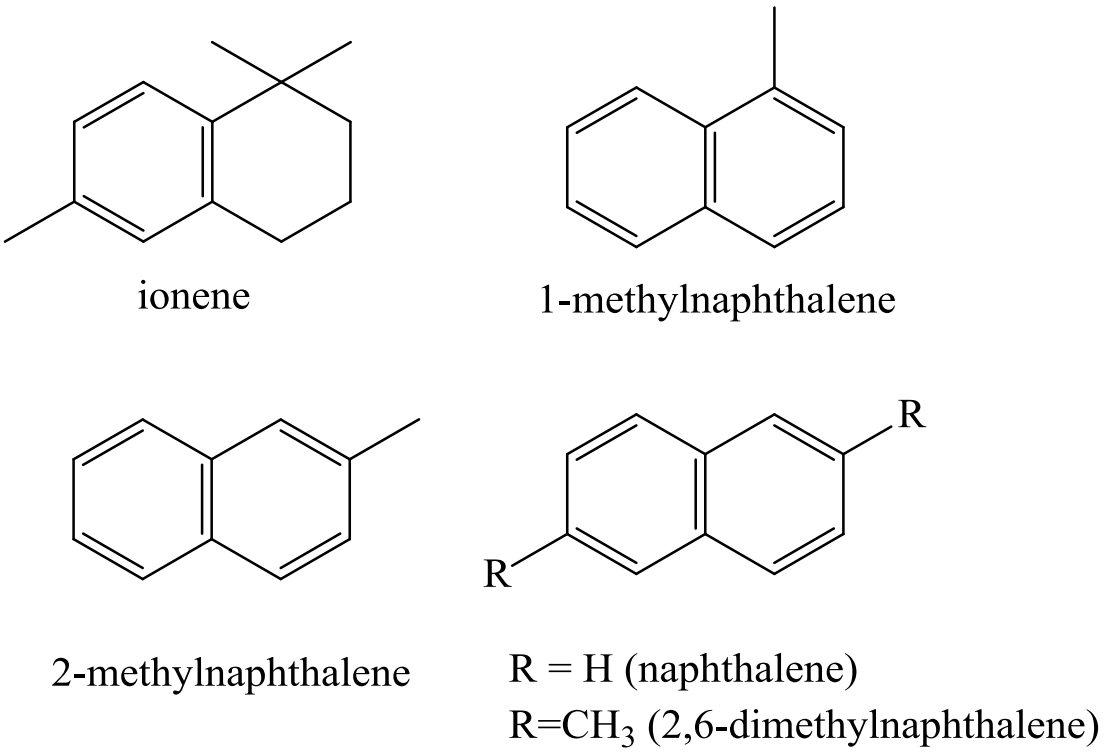


Figure 1.5 Pyrolysis products from sporopollenin & the β-carotene polymers.

1.3.4 Inhibition and radiolabelling studies on sporopollenin biosynthesis

1.3.4.1 Effects of inhibitors of carotenoid synthesis on sporopollenin

In contradiction to the findings by Shaw, Prahl *et al* (1985)[29] demonstrated that an inhibition of the formation of carotenoids did not block sporopollenin biosynthesis. Sandoz 58-035, a known inhibitor of carotenoid synthesis, was applied to the plants of the *Cucuribita* (pumpkin) and it was found to influence carotenoid metabolism at the site of sporopollenin synthesis by hindering the desaturation of carotenoids and therefore saturated precursors accumulated. Upon isolation of sporopollenin from the pollen, it was found that biosynthesis was only slightly affected. Saturated carotenoids or related precursors of sporopollenin can still take part in sporopollenin biosynthesis as Sandoz only affects the final stages of the biosynthesis.

1.3.4.2 Tracer experiments on sporopollenin biosynthesis

Gubatz *et al*[19] suggested that a phenyl propane unit such a *p*-coumaric acid could be a monomer involved in the structure of sporopollenin. They carried out tracer experiments with a number of radiolabelled substances as precursors on *Tulipa* and *Cucuribita* species. Phenylalanine was incorporated into the sporopollenin at a higher rate than any other precursor used. ^{14}C-Phenylalanine and *p*-hydroxybenzoic acid were the main labelled products following potassium hydroxide fusion. It was suggested that it was unlikely that these acids formed a genuine structure of the sporopollenin skeleton as they were formed under forcing conditions. As a result it is more likely that phenyl propyl unit is the more likely candidate. The structures of phenylalanine and the phenolic acids are shown in Figure 1.6.

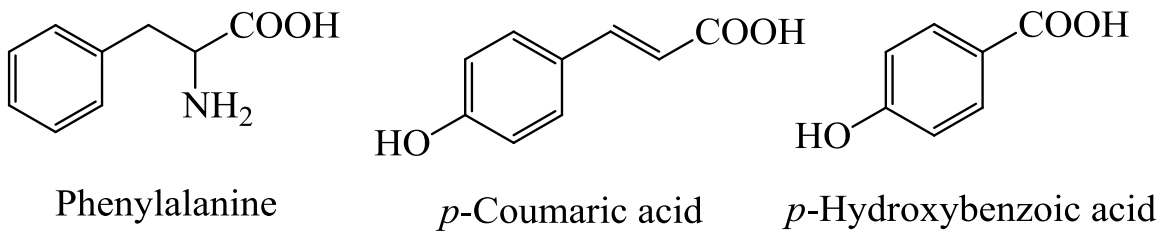


Figure 1.6 Structures of Phenylalanine and Phenolic acids

1.3.4.3 Incorporation of Oleic acid into sporopollenin[18]

Studies were undertaken on the green alga *Scenedesmus acutus* whose outer cell wall is composed of sporopollenin. The incorporation of ^{14}C oleic acid into the green alga was inhibited by the chloroacetamide herbicide Metazachlor. The sporopollenin was isolated after exposure of the green alga to ^{14}C oleic acid and it was found to be radioactive. The incorporation of ^{14}C oleic acid was inhibited by 98% after treatment of the cells with Metazachlor. These results propose that oleic acid, with the structure CH_3-$(CH_2)_7$-CH_2=CH_2-$(CH_2)_7CO_2H$, is a precursor of sporopollenin and that the biosynthetic pathway is targeted by Metazachlor.

1.3.4.4 Determination of the hydroxyl content of sporopollenin[30]

L. clavatum has a hydroxyl content of 6.6 hydroxyls per C_{90} fragment. The value for sporopollenins from other sources is in the range of 5.9-9.6 hydroxyls per C_{90} fragment. The number was determined by refluxing the sporopollenin with 1-C^{14}acetic anhydride. The incorporation of acetyl groups appeared to reach a maximum after 4 hours under refluxing conditions. Synthetic polymers prepared from oxidative polymers of carotenoids and fossil sporopollenin had lower values of around 1.7-1.9 hydroxyls per C_{90} unit.

1.3.5 Phenols as integrated components of sporopollenin

Shaw and Yeadon[23] discovered that phenolic acids were produced from potassium hydroxide fusion of *L. clavatum* and *L. Henryi* membranes. Schulze-Osthoff and Wiermann[19] carried out investigations on sporopollenin isolated from pine pollen and found that large amounts of phenols were obtained after nitrobenzene oxidation with *p*-coumaric acid being the main product. Following fusion with potassium hydroxide, *p*-hydroxybenzoic acid was the main component in the mixture of phenolic compounds obtained. The large amount of phenolic acids produced suggested that phenols are integral parts of sporopollenin. It was noted that nitrobenzene oxidation of sporopollenin did not give characteristic lignin degradation products. This was further evidence that sporopollenin differs in structure to lignin.[20]

In addition to these studies, Wehling *et al*[2] use AlI_3 (Aluminium iodide) to degrade sporopollenin. AlI_3 is a gentle degradative method and is noted to be capable of splitting ether bonds. As a result of this treatment the major product of degradation was *p*-coumaric acid and Wehling *et al* suggested that this was evidence to prove that *p*-coumaric acid is a genuine component of sporopollenin which may be bound by ether linkages. The oxidative polymer of β-carotene was treated with AlI_3 and no *p*-coumaric acid or any other phenolic products were detected. This suggested that it was highly likely that the β-carotene polymer differed structurally to the natural sporopollenin.

1.3.6 The use of spectroscopy in sporopollenin analysis

1.3.6.1 NMR Spectroscopy

^{13}C Solid State NMR. This has been used to examine the structure of a number of pollen and spore exines including *Betula, Pinus*, *Lycopodium* and two fossil spores, *Lagenicula* and *Parka*. All of the pollen and sproe exines all share broadly similar structural characteristics, but there are still some significant differences between them, especially the living and fossil material.

Shaw and Yeadon[23] illustrated that this technique was capable for detecting variation in a wide range of compounds included under the general term sporopollenin. This led to their viewpoint that sporopollenin was a polymerised fatty acid, based on the high proportion of aliphatics shown by their analysis.

Brooks and Shaw[4] also showed that the sporopollenins of higher plants, for example lycopods and algae, are similar in composition with some small differences.
Three spectra from *Betula, Pinus* and *Lycopodium* are shown in Figure 1.7, and Figure 1.8 shows the spectra from two fossil sources, *Lagenicula* and *Parka*.[31] The marked *Lycopodium* spectra showed the approximate positions of some of the functional groups. Distinct differences exist between the modern and fossil sporopollenin, there are wider ranges of relatively defined peaks in the modern fossil, whereas the fossil material has fewer and broader peaks. A prominent peak in the 15-40 ppm range, representing methyl groups could be observed in all spectra. Another smaller peak occurs at around

15-20 ppm represents aliphatic groups. The high number of aliphatic groups along with the presence of COOH or COOR groups at around 172 ppm and the low number of methyl components in the modern sporopollenins is important criteria for backing up the suggestion that fatty acids may be the initial precursor of sporopollenins. All spectra show peaks at 120-130 ppm that are likely to be aromatics and peaks at 140 ppm, which suggests the presence of phenols. The exine of the modern sporopollenins show peaks at 100 and 110 ppm, which are characteristic of unsaturated carbons. The peaks do not show up in fossil material, but this is to be expected, as they are relatively unstable so that they might be oxidized over the time. It was observed that the spectra of different workers varied. It was most likely that the variations are due to the methods of isolation and strict controls (including the reaction time, temperature and the amount of reagents used) should be exercised over preparatory techniques.

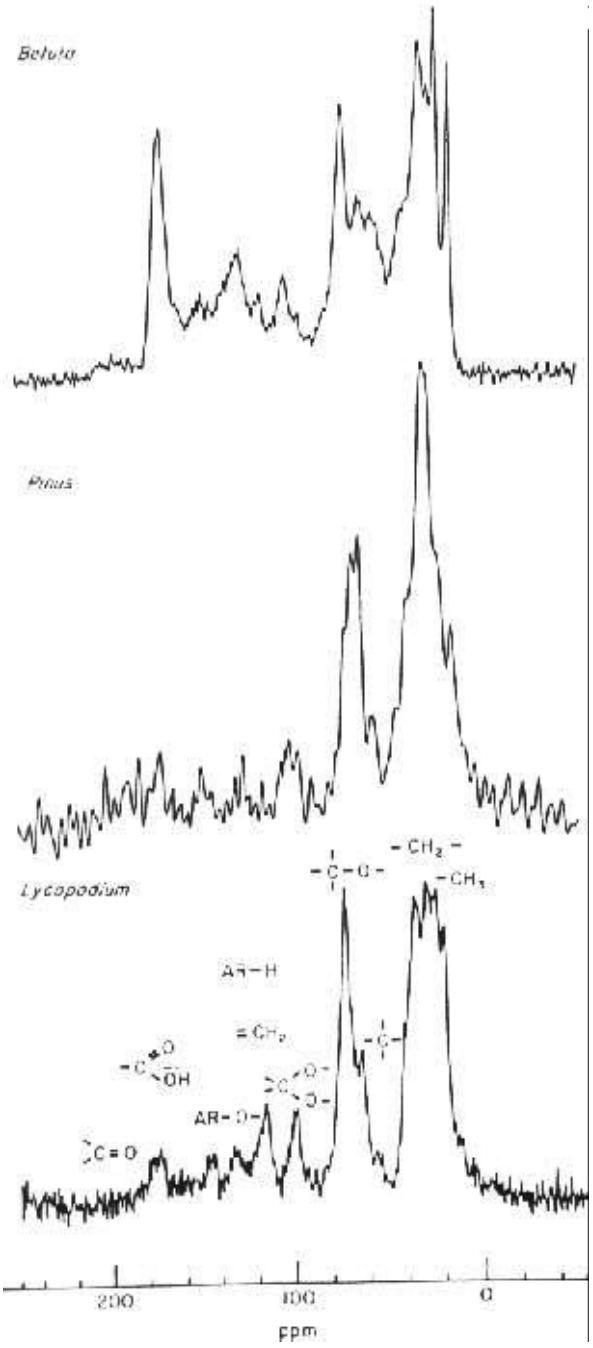


Figure 1.7 ^{13}C solid state NMR spectra of three modern sporopollenins from *Betula*, *Pinus* and *Lycopodium*. (Reproduced from *Annals of Botany*, **1992**, *69*, 545-549)[32]

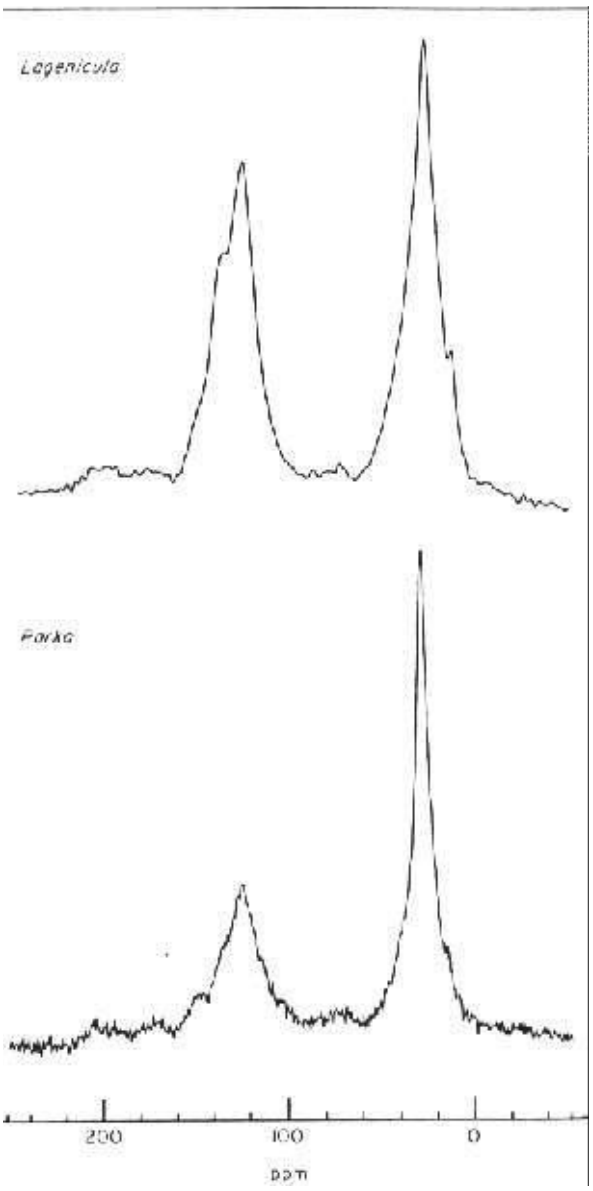


Figure 1.8 ^{13}C solid-state NMR spectra obtained from two fossil spores, *Lagenicula* and *Parka*. (Reproduced from *Annals of Botany*, **1992**, *69*, 545-549)**Error! Bookmark not defined.**

^{1}H NMR. The ^{1}H NMR spectra of some sporopollenins have been recorded,[21] with 2-aminoethanol as the solvent. The spectra showed the presence of 1,4-disubstituted or 1,2,3,4-tetrasubstituted benzene systems. These systems contained various oxygen containing functionalities such as hydroxyls, carboxylic acids and esters.

1.3.6.2 Infrared Spectroscopy

It has been proved that the Infrared spectra of sporopollenin from modern and fossil sources are similar by Shaw *et al's* work.[3] A number of sporopollenins including *L. clavatum, Pinus montana* and *Lilium henryii* have been measured. The spectra are shown in Figure 1.9 and results of these studies are summarized in Table 1.3. Other works undertaken by Kawase *et al*[33] also give similar results to Shaw's. Their work showed that sporopollenin contained hydroxyl groups, ethers, carbonyl groups and possibly the structure R-CO (R = aromatic, aliphatic or conjugation). Kawase proposed that the main structure of sporopollenin is a simple aliphatic polymer with aromatic and conjugated side groups. These studies suggest that sporopollenin consists of a main structure or backbone with side chains that vary between species.

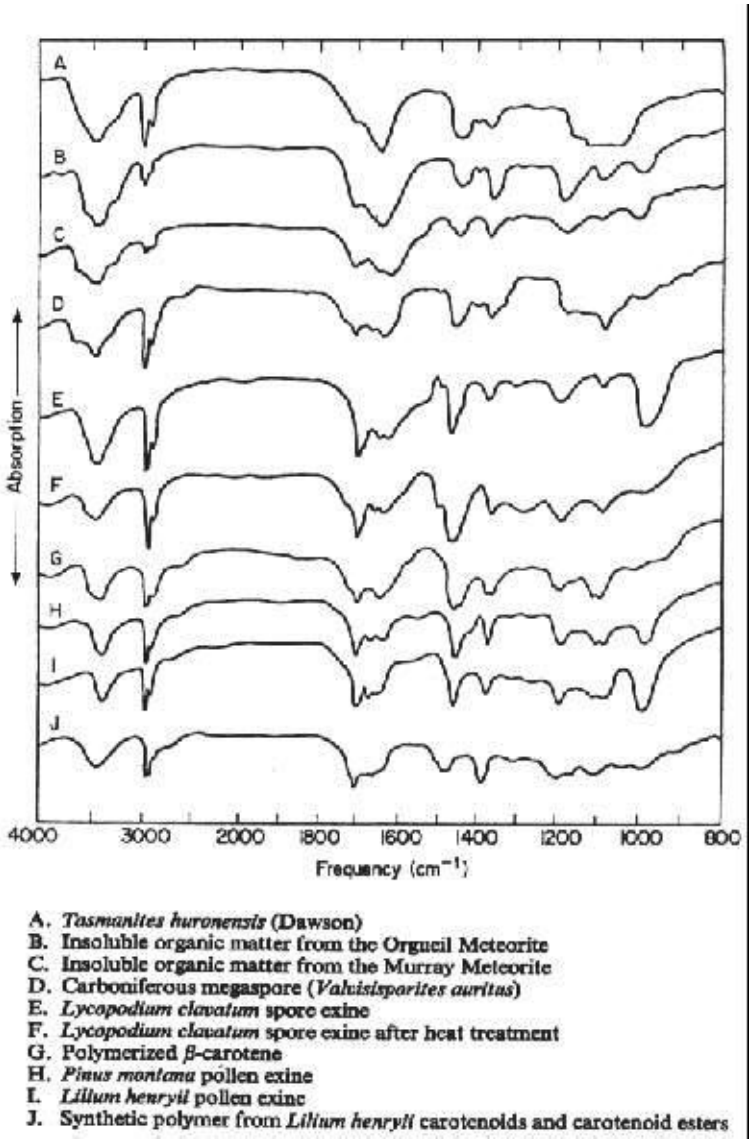


Figure 1.9 Infrared spectra of various sporopollenins. (Reproduced from *Sporopollenin in Phytochemical Phylogeny*, **1970**, *3*, 31-58)[3]

Region (cm^{-1})	Information gained from Infrared spectra of sporopollenin
4000-2000	Absorptions present at around: • 3400 cm^{-1} (strong, broad) indicates the presence of hydroxyl groups. After reaction with acetic anhydride the intensity of the peak is reduced. • 2930 cm^{-1} (strong, sharp) and 2860 cm^{-1} (strong, sharp but less intense). Likely to be due to CH_2 stretching frequencies
1780-1640	• All showed broad peak in this region which indicates the presence of C=O containing groups. • No absorption around 1750-1735 cm^{-1}, the region characteristic of ester groups, give the evidence for absence of esters. And *L. clavatum* membranes after methylation with diazomethane showed no absorption at 1780-1710 cm^{-1}, suggesting a lack of carboxylic groups.
1600-1500	• All membranes showed absorption bands in this region. The peaks around 1510 cm^{-1} showed an increase in intensity after diazomethane treatment. This indicates the presence of phenolic groups.
1500-700	• A broad absorption near 1100 cm^{-1} may be due to ether groups.

Table 1.3 Results of infrared spectra of some sporopollenin[24]

1.3.6.3 X-Ray photoelectron spectroscopy (XPS)[33]

XPS gave the results with similar conclusions to those obtained by Infrered spectroscopy. They showed that sporopollenin was made up of C, H and O and that the basic structure was nearly the same, with only some small variations between *Magnolia grandiflora* and *Hibiscus syriacus.* The results also suggested that there are main structural features present in all sporopollenin with varying side chains.

1.3.6.4 UV-VIS Spectroscopy[33]

Kawase *et al* determined the UV-VIS spectra of *M. grandiflora* and *H. syriacus* using 2-aminoethanol as solvent. Aromatic groups and conjugated side chains were presented in the spectra. Small structural variations were also shown between the two plant groups.

1.4 Applications of Sporopollenin Exine Capsules

1.4.1 The use of sporopollenin exine capsules in solid phase peptide synthesis (SPPS)

The basic principle of SPPS is the sequential addition of protected amino acids to growing peptide chain attached to an insoluble solid support until the required length of polypeptide chain is obtained. The synthesized peptide is then removed from the support. The repetitive nature of peptide synthesis means that the work up at each stage is reduced to simple filtration and washing of the solid supported peptide.

Mackenzie and Shaw[34] described preliminary work using sporopollenin from *Lycopodium clavatum* in SPPS. By reaction with chlorodimethyl ether and stannic chloride, chloromethyl groups could be added to the surface of the material. The loading of chlorine was found to be about 1 mmol/g as shown by removal with reaction with pyridine. Then they found that amino acids could be attached to the chloromethylated sporopollenin and then removed using HBr in TFA (Trifluoroacetic acid). Simple tripeptides could be prepared this way and the isolation was easy. After these reactions, it was also found the morphology of the sporopollenin was retained.

Adamson *et al*[35] prepared a tetra peptide on base of functionalized sporopollenin also from *Lycopodium clavatum*. First, they aminated the sporopollenin with 1,3-diaminopropane. Amination of sporopollenin with 1,3-diaminopropane resulted in a loading of 1.2 mmol/g of base (determined using the picric acid assay) after refluxing for 16 hours and a maximum loading of 1.6 mmol/g of base after 24 hours.

Adamson found that the NH group attached to sporopollenin was either only weakly basic (nucleophilic) or inaccessible. Evidence to support this point came from the results of the following experiments:[35]

1) Sporopollenin aminated with triethoxy aminopropyl silane, which attaches to silica gel at the ester end with the production of a basic amino gel. It gave a loading on to sporopollenin of 0.8 mmol/g of base by assay with picric acid. However attempts to acylate the basic group with acetic anhydride, succinic anhydride or Fmoc- alanine anhydride proved completely negative. This suggested that the amino end of the silylamine had attached and not the ester end.
2) When sporopollenin aminated with 1,3-diaminopropane was reacted with either acetic anhydride or chloroacetic anhydride, the amount of base was found to be halved according to the picric acid assay.
3) Treatment of sporopollenin aminated with 1,3-diaminopropane or triethoxy aminopropyl silane with acetyl chloride showed that no basic groups were present according to the picric acid assay. This implied that both ends of the attached diamine had reacted with the acid.
4) Sporopollenin after amination with 1,3-diaminopropane followed by reaction with either chloroacetic anhydride or chloroacetyl chloride, was reacted with the sodium salt of the 4-hydroxybenzyl alcohol linker to give a loading of 0.8 mmol/g, determined by chloride displacement. This implied that only the outer NH-chloroacetyl group had reacted and the inner chloroacetyl group attached to the nitrogen had been removed. From these results, Shaw suggested that the free end of an unattached diamine was more reactive than the NH attached (inner) to the surface

of sporopollenin.

These results suggested that the NH end of the diamine attached to sporopollenin was not as reactive as the free amine end of the diamine.

The sporopollenin with 4-hydroxybenzyl alcohol linker attached was then used for the preparation of the tetrapeptide which was built up by the addition of individual Fmoc-amino acid anhydrides. After each addition, the sporopollenin was reacted with acetic anhydride to cap any unreacted hydroxyls. After addition of the last amino acid, the final loading of the tetrapeptide was found to be 0.23 mmol/g (19 % yield based on initial loading of linker). Figure 1.10 outlines the strategy used to obtain the tetra peptide.

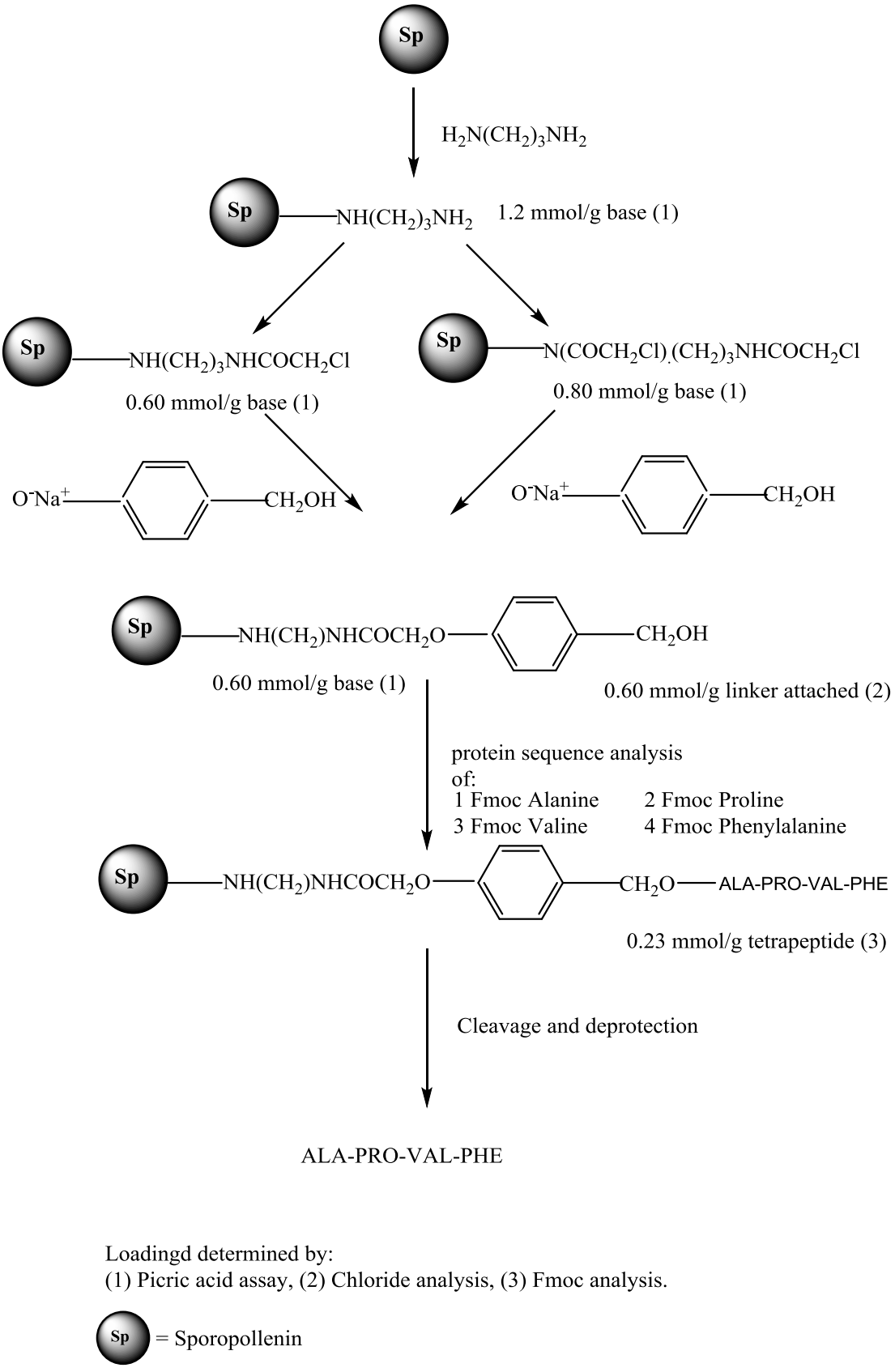


Figure 1.10 The use of functionalized sporopollenin in peptide synthesis[35]

1.4.2 The use of sporopollenin as a new chelating resin in ion and ligand exchange

Ion exchange is a process of exchange of ions between two electrolytes or between an electrolyte solution and a complex. Ligand exchange is a type of chemical reaction in which a ligand in a compound is replaced by another. Functionalized sporopollenin from *Lycopodium clavatum* has been used for both ion and ligand exchange chromatography and for the successful separation of various nucleosides, nucleotides, α-amino acids and transition metals.[36,37]

Sporopollenin has been modified with 1,2-diaminoethane, followed by either chlorosulphonic acid or by bromoacetate and $CuCl_2$. The results are summarised in Table 1.4**.**

Reagent	Sporopollenin Derivative	Medium	Compounds separated	Loading (mmol/g)
1,2-Diaminoethane	Sp-$NHCH_2CH_2NH_2$	Anion Exchanger	Ribonucleotides	1.59 (1)
Chlorosulphonic acid	Sp-$NHCH_2CH_2NHSO_3H$	Cation Exchanger	α-amino acids	1.60 (2)
Bromoacetate and $CuCl_2$	Sp-$NHCH_2CH_2N(CH_2CO_2)_2^{2-}Cu^{2+}$	Ligand Exchanger	Ribonucleosides	1.40 (3)

Loadings determined by (1) Picric acid assay, (2) Titrimetric analysis, (3) Method of analysis are not given

Table 1.4 Functionalized sporopollenin used in ion- and ligand-exchange processes.[36,37]

Metal ligand complexes based on aminated sporopollenin from *Lycopodium clavatum* have also been used for the adsorption of heavy metal ions from aqueous solution.[38] As such they could be potentially used in the treatment of water contaminated with heavy metals.

1.4.3 The use of sporopollenin in drug delivery

There have been a number of methods of drug delivery to the body. Traditional methods of drug delivery involve the use of injections or oral formulations, such as tablets, and whilst these methods are the most common modes they suffer from some disadvantages. One major disadvantage of these two methods is that after administration there is a rise of drug levels in the blood which can create toxic side effects.[39] This level then decreases until the next administration. Ideally it may be better for the drug to remain at a constant level in the blood for an extended period of time, and this can be achieved by controlled drug delivery.[39]

Controlled drug delivery systems involve the combination of a drug or bioactive agent with a polymer.[39,40] When this system is placed in the body the material is released from the polymer at a pre-determined rate.

A range of synthetic and natural polymers have been investigated for use in drug delivery.[39] The ideal solid support for drug delivery has the following properties

1 Physical and mechanical stability.

2 The support should be easily functionalised and have a sufficiently high drug loading.

3 Biodegradability.

4 Degradation of the support should occur within a reasonable time.

5 The support and the degradation products should be non-toxic.

6 Easy and cheap to prepare

Sporopollenin is found to have these essential qualities and it has been investigated in drug delievery field.

1.4.4 Other researches of sporopollenin exine capsules

Yilmaz *et al* have reported the use of sporopollenin as additives in lipase immobilization by a sol-gel process. They explored the effect of these materials in the enantioselective hydrolysis of (*R, S*)-naproxen methyl ester.[41]

Paunov *et* report a simple and robust technique for loading of sporopollenin microcapsules from *Lycopodium clavatum* with a range of inorganic and organic nanomaterials based on *in situ* preparation of nanoparticles by a chemical reaction in the sporopollenin exine capsule interior.[42]

Hamad *et al* demonstrated that living cells can be encapsulated inside sporopollenin microcapsules derived from *Lycopodium clavatum*.[43]

Wakil *et al* investigated whether encapsulating the ethyl ester form of fish oil and found that sporopollenin leads to enhanced bioavailability of eicosapentaenoic acid (EPA) ethyl ester compared to EPA ethyl ester alone.[44]

1.5 Summary of previous modification of sporopollenin

Sporopollenin from *Lycopodium clavatum* has been successfully functionalized by previous workers and the resulting modified exines used as solid phase 'functional materials'. Their research showed that sporopollenin has a number of advantages over commercial resins which is beneficial for chemical functionalization, namely:

1) Constant chemical structure

Sporopollenin is homogeneous and non-variable from grain to grain as the pollen and spores have a precise and constant morphology,[36,45] which means that sporopollenin prepared by different workers and on different occasions would have the same composition. As a result, the loadings after functionalization would be similar. However, it has been noticed from earlier studies that sporopollenin prepared by different methods shows a slight variation in composition.[10,11] Therefore, it is essential that sporopollenin should be prepared by only one method for the production of chemically and structurally consistent samples.

2) Constant spore/pollen size

Sporopollenin from *Lycopodium clavatum* has an average diameter of 20 μm, which is constant for each spore.[42] As most commercial resins have a variable particle size, this is a good advantage because columns of this material were free flowing and the flow rate was unaltered over a period of use of six months.[42] It may also possible to use spores and pollen grains from other species as they have exine capsule sizes of with diameter varying between 10 and 250 μm, though again being constant for each grain from a given species.[42]

3) Chemical and physical stability

The stability of sporopollenin is best demonstrated by the survival of pollen and spore exines in ancient sedimentary rocks, up to 500 million years old.[4]

Sporopollenin is also stable to organic and aqueous solvents and to chemical reagents including hot alkali and acid, although it is degraded by prolonged exposure to oxidising agents such as ozone.[3,4]

The stability of sporopollenin can also be demonstrated, as described earlier, by its ability to be used in peptide synthesis and ion and ligand exchange without any apparent degradation. It remained unchanged and retained the full morphology of the original spore.[42] It showed no signs of fragmentation and solvents flowed freely through it.[37,42]

4) Commercial availability

Lycopodium clavatum spores are inexpensive and are commercially available from a number of sources. There is around 20% sporopollenin in each *Lycopodium* spore membrane.[3,4] Sporopollenin is also convenient and easy to prepare from *Lycopodium clavatum* spores.[3,4]

However, there have been a number of problems and drawbacks encountered with this earlier work which include the following:

1 There was no stability studies carried out to determine how stable the linkages were which attached the functional groups to sporopollenin.

2 There has been no previous work to examine the filterability and stability of sporopollenin in various solvents.

3 There were no explanations given or investigations undertaken into the type of attachments which resulted after functionalization with the various reagents.

4 It was not clear how reproducible the loadings of functional groups attached to the sporopollenin were or how many times the functionalization reactions were carried out.

5 The overall yield of the tetrapaptide prepared by Adamson *et al*[20] using sporopollenin was very low. No optimisation studies or further work have been reported in order to increase the yields.

6 There have been no attempts to investigate the use of the naturally occurring hydroxyls of sporopollenin as points of further attachment or modification.

7 No attempts to determine if there were are any other naturally occurring functionalities present on the sporopollenin surface which could be used or if these functional groups would interfere with any further synthesis.

1.6 Project Aims

1.6.1 Application of functionalized sporopollenin in solid phase organic synthesis

Solid phase organic synthesis relies on the use of a solid support, and the early peptide work described above revealed that sporopollenin could be an alternative to the support materials currently in use. In this research, the aim was to functionalize sporopollenin from *Lycopodium clavatum* by amination and sulfonation and to investigate the use of these functionalized sporopollenin capsules in solid phase organic synthesis. A further aim was to exploit their stability by investigating reactions under harsher conditions than the room temperature used in the peptide synthesis / ion-exchange processes already described above.

1.6.2 Application of aminated sporopollenin as an emulsifier

Binks *et al* previously studied the behavior of naturally occurring spore particles and sporopollenin capsules at fluid interfaces and in emulsions (see Chapter 3). A second aim of this project was to investigate the property of the particle-stabilised emulsions using sporopollenin aminated with 1,6-diaminohexane and other 1,n-diaminoalkanes.

CHAPTER 2 Applications of surface modified sporopollenin as solid support in organic synthesis

2.1 Introduction

Solid supports are applied in organic synthesis in several of ways which include:

a) Solid phase organic synthesis (SPOS).[46-52]

b) The use of immobilized scavengers, reagents and catalysts to support solution phase chemistry.[46,47,53-58]

SPOS is a method where molecules are synthesized by attaching to an insoluble support. It has been used for almost 50 years and Merrifield[59] first developed this concept in 1963 for the peptides synthesis (as discussed in Chapter 1, page 28). It was also developed independently by Letsinger.[60] Merrifield introduced the solid phase in the preparation of peptides, by using a functionalized polystyrene (PS) support cross-linked with 2% divinylbenzene (DVB) in spherical beads form. *N*-Protected amino acids were linked and remained bound to the support throughout the synthesis. The product was then purified conveniently by simple filtration and washing of the solid support to remove excess reagents and any by-products after the synthesis. The final peptide was then detached from the support. Merrifield found that the peptide synthesis was simplified and shortened considerably by this procedure.

The solid phase synthesis method has also been developed and used for the synthesis of oligonucleotides and oligosaccharides.[61-63] In the 1970s, it was realized that insoluble polymers could be used for organic molecules synthesis in general and a number of

papers of early organic syntheses on solid supports were published.[55,64,65] One early application of SPOS was the preparation of insect sex attractants by Leznoff and co-workers in 1976, Figure 2.1.[66]

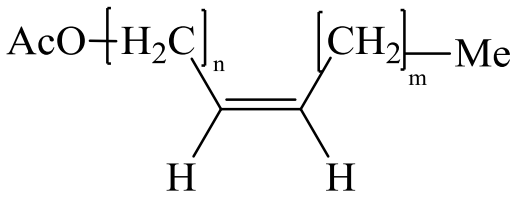


Figure 2.1 General formula of the insect sex attractants

However, solid phase organic synthesis never became popular until 1990s when an explosion of interest occurred. A large numbers of papers about SPOS have been published, which show the wide range of reactions which can be carried out on the solid phase and demonstrates the versatility and popularity of this method.[46,47,48,50,53,67,68,69,70]

Solid supported reagents, scavengers and catalysts have been used in organic chemistry since the 1960s.[46,47,54,55,56,57,58,71,72] Compared to SPOS, here the substrates are in solution whilst reagents are immobilized onto the support all the times.

Solid support reagents transform a substrate (or substrates) to a new chemical product (or products) and the excess or spent reagent may be removed by filtration.

Solid supported scavengers are solid supported reagents with reactive functional groups which can remove known impurities and quench excess reagents from the solution phase reactions.

Solid supported catalysts are simply solid phase reagents used in sub-stoichiometric amounts to effect transformation of a substrate.

All these immobilized reagents can be removed by simple filtration after the reaction. Now, this field has received renewed attention and a wide range of these agents have been developed for applications in solution phase synthesis.

2.2 Solid Phase Organic Synthesis (SPOS)

2.2.1 Advantages of SPOS

SPOS has a number of advantages over conventional solution phase synthesis which include:

a) Reactions can be driven to completion at high yields by using excess reagents.[46,48,49,52,59,68,70,72]

b) The intermediates and final products in the reaction remain attached to the solid support all the time. This makes purification simple and quick by filtration and washing to remove soluble excess reagents and by-products.[46-48,59,72]

c) Physical losses during the isolation and purification are reduced.[47]

d) Toxic or hazardous substances can be attached to a solid support which reduces their toxicity. It is friendlier to users and the environment.[47,71]

2.2.2 Solid Supports and Linkers

A series of different types of solid supports have been exploited for solid phase synthesis. The most common supports are usually synthetic cross-linked polymeric supports in the shape of spherical beads with typical diameter of 38 to 300 μm.[46,47,73]

Polystyrene (PS) beads cross-linked with 1-2% divinylbenzene (DVB),[46,47] which was first introduced by Merrifield in 1963[59] is still most widespread and commercially

available. Organic molecules are normally attached to the resin by an insoluble linker, immobilized protecting groups for solid phase synthesis,[48] Figure 2.2.

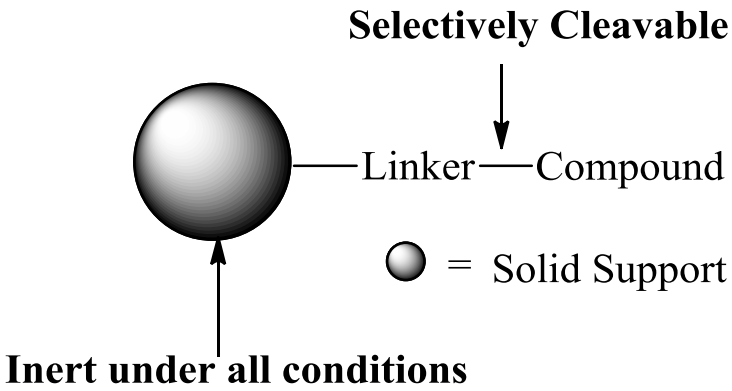


Figure 2.2 Illustration of a linker

2.2.2.1 Types of Solid Supports

a) Cross-Linked PS resins

Merrifield first used a chloromethylated-nitrated copolymer of styrene cross-linked with 2% DVB for solid phase peptide synthesis in 1963.[59] PS resins with varying amounts of cross-linking are now available and most commonly PS resins are micro- and macro-porous type resins.

Micro-porous PS-DVB resins possess a porous gel-type structure which swells up in polar organic solvents, for example, toluene, DCM or DMF, allowing easy penetration of reagents and solvents.[59] An example, in DCM, PS resin with 1% DVB will swell 4-6 times of its original volume.[47] These resins are usually prepared by polymerization of styrene and DVB, the cross-linking agent, suspended in water.[47,51,57,71] The beads produced can then be functionalized by chemical modification. Resin beads produced in

this way usually have different loadings which vary from batch to batch.[47] The degree of cross-linking is important as it affects the swelling, pore size and the mechanical stability of the beads.[59] Beads with low loading (less than 1 %) are too fragile to be stable and can make the filtration more difficult. High loading beads (8-16 %) are mechanically stronger but swell poorly and are too rigid to allow easy penetration of reagents and solvents into the beads causing slower and incomplete reactions.[59] PS resins cross-linked with 1-2% DVB are usually used and are commercially available with a wide range of functional groups and linkers attached, with a range of diameter usually in 5-400 μm.[46,47,74c] The most common sizes of the beads are 100-200 mesh (150-75 μm) and 200-400 mesh (75-37 μm).[46,47] Another method to prepare the functionalized PS-DVB resin is by adding a monomer containing the desired functionality to the styrene/DVB mixture during polymerisation.[47,57,71] Beads produced by this method give more control over loadings, particle size and swelling properties.[47,75] The loadings of linkers and functional groups attached to these resins are usually around 1 mmol/g but can reach up to 5 mmol/g.[46,47] The structure of the copolymer of styrene and 1% DVB is shown in Figure 2.3.[76]

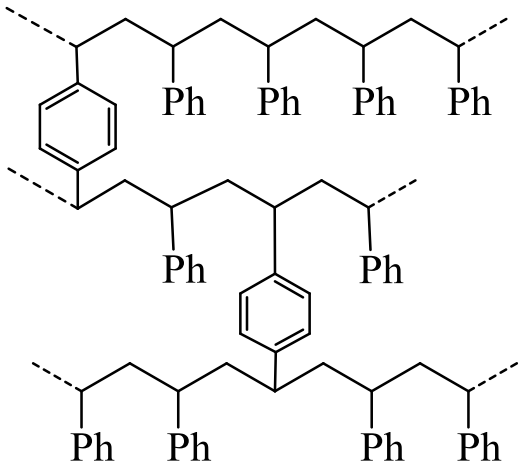


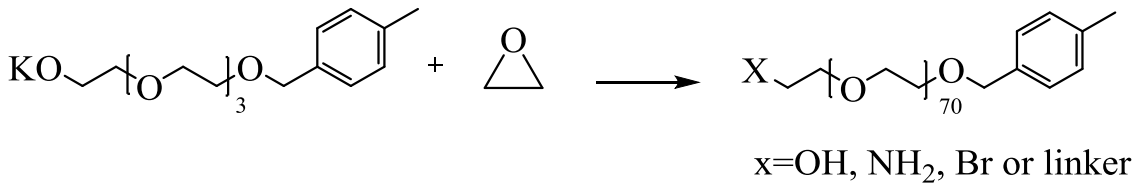
Figure 2.3 Structure of PS-DVB (1%) resin

Different from Micro-porous PS-DVB resins, **Macro-porous PS-DVB resins** possess permanent porosity even in the dry state. To prepare these resins, suspended dissolved monomers are polymerized in the presence of an organic solvent.[51,71,77] Solvent droplets called 'porogens' are trapped within the cross-linked polymer to produce large pores during the polymerization process. After the polymerization, the porogens are removed by washing or evaporation. Higher amounts of cross-linking agents are added (over 10% DVB) so that the porous structure of the resulting polymer remains stable and swelling of the resin will not occur before use.[51] Argopore® macro-porous resins supplied by Argonaut Technologies have a range of functionalities and linkers attached. The loading of these resins are between 0.6-1.1 mmol/g.[78b]

b) **PS-Poly (ethylene glycol) (PEG) Resins**

Bayer and Rapp first developed these PEG grafted PS resins for solid phase peptide synthesis.[79,80] These resins marketed under the name of TentaGel® are commercially available. TentaGel supports contain about 30% of a 1-2 % cross-linked PS substrate

with long PEG side chains (around 70 %) grafted onto the PS.[78] The PEG chains are immobilized by anionic graft copolymerization of ethylene oxide onto an immobilized alcohol on the support. The preparation and structure of TentaGel resin is shown in Scheme 2.1.



Scheme 2.1 Preparation of Tentagel resin

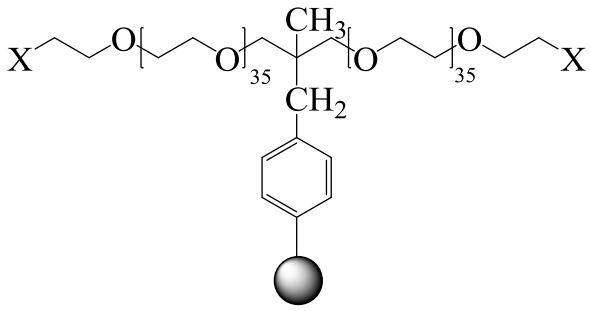
Compared to PS resins, PS-PEG composite supports swell in a wider range of solvents.[51,79,80] The PEG chains and their functional groups are flexible and have mobility so that they are completely solvated.[80] The loading of the TentaGel resins is between 0.15-0.30 mmol/g. The main problem of the TentaGel resins is the low stability in acid condition, when PEG is grafted to PS by using benzylic ether, it can be cleaved and release PEG which will contaminate products.[51,74c,78,81] Second generation PS-PEG composite supports have been developed which are more stable in acid and are available from a number of commercial sources derivatized with a variety of linkers.[46,47,78b] These include the following and are illustrated in Figure 2.4.

Argogel® resins (Argonaut Technologies)[78b,82] use an aliphatic linkage instead of the benzylic ether linkage. These resins are more stable in acid and are available for more choices of functional groups and linkers. The traditional TentaGel PS-PEG resins are prepared by grafting ethylene oxide onto a mono-alcohol immobilized on the PS

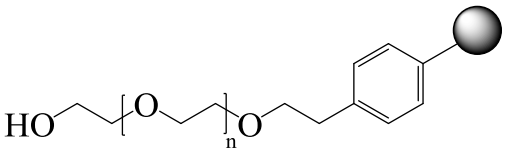
support. Argogel resins used diol to functionalize PS intermediate which results in twice the loading amount (0.4-0.5 mmol/g). The resin beads are 170 μm in average diameter and the size distribution is 120-230 μm.

NovaSyn® TG Resins (Novabiochem)[47] are prepared from low cross-linked hydroxyethyl PS and 3000 to 4000 MW functionalized PEG. The PEG is anchored to the PS backbone through and acid stable ethyl ether. NovaSyn resins are available on 90 μm and 130 μm beads and the loadings are between 0.1-0.40 mmol/g depending on the linker.

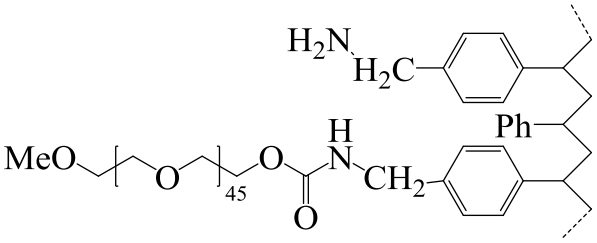
NovaGel™ Resins (Novabiochem) [47] are prepared from special high swelling aminomethyl resin, which has been partially derivatized with methyl-PEG-p-nitro-phenyl carbonate. The urethane linkage between the PEG and the base PS resin is also acid stable which minimizes the loss of PEG during a synthesis. Because the linkers are not attached to the end of the PEG chains, no loss of the substitution will happen even there is a leach of the PEG resins.



(a) ArgoGel resin



(b) NovaSyn resin

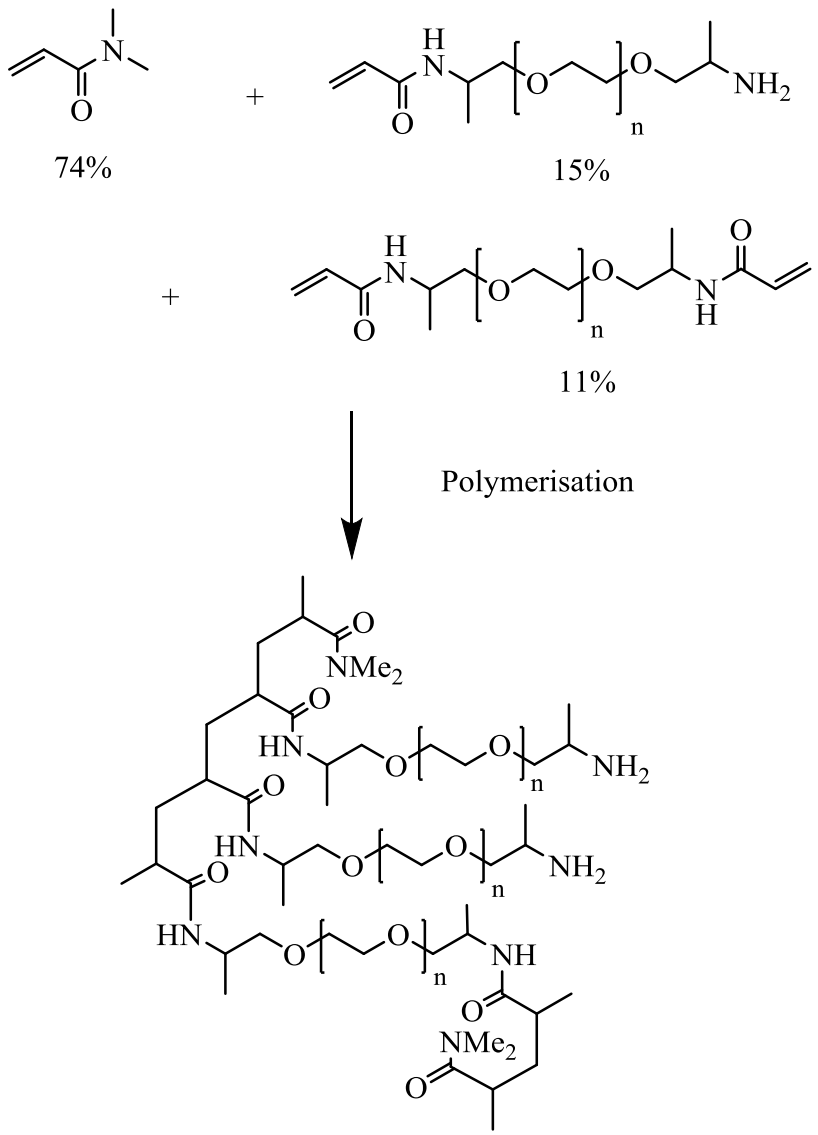


(c) NovaGel resin

Figure 2.4 Structures of the second generation of PS-PEG resins.

c) **PEGA resins**

At first, PEGA (acrylamidopropyl-PEG-*N*,*N*-dimethylacrylamide) resins were developed for solid phase peptide synthesis, but now they are used more frequently in solid phase organic synthesis.[57,83] These resins are prepared from 2-acrylamidopropyl-PEG_{800} and *N*, *N*-dimethylacrylamide cross-linked with *bis*-2-acrylamidopropyl-PEG_{800}. The preparation of PEGA resin using the three monomers is shown in Scheme 2.2.[47,51]



Scheme 2.2 Preparation of PEGA resin.

The advantages and disadvantages of the different solid supports used in solid phase synthesis are summarized in the following tables.

RESIN TYPE	ADVANTAGES	DISADVANTAGES
Micro-porous PS-DVB	Easy to handle	Not compatible with highly polar protic solvents such as water and MeOH.[47,51,74c]
	Commercially available with a broad range of functionalities and linkers.[46,47,84]	Thermal instability over 130 °C.[47,70]
	Chemical stability to a wide range of reaction conditions, for example, acids, bases, reducing agents, and organometallics.[47,70]	Prolonged use and stirring can cause mechanical breakdown of the resin making filtration difficult.[70]
	Can withstand temperatures of -78 °C to 110 °C.[47]	It has been found that during synthesis on PS-DVB resin; impurities were detected arising from the resin itself. Impurities resulted (up to 35% by weight) from trapped solvents or by-products from co-polymerization. Can contaminate products or reduce loadings.[85]
	Swells well in DCM, THF and DMF.[47,78b]	Not suitable for use in packed columns for continuous flow processes as the resin is soft and compressible.[86]
		This resin type can suffer from osmotic shock. This can occur when the resin is placed in a solvent which makes it swell and then introduced into a solvent which makes it shrink causing high levels of stress. This can cause the resin particles to fracture or burst.[86]
		Sizes of beads vary within the same batch.[46,47,78b]

Table 2.1 Advantages and disadvantages of using micro-porous PS-DVB resin.

RESIN TYPE	ADVANTAGES	DISADVANTAGES
Macro-porous PS-DVB	Large interior surface area which allows easy and rapid access of reagents.[71]	Lower reactivity than microporous resins.[71]
	Do not need to swell the resin prior to use.[71]	Lower loading capacity than microporous resins (0.6-1.1 mmol/g).[51,71,78b]
	Compatible with a variety of solvents including alcohols and water.[51,71]	Poor mechanical stability-breaks up easily with the formation of 'fines'.[71] Magnetic stirring is not recommended.[78b]
	Can be used for continuous flow reactions in columns as no volume change and little swelling of the resin occur.[51,71,86]	
	Rapid removal of solvents and reagents and ease of drying.[78b]	
	Resistance to bead cracking (osmotic shock)[78b]	
	Commercially available from Argonaut Technologies under the name of ArgoPore® with a range of attached linkers.[78b]	

Table 2.2 Advantages and disadvantages of using macroporous PS-DVB resin.

RESIN TYPE	ADVANTAGES	DISADVANTAGES
PS-PEG	The swollen PS-PEG chains are more mobile than cross-linked PS and provide a more 'solution-like environment resulting higher reaction rates.[51]	Lower loadings are observed than PS resins: (0.2-0.3 mmol/g).[80] 2nd generation resins have slightly higher loadings (0.4-0.6 mmol/g).[47,78b]
	Compatible and swell in a wide range solvents including water, MeOH, EtOH, DMF, THF.[46,47,78b]	Because of high PEG content, the beads are sticky, adherent and difficult to dry.[51,86]
	Have a narrower size distribution than PS resins: (ArgoGel = 120-250 μm beads) (NovaSyn = available as 90 μm or 130 μm beads.)	Problems of PEG leakage due to: → Acid instability of PS-PEG linkage. Although the 2nd generation resins are much more stable.[47,71,78b] → All types of PS-PEG resins have problems with PEG leakage due to the formation of PEG peroxides by the action of light and oxygen during long term storage.[47]
	Pressure stable so suitable for both continuous flow as well as batch synthesis.[80]	Are more expensive than other commercially available resins.[46,47]
		Suffer from mechanical instability. Stirring or vigorous shaking results in significant loss of material from the resin.[72]

Table 2.3 Advantages and disadvantages of using PS-PEG resin.

RESIN TYPE	ADVANTAGES	DISADVANTAGES
PEGA	Swells in a wide range of solvents including water, DMF, DCM, THF, and methanol.[47]	Limited mechanical stability. Can cause problems with the generation of fines which can cause problems during filtration.[51]
	Has sufficient pressure stability for use in continuous flow synthesis.[78 b]	PEGA resins have a low loading capacity of (0.2-0.4 mmol/g)
	Have a narrow size distribution, 50-100 mesh (300-150 μm).[47]	
	Commercially available from Novabiochem derivatised with the TFA labile 4-hydroxymethyl phenoxyacetic acid linker and the base labile 4-hydroxymethylbenzoic acid linker.[47]	

Table 2.4 Advantages and disadvantages of the use of PEGA resins.

d) Other Solid Supports

Other support materials have been used for solid phase peptide or oligonucleotide synthesis, which include polyacrylamide based resins, silica and cellulose. The properties and limitations of these supports are shown in Table 2.5.

RESIN TYPE	ADVANTAGES	DISADVANTAGES
Pepsyn[51] A poly (dimethylacrylamide resin) prepared from *N*,*N*-dimethylacrylamide, *N*-acrolylsarcosine methyl ester and bisacryloylethylenediamine.	More hydrophilic than PS resins and are compatible with a wide range of solvents.	Mechanically unstable. Cannot be used for continuous flow synthesis.[85]
		Low loading (0.1-0.2 mmol/g).
Pepsyn K[86,87,88] Pepsyn polymerised within the pores of rigid, inorganic kieselghur particles	The rigid, non-compressible framework makes it suitable for continuous flow synthesis.[86,87]	Low levels of substitution (0.1-0.2 mmol/g).
	Porous, framework of kieselghur allows rapid diffusion of reactants in and out of the gel matrix.[87]	Physical stability is poor leading to the generation of fines.[88]
		Escape of the gel during solvation can occur.[88]

PolyHipe[88] Consists of macroporous PS-DVB resin in which a poly (dimethylacrylamide) gel (Pepsyn) has been chemically bound.	PS matrix is highly branched with 90% porosity. Gives good solvent flow properties and accessibility under low pressure.[88]	Has more physical stability than Pepsyn K but still suffers from mechanical instability and breakdown from continuous use.
	Compatible with a wide range of solvents.[88]	
CPG[51] (Controlled pore glass) Composed of silica with large pore sizes (25-300 nm).	Has been used successfully in the synthesis of oligonucleotides.	Low loadings (~0.2 mmol.g).
	Has a more regular particle size and greater stability than Pepsyn K.	High cost.
		Hydrophilic nature of CPG makes water difficult to remove from the support.
Cellulose Used in the form of paper or cotton.[51,89]	Hydroxyl groups can be modified for peptide synthesis.[51]	Chemically less stable than the other supports mentioned.[51]
		Chemical modification of cellulose can increase the solubility or change the mechanical properties. This can lead to deterioration and partial loss of the support.[51]

Table 2.5 A selection of some less common supports used in solid phase synthesis.

Although these solid supports mentioned above have been used successfully in solid phase synthesis, they still have some common disadvantages, such as the poor mechanically or chemical stability and the high price. So, there is a need for new types of solid supports which will avoid these problems. The ideal solid support for solid phase synthesis should have the following properties:

1. Good Mechanical Stability

A physical stable support will ensure there is no breakdown of the support during synthesis. This will aid filtration and washing of the support and will improve efficiency and aid synthesis.

2. Chemical Stability

The solid support should be able to withstand a wide range of reagents and reaction conditions.

3. Easily Functionalized

The solid support should be easily derivatized with a wide range of functionalities and linkers. The loading of these groups should be high enough for the synthesis. A good working loading is around 1 mmol/g.[78c]

4. Economical to use

Solid supports which are inexpensive to prepare and use would improve the cost especially when carrying out large scale reactions.

2.2.2.2 Types of the linkers

The choice of linker is important in the solid phase organic reactions. A good linker should have properties as follow:

1. The linker to the solid support should be chemically stable to all conditions during the synthesis and cleavage process.[48,51,78c,90]

2. The linker to the compound should allow reversible release of the compound under carefully controlled and selected conditions without damage to the final product.[46,78c,91]

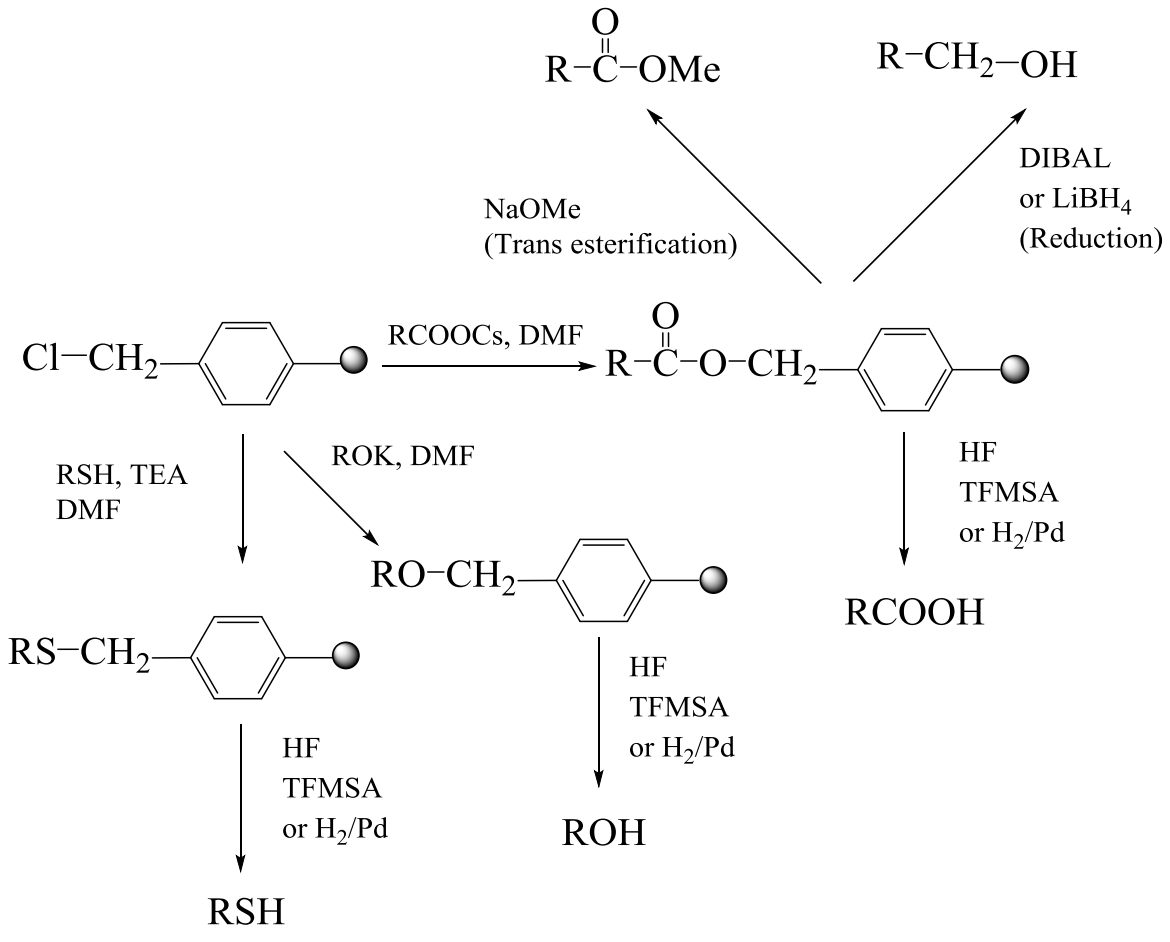
3. The linker should allow easy and quantitative attachment of the starting materal.[48,51,91,92]

4. The linker should allow quantitative cleavage of the product.[90]

Most of the linkers designed were used originally for the synthesis of peptides and were bound to the support by an amide or ester bond. However, cleavage of these molecules usually gave products which terminated with either carboxylic acid or amide functionalities.[50,68] To prepare a wider range of organic molecules, different linkers and cleavage methods have been introduced which allow release of a broad range of other functionalities, like hydroxyls, phenols, amines, aldehydes and alkenes.[48,91,93] Over the past 25 years, the range of linkers developed for solid phase synthesis is more widespread and it has been reported that more than 200 linkers have been evolved.[48] The most popular types of cleavage strategies involve the use of acidic or basic conditions.[46-48,91,94] Photocleavable and traceless linkers and linkers which induce cyclative cleavage are also available.[48,91,94] As a whole range of functional groups can

be liberated from the same linker using different reagents, the terminal variation of the Linkers become possible.[46,47,91]

One common example, Merrifield resin was developed for the solid phase synthesis of peptides.[59] Functional groups such as carboxylic acids, phenols, alcohols and thiols can be modified, and cleavage of these groups can be carried out using various reagents,[46,47,95,96] Scheme 2.3.



Scheme 2.3 Attachment and cleavage methods using Merrifield resin.[46,47,95,96]

2.3 Solid Supported Reagents, Scavengers and Catalysts.

2.3.1 Limitations of Solid Phase Organic Synthesis

In spite of the obvious advantages of solid supported organic synthesis, there are some limitations by using this method:

a) The speed of the reactions can be slow on the solid phase compared with the same reaction in solution.[54]

b) It is difficult to monitor reaction progress during SPOS. Monitoring techniques like TLC and NMR cannot be used, because of the reagent may degradated during the reaction and the NMR range for the solid support is always broad.[54] Although there are a number of methods available for monitoring solid phase reactions, such as colorimetric assays,[93] FTIR,[97] elemental analysis[98] and Gelphase NMR,[99] it has been reported that they do not provide the same quality of analysis as the traditional monitoring techniques.[54] Problems like time consuming and less sensitive also occurred in most of the monitoring methods used.

c) Additional steps for attaching and removing the materials from the solid support are required.

d) The solid support and linker type limits the possible chemistry which can be performed.[68]

e) The optimization involved from transferring the reaction from solution to solid phase can be tedious.[54]

Solution phase synthesis avoids these disadvantages, but a major drawback is the difficulty in purifying the final products. However, the development of polymer bound reagents and scavengers can overcome this problem. The use of insoluble polymers and other solid supported reagents to remove by-products and excess reactants have been used for purification of the product in solution.[54]

2.3.2 The use of solid support reagents and scavengers in organic synthesis

Solid support reagents and scavengers have been used in organic synthesis from 1946,[100] but in the early time they were not popular because of their high cost and difficulty in recycle, and the extreme long reaction time.

However, in 2000s, many new and several improved solid-supported reagents have been developed, due to the need to generate large numbers of new compounds in a cleaner, faster and more efficient manner, and an ever increasing number of which are becoming commercially available.[54]

A key advantage of using solid support reagents is that the reaction can be driven to competition by using excess reagents; the product can be obtained by a simple filtration. Another attractive aspect is that all the harmful reagents and their by-products can be can be handled safely with improved acceptability and utility once immobilized onto the solid support. More than one reagent can be used at the same time, and even species that are incompatible in solution may be used together to achieve one-pot transformations.

Furthermore, scavengers can isolate pure products easily if the reactions proceed poorly or generate by-products and impurities, and reactions can be optimized and scaled up readily and can be constantly monitored using conventional methods such as TLC, LC-MS, GC-MS, SFC-MS, NMR etc. Also, because the chemistry is carried out in solution, they often require only minimal optimization when compared to those involving the transfer of a solution phase reaction onto a polymer bound substrate.[54]

Usually to prepare of biologically active and many other functional materials from small, commercially available building blocks, more than one synthetic step is required. For example, at least ten transformations steps are required for the synthesis of most modern drugs, and even twenty for more complex molecules. Although there are a large number of simple starting materials currently available for synthesis, chemists prefer using much more complex molecules as these lead more rapidly to drug-like molecules. A practical solution for the preparation of large chemical libraries is to use solid supported reagents in a designed, sequential, and multi-step fashion.[54]

2.3.3 Examples of Some Commercially Available Immobilized Scavengers and Reagents.

2.3.3.1 Scavenger Resins

a) Tris(2-aminoethyl)amine Resin 1

This nucleophilic resin (See Figure 2.5) has been used as a scavenger to remove acid chlorides, sulfonyl chlorides, isocyanates, isothiocyanates and acids.[47,58,78b] The quenching reaction is normally carried out at room temperature in polar solvents such as DCM, DMF and THF with the reaction complete between 0.5-4 hours.[47] 1.5-3 equivalents of the resin relative to the electrophile to be quenched are added. With acid and sulfonyl chlorides 3-6 equivalents are added to neutralize the HCl generated unless an additional base is used.[47,78b]

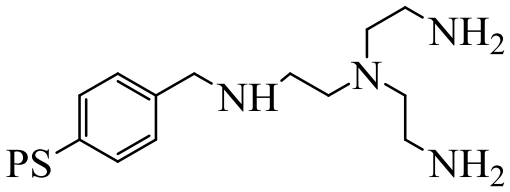
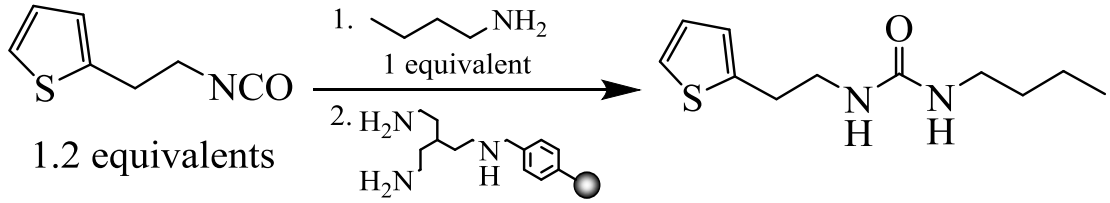


Figure 2.5 Tris(2-aminoethyl)amine scavenger resin.

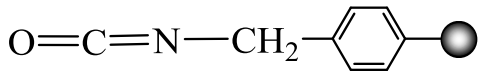
An example of a reaction using the scavenger resin **1** is the reaction between an isocyanate and an amine shown in Scheme 2.4.[58] Excess of the isocyanate is reacted with the amine, then the scavenger **1** is added which reacts and quenches the excess isocyanate, then filtration and evaporation of the solvent then gives the product. TLC and ^{1}H NMR are used to monitor the reaction and the quenching procedure, showing good purity of the product.



Scheme 2.4 Synthesis using tris(2-aminoethyl) scavenger resin.

b) Methyl isocyanate resin 2

This electrophilic scavenger (see Figure 2.6) removes nucleophiles such as amines and alkoxides without releasing any by-products.[47,58] The scavenger **2 (**1.5-3 equivalents**)** is added relative to the nucleophile to be removed. The reaction with primary and secondary amine occurs rapidly at room temperature over 1-3 hours. Alcohols are less reactive under these conditions; therefore the amine can be selectively removed in the presence of hydroxyl containing compounds.[47] Aromatic amines are even less reactive but can be removed at 60 °C over 18 hours.



Methyl isocyanate resin

Commercial source	Base Resin	Loading (mmol/g)	Mesh size
Novabiochem	PS-DVB (2%)	1.0-1.3	200-400
Argonaut	PS-DVB (1%)	1.0-1.7	100-200

Figure 2.6 Methyl isocyanate scavenger resins.

2.3.3.2 Immobilized Reagents

a) Morpholinomethyl polystyrene 3

This polymer bound tertiary amine (see Figure 2.7) is useful for a variety of reactions which involves the use of a tertiary base.[47,58]

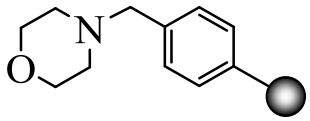
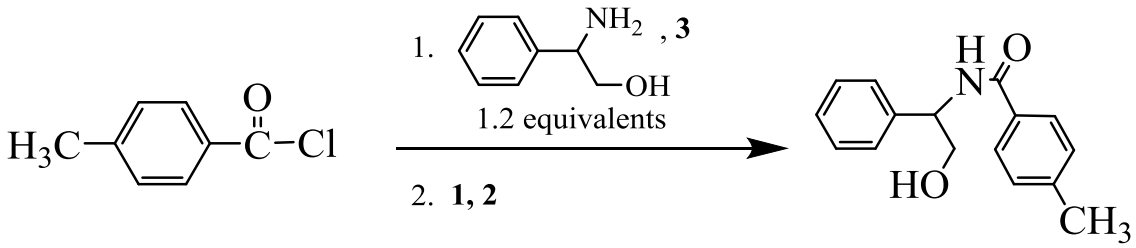


Figure 2.7 Morpholinomethyl polystyrene.

The need to separate the amine salts at the end is eliminated.[47] This reagent can be used in conjunction with the tri-amine scavenger and the isocyanate resin for the preparation of amides.[58] This reaction is exemplified in Scheme 2.5 where an 81 % yield was obtained.[58] Novabiochem supply this resin with a loading of 3.2-3.8 mmol/g with the base resin consisting of PS with 2 % DVB cross-linking with a size of 200-400 mesh.[47]



Scheme 2.5 Amide synthesis using polymer supported reagents and scavengers.

b) Polystyrene immobilised DMAP 4

This acylation catalyst (See Figure 2.8) has been immobilized onto 4% crosslinked (DVB) polystyrene resin and has been used to accelerate the etherification of tertiary alcohols.[78b] Usually 0.1 equivalents of the immobilized catalyst is added to the reaction

mixture overnight at 110°C. The resin beads are 30-80 mesh with loadings in the range 1.1-1.8 mmol/g.[78b]

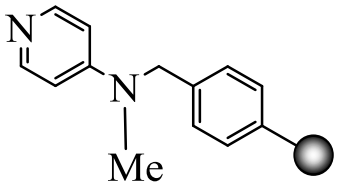


Figure 2.8 Polymer bound DMAP.

2.3.4 Properties of an ideal Solid Support for the Immobilization of Reagents and Scavengers

The solid supports used to immobilize scavengers and reagents still have their limitations. Take microporous gel-type DVB cross-linked PS resin, which is common commercially available base resin as an example, the major disadvantage of this resin is that it swells considerably in the solvents used for chemical synthesis. This is a problem when these resins are used for the purification of small scale reactions. Resins which do not swell are considered to be the best. Some non-swelling resins such as macro-porous resin and silica have been developed and used but suffer from mechanical stability. (See Chapter 2.2.2.1) It is recommended by the manufacturers that the resins should be agitated gently.[47,78b] This is because conventional stirring techniques such as magnetic stirring would degrade the resins.

The ideal base resin for the immobilization of scavengers and reagents should have the qualities listed below:

1. Non-swellable in common organic solvents.

2. High loading of functional groups.

3. Mechanical and chemical stability.

2.4 Aims and objectives for the application of sporopollenin as a solid supported reagent

The application of sporopollenin as a solid supported reagent was to be investigated. The standard required for sporopollenin to be a suitable solid support is shown as follows:

1. Sporopollenin needs to be conveniently derivatised, either by direct functionalization on an existing functional group on the sporopollenin or by attachment of new anchoring functional group and a linker.

2. The loadings of these functional groups need to be at a level such that they are competitive with the loadings of commercial resins. The functional groups should be stable to the conditions in which they may be required to perform.

3. In spite of previous literature demonstrating that attachments to sporopollenin are feasible, there still remain a number of questions covering the nature and stability of attachment.

Therefore objectives pursued in this thesis to address these issues are outlined as follows:

1. Reacting the surface with a range of reagents and investigate which functional groups are generated.

2. Assess the stability of the attachments with a wide range of conditions that are likely to be used in synthesis.

3. Assess the loadings of these functional groups attached to sporopollenin and verify their reproducibility and consistency.

4. To explore the performance of surface modified sporopollenin exines in some organic reactions.

2.5 Result and Discussion

2.5.1 Preparation of sporopollenin from Lycopodium clavatum spores powder

A number of different methods have been reported in the literature for the preparation of sporopollenin exine capsules from *Lycopodium clavatum* (see chapter 1.2). They all involve the following steps but vary slightly by additional treatments, e.g. reaction with ninhydrin and sulphuric acid:

a) Spores were treated with hot organic solvents to remove surface coatings and small amounts of fat.

b) A KOH reflux was used to release the cytoplasmic contents. These include proteins, fats and waxes.

c) The intine (cellulose layer) was removed with 85 % H_3PO_4.

d) The breakdown products were removed with solvent and aqueous washes.

In this work, raw spores were first stirred in acetone under reflux to remove their lipidic content, such as tryphine, pollenkitt, and cell membranes. The spores were then treated with hot aqueous potassium hydroxide, which hydrolysed the proteins, nucleic acids or other nitrogenous products. Then 85 % ortho-phosphoric acid was used to remove the polysaccharides and cellulose, which largely make up the intine. The phosphoric acid may form a mixed phosphoric-carboxylic anhydride with the carboxylic acid functions on the surface of the sporopollenin, which leaves a good leaving group for the subsequent amination process. Lactones may also be formed under these conditions.

After these procedures, the sporopollenin was dried under a vacuum to a constant weight.

The extraction procedure was repeated four times over the course of this project and the result of elemental analyses is shown in Table 2.6:

Sample No.	Elemental Analysis			
	C%	H%	O%	N%
SECs-1	60.71	7.56	31.73	0.00
SECs-2	65.00	8.26	26.74	0.00
SECs-3	62.36	9.63	28.01	0.00
SECs-4	57.06	8.91	34.03	0.00
Note: The date in the O% column were not detected by the equipment, the total value of C%, H% and O% shouled be 100, so the value of O% can be calculated after the other two values are confirmed.				

Table 2.6 Result of elemental analysis of extracted sporopollenin.

The results showed that there were some differences in the contents of carbon, hydrogen and oxygen in the four batches, but there was no nitrogen in the product (within limits of detection). This was in line with previous work in the group and emphasized the need to carefully record the origin of each extracted sample and its subsequent derivatisations.

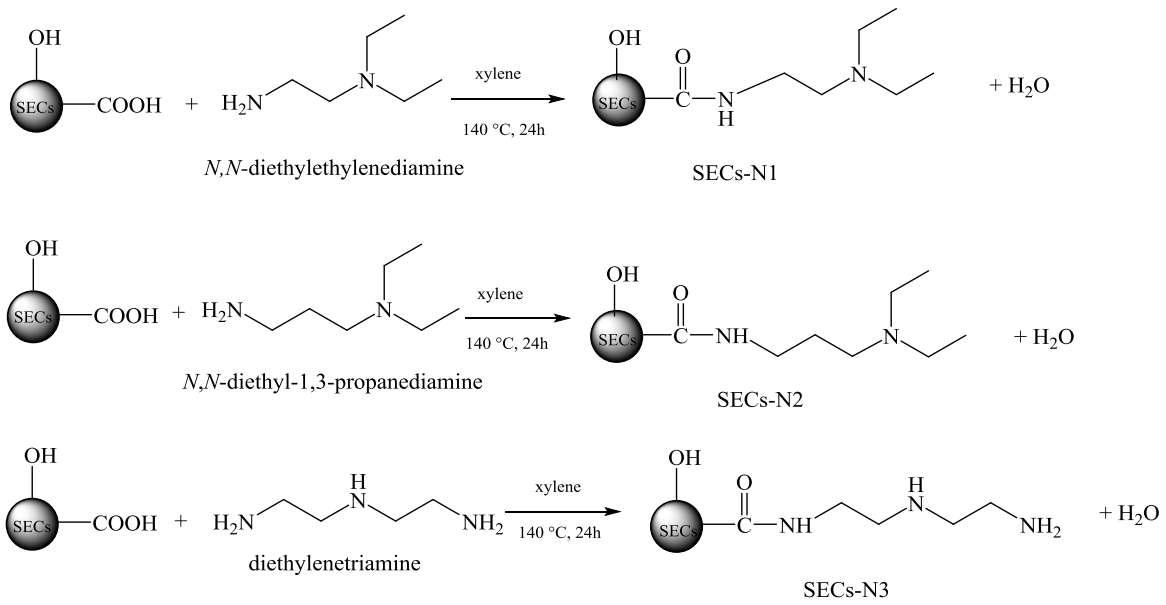
2.5.2 Amination of the sporopollenin and analysis

2.5.2.1 Amination

Amination of sporopollenin has been carried out by previous workers[101] with attachment of 1,3-diaminopropane achieving a loading of 1.6 mmol of base/g, as determined using the picric acid assay. However, the results from the picric acid assay and elemental analysis of previous work varied considerably,[101] the explanation was because of the $–NH_2$ groups were not fully reacted with the picric acid due to steric hindrance or that cross-linking of the diamine was occurring. (In this work, chloride analysis was used, explain in Chapter 2.5.2.2)

Diamine attached linkers could be useful for solid phase synthesis or drug delivery, however a tertiary amine would be a more attractive scavenger or organic base catalyst in organic synthesis.

Thus amination of sporopollenin using an α,ω-diamine was investigated further as a method of attaching a basic functional group to the surface of the material. The diamines first tried in this study were *N*,*N*-diethylethylenediamine, *N*,*N*-diethyl-1, 3-propanediamine, and diethylenetriamine. (See Scheme 2.6)



Scheme 2.6 Amination of sporopollenin with α,ω-diamine.

The sporopollenin was treated with each of the amines in xylene under reflux for 24 h using a Dean-Stark trap. After cooling, the product was washed successively with same amount hydrochloric acid and sodium hydroxide, and then washed with distilled water (until the filtrate was neutral and uncolored), methanol then dichloromethane. The product was dried in a desiccator to a constant weight (see Table 2.7)

Sample No.	amine	Yield%*	Elemental Analysis			
			C%	H%	O%	N%
SECs-N1	*N, N*-diethyl-ethylenediamine	93.6	63.26	8.09	25.69	2.96
SECs-N2	*N,N*-diethyl-1,3-propanediamine	93.9	63.90	8.36	24.12	3.62
SECs-N3	diethylenetriamine	99.9	59.83	8.04	26.25	5.88
Note: * Loss of weight occurs from transfer. The data in the O% column were calculated in the same method as introduced in Table 2.6.						

Table 2.7 Elemental analyses and yields from the amination of sporopollenin.

The elemental analysis showed that the sporopollenin reacted with diethylenetriamine had higher nitrogen content than other amines. This was anticipated because diethylenetriamine has three nitrogen atoms compared to two for the other amines.

2.5.2.2 Gravimetric analysis of hydrochloride salts

The nitrogen atoms in the sporopollenin derivative which are not linked to the carboxyl group should retain their basicity and so were referred to as "basic nitrogens" (See Figure 2.9), and the tertiary amino groups are intended to play the key role in the use of these materials as solid phase reagents in organic synthesis.

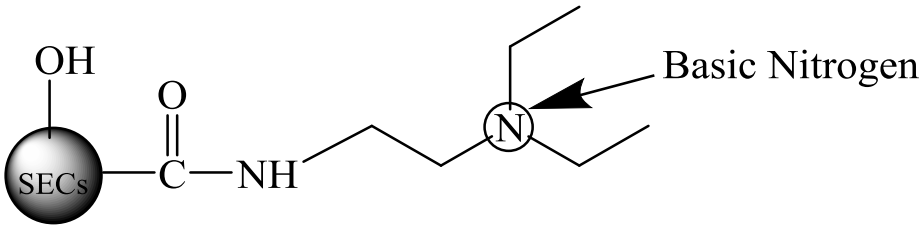
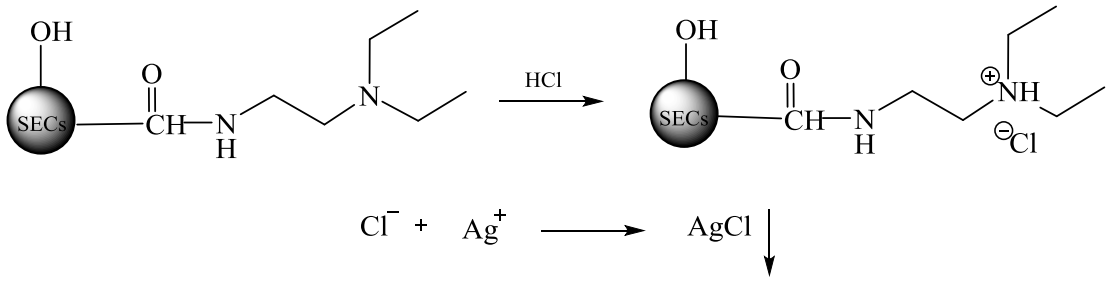


Figure 2.9 Basic nitrogen.

As it shown in the figure above, the amount of "N^{basic}" should be half of "N^{total}", however, some nitrogen might attached to the surface of sporopollenin in some other form, or be inaccessible to protonation – for example by formation of internal salt bridges. Therefore gravimetric analysis of the hydrochloride salts was used as an experimental method to confirm whether the calculated value of basic nitrogen of the aminated sporopollenin was in accordance with the result from elemental analysis.

Derivatised sporopollenin was suspended in excess hydrochloric acid overnight (2 M, 10 cm^3 for 0.1 g sporopollenin), then filtered, washed thoroughly with deionised water, then filtered again. Next the oven dried solid particles (at 60°C, 3 h) were suspended with stirring in aqueous NaOH (2 M, 10 cm^3) with heating for 2 h. The filtrate was carefully collected and acidified with nitric acid (6 M, 5 cm^3), then treated with silver nitrate solution (0.1 M, 5 cm^3). The mixture in the flask was covered with tinfoil to keep the precipitate away from light immediately to avoid decomposition and set aside overnight. The next day, silver chloride was coagulated by heating at 100°C with constant stirring, after cooling down for 1h; the precipitate was collected by washing with dilute nitric acid (6 M, 10 cm^3 × 3) and filtered through an oven-dry, pre-weighed Gooch funnel. The funnel with precipitate was dried in a 120°C oven for 3 h. Then the mass of precipitate was determined and converted into moles. The amount of silver chloride was compared to the calculated amount of N^{tot} and N^{basic} from elemental analysis. The mechanism of chloride analysis is shown in Scheme 2.7



Scheme 2.7 Mechanism of Gravimetric analysis of hydrochloride salts.

The analyses for sporopollenin aminated with *N*, *N*-diethylenediamine (SECs-N1), *N*, *N*-diethyl-1, 3-propanediamine (SECs-N2) and diethylenetriamine (SECs-N3) were carried out three times, and the results are shown in Table 2.8:

Sample No.	Elemental Analysis (N %)	N^{tot} (mmol/g)	N^{basic} calculated (mmol/g)	chloride (mmol/g)		
				1	2	3
SECs-N1	2.96	2.11	1.06	1.11	1.11	1.09
SECs-N2	3.62	2.58	1.29	1.37	1.60	1.48
SECs-N3	5.88	4.20	1.40 - 2.80	2.34	2.79	2.31

Table 2.8 Result of gravimetric analysis of hydrochloride salts

The conversion of N% to N^{tot} is given by:

N^{tot} (mmol/g) $=$ 1 g × N % ÷ 14 (g/mol) ×1000, and N^{basic} (mmol/g) = N^{tot} (mmol/g)/2.

For sample SECs-N3, we were not sure whether one or both of the primary amino groups would be attached to the surface of the sporopollenin, so the amount of N^{basic} was expected to be the range from 1.40 mmol/g (4.20 × 1 / 3) (cross linked) to 2.80 mmol/g (4.20 × 2 / 3) (mono attachment). The result of gravimetric analysis indicated that the number is close to 2.80 mmol, which indicates that only one amino group was attached to the surface of the sporopollenin.

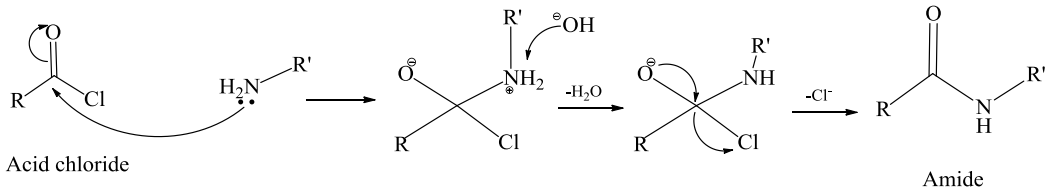
At the beginning of the gravimetric analysis, the treatment with sodium hydroxide and the collection of silver chloride was performed at room temperature. However the yield of silver chloride was quite poor as the fine precipitate was difficult to collect by filtration. The temperature was increased to 100 °C for the sporopollenin derivative to react with sodium hydroxide to be taken to completion. In addition, to assist in the

coagulation of the silver chloride, the reaction was heated to boiling. This modification gave results that were in better agreement with the expected loading of basic nitrogen, but there was still not a perfect match with this value. However, it must be taken into account that it was difficult to avoid losses on such small scale reactions using several transfer steps.

2.5.3 Application of aminated sporopollenin in organic synthesis

2.5.3.1 Schotten-Baumann type acylation

The Schotten-Baumann reaction is an organic reaction used to convert an acyl halide or anhydride to an amide if reacted with an amine and base, or an ester if reacted with an alcohol and base. The reaction with the amine begins with the nitrogen attacking the carbonyl carbon of the acyl halide which then eliminates the halide from a tetrahedral intermediate. Deprotonation with the base then provides the final amide product. The reaction with the alcohol would happen in a similar fashion.[102,103]

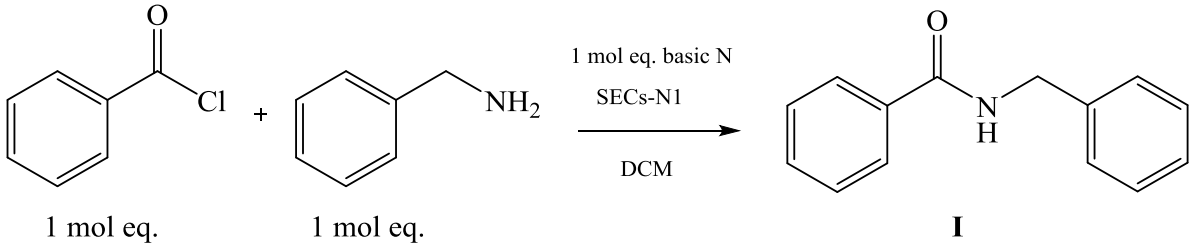


Scheme 2.8 Mechanism of Schotten-Baumann reaction.

From the mechanism (Scheme 2.8), we can see that an equivalent amount of hydrochloric acid is produced as a by-product, which will create a salt with the unreacted amine and therefore reduce the yield to a maximum of 50%. Sporopollenin loaded with a tertiary amine has the ability to remove this acidic by-product from the amide forming reaction and thus allow the reaction reach completion. Unlike using a soluble amine base the work up for the reaction would be reduced to a simple filtration and evaporation of the solvent. Also, the sporopollenin were reusable after a sequence of basification, washing with dichloromethane and then drying in a dessicator.

Initial attempt

In this study, we investigated the reaction of benzoyl chloride and benzylamine with sporopollenin aminated with *N*,*N*-diethylethylenediamine (SECs-N1, N_{basic} loading=1.15 mmol/g) as supported scavenger (see Scheme 2.9). This choice was made since the reactant and reagents could be readily followed by gas chromatography–mass spectrometry (GC-MS), hence giving a convenient means to assess the potential of the derivatised sporopollenin in the role of a stable and solid supported base.



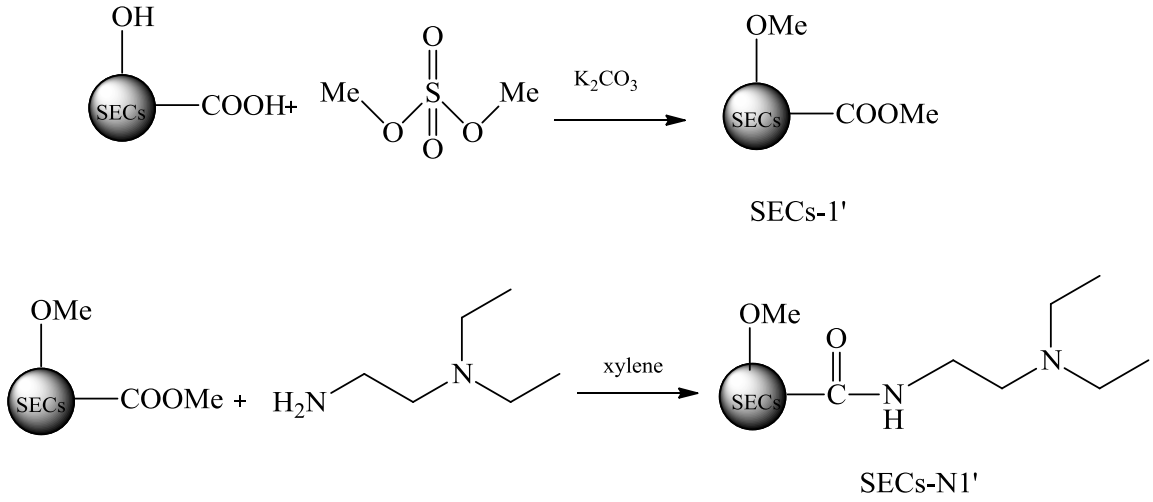
Scheme 2.9 Amide formation with aminated sporopollenin as a support scavenger.

Equivalent benzoyl chloride, benzylamine and aminated sporopollenin was stirred in a flask with DCM at room temperature for 3 hours then reaction was stopped (tested by TLC), and the sporopollenin was removed by washing with dichloromethane and filtered. The residue was examined by Gas Chromatography–Mass Spectrometry (GC-MS), showing that the product **I** had a high purity.

The reaction was repeated three times using the same recycled sporopollenin sample for reactions two and three. The sporopollenin was recycled by washing with aqueous NaOH (2 M, 30 cm^3) and then water until the filtrate was neutral, then dried under vacuum to a constant weight. However, the yield of **I**, was quite low (about 50%).

“Capping” sporopollenin before amination

The reason why the yield was so low was assumed to be because the benzoyl chloride had reacted with the free hydroxyl groups on the surface of sporopollenin (as indicated an increase in the percentage of C as shown by elemental analysis), therefore, we thought to ‘cap’ the free hydroxyl groups of original extracted sporopollenin with methyl groups by reacting the sporopollenin with dimethyl sulfate, and the weight of methylated sporopollenin increased 0.1 g for 3 g of the original sporopollenin. Then the methylated sporopollenin would be aminated with the diamine (*N*,*N*-diethylethylenediamine) (see Scheme 2.10) to produced a ‘capped’ solid base.



Scheme 2.10 Methylation of the sporopollenin.

The result of elemental analysis methylated sporopollenin is shown in Table 2.9:

Sample No.	Elemental Analysis			(Ratio)N/C	Basic N
	C%	H%	N%		mmol/g
SECs-1	60.71	7.56	0.00	/	/
SECs-1′	67.29	7.98	0.00	/	/
SECs-N1’	66.40	6.80	3.57	0.054	1.27
SECs-2	65.00	8.26	0.00	/	/
SECs-2′	66.96	8.13	0.13	/	/
SECs-N2’	67.16	7.69	4.14	0.062	1.48
SECs-N1	63.26	8.09	2.96	0.048	1.06

Note: Samples SECs-1 and SECs-2 (Table 2.6), after methylation with dimethyl sulfate are designated SECs-1′, SECs-2′. After amination with *N*, *N*-diethylethylenediamine the capped samples are referred to as SECs-N1’ and SECs-N2’. Sample SECs-N1 (Table 2.7) is ‘uncapped’ sporopollenin reacted directly with *N*, *N*-diethylethylenediamine.

Table 2.9 Elemental analysis of methyl–capped sporopollenin.

It was found that after the reaction with dimethyl sulfate, the percentage of carbon of the new sporopollenin (SECs-1′, SECs-2′) was higher than the non-methylated form (SECs-1, SECs-2), which was expected on the basis that the hydroxyl and carboxyl were capped with methyl groups thus increasing the proportion of carbon in the polymer. Compared to SECs-N1, the uncapped aminated sporopollenin, the capped aminated sporopollenin (SECs-N1’, SECs-N2’) had a higher ratio of N/C, which implied that there was a higher loading of basic nitrogen on the surface of the sporopollenin. Because of the higher loading, the sporopollenin used in this study were all methylated before amination, however this caused unexpected problems in the surfactant work (see Chapter 3).

Attempt with methylated sporopollenin in Schotten-Baumann acylation

The acylation of benzylamine with benzoyl chloride was attempted again but using the capped sporopollenin (SECs-N1', SECs-N2') and again the reaction was repeated. The results are shown in Table 2.10:

Supported base	Yield of **I***		
SECs-N1 not capped	57%	46%	47%
SECs-N1' capped	97%	90%	77%
SECs-N2' capped	93%	83%	/
Note:*In this thesis, all the yields of products were calculated by multiple the mass recovery of product and the purity of the product by GC-MS.			

Table 2.10 Acylation of benzylamine with benzoyl chloride using different sporopollenins loaded with *N*,*N*-diethylethylenediamine.

It was found when using methylated sporopollenin the yield of **I** was much higher compared to previous experiments, supporting the idea that reactive surface hydroxyl groups accounted for the lower yields obtained earlier using the non-methylated aminosporopollenin (SECs-N1). In addition, it was noticed that the yield of product a decreased slightly each time the sporopollenin (SECs-N1', SECs-N2') was re-used, however, they were still higher by at least 20% than in the original experiments.

2.5.3.2 Aminated sporopollenin in Knoevenagel Condensation

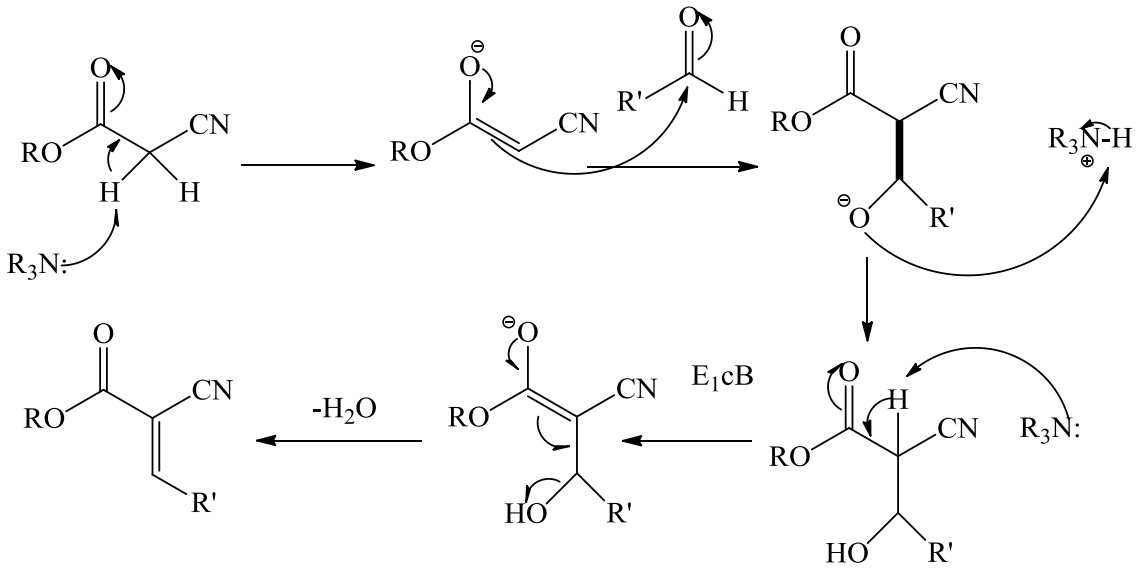
The Knoevenagel condensation of aldehydes with active methylene compounds is an important synthesis of olefins.[104] As a C=C bond forming reaction, the Knoevenagel condensation has found widespread application in the synthesis of polymerizable monomers with advantageous optical and electrical properties, natural products and pharmaceutically important molecules.[105] A large number of catalysts are used for this reaction, from conventional catalysts such as piperidine to ionic liquids and carbon nanotubes containing nitrogen.[106] A number of examples of supported catalysts used for Knoevenagel condensation can also be found in the literature, but many of them exhibit several drawbacks.[104] Some are time consuming for the reaction, some contain metals, and some need special laboratory techniques and inert atmospheres, and some need to use halogenated or aromatic solvents.[107]

Krishnan and Sreekumar used polystyrene-supported poly (amidoamine) (PAMAM) dendrimers as highly efficient and environmental friendly base catalysts for Knoevenagel condensation and proved that these PAMAM dendrimers are a very active and reusable basic organocatalyst capable of promoting Knoevenagel condensations in an environmentally friendly way with high efficiency.[107]

Wiles *et al* successfully demonstrated a series of silica-supported bases within an EOF-based micro-fabricated device, enabling the synthesis and characterization of eight condensation products.[104]

Macquarrie and Jackson prepared aminopropyl-functionalised Mobil Crystalline Materials (MCMs) *via* a one-pot method and used it as a novel effective base catalyst for the Knoevenagel reaction, with significant improvements in terms of turnover number and solvent dependence to the ostensibly similar aminopropylsilica.[108,109] Kaupp *et al* reported that Knoevenagel condensations could be promoted successfully in the solid state or solvent-, catalyst-, and support-free in the stoichiometric melt. The reactions are waste-free, and no microwave irradiation is required.[110]

In Kaupp and Wiles's study[105,111], only aldehydes were used in the reaction, Krishnan and Macquarrie[109] also investigated the behavior of ketones, the results showed that aldehydes are more reactive than ketones in Knoevenagel condensation with the catalysts.



Scheme 2.11 Mechanism of Knoevenagel condensation.

As the mechanism (Scheme 2.11) shows, tertiary amine may be used as the catalyst for Knoevenagel condensation. The aim in our study is to investigate the capability of sporopollenin aminated with *N*, *N*-diethyl-1, 3-propanediamine (SECs-N2, N^{basic} =1.29 mmol/g) in Knoevenagel condensation between aldehyde and ketone under different reaction conditions.

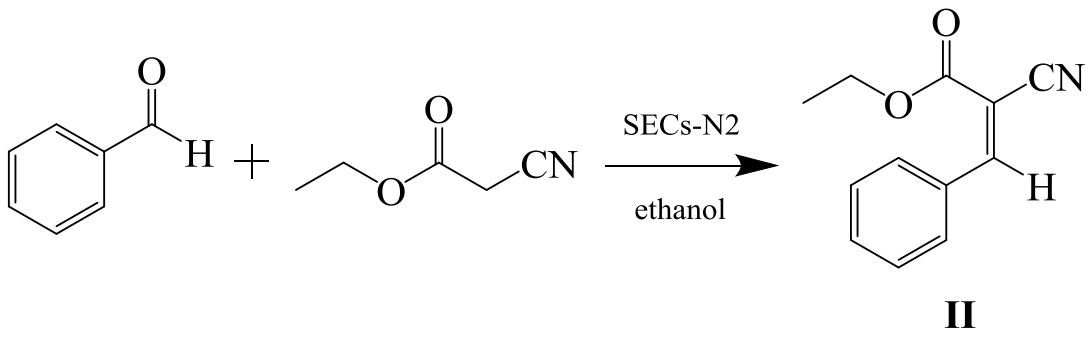
Initial attempt

Initially, equimolar amounts of benzaldehyde and either diethyl malonate or ethyl cyanoacetate were reacted in the presence of sporopollenin (SECs-N2) in ethanol. After simple filtration and evaporation the product was analyzed using ^{1}HNMR to detect whether the reaction had taken place. The reactions were carried out in different conditions, Table 2.11.

entry	Carbonyl compound	Active methylene compound	Amount of N^{basic}, X%	T/°C	Time/h	Yield%*
1	benzaldehyde	diethyl malonate	1	25	2	0
2	benzaldehyde	diethyl malonate	5	25	3	0
3	benzaldehyde	diethyl malonate	10	25	5	0
4	benzaldehyde	ethyl cyanoacetate	1	25	3	90
5	benzaldehyde	ethyl cyanoacetate	5	25	2	93
6	benzaldehyde	ethyl cyanoacetate	10	25	5	96
Note: Aminated sporopollenin SECs-N2 was used in these reactions. The 4th column, X%, represents the amount of basic nitrogen present with the loading N^{basic} = 1.29 mmol/g used to calculate the mass of exines.						

Table 2.11 Reaction between benzaldehyde and diethyl malonate/ethyl cyanoacetate catalyzed by aminated sporopollenin (SECs-N2) in different conditions.

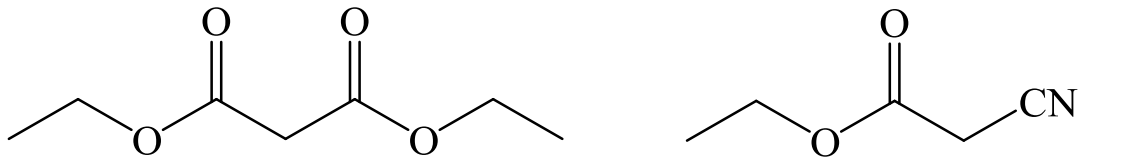
From the spectrum of ^{1}H-NMR, the methylene group was still observed after the reaction of benzaldehyde and diethyl malonate, which showed that the condensation had not occurred. Even when more base catalyst was used and the reaction time increased, still no reaction took place. For the reaction of benzaldehyde and ethyl cyanoacetate (see Scheme 2.12), the spectrum of ^{1}H-NMR showed that the product **II** was obtained, even with less catalyst and shorter reaction time compared to that investigated for diethyl malonate.



II

Scheme 2.12 Reaction between benzaldehyde and ethyl cyanoacetate catalyzed by aminated sporopollnein (SECs-N2).

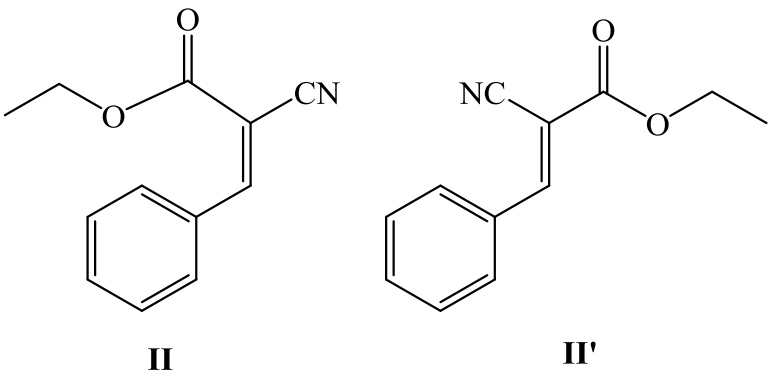
The p*K*a of the acidic hydrogen in both species are different (Figure 2.10). The ethyl cyanoacetate, which methylene group is more acidic, make a better Knoevenagel donor than diethyl malonate.



Diethyl malonate pK_a=13.0 Ethyl cyanoacetate pK_a=9.0

Figure 2.10 pK_a of carbonyl compounds investigated (in water).

When the reaction was carried out between benzaldehyde and ethyl cyanoacetate under reflux, a more complex ^{1}H NMR spectrum with different phenyl hydrogen signals indicates that isomers of the product were obtained (Figure 2.11),



II **II'**

Figure 2.11 Isomers of the product arising from reaction between benzaldehyde and ethyl cyanoacetate catalyzed by aminated sporopollenin (SECs-N2).

To show that it was the aminated sporopollenin that was catalysing the condensation, rather than the sporopollenin itself, the reaction was repeated with unmodified sporopollenin pre-washed with either sodium hydroxide to generate it in a basic form. In the reaction of benzaldehyde and ethyl cyanoacetate it was found that the reactions still proceeded but very much more slowly, about 60% product was obtained after reaction for 24 h.

Scope of reaction

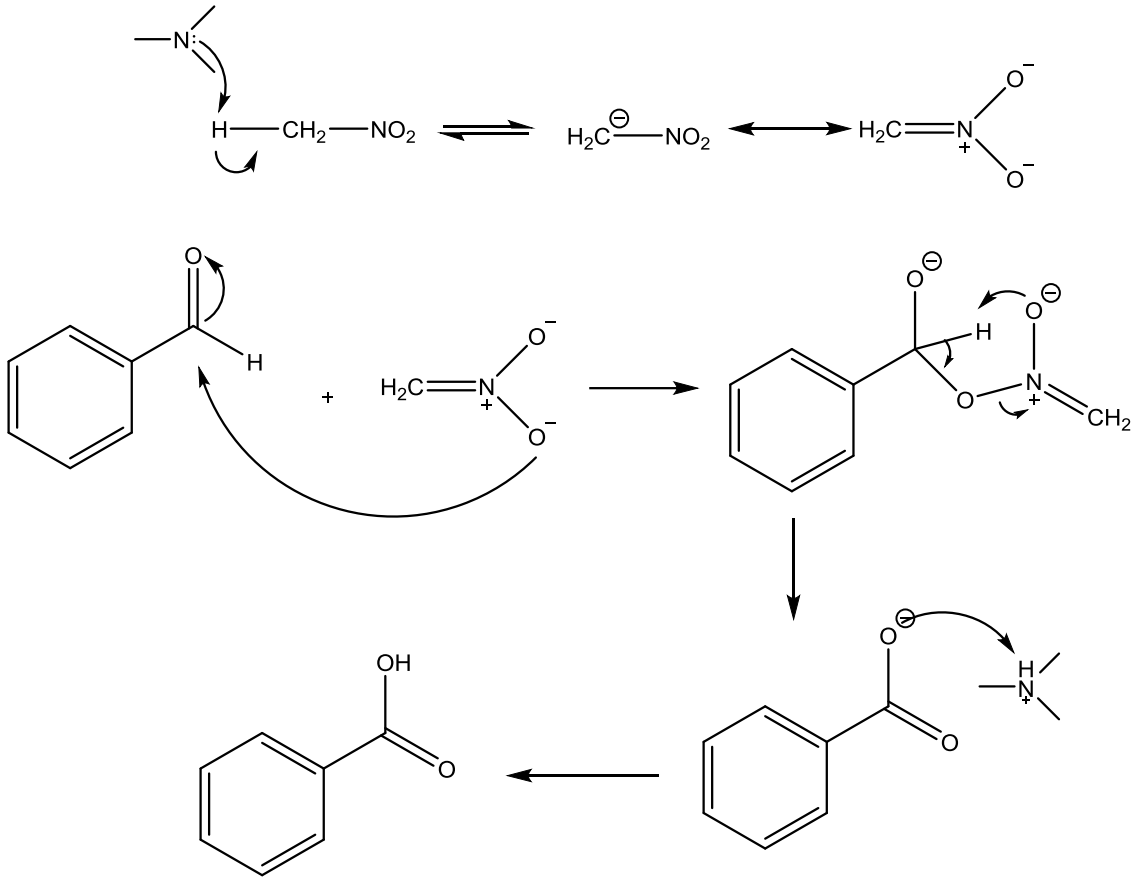
After the initial attempt, the reaction of more carbonyl compounds and active methylene compounds were explored with the aminated sporopollenin (SECs-N2) under variety different conditions. (See Table 2.12)

entry	Carbonyl compound	Active methylene compound	Amount of N^{basic} , X%	T/°C	Time/h	Yield%
1	benzaldehyde	diethyl malonate	1	80	1	0
2	benzaldehyde	diethyl malonate	1	80	3	0
3	benzaldehyde	diethyl malonate	5	80	3	0
4	cyclohexanone	diethyl malonate	1	80	1	0
5	Isatin	diethyl malonate	1	80	1	0
6	Isatin	ethyl cyanoacetate	10	80	5	0
7	Acetophenone	diethyl malonate	1	80	1	0
8	Acetophenone	ethyl cyanoacetate	10	80	5	0
9	benzophenone	diethyl malonate	1	80	1	0
10	benzophenone	ethyl cyanoacetate	10	80	5	0
11	benzophenone	di-isopropyl malonate	10	80	18	0
12	benzaldehyde	1,3-cyclohexanedione	5	80	1	0
13	benzaldehyde	1,3-cyclohexanedione	10	80	3	0
14	cyclohexanone	ethyl cyanoacetate	10	80	5	86
15	benzaldehyde	Acetylacetone	10	80	5	0
16	benzaldehyde	Ethyl acetylacetate	10	80	5	0
17	benzaldehyde	Nitromethane	10	80	5	60

Table 2.12 Further attempts of Knoevenagel condensation catalyzed by aminated sporopollenin (SECs-N2).

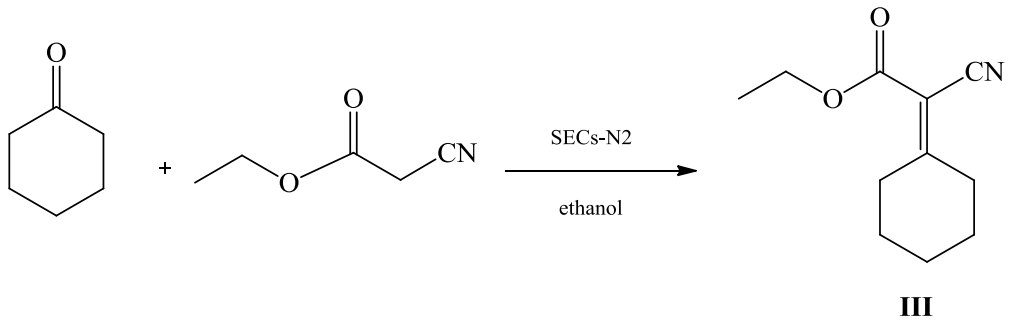
From the ^{1}H-NMR spectra of the isolated material, it was observed that for most reactions no product was found, even if the reaction time and temperature were increased and more catalyst was added. The reason for this is most probably due the fact that the electron-withdrawing groups connected to the carbonyl carbon will make the carbonyl compound less reactive, or the pK_a of the active methylene compounds is too high. Except for acetylacetone (entry 15), which has a similar pK_a compare to ethyl cyanoacetate. The reason might be that the reactivity of the carbonyl groups in acetylacetone is higher than the cyano group and ester group in ethyl cyanoacetate, which makes acetylacetone react with the aminated sporopollenin. An experiment of acetylacetone and sporopollenin was carried out and it was found that the amount of acetylacetone was reduced after the reaction, which supports the assumption.

Surprisingly, for the reaction between benzaldehyde and nitromethane (entry 17), it was found that instead of the Knoevenagel condensation product, benzoic acid was found as the main product at a yield about 60%, which might be caused by a competing Hass-Bender oxidation, see Scheme 2.13.[111]



Scheme 2.13 Reaction between benzaldehyde and nitromethane catalyzed by aminated sporopollenin (SECs-N2).

Only the reaction of benzaldehyde and cylohexanone (see Scheme 2.14) worked well with ethyl cyanoacetate.



III

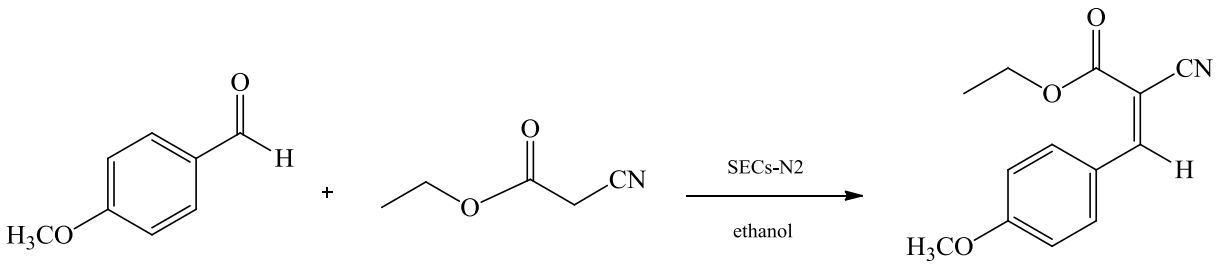
Scheme 2.14 Reaction between cyclohexanone/benzaldehyde and ethyl cyanoacetate catalyzed by aminated sporopollenin (SECs-N2).

The yield of **III** was not very satisfactory. The reactivity of ketone is weaker than an aldehyde; therefore the intial reaction conditions were changed to doing them at room temperature and with an increase in reaction time but this did not improve the yield. Then the amount of cyclohexanone was doubled to keep the balance of the reactant and product, which resulted in an improved yield increased. (See Table 2.13)

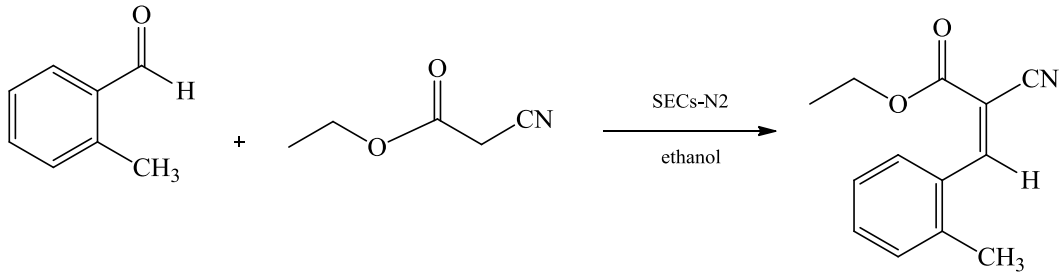
entry	Carbonyl compound	Active methylene compound	Amount of N^{basic} , X%	T/° C	Time/h	Yield%
1	cyclohexanone	ethyl cyanoacetate	10	80	5	86
2	cyclohexanone	ethyl cyanoacetate	10	25	18	88
3	cyclohexanone	ethyl cyanoacetate	10	80	5	99
Note: In entries 1 and 2 the ratio of reactants were both 1:1, but in entry 3, 10 mmol cyclohexanone and 5 mmol ethyl cyanoacetate were used. And the yield increased obviously compared to entry 1 and entry 2.						

Table 2.13 Reaction between cyclohexanone and ethyl cyanoacetate catalyzed by aminated sporopollenin (SECs-N2) under different condtions.

To give a further support for the catalytic effect of the aminated sporopollenin (SECs-N2) in the synthesis, we also investigated the reaction of *p*-anisaldehyde and *o*-tolualdehyde with ethyl cyanoacetate. The analysis of GC-MS showed that the reaction worked well (See Scheme 2.15)



IV

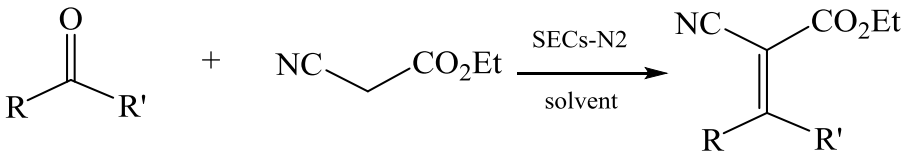


V

Scheme 2.15 Reaction between *p*-anisaldehyde/ *o*-tolualdehyde and ethyl cyanoacetate catalyzed by aminated sporopollenin (SECs-N2).

Optimizing the reaction conditions

In attempt to obtain the best possible yields we investigated a series of reactions under different conditions (Table 2.14).



II, III, IV, V

R	R′	Amount of N^{basic}, X%	solvent	T/°C	t/h	yield
Ph	H	1	ethanol	25	3	94
Ph	H	10	ethanol	25	5	99
p-$CH_3OC_6H_4$	H	10	ethanol	25	5	94
o-$CH_3C_6H_4$	H	10	ethanol	25	5	93
c-C_5H_{10} (cyclohexanone)		2	cyclohexane	82	1	92*
c-C_5H_{10}		5	ethanol	80	5	97
c-C_5H_{10}		10	ethanol	80	5	99
Ph	Me	10	cyclohexane	82	24	0
Ph	Me	10	toluene	112	24	0
Ph	Ph	10	cyclohexane	82	72	0

Note:*the reaction was carried out with a Dean-Stark trap to remove water.

Table 2.14 Optimisation of conditions for the Knoevenagel condensation catalysed by aminated sporopollenin (SECs-N2).

The results showed that benzaldehyde and its derivatives could be reacted successfully with ethyl cyanoacetate at room temperature in the presence of aminated sporopollenin (SECs-N2) as the catalyst, and when more sporopollenin was used and reaction times were longer, a higher yield was obtained. For the reaction between cyclohexanone and ethyl cyanoacetate the result was the same, only the reaction temperature need to be at reflux. These optimized conditions were investigated for the reaction between acetophenone or benzophenone and ethyl cyanoacetate again, but again this still failed to give any product. This may be due to the phenyl groups are electron-withdrawing and make carbonyl carbon less reactive. The benzene rings prevent the carbonyl group interacting with the amino groups on the surface of the catalyst.[107]

The impact of water on the progress of the reaction between cyclohexanone and ethyl cyanoacetate was tested. In a series of reactions water was removed continuously by a Dean-Stark trap, and it was found that the reaction rate, conversion and yield all increased significantly. This effect was not investigated for reactions with benzaldehyde as its reactivity was already high enough such that the reaction could take place at room temperature and give an excellent yield. The effect of water removal can be seen in the reaction between cyclohexanone and ethyl cyanoacetate, with cyclohexane as the solvent for 1 h. (See Figure 2.11)

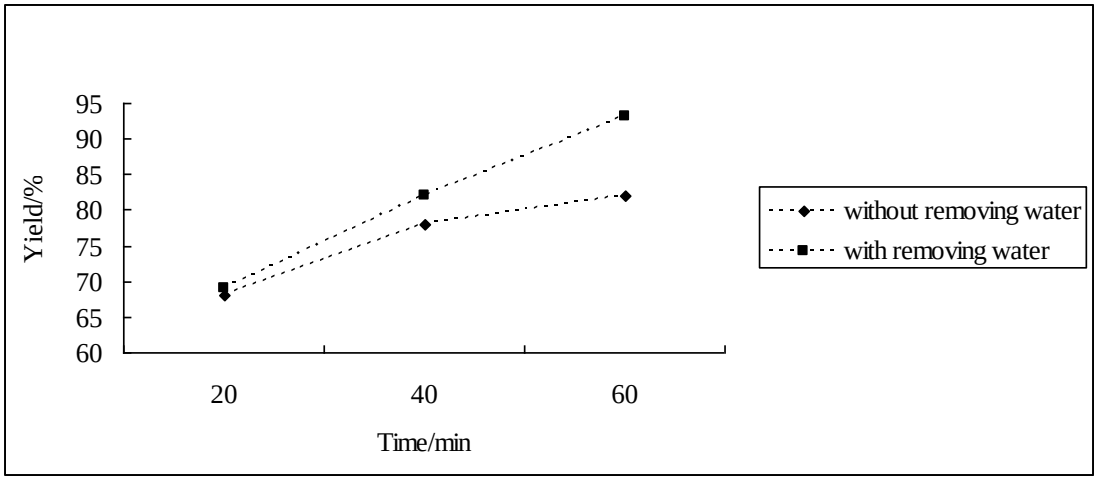
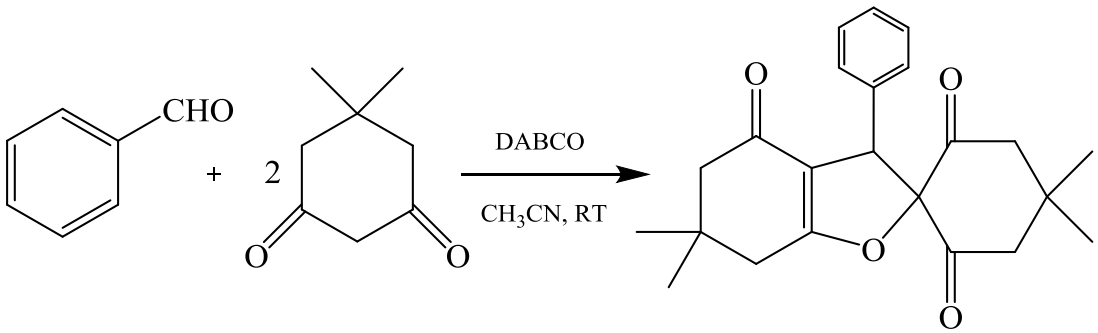


Figure 2.11 Impact of water in Knoevenagel Condensation using cyclohexane as a solvent at 82°C for 1 h.

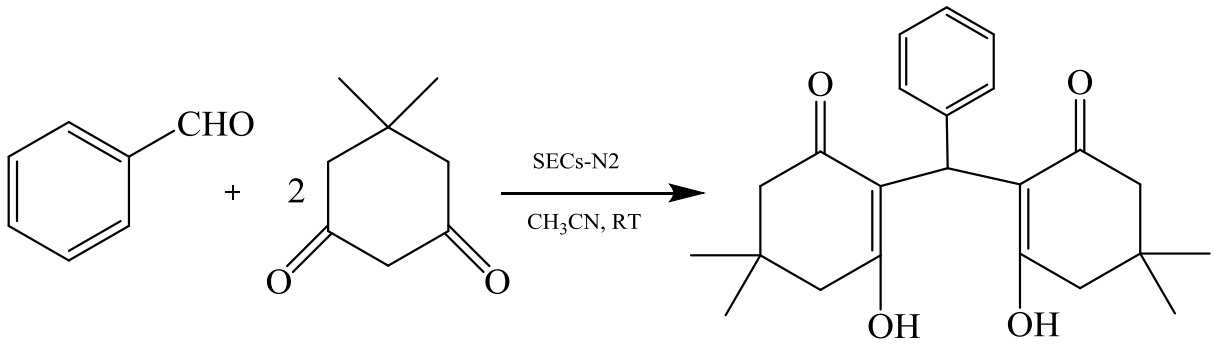
2.5.3.3 Reaction of benzaldehyde and dimedone/1,3-cycohexanedione catalyzed by aminated sporopollenin (SECs-N2)

Carbonyl compounds such as 5,5-dimethyl-cyclohexane-1,3-dione (dimedone) have been widely used in organic synthesis,[112,113] not only as a reagent in Knoevenagel condensations but also in reactions with electron-deficient alkenes *via* a Michael addition reaction. On the other hand, one or both of its carbonyl groups could take part in substitution and cyclization reactions through the tautomerized enolate form. Thus cascade reactions of addition, elimination and substitution can be achieved in many reactions involving dimedone. Chen *et al*[114] used 1,4-diazabicyclo[2.2.2]octane (DABCO) as a catalyst in the reaction of dimedone with various aldehydes and got a new spiro dihydrofuran as a sole product, and the reactions of 1,3-cycohexanedione with aromatic aldehydes under DABCO were also examined, all the aldehydes gave 9-aryl-1,8-dioxo-xanthenes as main products instead of the tetraketone. (See Scheme 2. 16)



Scheme 2.16 Reaction between benzaldehyde and dimedone with DABCO as a catalyst.[114]

In our study, the reaction between benzaldehyde and dimedone/1,3-cycohexanedione using aminated sporopollenin (SECs-N2) instead of DABCO was investigated under different conditions, with ethanol as the solvent. It is found that no reactions occurred between benzaldehyde and 1,3-cycohexanedione. Also in the reaction between benzaldehyde and dimedone, the ^{1}H-NMR and GC-MS of the product isolated in each case showed that irrespective of the reactant ratio, the only material isolated was the tetraketone (See Scheme 2.17), the reactant ratio only has impact on yield (See Table 2.15).



VI

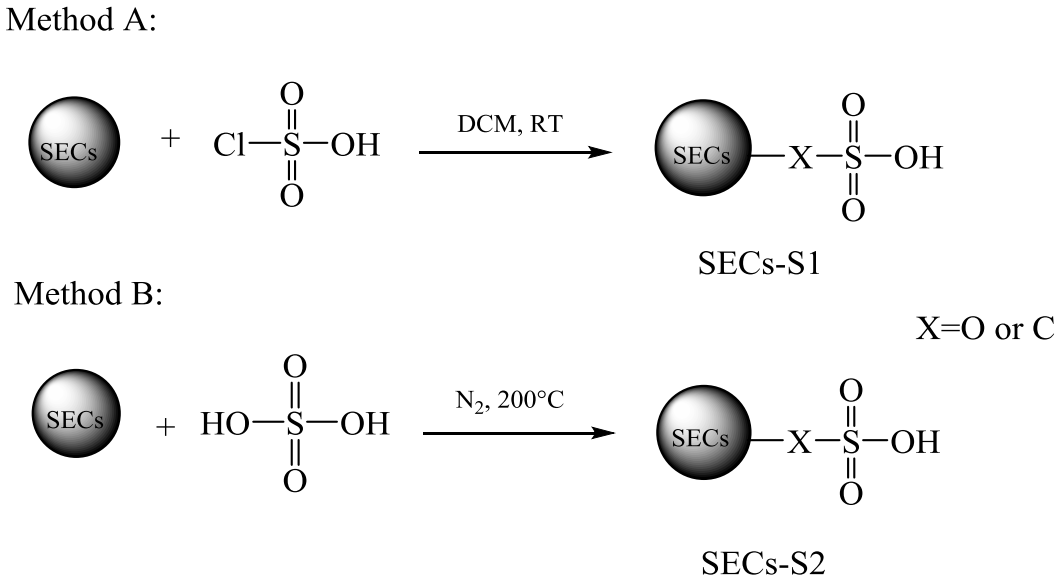
Scheme 2.17 Reaction between benzaldehyde and dimedone catalyzed by aminated sporopollenin (SECs-N2).

entry	Ratio of benzaldehyde and dimedone	Amount of N^{basic} , X%	T/°C	Time/h	Yield%
1	1:1	10	25	5	51
2	1:2	10	25	5	85
3	2:1	10	25	5	47

Table 2.15 Reaction of benzaldehyde and dimedone in different ration catalyzed by aminated sporopollenin (SECs-N2).

2.5.4. Sulfonation of the sporopollenin and analysis

The aim of sulfonating the sporopollenin was to introduce acid groups on the surface of the particles, and then to be used as a solid reagent in organic synthesis. Sporopollenin has been successfully sulphonated with chlorosulphonic acid by Shaw and Sykes.[115] Another method for sulfonation is to heat the sporopollenin in sulfuric acid (64%) at 200 °C under a flow of nitrogen.[116] (See Scheme 2.18) We also tried chlorosulphonic acid with the presence of trifluoroacetic acid without solvent, by adding chlorosulfonic acid slowly to a flask with sporopollenin and trifluoroacetic acid with ice water to keep the flask cool, and then the mixture were heated to 60 °C for 2 h then quenched with toluene. But the trifluoroacetic acid is highly corrosive and it is difficult to control the temperature of the container. Furthermore, the quenching and filtration were complicated, so we abandoned this method.



Scheme 2.18 Sulfonation of sporopollenin.

To detect the acidity of the modified sporopollenin, SECs-S1 and SECs-S2 were treated with sodium hydroxide to produce the corresponding sodium salt, and then the filtrate was titrated with hydrochloric acid using methyl orange as indicator. However, a problem arose with the titration of SECs-S2, the filtrate was a dark brown color, which made it impossible to see the color change using methyl orange, and the product was difficult to filter and took on a charcoal colored appearance, which suggested that the reaction condition may cause degradation of the sporopollenin.

For SECs-S1, the titration results (repeat three times) showed that the sulfonated sporopollenin has an H^+ concentration of 11.2mmol/g.

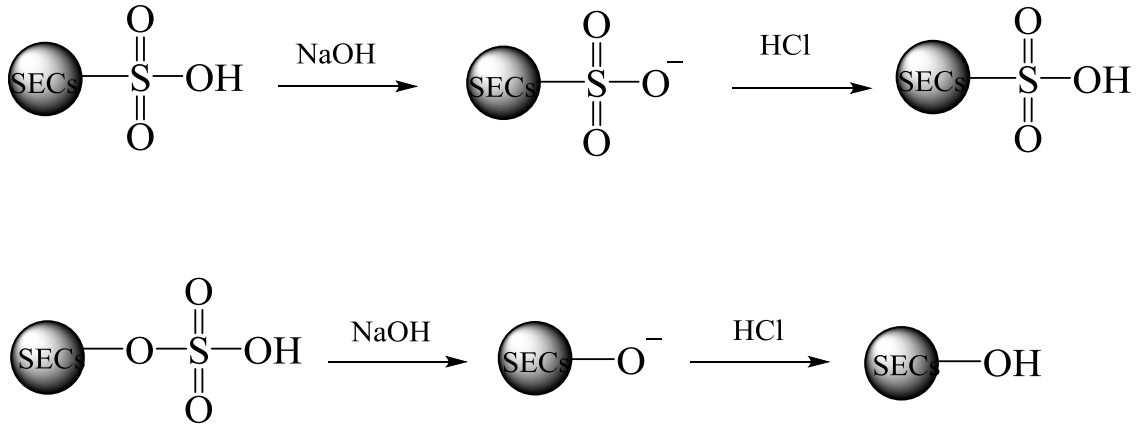
Then the samples were taken for elemental analysis and ICP-MS (Inductively coupled plasma mass spectrometry) analysis, the results are shown in Table 2.16

Sample No.	Elemental analysis		ICP-MS analysis	
	S%	S mmol/g	S%	S mmol/g
SECs-S1	10.7	3.34	9.64	3.01
SECs-S2	0.00	0.00	2.48	0.775

Table 2.16 Result of elemental analysis for the sulfonated sporopollenin.

According to previous experience, ICP-MS has a higher accuracy in sulfur detecting than elemental analysis, hence the data from ICP-MS is more credible. The result showed that the loading of sulfur in SECs-S1 is much higher than SECs-S2.

In Scheme 2.18, the "X" which connected "SO_3H" and sporopollenin could be C or O, or both of them. To detect the unknown factor in the sulfonated sporopollenin, a few samples were immersed in excess sodium hydroxide (2M) and hydrochloric acid (2M) overnight successively, and then the pH of the samples was tested with pH meter. (See Scheme 2.19)



Scheme 2.19 Test of "X" in the sulfonated sporopollenin.

As the Scheme shows, if "X" is O, after the process, the particle should be neutral. In another situation, "X" is C or both C and O exist, the particle should be acidic again. After 3 times of the process, pH of SECs-S1 changes from 1.35 to 3.17, and pH of SECs-S2 changes from 2.35 to 3.68. Both of the washed samples were still acid, and the acidity decreased, so the "X" should be both C and O. The dried samples were also tested by elemental analysis and ICP-MS, the results showed that the sulfur still existed but had a decrease in quantity, which supported the conclusion. (See Table 2.17)

Sample No.	Elemental analysis		ICP-MS analysis	
	S%	S mmol/g	S%	S mmol/g
SECs-S1’	6.02	1.88	5.47	1.71
SECs-S2’	0.00	0.00	2.06	0.644

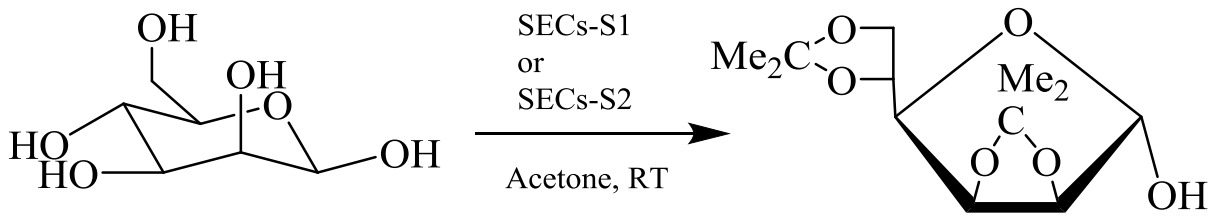
Table 2.17 Result of elemental analysis for the sulfonated sporopollenin

2.5.5 Application of sufonated sporopollenin in organic synthesis

2.5.5.1 Isopropylidene derivatives

Formation of *O*-isopropylidene derivatives of cyclic sugars by condensation of acetone has been widely used in synthetic, structural and conformational studies.[117] Several methods are available in the literature for the synthesis of *O*-isopropylidene derivatives of sugars. The common method consists of the condensation of a sugar with acetone in the presence of a catalyst under anhydrous conditions. In the last century, catalysts used for the isopropylidene derivative reaction can be found in the literature, such as anhydrous ferric chloride,[118] anhydrous aluminium chloride[119] and Zeolite HY.[120] However, these catalysts have some drawbacks, such as chemical toxicity, low yields, long reaction times, harsh reaction conditions and difficulties in purification. Hence, there is a need for new method to synthesis the isopropylidene derivatives. Recent years, Rajput and Mukhopadhyay reported a method for the synthesis of *O*-isopropylidene derivatives from free sugars using sulfuric acid immobilized on silica as an effective catalyst.[121]

In this research, sulfonated sporopollenin particles (sample SECs-S1, SECs-S2) are used as a catalyst for the synthesis of 2,3:5,6-Di-*O*-isopropylidene-α-D-mannofuranose from D-mannose and acetone. (See Scheme 2.20)



VII

Scheme 2.20 Synthesis of 2,3:5,6-Di-*O*-isopropylidene-α-D-mannofuranose

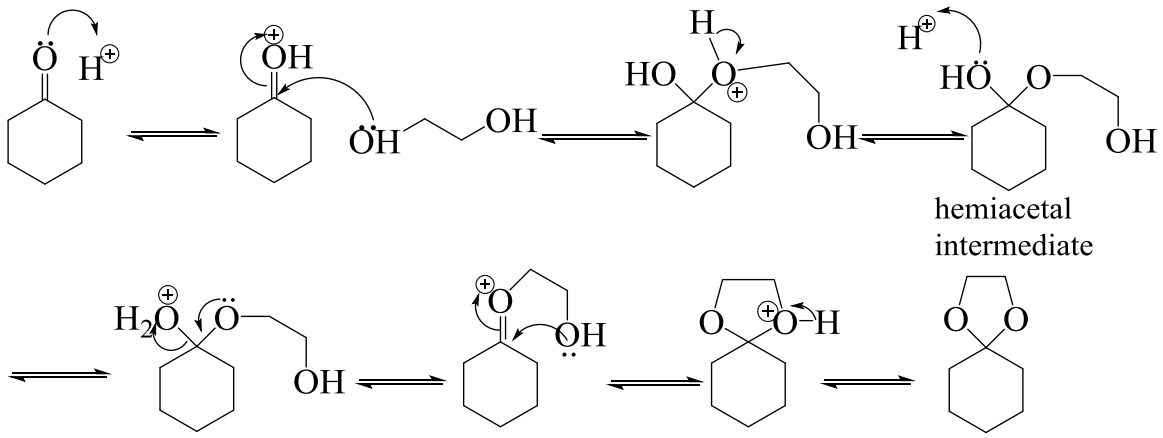
Both sulfonated sporopollenin particles (SECs-S1, SECs-S2) catalyzed this reaction effectively, obtaining a yellow solid as raw product with a high mass recovery at about 100%. The product was dissolved in a little diethyl ether and precipitated with the addition of hexane, to get white crystals of product. (See Table 2.18)

Entry	Catalyst 0.5g	T/°C	Time/h	Mass recovery%	$Yield_{recrystallization}$%
1	SECs-S1	25	18	94	66
2	SECs-S2	25	18	97	68

Table 2.18 Reaction between D-mannose and acetone catalyzed by sulfonated sporopollenin (SECs-S1, SECs-S2).

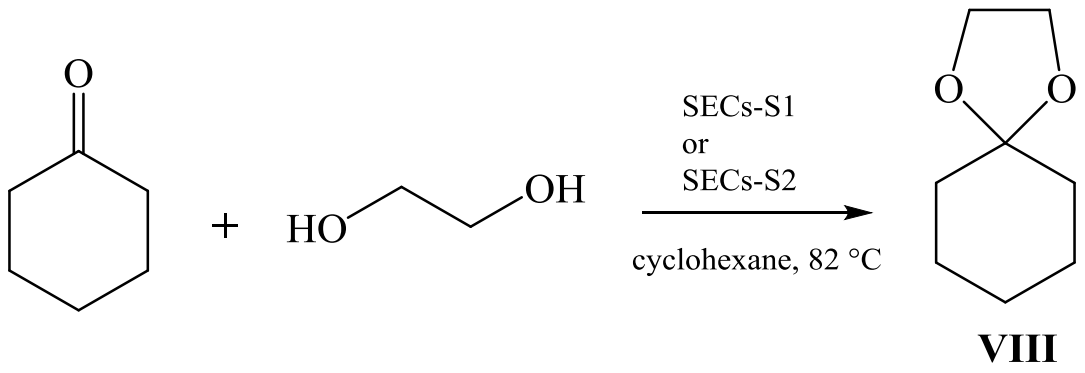
2.5.5.2 Preparation of Some Cyclic Acetals

Cyclic acetals have been the subject of numerous investigations. Acid catalyst is needed for this reaction. (See Scheme 2.21)



Scheme 2.21 Mechanism of formation of cyclic acetal

In this research, sulfonated sporopollenin particles (SECs-S1, SECs-S2) were used as a catalyst for the reaction between cyclohexanone and ethylene glycol respectively. Both sulfonated sporopollenin particles (SECs-S1, SECs-S2) catalyzed this reaction effectively, Scheme 2.22 and Table 2.19.



Scheme 2.22 Reaction between cyclohexanone and ethylene glycol catalyzed by sulfonated sporopollenin (SECs-S2).

Entry	Catalyst 0.5g	T/°C	Time/h	Yield%
1	SECs-S1	82	18	62
2	SECs-S2	82	18	66

Table 2.19 Reaction of cyclohexanone and ethylene glycol catalyzed by sulfonated sporopollenin (SECs-S2).

Acetophenone, with its bulky radicals was also tried in the preparing of cyclic acetal with the presence of sulfonated sporopollenin particles (SECs-S1, SECs-S2). However, no reaction happened between acetophenone and ethylene glycol, which may due to the phenyl rings decreasing the reactivity of the carbonyl group.

CHAPTER 3 Application of surface modified sporopollenin as an emulsifier

3.1 Introduction

3.1.1 General characteristics and stability of emulsions

When two or more immiscible liquids (e.g. oil and water) are mixed an emulsion is formed, with droplets of one liquid (the dispersed phase) within the other one (the continuous phase). Generally, emulsions exist as either an oil-in-water (o/w) type or water-in-oil (w/o) type, where the dispersed phase is oil and water respectively. The type of emulsion formed depends on the volume fraction of the phases and the emulsifier used. The boundary between the phases is called the interface. When light passes through the emulsion, it is scattered by the phase interfaces so making the emulsions often have a cloudy appearance. Normally, emulsions are inherently unstable and will not form spontaneously. Energy input such as shaking, stirring or homogenizing is needed to form an emulsion.

Emulsion stability refers to the ability of an emulsion to resist change in its properties over time[122] There are four types of instability in emulsions: flocculation, creaming/sedimentation, coalescence and Ostwald ripening. Flocculation occurs when an attractive force exists between the droplets. They gather together in aggregates that are distributed throughout the emulsion and this process is reversible. The flocculated droplets can be separated by gentle agitation if the attractive force is weak, or with more energy required to break up the aggregates when the interactive force is strong. In an

o/w emulsion, creaming is the migration of the oil drops to the top of the emulsion, while sedimentation is the droplets moving to the bottom of a w/o system. These phenomena are caused by gravity when there are differences in density between the continuous and dispersed phase. Creaming and sedimentation are also reversible. Coalescence is an irreversible process in which droplets combine together to form a larger droplet, making the average size of the droplet increase over time. Coalescence occurs when the film of continuous phase separating the droplets is thin enough to rupture. Ostwald ripening is another irreversible process to describe the oil dissolving from the small droplets and diffusing through the continuous phase to condense onto the larger ones. This is because the oil in small drops is more soluble in the continuous phase than oil in the larger droplets.[123] Ostwald ripening generally occurs in water-in-oil emulsions, while flocculation is found in oil-in-water emulsions.[124]

An appropriate emulsifier can increase the kinetic stability of an emulsion so that the size of the droplets does not change significantly with time, making the emulsion more stable. As a result, emulsions find widespread use in applications in food, medicine, firefighting and many other areas.

3.1.2 Solid particles stabilized emulsions (Pickering emulsions)

Emulsions that are stabilized by solid particles which adsorb onto the interface between the two phases are called a Pickering emulsion. The solid particles added to the mixture of oil and water will bind to the interface and prevent the droplets from coalescing, making the emulsion stable. The ability of solid particles to stabilize emulsions depends on their wettability (quantified by the particle contact angle) at the interface.[125] When a particle sits at a liquid interface, Figure 3.1, the contact angle is the angle between the tangents to the particle surface and the water-oil (air) interface at every point of the three-phase contact line.

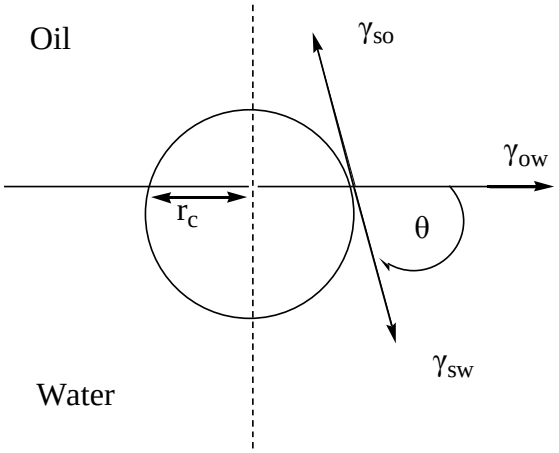


Figure 3.1 Contact angle of a spherical particle at an oil-water interface, γ_{so}, γ_{ow}, γ_{sw} are the interfacial tensions, and r_c is the radius of the three phase contact line.

The detachment energy of a spherical particle from the oil-water interface is given by the equation:[126,127]

$$E=\pi r^2\gamma_{ow}(1\pm\cos\theta)^2$$

where r is the particle radius and γ_{ow} is the interfacial tension. If the particle is wetted equally by both fluids, then the contact angle is 90° and E has the greatest value.[128]

For hydrophilic particles the contact angle is less than 90° and a contact angle bigger than 90° if found for hydrophobic particles. For equal volumes of oil and water, hydrophilic particles tend to stabilize o/w emulsions and hydrophobic particles tend to stabilize w/o emulsions.[129,130,131] Therefore, it is possible for a phase inversion if the particle wettability is changed from hydrophilic to hydrophobic (or the opposite).

However, for systems of unequal phase volumes, the emulsion type is decided by both the phase volumes and the wettability of particles. For example, when the volume of oil is greater than water, hydrophilic particles can stabilize water-in-oil emulsions.[132]

The concentration of particles is also important to the stability of the emulsions. When the concentration of particles is high, the particles will surround with the droplet surfaces to form a physical barrier to prevent droplet coalescence.[133] The wettability of solid particles is decided by the functional groups, and therefore charge or polarity, on their surface. For example, particles with large numbers of hydroxyl groups on the surfaces are hydrophilic and can be wetted well by water. The wettability of particles can be altered by chemical modification or by using a surfactant.

3.1.3 Previous work on behaviour of spore/sporopollenin particles at fluid interfaces

There is a lot of interest in the behaviour of solid particles, such as silica, clay, iron oxide and polymer, at fluid-fluid interfaces and in their ability to stabilize emulsions and foams without the use of other surfactants.[127,134-139] The free energy of adsorption to interfaces of the particles can be very high so that they can stabilize emulsions and foams for a long time. Coalescence and, in some cases, disproportionation or Ostwald ripening, can be prevented completely.[135,140,141]

In 2005, Binks *et al* investigated the behavior of spore particles from *Lycopodium clavatum* (See Chapter 1.1) at planar fluid interfaces and in emulsions. Differing from most particles used to stabilize emulsions, which are synthetic, the spore particles are naturally occurring. The unprocessed spore particles are more hydrophilic than hydrophobic, stabilizing o/w type emulsions with oils of a range of polarities efficiently.[142]

After the initial investigations, sporopollenin capsules extracted from the spores of *Lycopodium clavatum* (See Chapter 1.1-1.3) were studied for their ability to stabilize emulsions.[143] These sporopollenin exine capsules showed interesting properties compared to the unprocessed spores. For example, in emulsions of isopropyl myristate (IPM) and water stabilized by 0.5 wt% sporopollenin particles, the emulsion type could be changed from w/o to o/w by increasing the pH, Figure 3.2.

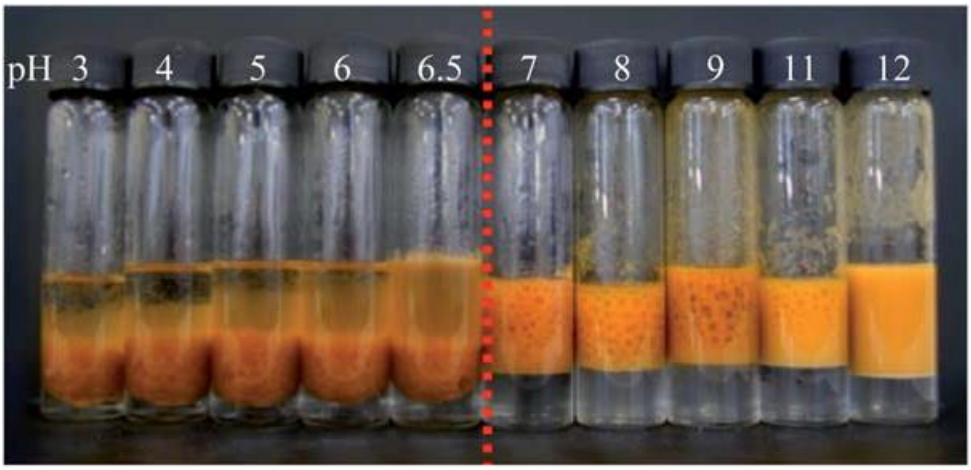


Figure 3.2 IPM-water emulsions stabilized by 0.5 wt% sporopollenin particles. Photograph of vessels at different pH after 1week. Left of dotted line the emulsion type is w/o and o/w to the right.[143]

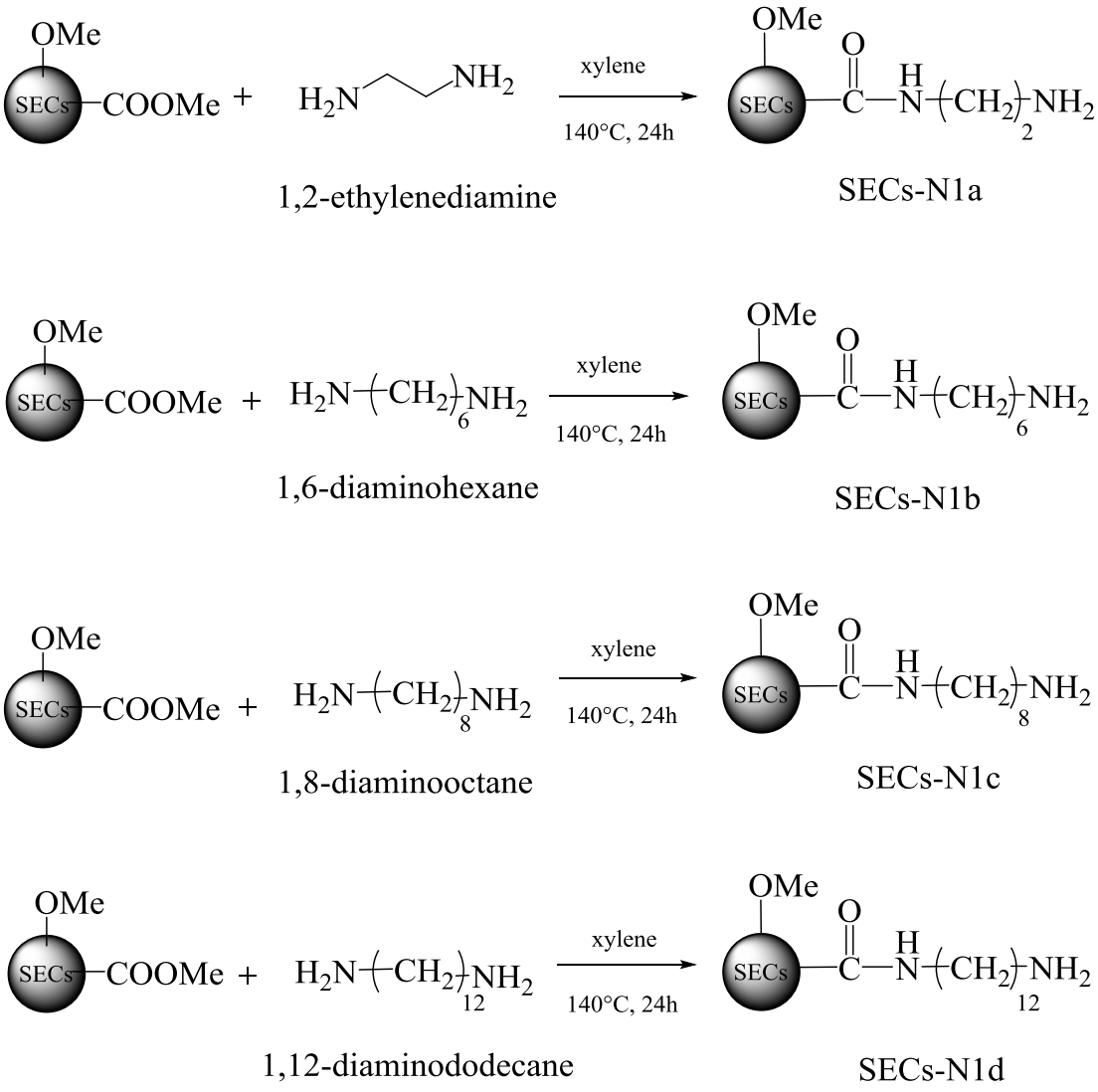
It was proposed that the reason for this inversion of emulsion type was due to a change in charge of the functional groups on the surface of the capsules. At low pH the particles (with carboxyl groups on the surface) are uncharged. The relative hydrophobicity leads the particles preferring stabilize to w/o emulsions. As the pH was increased, the particles became highly negatively charged due to the ionization of the carboxylic acids. The particles therefore became more hydrophilic and favoured stabilization of o/w emulsions.[144,145] Different conditions were investigated by increasing the concentration of the particles and by using different oils (dodecane or tricaprylin) respectively, but inversion of the emulsion type still was observed.[143]

As the wettability of particles could be altered by chemical modification, one aim of this work is to investigate the different behaviour of aminated sporopollenin particles in vary extent of coating in stabilizing emulsions.

3.2 Results and Discussion

3.2.1 Amination of sporopollenin particles

Carboxylic acids groups on the surface of sporopollenin can be modified by amination and if a primary diamine was used then a ‘primary amino sporopollenin’ could be made. Sporopollenin exine capsules (SECs) were first extracted from raw spores of *Lycopodium clavatum* and then methylated using dimethyl sulphate to get a higher loading of N in amination (See Chapter 2.5.1 and Chapter 2.5.3.1). Next, a series of straight chain diamines with different number of carbon atoms were reacted with the methylated SECs to produce a series of aminated SECs (Scheme 3.1). Elemental analysis and gravimetric chloride analysis then revealed the loading of basic nitrogen (Chapter 2.5.2.2) in each case, and the results are shown in Table 3.1.



Scheme 3.1 Amination of sporopollenin with different diamines

Code	Chain length of diamine	Time / h	N^{tot} mmol/g	N^{basic} mmol/g
SECs-N1a	2	24	3.03	1.52
SECs-N1b	6	24	2.43	1.22
SECs-N1c	8	24	2.90	1.45
SECs-N1d	12	24	2.88	1.44

Table 3.1 Loading of N^{basic} (half value of N^{tot}, modified on the surface of SECs), as determined by elemental analysis. Reactions used 1.53 g SECs and 30 mL of xylene and 0.100 mmol of diamine.

The result showed that there is no big difference of amount of basic nitrogen on the surface of modified sporopollenin using diamines of different chain length. So 1, 6-diaminohexane, which is cheap and readily available, was used for a further study to consider the effect of amine concentration and reaction time on the loading of amine.

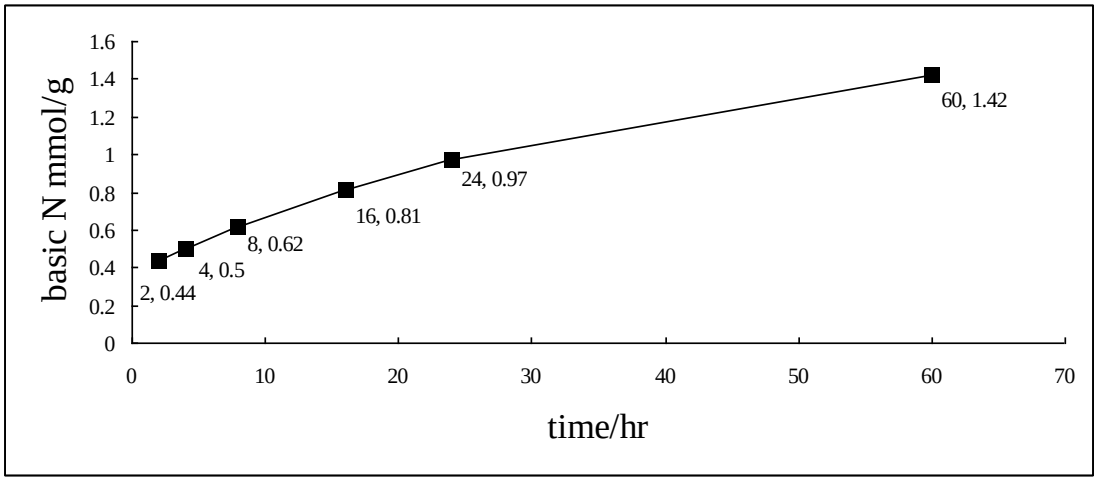
Firstly the reactions were carried out using a fixed amount of diamine and solvent changing the reaction time, and secondly reaction conditions were changed to use different amounts of diamine relative to the amount of SECs and solvent. The results are shown in Table 3.2.

Code	[diamine]/ mmol	Time/h	N^{tot} mmol/g	N^{basic} mmol/g
SECs-N2a	0.133	2	0.87	0.44
SECs-N2b	0.133	4	1.01	0.50
SECs-N2c	0.133	8	1.25	0.62
SECs-N2d	0.133	16	1.62	0.81
SECs-N2e	0.133	24	1.95	0.97
SECs-N2f	0.133	60	2.84	1.42
SECs-N3a	0.067	16	1.34	0.67
SECs-N3b	0.200	16	1.80	0.90
SECs-N3c	0.267	16	2.03	1.02

Table 3.2 Loading of basic nitrogen (N^{basic}) on the surface of aminated sporopollenin particles when aminated with 1,6-diaminohexane using different reaction conditions. Each reaction used 2.00 g SECs and 30 mL of xylene for the amount of diamine indicated.

The result showed, not surprisingly, that more reactant used and longer reaction time employed leads to an increase of the amount of basic N on the surface of sporopollenin, Figure 3.3.

(a)



(b)

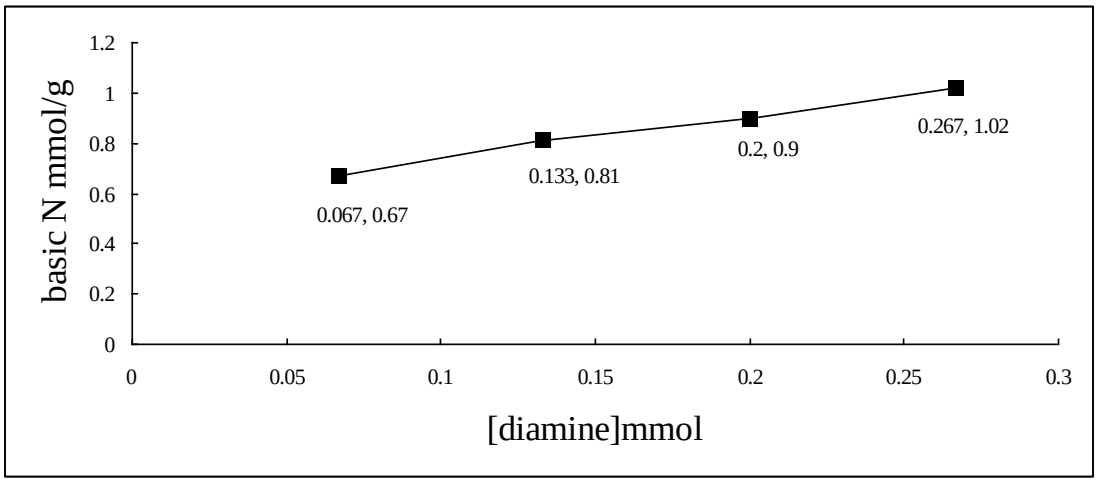


Figure 3.3 (a) Relationship between reaction time and loading of basic N in reaction with 2.00 g SECs and 0.133 mmol 1, 6-diaminohexane.
(b) Relationship between amount of 1, 6-diaminohexane used and loading of basic N in reaction with 2.00 g SECs at 16h.

The appearance of extracted sporopollenin compared to the aminated sporopollenin can be seen in the SEM images in Figure 3.4. After amination the SECs are still intact, monodisperse and retain the rough outer surface seen in the un-processed spores. It apprears that the amination doesn't make any visible difference in the morphological appearance of the particles.

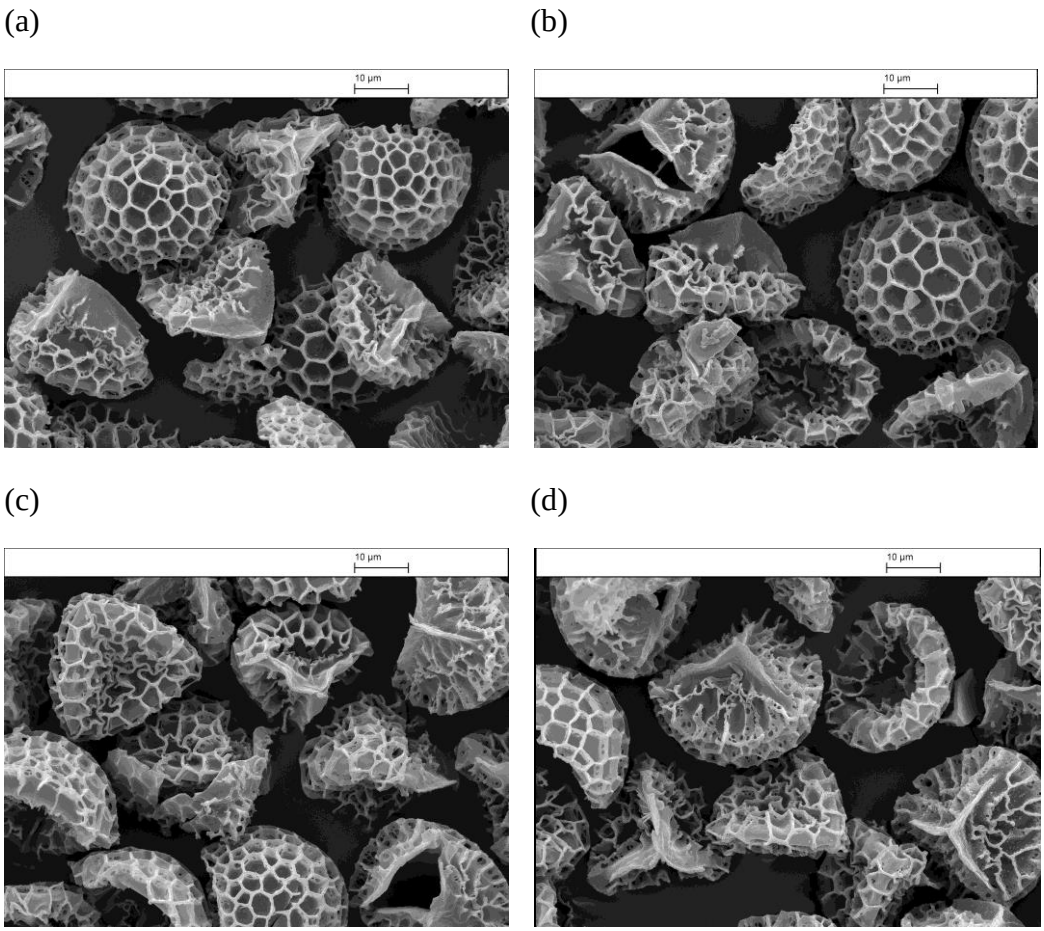


Figure 3.4 SEM images of dry sporopollenin particles (a) newly processed SECs, (b) after amination for 2 hr, (c) after amination for 16 hr, (d) after amination for 60 hr.

3.2.2 Wettability of modified and unmodified sporopollenin particles

In order to evaluate the changes in surfactant properties that amination brings about, the wettability of the particles was examined. First, a small amount (10 mg) of the extracted sporopollenin particles (SECs-3, Table 2.6) and aminated sporopollenin particles (SECs-N2a---SECs-2f, Table 3.2) were dispersed in pure water, a mixture of methanol and water, and dodecane (5 mL) respectively without hand shaking. Orange-brown dispersions were observed. The time for the particles to pass through the air-liquid interface was recorded as a simple way of measuring the wettability of the different particles, Table 3.3.

liquid/solid	SECs-3	SECs-N2a	SECs-N2b	SECs-N2c	SECs-N2d	SECs-N2e	SECs-N2f
water	30s	More than 10 min					
dodecane	Less than 3 sec						
methanol	5s	5s	5s	6s	6s	7s	7s
80% methanol in water	5s	6s	6s	6s	6s	7s	7s
60% methanol in water	5s	5s	6s	6s	6s	7s	7s
40% methanol in water	8s	8s	8s	7s	7s	9s	9s
20% methanol in water	11s	10s	12s	12s	11s	11s	12s

Table 3.3 Wettability test for various modified sporopollenin particles

The results showed that the aminated sporopollenin particles are more hydrophobic than the unmodified ones. This was unexpected but it was suspected that this might be due to the methylation step before amination. So another series of tests was undertaken in water using methylated sporopollenin particles and aminated sporopollenin particles made *via* a direct route which did not involve the methylation step. These results showed that the methylated sporopollenin is very hydrophobic but the latter aminated particles are similar to the sporopollenin particles as first extracted from the raw spores

(immersed in water within 30s). Clearly the methyl groups modifying the surface of sporopollenin increased the hydrophobicity of the particles considerably, and this outweighed the effect of the subsequent amination.

3.2.3 Emulsions stabilized by aminated sporopollenin particles

From the previous work by Binks *et al* it was shown that raw spore particles stabilized o/w emulsion for a range of oils at different pH and particle concentrations, and it was also possible for extracted sporopollenin particles to stabilize emulsions in which the emulsion type (o/w or w/o) depended on the pH.[142,144]

In a similar fashion we set out to examine the emulsifying properties of the aminated sporopollenin particles, using first unmodified sporopollenin particles (SECs-3) and aminated sporopollenin particles (SECs-N2a---SECs-N2f) at different concentrations (0.2, 0.5, 1.0, 1.5, 2.0 wt%) using isopropyl myristate (IPM) and water (1:1 by volume), Figure 3.5.

(a)


(b)


(c)


(d)


(e)


(f)


(g)


Figure 3.5 IPM-water emulsions (o/w) stabilised by sporopollenin: Left to right: pure oil and water, sporopollenin particle concentrations of 0.2 wt%, 0.5 wt%, 1.0 wt%, 1.5 wt%, 2.0 wt%

(a) unmodified sporopollenin (b) aminated sporopollenin for 2 hr
(c) aminated sporopollenin for 4 hr (d) aminated sporopollenin for 8 hr
(e) aminated sporopollenin for 16 hr (f) aminated sporopollenin for 24 hr
(g) aminated sporopollenin for 60 hr
Photos were taken when the emulsions were made after 1week.

The conductivity and pH of the sporopollenin particles stabilised emulsions increase with the particle concentration. The relative high values of emulsions stabilised by unmodified sporopollenin particles (SECs-3) is due to the acid groups deprotonated and ionised, Table 3.4. (Values were measured immediately after the emulsions were made)

(a)

Conductivity/ μS/cm						
Sample No.	Wt %	0.2	0.5	1.0	1.5	2.0
SECs-3		28.50	46.60	83.10	104.30	133.10
SECs-N2a		24.10	8.63	23.3	29.5	46.30
SECs-N2b		6.05	8.68	23.90	32.20	53.70
SECs-N2c		2.25	6.90	13.81	20.10	30.30
SECs-N2d		2.36	7.86	20.20	30.10	50.50
SECs-N2e		3.93	7.02	20.00	25.00	43.20
SECs-N2f		4.31	11.92	20.40	26.80	28.40

(b)

pH						
Sample No.	Wt %	0.2	0.5	1.0	1.5	2.0
SECs-3		10.23	10.80	11.06	11.14	11.28
SECs-N2a		8.57	7.19	8.54	9.80	8.68
SECs-N2b		5.48	5.03	5.65	5.57	5.70
SECs-N2c		7.59	7.78	8.23	8.05	8.15
SECs-N2d		8.22	8.39	8.89	9.49	9.62
SECs-N2e		8.25	8.79	9.15	9.35	8.81
SECs-N2f		10.23	10.80	11.06	11.14	11.28

Table 3.4 (a) Conductivity, (b) pH of the IPM-water emulsions stabilized by sporopollenin particles

Also, the stability of the emulsions to creaming and coalescence increases with particle concentration by calculating the $f_{aq(oil)}$, where $f_{aq(oil)}=h_{aq(oil)}/0.5h_t$, $h_{aq(oil)}$ is the total height of the water (oil) phase resolved at any time and h_t is the height of the liquid mixture. When the value of $f_{aq(oil)}$ is 0, the emulsion is complete stable, and the value equals to 1 means complete coalescence (creaming/sedimentation), and as the particle concentration is higher, the droplet size of the emulsion decreases, Table 3.5. When measuring the size of emulsion droplet, it is found that the droplet sizes of emulsions stabilized by sporopollenin particles in 0.2 wt% (see Figure 3.5) are too uneven to measure, and for the milky appearance of the unmodified sporopollenin (SECs-3), it is difficult to measure f_{oil} and droplet diameter. (Values were measure after the emulsions were made after 1 week)

(a)

F_{aq}/ F_{oil}						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3						
SECs-N2a		0.88/0.27	0.78/0.24	0.71/0.18	0.59/0.16	0.48/0.14
SECs-N2b		0.83/0.36	0.77/0.34	0.63/0.23	0.52/0.17	0.27/0.12
SECs-N2c		0.80/0.40	0.73/0.22	0.62/0.17	0.58/0.18	0.47/0.12
SECs-N2d		0.74/0.32	0.71/0.24	0.64/0.20	0.60/0.10	0.67/0.08
SECs-N2e		0.79/0.22	0.76/0.21	0.51/0.18	0.55/0.13	0.38/0.13
SECs-N2f		0.78/0.23	0.76/0.25	0.60/0.17	0.46/0.15	0.46/0.14

(b)

Average drop diameter/mm						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3						
SECs-N2a		/	1.9	0.99	0.69	0.55
SECs-N2b		/	1.9	1.2	0.75	0.58
SECs-N2c		/	1.7	1.1	0.78	0.56
SECs-N2d		/	1.8	0.97	0.73	0.57
SECs-N2e		/	1.7	0.99	0.69	0.57
SECs-N2f		/	1.9	0.98	0.71	0.57

Table 3.5 (a) $f_{aq(oil)}$ (b) average drop diameter of the IPM-water emulsions stabilized by sporopollenin particles

Next the same set of experiments was carried out with different oils, namely dodecane (Figure 3.6 and Table 3.6) and tricaprylin (Figure 3.7 and Table 3.7). The results for emulsion type and the relationship between conductivity, pH, stability and drop size of the emulsion with particle concentration is the same as that trend found for emulsions made with IPM.

(a)


(b)


(c)


(d)


(e)


(f)


(g)


Figure 3.6 Dodecane-water emulsions (o/w) stabilised by sporopollenin: Left to right: pure oil and water, sporopollenin particle concentrations of 0.2 wt%, 0.5 wt%, 1.0 wt%, 1.5 wt%, 2.0 wt%

(a) Unmodified sporopollenin (b) aminated sporopollenin for 2 hr
(c) aminated sporopollenin for 4 hr (d) aminated sporopollenin for 8 hr
(e) aminated sporopollenin for 16 hr (f) aminated sporopollenin for 24 hr
(g) aminated sporopollenin for 60 hr
Photos were taken when the emulsions were made after 1week.

(a)

Conductivity/ μS/cm						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3		29.90	58.40	85.40	115.70	144.50
SECs-N2a		13.38	21.00	30.10	35.90	62.40
SECs-N2b		6.30	20.50	31.10	66.50	90.80
SECs-N2c		3.14	11.88	19.18	24.10	32.00
SECs-N2d		5.91	17.54	21.20	35.00	/
SECs-N2e		4.28	11.87	20.04	34.30	41.50
SECs-N2f		6.18	13.90	20.40	43.60	72.50

(b)

pH						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3		8.15	8.94	9.63	9.99	10.08
SECs-N2a		7.88	7.91	7.62	7.81	7.98
SECs-N2b		7.92	6.70	6.87	6.78	6.88
SECs-N2c		7.01	7.67	7.81	8.05	8.04
SECs-N2d		7.59	7.72	7.91	8.12	/
SECs-N2e		7.93	8.05	8.23	8.51	9.06
SECs-N2f		7.85	7.93	7.90	8.02	8.38

(c)

F_{aq}/F_{oil}						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3						
SECs-N2a		0.85/0.20	0.72/0.22	0.68/0.14	0.66/0.10	0.42/0.08
SECs-N2b		0.74/0.28	0.66/0.14	0.60/0.12	0.54/0.09	0.46/0.06
SECs-N2c		0.73/0.28	0.71/0.14	0.63/0.09	0.56/0.07	0.49/0.06
SECs-N2d		0.81/0.06	0.87/0.05	0.73/0.05	0.60/0.04	/
SECs-N2e		0.81/0.10	0.83/0.11	0.58/0.03	0.50/0.02	0.47/0.02
SECs-N2f		0.78/0.12	0.81/0.09	0.64/0.08	0.60/0.03	0.48/0.02

(d)

Average drop diameter/mm						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3						
SECs-N2a		/	1.5	0.99	0.79	0.61
SECs-N2b		/	1.4	0.93	0.75	0.57
SECs-N2c		/	1.3	0.96	0.76	0.61
SECs-N2d		/	1.3	1.0	0.81	/
SECs-N2e		/	1.4	1.2	0.80	0.54
SECs-N2f		/	1.5	1.1	0.85	0.58

Table 3.6 (a) Conductivity, (b) pH, (c) $f_{aq(oil)}$ (d) average drop diameter of the dodecane-water emulsions stabilized by sporopollenin particles

(a)


(b)


(c)


(d)


(e)


(f)


(g)


Figure 3.7 Tricaprylin-water emulsions (o/w) stabilised by sporopollenin: Left to right: pure oil and water, sporopollenin particle concentrations of 0.2 wt%, 0.5 wt%, 1.0 wt%, 1.5 wt%, 2.0 wt%

(a) Unmodified sporopollenin (b) aminated sporopollenin for 2 hr
(c) aminated sporopollenin for 4 hr (d) aminated sporopollenin for 8 hr
(e) aminated sporopollenin for 16 hr (f) aminated sporopollenin for 24 hr
(g) aminated sporopollenin for 60 hr
Photos were taken when the emulsions were made after 1week.

(a)

Conductivity/ μS/cm						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3		27.50	49.60	65.20	113.20	140.20
SECs-N2a		25.50	22.10	27.60	30.00	18.91
SECs-N2b		7.99	20.50	47.00	64.20	83.80
SECs-N2c		3.61	6.75	19.19	37.30	57.10
SECs-N2d		5.51	10.23	22.90	23.30	27.20
SECs-N2e		9.01	15.90	21.20	40.30	58.30
SECs-N2f		10.04	14.82	20.50	38.20	52.20

(b)

pH						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3		8.60	9.47	10.19	10.50	10.53
SECs-N2a		8.74	8.38	7.99	8.52	8.41
SECs-N2b		6.86	6.77	6.66	6.70	6.59
SECs-N2c		7.23	8.31	8.88	9.20	9.31
SECs-N2d		7.31	7.89	8.11	8.36	8.88
SECs-N2e		7.69	8.23	8.95	9.54	9.68
SECs-N2f		7.70	8.21	8.43	9.04	9.22

(c)

F_{aq}/F_{oil}						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3						
SECs-N2a		0.79/0.11	0.54/0.06	0.63/0.06	0.51/0.10	0.44/0.09
SECs-N2b		0.69/0.10	0.64/0.10	0.46/0.12	0.53/0.10	0.38/0.09
SECs-N2c		0.87/0.15	0.76/0.13	0.60/0.13	0.58/0.10	0.35/0.10
SECs-N2d		0.81/0.14	0.59/0.12	0.54/0.11	0.47/0.10	0.44/0.08
SECs-N2e		0.78/0.12	0.71/0.12	0.64/0.09	0.46/0.08	0.32/0.07
SECs-N2f		0.77/0.12	0.67/0.10	0.60/0.11	0.59/0.06	0.44/0.05

(d)

Average drop diameter/mm						
Sample No.	Wt%	0.2	0.5	1.0	1.5	2.0
SECs-3						
SECs-N2a		/	1.2	0.90	0.76	0.61
SECs-N2b		/	1.4	0.86	0.67	0.62
SECs-N2c		/	1.2	0.89	0.69	0.62
SECs-N2d		/	1.3	0.91	0.72	0.61
SECs-N2e		/	1.2	0.90	0.73	0.62
SECs-N2f		/	1.3	0.88	0.76	0.63

Table 3.7 (a) Conductivity, (b) pH, (c) $f_{aq(oil)}$ (d) average drop diameter of the tricaprylin-water emulsions stabilized by sporopollenin particles

The results showed that the f_{aq}/f_{oil} values of emulsions stabilized with dodecane and tricaprylin are smaller than the values in IPM-water emulsions, which reveals that dodecan-water emulsions and tricaprylin-water emulsions are more stable than IPM-water emulsions. These maybe caused by the different of oil properties and it would affect the particles wettability at their interface.

The appearance of the aminated sporopollenin particles stabilized emulsion was the same as previous work by Binks.[143] However, it was found that the unmodified sporopollenin particle-stabilized emulsions, used as a reference, appeared very different. These emulsions contained brown emulsion droplets but within a ‘milky’ white liquid. The last step of the extraction of this sample of unmodified sporopollenin was washing using a PBS buffer (pH = 7.4). It was therefore assumed that residual phosphate salts in the sporopollenin sample gave rise to this milky white emulsion as a result of low molecular weight ‘contaminants’. A further sporopollenin sample was prepared in ‘acid form’, using the extracted sporopollenin as above. Thus sporopollenin SECs-3 was stirred in HCl overnight, washed with deionized water, until the pH of the filtrate was 7, then dried with a dessicator overnight. The photographs of the new emulsions are shown in Figure 3.8.

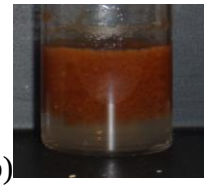
(a)  (b) 

Figure 3.8 (a) A 1.0 wt% sporopollenin particle (acid form) stabilized emulsion of IPM-water, (b) 1.0 wt% sporopollenin particle (acid form) stabilized emulsion of tricaprylin-water.

The appearance of the emulsions formed by the particles in acid form was no longer milky. It was noted that the color of the emulsion in Figure 3.8(a) is lighter than the one in Figure 3.8(b), but it was presumed that this was due to the difference in the oil phase.

The results of the experiments undertaken revealed that the use of higher particle concentrations to a decrease in the droplet size of the emulsion with all the three oils investigated (see Table 3.5, Table 3.6, and Table 3.7). At low particle concentration (0.5 wt %), the size of emulsion droplet from the different oils was IPM > dodecane > tricaprylin (see Table 3.5, Table 3.6, and Table 3.7). When the particle concentration was higher, the difference between the droplet sizes was very small.

For sporopollenin particles with different amine loadings (i.e. aminated for different times) the emulsion droplet sizes were similar when using the same particle concentration and the same oil. Because the effect of amination time of the sporopollenin particles on the emulsion properties was small, one group of the aminated sporopollenin (SECs-N2b, aminated for 4 hrs) was investigated under acidic conditions (pH = 2). It was hoped that full protonation of the amine groups on the surface of the sporopollenin particles would be a sufficient change in particle properties to affect the emulsion type and/or properties. These particles were investigated at pH 2 in tricaprylin-water, and the appearance of the emulsion formed is shown is Figure 3.9. Emulsion properties for this system are shown in Table 3.8.

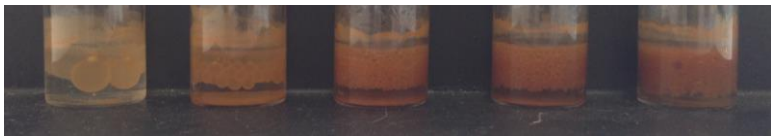


Figure 3.9 Sporopollenin particles aminated for 4 hr (SECs-N2b) stabilizing tricaprylin & water emulsions at pH=2

Wt%	0.2	0.5	1.0	1.5	2.0
Conductivity/μS cm^{-1}	22.8	39.8	49.0	73.4	89.5
pH	2.30	2.10	2.42	2.77	3.14
F_{aq}/F_{oil}	0.83/0.23	0.72/0.18	0.53/0.14	0.43/0.10	0.38/0.05
Drop diameter/mm	/	2.91	1.58	1.33	0.76

Table 3.8 Conductivity, pH, $f_{aq(oil)}$ and average drop diameter of the emulsions stabilized by sporopollenin particles aminated for 4 h (SECs-N2b) in tricaprylin & water at pH=2

The aminated sporopollenin particles stabilized emulsion at low pH is an o/w type emulsion, by a test drop. Under these conditions, the particles are positively charged; the increased hydrophilicity leads to the stabilization of o/w emulsions, and this result corresponds to the conclusion of the work by Binks *et al.*[143]

CHAPTER 4 Experimental Procedures

4.1 Material Evaluation

Nuclear Magnetic Resonance Spectroscopy

Structure of products was verified by ^{1}H NMR spectroscopy using a JEOL Eclipse 400 MHz spectrometer. Deuterated chloroform with an internal standard of tetramethylsilane was typically used as the solvent. The following abbreviations are examples to describe the splitting patterns:

s---singlet; d---doublet; t---triplet; quart---quartet; m---multiplet.

Gas Chromatography-Mass spectrometry

The purity of the products and the relevant mass spectrum were verified using a Perkin Elmer TurboMass machine, using acetone as sample solvent, injector temperature 200 °C, helium flow rate 1 ml min^{-1}, oven temp 50 °C for 4 min then ramped to 250 °C at 30 °C min^{-1} and held for 30 min with a 3.5 min filament delay.

Inductively Coupled Plasma-Mass Spectrometry

The sulfur analysis of sulfonated sporopollenin was conducted using a Perkin Elmer ELAN DRC II machine.

Elemental Analysis

The elemental analysis of sporopollenin was conducted using a Fisons EA 1108 CHN machine.

Conductivity and pH

Conductivity of the emulsions stabilized by sporopollenin particles was measured using a Jenway 4510 Conductivity Meter. pH of the emulsions stabilized by sporopollenin particles was conducted using a Hanna HI 2210 pH Meter.

Reagents and Reaction solvents

Reagents and reaction solvents were used without further purification unless stated otherwise. Nitromethane was distilled before use. Acetone, used in the preparation of isopropylidene derivatives as a reactant, and ethylene glycol were dried over anhydrous potassium carbonate before use. Dodecane was passed twice through a basic alumina column to remove polar impurities

4.2 Experimental procedures in solid phase organic synthesis

4.2.1 Preparation of sporopollenin from Lycopodium clavatum spores powder

Method A (SECs-1, SECs-2, SECs-4)

Lycopodium clavatum spores (125 g) were stirred in acetone (375 cm^3) under reflux for 4 h at 60 °C. The defatted spores were recovered by filtration and then dried under a vacuum pump overnight. The spores were then stirred under reflux with 6% KOH (560 cm^3) for 6 h at 80 °C. Then the KOH solution was refreshed and heating for a further 6 h at 80 °C. After cooling, the spores were recovered by filtration and washed with water until the filtrate were neutral and uncolored. The spores were then dried with vacuum overnight. The spores were then suspended in *ortho*-phosphoric acid (85%, 600 cm^3) and stirred for 7 days at 60 °C. The acid-hydrolyzed spores were recovered by filtration, washed successively with water (5 × 200 cm^3), acetone (200 cm^3), HCl (2 M, 200 cm^3), NaOH (2 M, 200 cm^3), water (5×200 cm^3), acetone (200 cm^3) and ethanol (200 cm^3), then re-suspended in ethanol (750 cm^3) and stirred under reflux for 2 h at 80 °C, Finally the particles were recovered by filtration, washed with ethanol (3× 200 cm^3), dried with vacuum overnight to a constant weight.

Method B (SECs-3)

The defatted process is the same with method A, only different for the washing procedure. The acid-hydrolyzed spores were recovered by filtration, washed successively with water (3 × 1000 cm^3), NaOH (2 M, 2 × 1000 cm^3), water (3 × 1000 cm^3), phosphate buffer solution (pH=7.4 2 × 1000 cm^3) and water (3 × 1000 cm^3). The remaining material was dried with vacuum overnight and then heated under reflux with ethanol (2000 cm^3) for 2 hours at 80 °C. Finally the particles were recovered by filtration, washed with ethanol (3× 1000 cm^3), dried with vacuum overnight to a constant weight.

Elemental analysis was carried out for the sporopollenin prepared by these methods. And the results are shown in Table 2.6.

4.2.2 Amination of the sporopollenin and analysis

4.2.2.1 Preparation of aminated sporopollenin particles (SECs-N1, SECs-N2 and SECs-N3)

SECs-N1: Sporopollenin particles (SECs-1, 3.3 g) and *N*, *N*-diethylethylenediamine (26 cm^3) in xylene (10 cm^3) were stirred under reflux using a Dean-Stark trap for 24 h at 140 °C. The cooled aminated sporopollenin was then washed successively with xylene (3 × 10 cm^3), HCl (2 M, 2 × 10 cm^3), NaOH (2 M, 2 × 10 cm^3), distilled water (10 × 20 cm^3, until the pH of filtrate was 7), methanol (3 × 10 cm^3) and dichloromethane (3 × 10 cm^3). The aminated sporopollenin particles were then dried in desiccator over phosphorus pentoxide (P_2O_5) for 5 days to a constant weight.

SECs-N2: The procedure followed was the same as for the preparation of SECs-N1, using *N*,*N*-diethyl-1,3-propanediamine (33.6 cm_3) instead of *N*,*N*-diethylethylenediamine.

SECs-N3: The procedure followed was the same as for the preparation of SECs-N1, using diethylenetriamine (30 cm^3) instead of *N*, *N*-diethylethylenediamine.

Reactions are shown in Scheme 2.6 and the elemental analysis results are shown in Table 2.7.

4.2.2.2 Gravimetric analysis of aminated sporopollenin

Derivatised sporopollenin (SECs-N1, 0.1 g) was suspended in excess hydrochloric acid overnight (2 M HCl, 10 cm^3), then filtered, washed thoroughly with deionised water (3×10 cm^3) and ethanol (10 cm^3), then filtered again. Next the oven dried (60 °C, 3 h) solid particles (mass $w_0 \sim 0.1$ g accurate to 1 mg) was suspended with stirring in sodium hydroxide (2 M, 10 cm^3) with heating for 2 h. The filtrate was carefully collected and acidified with nitric acid (6 M, 5 cm^3), then treated with silver nitrate solution (0.1 M, 5 cm^3). The mixture in the flask was covered with tinfoil to keep the precipitate away from light immediately to avoid decomposition and set aside overnight. The next day, silver chloride was coagulated by heating at 100 °C with constant stirring. After cool for 1h the precipitate was collected, washed with dilute nitric acid (6 M, 3×10 cm^3) and filtered through an oven-dry, pre-weighed Gooch funnel. The funnel with precipitate was dried in a 120 °C oven for 3h. Then the mass of precipitate was determined and converted into moles.

Loading of chloride in the original sample ($mmol.g^{-1}$) was calculated as such: loading = $W_{AgCl} / (M_{AgCl} \times w_0)$, with W_{AgCl} the weight of precipitate (mg) and M_{AgCl} the molecular mass of silver chloride (i.e. $143.3 g.mol^{-1}$)

The procedure to analysis of SECs-N2 and SECs-N3 was the same as for the analysis of SECs-N1, Table 2.8.

4.2.3 Schotten-Baumann type acylation

4.2.3.1 General procedure for the preparation of I

Benzylamine (0.33 cm^3, 3 mmol), benzoyl chloride (0.36 cm^3, 3 mmol) and the aminated sporopollenin (sample SECs-N1, N_{basic} loading=1.06 mmol/g, 2.83 g, 3 mmol N_{basic}) in dry dichloromethane (20 cm^3) were stirred for 3 h at room temperature. The sporopollenin was recovered by filtration and washed with dichloromethane (2 × 50 cm^3) to remove any remaining amide from the sporopollenin. The solvent was removed by rotary evaporator and then the white powder was dried over P_2O_5 in a desiccator to a constant weight.

4.2.3.2 Methylation of sporopollenin (SECs-1', SECs-2')

To a stirred solution of sporopollenin (sample SECs-1, 5 g) in acetone (100 cm^3) was added anhydrous powdered potassium carbonate (13.8 g). Dimethyl sulfate (4.75 cm^3) was added in portions over 10 mins at room temperature. After the addition was completed, the solution was heated to reflux at 58 °C and maintained for 3 h. The solution was cooled to room temperature and then washed successively with water (100 cm^3), ethyl acetate (3 × 50 cm^3), methanol (3 × 20 cm^3), and dichloromethane (3 × 20 cm^3) and then dried over P_2O_5 in a desiccator to a constant weight. Suspended the dried product (3.3 g) and *N*,*N*-diethylethylenediamine (30 cm^3) in xylene (10 cm^3), stirred under reflux at 140 °C using a Dean-Stark trap for 24 h. The cooled aminated sporopollenin were then washed successively with xylene (3×10 cm^3), HCl (2 M, 2 × 10 cm^3), NaOH (2 M, 2 × 10 cm^3), distilled water (till the pH of filtrate=7), methanol (3 ×

10 cm^3) and dichloromethane (3 × 10 cm^3). The aminated sporopollenin particles were then dried in a desiccator over P_2O_5 for 5 days to a constant weight.

Reactions of methylation are shown in Scheme 2.10 and the elemental analysis results are shown in Table 2.9.

4.2.3.3 Preparation of I by using methylated sporopollenin

The general procedure was the same as described in **4.1.3.1**, only by using methylated sporopollenin (SECs-N1', N_{basic} loading=1.27 mmol/g, 2.36 g, 3 mmol N_{basic} / SECs-N2', N_{basic} loading=1.48 mmol/g, 2.03 g, 3 mmol N_{basic})

***N*-Benzyl-benzamide I** as a white solid, yield 97% (mass recovery × purity, 97.0 × 99.9), Table 2.10.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ_H= 4.64 (2H, d, J=5.7 Hz, CH_2), 7.42 (10H, m, Ar), 7.79 (1H, s, NH).

GC-MS: m/z 211 (M^+), t_R 10.7 mins.

4.2.4 Knoevenagel Condensation

4.2.4.1 General procedure for the preparation of II

Ethyl cyanoacetate (0.53 cm^3, 5 mmol) and benzaldehyde (0.51 cm^3, 5 mmol) were added to a 10 cm^3 round-bottomed flask with aminated sporopollenin (sample SECs-N2, N_{basic} loading=1.29 mmol/g, 0.38g, 10% N_{basic}) and ethanol (5 cm^3), stirred at RT for 3h. Then the mixture was filtered and washed with ethyl acetate (30 cm^3), the filtrate was evaporated by a rotary evaporator for 30min and the product was dried in a vacuum to give a constant weight.

Preparation of **II** were carried out in same procedure only by using different conditions, Table 2.14.

2-Cyano-3-phenyl-acrylic acid ethyl ester II as a yellow solid, yield 99% (mass recovery × purity, 99.0 × 99.9), Table 2.14.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ_H= 1.38 (3H, t, *J*=7.1 Hz, CH_3), 4.36 (2H, q, *J*=7.1 Hz, CH_2), 7.51 (3H, m, Ar), 7.96 (2H, m, Ar) and 8.24 (1H, s, CH).

^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ_C= 162.5, 155.1, 133.3, 131.4, 131.0, 129.3, 115.5, 102.9, 62.7, 14.1.

GC-MS: m/z 201 (M^+), t_R 7.58 mins.

4.2.4.2 General procedure for the preparation of III

Ethyl cyanoacetate (0.53 cm^3, 5 mmol) and cyclohexanone (1.04 cm^3, 10 mmol) were added to a 10 cm^3 round-bottomed flask with aminated sporopollenin (sample SECs-N2, N_{basic} loading=1.29 mmol/g, 0.38g, 10% N_{basic}) and ethanol (5 cm^3), stirred refluxing with a Dean-Stark trap at 80 °C for 5h. Then the mixture was filtered and washed with ethyl acetate (30 cm^3), the filtrate was evaporated by a rotary evaporator for 30min and the product was dried in a vacuum to give a constant weight.

Preparation of **III** were carried out in same procedure only by using different conditions, Table 2.14.

Cyano-cyclohexylidene-acetic acid ethyl ester III as a yellow liquid, yield 99% (mass recovery × purity, 99.0 × 99.9), Table 2.14.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): $\boldsymbol{\delta_H}$**=** 1.35 (3H, t, *J*=6.9 Hz, CH_3), 1.62-1.89 (6H, m, CH_2), 2.66 (2H, t, *J*=6.1 Hz, CH_2), 2.98 (2H, t, *J*=6.1 Hz, CH_2), 4.26 (2H, q, *J*=6.9 Hz, CH_2).

GC-MS: m/z 193 (M^+), t_R 9.25 mins.

4.2.4.3 General procedure for the preparation of IV

Ethyl cyanoacetate (0.53 cm^3, 5 mmol) and *p*-anisaldehyde (0.61 cm^3, 5 mmol) were added to a 10 cm^3 round-bottomed flask with aminated sporopollenin (sample SECs-N2, N_{basic} loading=1.29 mmol/g, 0.38g, 10% N_{basic}) and ethanol (5 cm^3), stirred at RT for 5h.

Then the mixture was filtered and washed with ethyl acetate (30 cm^3), the filtrate was evaporated by a rotary evaporator for 30min and the product was dried in a vacuum to give a constant weight.

2-Cyano-3-(4-methoxy-phenyl)-acrylic acid ethyl ester IV as a yellow solid, yield 94% (mass recovery × purity, 99.0 × 94.4), Table 2.14.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ_H= 1.37 (3H, t, *J*=7.1 Hz, CH_3), 3.88 (3H, s, OCH_3), 4.36 (2H, q, *J*=7.1 Hz, CH_2), 6.99 (2H, d, Ar), 7.99 (2H, d, Ar), 8.18 (1H, s, H).

^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ_C= 163.7, 163.1, 154.4, 133.6, 124.3, 116.2, 114.7, 99.3, 62.4, 55.6, 14.2.

GC-MS: m/z 231 (M^+), t_R 9.28 mins.

4.2.4.4 General procedure for the preparation of V

Ethyl cyanoacetate (0.53 cm^3, 5 mmol) and *o*-tolualdehyde (0.59 cm^3, 5 mmol) were added to a 10 cm^3 round-bottomed flask with aminated sporopollenin (sample SECs-N2, N_{basic} loading=1.29 mmol/g, 0.38g, 10% N_{basic}) and ethanol (5 cm^3), stirred at RT for 5h. Then the mixture was filtered and washed with ethyl acetate (30 cm^3), the filtrate was evaporated by a rotary evaporator for 30min and the product was dried in a vacuum to give a constant weight.

2-Cyano-3-o-tolyl-acrylic acid ethyl ester V as a yellow solid, yield 93% (mass recovery × purity, 93.0 × 99.9), Table 2.14.

1H NMR (400 MHz, $CDCl_3$, 25 °C): δ_H= 1.41 (3H, t, *J*=7.1 Hz, CH_3), 2.46 (3H, s, CH_3), 4.39 (2H, q, *J*=7.1 Hz, CH_2), 7.26-7.44 (4H, m, Ar), 8.16 (1H, s, H).

^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ_C=162.4, 153.4, 139.7, 132.8, 130.9, 130.5, 128.6, 126.7, 115.4, 104.4, 62.7, 19.8, 14.1.

GC-MS: m/z 225 (M^+), t_R 8.02 mins.

4.2.4.5 General procedure for the preparation of VI

Dimedone (1.4 g, 10 mmol) and benzaldehyde (0.51 cm^3, 5 mmol) were added to a 10 cm^3 round-bottomed flask with aminated sporopollenin (sample SECs-N2, N_{basic} loading=1.29 mmol/g, 0.38g, 10% N_{basic}) and ethanol (20 cm^3), stirred at RT for 5h. Then the mixture was filtered and washed with ethyl acetate (30 cm^3), the filtrate was evaporated by a rotary evaporator for 30min and the product was dried in a vacuum to give a constant weight.

Preparation of **VI** were carried out in same procedure only by using different amount of benzaldehyde, Table 2.15.

2,2'-(phenylmethanediyl)bis(3-hydroxy-5,5-dimethylcyclohex-2-en-1-one) VI as a yellow solid, yield 85% (mass recovery × purity, 85.0 × 99.9), Table 2.15.

^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ_H= 1.10 (6H, s, CH_3), 1.24 (6H, s, CH_3), 2.28-2.49 (8H, m, CH_2), 5.54 (1H, s, CH), 7.10-7.26 (5H, m, Ar), OH not observed.

^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ_C= 190.5, 189.4, 138.0, 128.2, 126.8, 125.8, 115.6, 47.0, 46.4, 32.7, 31.4, 29.6, 27.4.

GC-MS: m/z 368 (M^+), t_R 10.18 mins.

4.2.4.6 General procedure for other reactions without product

Compounds with active methylene groups A and aldehydes/ketone B were added to a 10 cm^3 round-bottomed flask with aminated sporopollenin (sample SECs-N2, N_{basic} loading=1.29 mmol/g, Xg) and ethanol (5 cm^3), stirred refluxing at 80 °C for Y h. Then the mixture was filtered and washed with ethyl acetate (30 cm^3), the filtrate was evaporated by a rotary evaporator for 30min and the product was dried in a vacuum to give a constant weight.

The reactant A and B, amount of aminated sporopollenin used X g and reaction time Y h are shown in Table 2.12.

4.2.5 Sulfonation of the sporopollenin and analysis

4.2.5.1 Preparation of sulfonated sporopollenin particles (SECs-S1, SECs-S2)

Method A: Sporopollenin (sample SECs-3, 10 g) and dichloromethane (200 cm^3) were added to a 0.5 L round-bottomed flask which was cooled with ice water. Chlorosulfonic (40 cm^3) was added slowly and then stirred for 4h at room temperature. Then the mixture was washed with water until the pH of the filtrate was 7 and dried by vacuum to a constant weight.

Method B: Sporopollenin (sample SECs-3, 10 g) and sulfuric acid (64%, 200 cm^3) were added to a 0.5 L round-bottomed flask, stirring and heating at 200 °C under a flow of nitrogen for 24 h. Then the mixture was washed with water until the pH of the filtrate was 7, dried by vacuum to a constant weight.

Results of elemental analysis and ICP-MS are shown in Table 2.16.

4.2.5.2 Acidity test of sulfonated sporopollenin particles (SECs-S1, SECs-S2)

Sulfonated sporopollenin particles (Sample SECs-S1, 0.1 g) were added to a 50 mL round-bottle flask with aqueous sodium hydroxide (0.1 M, 20 cm^3) and stirred overnight, and then the collected filtrate was titrated with hydrochloric acid (0.1 M, 20 cm^3) with methyl orange as an indicator.

The procedure of testing SECs-S2 was the same as the method to test SECs-S1.

The H^+ concentration of sulfonated sporopollenin particles was calculated by the equation:

$$n(H^+)+c(HCl) \times V(HCl)=c(NaOH) \times v(NaOH)$$

Results of the sulfonated sporopollenin acidity test are shown in section 2.5.4.

4.2.5.3 Structure test of sulfonated sporopollenin particles (SECs-S1, SECs-S2)

Sulfonated sporopollenin particles (SECs-S1, 0.5 g) were immersed with water (20 cm^3) and the pH of the solution was tested by pH meter.

Sulfonated sporopollenin particles (SECs-S1, 0.5 g) were added to a 50 mL round-bottom flask with aqueous sodium hydroxide (2 M, 20 cm^3) and stirred overnight, then filtered and washed with water (10 × 20 cm^3). The base treated particles were suspended in Hydrochloric acid (2 M, 20 cm^3) and stirred overnight, and then the solid were filtered and washed with water (10 × 20 cm^3). After dried under a vacuum, the dried particles were immersed with water (20 cm^3) and the pH of the solution was tested by pH meter.

The procedure of testing SECs-S2 was the same as the method to test SECs-S1.

Results of pH change are shown in section 2.5.4, and results of elemental analysis and ICP-MS are shown in Table 2.16.

4.2.6 General procedure for the preparation of VII

D-mannose (1 g) and sulfonated sporopollenin (SECs-S1, 0.5 g) were added to a 50 cm^3 round-bottomed flask with acetone (dried with potassium carbonate before use, 30 cm^3) and stirred for 18 h at RT. Then the mixture was filtered and washed with acetone (dried with potassium carbonate before use, 30 cm^3), the filtrate was evaporated by rotary evaporator for 30min. Then product was dissolved with diethyl ether (5 cm^3) and precipitated with hexane (20 cm^3). The recrystallized solid was dried in a desicator with a vacuum to a constant weight.

Preparation of **VII** was carried out by using sulfonated sporopollenin (SECs-S2, 0.5 g) in the same procedure as described above.

2,3:5,6-Di-*O*-isopropylidene-α-D-mannofuranose VII as a white solid, yield 68% (mass recovery × purity, 68.0 × 99.9), Table 2.18.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ_H= 1.30 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1,43 (3H, s, CH_3), 1.44 (3H, s, CH_3), 3.44 (1H, d, *J*=8.2 Hz, OH), 4.05 (2H, m, CH_2), 4.15 (1H, q, *J*=3.7 Hz, CH), 4.37 (1H, q, *J*=4.7 Hz, CH), 4.58 (1H, d, *J*=5.9 Hz, CH), 4.78 (1H, q, *J*=3.7 Hz, CH), 5.35 (1H, s, CH).

GC-MS: m/z 244 (M^+), t_R 9.65 mins.

4.2.7 General procedure for the preparation of VIII

Ethylene glycol (0.56 cm^3, 10 mmol) and cyclohexanone (1.04 cm^3, 10 mmol) were added to a 10 cm^3 round-bottomed flask with sulfonated sporopollenin (SECs-S1, 0.5 g) and cyclohexane (5 cm^3), stirred refluxing with a Dean-Stark trap at 80 °C for 3h. Then the mixture was filtered and washed with ethyl acetate (30 cm^3), the filtrate was evaporated by a rotary evaporator for 30min and the product was dried in a vacuum to give a constant weight.

Preparation of **VIII** was carried out by using sulfonated sporopollenin (SECs-S2, 0.5 g) in the same procedure as described above.

1,4-Dioxa-spiro[4.5]decane VIII as a colorless liquid, yield 66% (mass recovery × purity, 66.0 × 99.9),. Table 2.19.

^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ_H= 1.40 (4H, s, CH_2), 1.58 (6H, s, CH_2), 3.93 (4H, s, CH_2).

GC-MS: m/z 142 (M^+), t_R 7,51 mins.

4.3 Experimental procedures in particle-stabilized emulsions

4.3.1 Preparation of aminated sporopollenin particles

4.3.1.1 Aminated sporopollenin particles used to stabilize emulsions. (SECs-N2a, SECs-N2b, SECs-N2c, SECs-N2d, SECs-N2e and SECs-N2f)

SECs-N2a: The sporopollenin (SECs-3) was methylated first. To a stirred solution of simply extracted sporopollenin (SECs-3, 20 g) in acetone (300 cm^3) was added anhydrous powdered potassium carbonate (110 g). Dimethyl sulphate (38.0 cm^3) was added in portions for about 10 min at room temperatue. After the addition was completed the solution was heated to reflux and maintained for 3 h. After cooling, the mixture was filtered and washed with water (3 × 100 cm^3) to dissolve potassium carbonate, and then washed with methanol (3 × 100 cm^3) and DCM (3 × 100 cm^3), the particles were dried in a dessicator overnight to give a constant weight.

Methylated sporopollenin (2 g) and 1, 6-diaminohexane (18.0 cm^3, 0.134 mmol) were suspended in xylene (30 cm^3) and stirred under reflux with a Dean-Stark trap for 2h. The cooled aminated sporopollenin was washed with xylene (3 × 30 cm^3), HCl (2 M, 2 × 20 cm^3) and NaOH (2 M, 2 × 20 cm^3) successively, then the washing procedure was continued by using large scale water (5 × 200 cm^3) to reduce the conductivity of the filtrate until the value was less than 20 μS cm^{-1} . Finally the sporopollenin was washed with methanol (3 × 30 cm^3) and DCM (3 × 30 cm^3) successively; the particles were dried in a dessicator overnight to a constant weight.

The process for preparation of **SECs-N2b**, **SECs-N2c**, **SECs-N2d**, **SECs-N2e**, **SECs-N2f** were broadly consistent with the preparation of **SECs-N2a**, the only differences were the reaction time, SECs-N2b for 4 h, SECs-N2c for 8 h, SECs-N2d for 16 h, SECs-N2e for 24 h and SECs-N2f for 60 h.

Results of elemental analysis of these aminated sporopollenin particles are shown in Table 3.2.

4.3.1.2 Aminated sporopollenin particles made as reference (SECs-1a, SECs-1b, SECs-1c, SECs-1d, SECs-3a, SECs-3b and SECs-3c)

SECs-1a: Methylated sporopollenin (1.53 g) and 1,2-ethylenediamine (6.7 cm^3, 0.100 mmol) were suspended in xylene (30 cm^3) and stirred under reflux with a Dean-Stark trap for 24h. The cooled aminated sporopollenin was washed with xylene (3 × 30 cm^3), HCl (2 M, 2 × 20 cm^3) and NaOH (2 M, 2 × 20 cm^3) successively, then the washing procedure was continued by using large scale water (5 × 200 cm^3) to reduce the conductivity of the filtrate until the value was less than 20 μS cm^{-1} . Finally the sporopollenin was washed with methanol (3 × 30 cm^3) and DCM (3 × 30 cm^3) successively; the particles were dried in a dessicator overnight to a constant weight.

The process for preparation of **SECs-N1b**, **SECs-N1c**, **SECs-N1d** were broadly consistent with the preparation of **SECs-N1a**, the only differences were the reactants, SECs-N1b with 1,6-diaminohexane (13.8 cm^3, 0.100 mmol), SECs-N1c with

1,8-diaminooctane (14.4 g, 0.100mmol) and SECs-N1d with 1,12-diaminododecane (20.0 g, 0.100mmol).

All the aminations above follow the reactant ratio: 1.53 g of sporopollenin particles with 0.100 mmol of diamine, which is in consistent with the ratio used in the preparation of SECs-2a.

Results of elemental analysis of these aminated sporopollenin particles are shown in Table 3.1.

SECs-3a: Methylated sporopollenin (2 g) and 1,6-diaminohexane (9.0 cm^3, 0.067 mmol) were suspended in xylene (30 cm^3) and stirred under reflux with a Dean-Stark trap for 16 h. The cooled aminated sporopollenin was washed with xylene (3 × 30 cm^3), HCl (2 M, 2 × 20 cm^3) and NaOH (2 M, 2 × 20 cm^3) successively, then the washing procedure was continued by using large scale water (5 × 200 cm^3) to reduce the conductivity of the filtrate until the value was less than 20 μS cm^{-1} . Finally the sporopollenin was washed with methanol (3 × 30 cm^3) and DCM (3 × 30 cm^3) successively; the particles were dried in a dessicator overnight to a constant weight.

The process for preparation of **SECs-N3b**, **SECs-N3c** were broadly consistent with the preparation of **SECs-N3a**, the only differences were the scale of amine used, SECs-N3b with 1,6-diaminohexane (27.0 cm^3, 0.200 mmol) and SECs-N3c with 1,6-diaminohexane (36.0 cm^3, 0.267 mmol).

Results of elemental analysis of these aminated sporopollenin particles are shown in Table 3.2.

4.3.2 Wettability test

The relevant sporopollenin particles (10 mg) were placed on the surface of the appropriate liquid (5 cm^3) in a glass vessel (height 6 cm, i.d. 2.6 cm). The time for the particles to pass through the air-oil/water interface was recorded, Table 3.3.

4.3.3 Preparation and characterization of emulsions.

Emulsions of equal volumes (5 cm^3) of oil and a sporopollenin particle dispersion in water were prepared in a glass vessel (height 6 cm, i.d. 2.6 cm) by stirring with an Ultra-turrax homogeniser (2 min at 13000 rpm). The emulsion type was determined by the drop test immediately after the emulsification. This was done by placing one drop of emulsion separately into a small amount of water and of oil. Then the conductivity and pH of the emulsion were measured with a conductivity meter and pH meter (calibration before measuring). The fraction of water, f_{aq}, or oil, f_{oil}, were calculated from $f_{aq(oil)}=h_{aq(oil)}/0.5h_t$, where $h_{aq(oil)}$ is the total height of the water (oil) phase and h_t is the height of the liquid mixture. When the value of $f_{aq(oil)}$ is 0, the emulsion is completely stable, and the value equals to 1 means complete coalescence.

The height of the water layer was observed with a ruler after 30 mins, 1 h, 1 day, 3 days and 1 week after emulsification. One week after the emulsion had been made a photo

was taken by camera (SONY, DSC-TZ100V). The mean diameter of the emulsion droplets were measured by using ImageJ to take the average value of 20 droplets. All measurements were carried at 20 ± 2 °C.

CHAPTER 5 Conclusions

Sporopollenin has been extracted from fresh pollen and spores. The sporopollenin was first modified with either *N*,*N*-diethylethylenediamine, *N*,*N*-diethyl-1,3-propanediamine or diethylenetriamine. Elemental analysis and gravimetric analysis of hydrochloride salts were used to calculate the content of basic nitrogen groups. Then the aminated sporopollenin was used in Schotten-Baumann acylations and in Knoevenagel condensation as a supported reagent. In the initial attempt of Schotten Baumann acylation, the yield of product was poor, and then the sporopollenin was methylated before amination to give a higher loading of basic N, at the same time some functional groups on the surface of sporopollenin may effect the acylation were protected, then better yields of product from Schotten-Baumann acylation were obtained. Then the Knoevenagel condensation catalyzed by aminated sporopollenin was investigated to get the best conditions for different reactants. The results showed that the reaction between aldehyde with high activity like benzaldehyde and compounds with active methylene groups could be catalyzed by the aminated sporopoollenin successfully, and ketone with lower activity compare to aldehyde like cyclohexanone can also undergo the condensation but a higher temperature is needed. The extracted sporopollenin was also made in acid form by sulfonation with two methods, and the sulfonated sporopollenin was used as an effective catalyst in the preparation of an isopropylidene derivative and a cyclic acetal.

Another application of the modified sporopollenin is to stabilize emulsions. The extracted sporopollenin particles were aminated with 1,6-diaminohexane and their

behavior in stabilizing emulsions with different oils have been investigated. The aminated particles stabilized o/w type emulsion in acid environment as expected. However, the sporopollenin particles were methylated first before amination, the methyl groups did affect the hydrophilicity of the particles. So new investigation for this work should be carried out by using aminated sporopollenin without methylation to study the relationship of emulsion type and pH varies.

CHAPTER 6 References

[1] F. Zetsche, P. Kalt, J. Liechti and E. Ziegler, *J. Prakt. Chem*. **1937**, *148*, 267-286.
[2] P. D. Moore, J. A. Webb and M. E. Collinson, *Pollen analysis*, 2nd edition, **1999**, Blackwell Scientific Publications.
[3] G. Shaw, *Sporopollenin in Phytochemical Phylogeny*, J. B. Harborne (Ed), **1970**, Academic Press, London and New York, Chapter 3, 31-58.
[4] G. Shaw, The Chemistry of sporopollenin, in: *Sporopollenin*, J. Brooks, M. Muir, P. Van Gijzel and G. Shaw, (Eds) Academic Press, London and New York, **1971**, 305-348.
[5] R. G. Stanley and H. F. Linskens, *Pollen: biology, biochemistry, management*, **1974**, Springer, Verlag, Berlin, Heidelberg and New York.
[6] I. Feagri and J. Iversen, *Textbook of pollen analysis*, **1964**, Blackwell London.
[7] F. Zetsche and K. Huggler, *Liebig's Ann. Chem*. **1928,** *461***,** 89.
[8] F. Zetsche, P. Kalt, J. Liechti and E. Ziegler, *J. Prakt. Chem.* **1937**, *148*, 67-84.
[9] J. Brooks and G. Shaw, *Origin and development of living systems*, **1973**, Academic Press London and New York.
[10] U. Brunner and R. Honegger, *Can. J. Bot*. **1985**, *63*, 2221-2229.
[11] G. Shaw and D. Apperley, *Grana*. **1996**, *35*, 125-127.
[12] G. Erdtman, *Svensk Botanisk Tidskrift.* **1960**, *54*, 561-564.
[13] E. Dominguez, J.A. Mercado, M.A. Quesada and A. Heredia, *Grana*, Supplement 1. **1993**, 12-17.
[14] N. M. Tarlyn, V. R. Franceschi, J. D. Everard and F. A. Loewus, *Plant science.* **1993**, *90*, 219-224.
[15] K. E. Espelie, F. A. Loewus, R. J. Pugmire, W. R. Woolfenden, B. G. Baldi and P. H. Given, *Phytochemistry*, **1989**, *28*, 751-753.
[16] F. A. Loewus, B. G. Baldi, V. R. Franceschi, L. D. Meinert and J. J. McCollum, *Plant Physiol*. **1985**, *78*, 652-654.
[17] M. Couderchet, J. Schmalfus and P. Boger, *Pestic. Biochem. Phys*. **1996**, *55*, 189-199.
[18] S. Gubatz, M. Rittscher, A. Meuter, A. Nagler, R. Wiermann, *Grana*, Supplement 1 **1993**, 12-17.
[19] K. Schulze Osthoff and R. Wiermann, *J. Plant Phys*. **1987**, *131*, 5-15.
[20] F. Ahlers, J. Lambert and R. Wiermann, *Z. Naturforsch*. **1999**, ***54c***, 492-495.
[21] F. Zetsche and O. Kalin, *Helv. Chim. Acta*. **1931**, *14*, 517-519.
[22] F. Zetsche, P. Kalt, J. Liechti, E. Ziegler, *J. Prakt*, *Chem*. **1937**, *148*, 267.
[23] G. Shaw and A. Yeadon, *J. Chem. Soc*. **1966**, 16-22.
[24] J. Brooks and G. Shaw, *Grana*, **1968**, *8*, 2-3.
[25] G. Shaw and A. Yeadon, *Grana*, **1964**, *5*, 247-252.
[26] J. Brooks and G. Shaw, *Nature*, **1968**, *219*, 532-533.
[27] R.G. Achari, G. Shaw and R. Hollyhead, *Chem. Geol*. **1937**, *12*, 267.
[28] P. Fawcett, D. Green, R. Holleyhead and G. Shaw, *Grana*. **1970**, *10*, 246-247.
[29] A.K. Prahl, H. Springstubbe, K. Grumbach and R. Wiermann *Z. Naturforsch*. **1985**, *40c*, 621-626.
[30] G. Shaw and A. Yeadon, *Nature*, **1973**, *244*, 215-217.
[31] F. Ahlers, J. Lambert and R. Wiermann, *Z. Naturforsch*. **1999**, *54c*, 492-495.
[32] A.R. Hemsley, W.G. Chaloner, A.C. Scott and C.J. Groombridges, *Annals of Botany*. **1992**, *69*, 545-549.
[33] M. Kawase and M. Takahashi, *Grana*, **1995**, *34*, 242-245.
[34] G. Mackenzie and G. Shaw, *Int .J. Peptide Protein Res*. **1980**, *15*, 298-300.
[35] R. Adamson, S. Gregson and G. Shaw, *Int. J. Peptide Protein Res*. **1983**, *22*, 560-564.
[36] G. Shaw, M. Sykes, R. W. Humble, G. Mackenzie, D. Marsden and E. Pehlivan,

Reactive Polymers. **1988**, *9*, 211-217.
[37] E. Pehlivan, M. Ertos, S. Yildiz and H. Duncan, *Separ. Sci. Technol*. **1994**, *29*, 1757-1768.
[38] A. J. Havinga, *Pollen et Spores*. **1984**, *26*, 541-558.
[39] L. Brannon-Peppas, *Med. Plastics and Biomaterials*. **1997**, 34-44.
[40] V. V. Ranade, *J. Clin. Pharmacol*. **1990**, *30*, 107-120.
[41] E. Yilmaz, M. Sezgin, and M. Yilmaz, *Journal of Molecular Catalysis B: Enzymatic*. **2010**, *62*, 162–168
[42] V. N. Paunov, G. Mackenzie and S. D. Stoyanov, J. Mater. Chem. **2007**, *17*, 609–612
[43] S. A. Hamad, A. K. Dyab, S. D. Stoyanov and V. N. Paunov, *J. Mater. Chem.* **2011**, ***21***, 18018
[44] A. Wakil, G. Mackenzie, A. D. Taboada, J. G. Bell and S. L. Atkin, *Lipids*. **2010**, *45*, 645–649
[45] A. J. Havinga, *Pollen et Spores*. **1984**, *26*, 541-558.
[46] W. D. Bennet, J. W. Christensen, L. K. Hamaker, M. L. Peterson, M. R. Rhodes and H. H. Saneii (Eds) *Advanced ChemTech Handbook of Combinatorial & Solid Phase Organic Chemistry*, **1998**.
[47] Novabiochem *2000 Catalogue Including Solid Phase Peptide Synthesis, Solid Phase Organic Synthesis and Polymer Supported Reagent Handbook.*
[48] F. Guiller, D. Orain and M. Bradley, *Chem. Rev.* **2000**, *100*, 2091-2157.
[49] B. Dorner, R. Steinauer and P. White, *Chimia*. **1999**, *53*, 11-17.
[50] J. Fruchtel and G. Jung, *Angew. Chem. Int. Ed. Engl*. **1996**, *35*, 17-42.
[51] F. Z. Dorwald, *Organic Synthesis on Solid Phase, Supports, Linkers, Reactions*, **2000**.
[52] A. R. Brown, P. H. H. Hermkens, H. C. J. Ottenheijm and D. C. Rees, *Synlett*. **1998**, 817-827.
[53] P. H. H. Hermkens, H. C. J. Ottenheijm and D. C. Rees, *Tetrahedron*. **1997**, *53*, 5643-5678.
[54] S. V. Ley, I. R. Baxendale, R. N. Bream, P. S. Jackson, AS. G. Leach, D. A. Longbottom, M. Nesi, J. S. Scott, R. I. Storer and S. J. Taylor, *J. Chem. Soc., Perkin Trans*. **2000**, *1*, 3815-4195.
[55] C. C. Leznoff, *Chem. Soc. Rev*. **1974**, *3*, 65-85.
[56] S. W. Kaldor and M. G. Siegal, *Curr. Opin. Chem. Biol*. **1997**, *1*, 101-106.
[57] A. Akelah, *Reactive Polymers*. **1988**, *8*, 273-274.
[58] R. J. Booth, *J. Am. Chem.Soc.* **1997**, *119*, 4882-4886.
[59] R. B. Merrifield, *J. Am. Chem. Soc*. **1963**, *85*, 2149-2154.
[60] R. Letsinger and M. J. Kornet, *J. Am. Chem. Soc*. **1963**, *85*, 3045-3046.
[61] R. Letsinger and V. Mahadevan, *J. Am. Chem. Soc*. **1965**, *87*, 3526-3527.
[62] J. M. Frechet and C. Schuerch, *J. Am. Chem. Soc*. **1971**, *93*, 492-496.
[63] R. D. Guthrie, A. D. Jenkins and J. Stehlicek, *J. Chem. Soc*. **1971**, 2690-2696.
[64] J. I. Crowley and H. Rapoport, *Acc. Chem. Res*. **1976**, *9*, 135-144.
[65] C. C. Leznoff, *Acc. Chem. Res*. **1978**, *11*, 327-333.
[66] C. C. Leznoff and T. M. Fyles, *J. Chem. Soc. Chem. Comm*. **1976**, 251-252**.**
[67] R. Brown, *Contemporary Organic Synthesis*. **1997**, *4*, 216-237.
[68] F. Balkenhol, C. Von dem Bussche-Hunnefeld, A. Lansky and C. Zechel, *Angew. Chem. Int. Ed. Engl*. **1996**, *35*, 2288-2337.
[69] S. E. Hall, *Molecular Diversity*. **1999**, *4*, 131-142.
[70] P. H. H. Hermkens, H. C. J. Ottenheijm and D. Rees, *Tetrahedron*. **1996**, *52*, 4527-4554.
[71] J. M. J. Frechet, *Tetrahedron.* **1981**, *37*, 663-683.
[72] L. A. Thompson and J. A. Ellman, *Chem. Rev.* **1996**, *96*, 555-600.
[73] M. Smith, *New Scientist*, **1996**, 22-25.

[74] RSC Residential School: Solid Phase Chemistry and its Incorporation into Pharmaceutical Strategy. Wed. 15 to Fri. 17 September, 1999, Southampton University. Lectures from:
a) G. Johnston (IRORI) Europe Ltd, Solid Phase Combinatorial Chemistry with Non-invasive Encoding of Compounds.
b) C. Ruddick (Mettler-Toledo Myriad Ltd), the Mettler-Toledo Myriad Personal Synthesiser.
c) M. Bradley, (Southampton University) Solid Phase Synthesis, Linkers, Scavengers and Reagents.
[75] Polymer Laboratories, *StratoSpheres™Range of Solid Phase Synthesis Resins, Scavenger Resins and Solid Supported Reagents*. Issue 2.
[76] R. B. Merrifield, *Angew. Chem. Int. Ed. Engl*. **1985**, *24*, 799-892.
[77] R. Kunin, E. Meitzner and N. Bortnick, *J. Am. Chem. Soc*. **1962**, *84*, 305-306.
[78] a) Argonaut Technologies, Trident Automated Synthesiser System, www.argotech.com
b) Argonaut Technologies, *Resins and Reagents for Accelerating Synthesis and Purification*. **2000**.
[79] E. Bayer, *Angew. Chem. Int. Ed. Engl*. **1991**, *30*, 113-129.
[80] G. Jung, *Comb. Pept. Nonpept. Libr.* **1996**, VCH, Weinheim, Germany.
[81] S. H. DeWitt, J. S. Kiely, C. J. Stankovic, M. C. Schroeder, D. M. R.Cody and M. R. Pavia, *Proc. Natl. Acad. Sci*. **1993**, *90*, 6909-6913.
[82] O. W. Gooding, S. Baudart, T. L. Deegan, K. Heisler, J. W. Labadie, W. S. Newcomb, J. A. Porco, JR. and P. Van Eikeren, *J. Comb. Chem*. **1999**, *1*, 113-122.
[83] M. Meldal, *Tetrahedron Lett*. **1992**, *33*, 3077-3080.
[84] Lancaster Chemical Catalogue, 2000-2001.
[85] E. Atherton, E. Brown and R. C. Sheppard, *J. Chem. Soc. Chem. Commun*. **1981**, 1151-1152.
[86] D. C. Sherrington, *Chem. Commun*. **1998**, 2275-2286.
[87] A. Dryland and R. C. Sheppard, *J. Chem. Soc. Perkin Trans*. **1986**, *1*, 125-137.
[88] P. W. Small and D. C. Sherrington, *J. Chem. Soc*. **1989**, 1589-1591.
[89] J. Eichler, M. Bienert, A. Stierandova and M. Lebl, *Peptide Research*. **1991**, *5***,** 296-307.
[90] B. J. Backes and J. A. Ellman, *Curr. Opin. Chem. Biol*. **1997**, *1*, 86-93.
[91] I. W. James, *Tetrahedron*. **1999**, *55*, 4855-4946.
[92] J. R. Morphy, *Curr. Opin. Drug Disc. Develop*. **1998**, *1*, 59-65.
[93] B. A. Bunin, *the Combinatorial Index*. **1998**. Academic Press, San Diego.
[94] H. Eggenweiler, *Drug Disc.Today*. **1998**, *3*, 552-560.
[95] B. F. Gisin, *Helv. Chim. Acta*. **1973**, *56*, 1476-1482.
[96] P. M. Worster, C. R. McArthur and C. C. Leznoff, *Angew. Chem. Int. Ed. Engl*. **1979**, *18*, 221-222.
[97] I. E. A. Deben, T. H. M. V. Wijjk, E. M. V. Doornum and P. H. H. Hermkens, *Mikrochim. Acta*. **1997**, *14*, 245-246.
[98] B. Yan, C. F. Jewell, Jr. and S. W. Myers, *Tetrahedron*. **1998**, *54*, 11755-11766.
[99] B. J. Egner and M. Bradley, *Drug Disc. Today*. **1997**, *2*, 102-109.
[100] S. Sussman, *Ind. Eng. Chem*. **1946**, *38*, 1228
[101] R. Adamson, S. Gregson and G. Shaw, *Int. J. peptide Protein Res*. **1983**, *22*, 560-564.
[102] C. Schotten. *Ber. Dtsch. Chem. Ges*. **1884**, *17*, 2544–2547.
[103] E. Baumann. *Ber. Dtsch. Chem. Ges*. **1886**, *19*, 3218–3222.
[104] C. Wiles, P. Watts and S. J. Haswell, *Tetrahedron*. **2004**, *60*, 8421-8427
[105] L. F. Tietze and N. Rackelman. *Multicomponent Reactions*. **2005**, 121-168. Wiley-VCH, Weinheim.

[106] G. Jone, *Organic Reactions*, **1967**, *15*, 204-599. Wiley, New York.
[107] G. R. Krishnan and K. Sreekumar, *Eur. J. Org. Chem*. **2008**, 4763-4768.
[108] D. J. Macquarrie and D. B. Jackson, *Chem. Commun*. **1997**, 1781.
[109] D. J. Macquarrie, J. H. Clark, A. Lamber, J. E. G. Mdoe, A. Priest, *Elsevier Science*. **1997**, *35*, 153-158.
[110] G. Kaupp, M. Naimi-Jamal and J. Schmeyers, *Tetrahedron*. **2003**, *59*, 3753-3760.
[111] H. B. Hass and M. L. Bender. *J. Am. Chem. Soc*. **1949**, *71*, 1767-1769.
[112] V. Singh and S. Batra. *Tetrahedron*. **2008**, *64*, 4511.
[113] A. Padwa and S. K. Bur. *Tetrahedron*. **2007**, *63*, 5341.
[114] J. Chen, J. Shi and C. G. Yan, *Chem. Res. Chinese Universities*. **2011**, *27*, 49-53.
[115] G. Shaws and M. Sykes. *Reactive Polymers*. **1988**, *9*, 211-217.
[116] W. Deng, M. Liu, Q. Zhang, X. Tan and Y.Wang, *Chem. Commun*. **2010**, *46*, 2668-2670
[117] J. A. Mills. *Adv. Carbohydr. Chem*. **1955**, *10*, 1-53.
[118] P. P. Singh, M. M. Gharia, F. Dasgupta and H. C. Srivastava. *Tetrohedron Lett*. **1977**, 439-440.
[119] B. Lal, R. M. Gidwani and R. H. Rupp, *Synthesis*. **1989**, 711-713.
[120] A. P. Rauter, F. Ramőa-Ribeiro, A. C. Fernandes and J. A. Figueiredo, *Tetrohedron*. **1995**, *51*, 6529-6540.
[121] V. K. Rajput and B. Mukhopadhyay. *Tetrahedron Letters*. **2006**, *47*, 5939-5941.
[122] E. A. Decker, *McClements Food hydrocolloids*. **1999**, *13*, 419-424.
[123] B. P. Binks, *Modern Aspects of Emulsion Science*. **1998**, 1-33, Royal Society of Chemistry (Cambridge).
[124] A. T. Hubbard, *Encyclopedia of Surface and Colloid Science*, **2007**, 4230.
[125] B. P. Binks and T. S. Horozov, *Colloidal Particles at Liquid Interfaces*, ed. **2006**, 1-74. Cambridge University Press, Cambridge.
[126] A. F. Koretsky and P. M. Kruglyakov. *Izv. Sib. Otd. Akad. Nauk USSR*, **1971**, ***2***, 139.
[127] B. P. Binks, *Curr. Opin. Colloid Interface Sci*. **2002**, ***7***, 21.
[128] T. S. Horozov, B. P. Binks, R, Aveyard and J. H. Clint. *Colloid. Surface. A*. **2006**, *377*, 282-283.
[129] B. P. Binks and S. O. Lumsdon, *Langmuir*. **2000**, *16*, 8622.
[130] N. W. Yan, M. R. Gray and J. H. Masliyah, *Colloids Surf. A*. **2001**, *193*, 97.
[131] S. Stiller, H. Gers-Barlag, M Lergenmueller, F. Pflücker, J. Schultz, K. P. Wittern and R. Daniels, *Colloids Surf. A*. **2004**, *232*, 261.
[132] R. Aveyard, B. P. Binks and J. H. Clint, *Adv. Colloid Interfac*. **2003**, *503*, 100-102.
[133] R. J. G. Lopetinsky, J. H. Masliyah and Z. Xu. *Colloidal Particles at Liquid Interfaces*. **2006**, 186-224. Cambridge University Press (Cambridge).
[134] B. R. Midmore, *Colloids Surf*. A **1998**, *132*, 257.
[135] D. E. Tambe and M. M. Sharma, *J.Colloid Interface Sci*. **1993**,*157*,244.
[136] B. P. Binks and S. O. Lumsdon, *Langmuir* **2000**,*16*,2539.
[137] N. Yan, J. H. Masliyah, Colloids Surf. A. **1995**, *96*, 229.
[138] S. Melle, M. Lask and G. G. Fuller. *Langmuir* **2005**, *21*, 2158.
[139] B. P. Binks and J. A. Rodrigues, *Angew. Chem. Int, Ed*. **2005**, *44*, 441.
[140] S. Levine, B. D. Bowen and S. J. Partridge. *Colloids Surf*. **1989**, *38*, 325.
[141] Z. Du, M. P. Bilbao-Montoya, B. P. Binks, E. Dickinson, R. Ettelaie and B. S. Murray, *Langmuir*. **2003**, *19*, 3106.
[142] B. P. Binks, J. H. Clint, G. Mackenzie, C. Simcock and C. P. Whitby, *Langmuir*, **2005**, *21*, 8161.
[143] B. P. Binks, A. N. Boa, M. A. Kibble, G. Mackenzie and A. Rocher, *Soft Matter*. **2011**, *7*, 4017-4024
[144] B. P. Binks and J. A. Rodrigues. *Angew. Chem. Int. Ed*. **2005**, *44*, 441.

[145] J. Li and H. D. H. Stőver, *Langmuir*. **2008**, *24*, 13237.