

Association of Public Analysts

Mastership in Chemical Analysis Examination

Training Guide

Microscopy (2nd Edition)

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FOREWORD

This study guide is one of a series produced by the Training Committee of the Association of Public Analysts for use in the profession. It is particularly directed at candidates preparing for the Mastership in Chemical Analysis (MChemA) examination. The Committee would welcome corrections to the text, if necessary, and constructive comment on ways of improving future editions. Correspondence should be sent to the secretary at the address given at the foot of the acknowledgements page.

Other training guides published by the Association of Public Analysts training committee are:-

Audio-Visual Resources

Candidate's Record of Professional Training and Experience

Certificate Writing

Food Complaints

Legislation

Study Guide for the MChemA

Microbiology (in preparation)

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March 1998.

INTRODUCTION

The first edition of this guide, issued in November 1990, comprised three lists and a microscopy bibliography. The lists were :

- (1) 'Range of Materials to be Studied'
- (2) 'Specimens available from the Association'
- (3) 'Materials Set for Examination 1970 - 1990'.

Following a review of the guide by the Training Committee it was decided to expand the contents to include a more detailed study guide on the subject of microscopy as well as updating the lists and bibliography previously published.

The syllabus for Part C of the examination for the Mastership in Chemical Analysis (MChemA) includes 'Microscopical examination of food, drugs and other materials, together with the identification of foreign and extraneous contaminants, this will include both qualitative and quantitative aspects'. The theory and practical application of microscopy may also be examined in the written papers of parts A, B1 & B2.

Candidates preparing for the MChemA are therefore facing a rather daunting task in preparing themselves to address this requirement for a very broad knowledge of microscopy and its application to the work of a public analyst. This training guide is intended to provide a structured approach to the acquisition of the skills and knowledge required. It cannot and does not attempt to be a definitive text on microscopy. There are many texts, some of which are included in the bibliography that will provide the detail needed by any student of the subject. This guide simply attempts to provide the signposts along the route for trainee microscopists and may also assist those who wish to become competent in microscopy for accreditation purposes .

Microscopy is not a subject that can be covered quickly. It requires time to be spent acquiring the body of knowledge that provides a personal library. It is therefore strongly recommended that a personal microscopy file (a lever arch file or bound notebook is ideal) is set up as soon as the first view down a microscope is taken, and that this file is updated every time the microscope is used. In this way a comprehensive training record will emerge and this personal aide memoir will very quickly become an invaluable tool.

Microscopy has a major role to play in the everyday work of a public analyst laboratory. It has several major advantages:

- ◆ requires little sample
- ◆ can be non-destructive
- ◆ portable
- ◆ time-saving
- ◆ economical
- ◆ interesting

THE ROUTE

1. Get to know your microscope

It is essential to obtain a general working knowledge of the light microscope. This is best achieved by obtaining a good basic text on the subject and sitting at a microscope with the book and the manufacturer's instruction manual. An excellent inexpensive book on the subject is published by the Royal Microscopical Society ¹. The following subject areas should be covered.

- ◆ types of microscope
- ◆ construction of the microscope
- ◆ function of parts
- ◆ types of illumination
- ◆ magnification
- ◆ setting up the microscope and optimization of the light path and resolution
- ◆ optical techniques - polarisation, phase contrast
- ◆ accessories - dispersion staining, oil immersion lens, quarter wave plates, rotating stage
- ◆ care and routine maintenance

In learning about the construction and use of the microscope most books will recommend various substances to demonstrate the capabilities of the technique. One of the best is an aqueous slurry of diatomaceous earth placed upon a slide with a coverglass laid gently on the surface. This will reveal hundreds of fascinating structures that grow in detail with increasing skill at setting up the microscope.

2. Looking at your specimen

For examination by a compound microscope, a specimen needs to be translucent. Translucency can be achieved in many ways, dependent on the article to be examined - powdered, smeared, squashed, sectioned, even moulded to leave an image e.g. hairs in polyvinyl alcohol (PVA) solution. There is a detailed account of the preparation of food for microscopical examination in Food Microscopy by Olga Flint ². Most microscopical examination requires the specimen to be mounted on a glass slide in a liquid medium with a protective cover glass slip. A vast amount of all observations can be made using water as the liquid medium, or a solution of 50% glycerol in water which tends to dry out less quickly. Other mounting liquids are available that provide better clarity by clearing the image e.g. chloral hydrate by dissolving starch and decolourising. Various dyes and staining techniques can also be used to highlight the presence of certain constituents e.g. iodine for starch.

It is important to remember that the processes involved in preparing and mounting a specimen will affect its characteristics and these changes must be taken into account when attempting an examination for identification or investigation. The most famous microscopical artefact - the electron micrograph of the bimolecular cell membrane structure was extensively documented in books and journals - but it was later shown to be an artefact created by the procedure used to prepare the material for examination.

¹ An Introduction to the optical microscope, 2nd edition, 1989, Savile Bradbury, OUP ISBN 0-19-856419-8

² Food Microscopy: a manual of practical methods, using optical microscopy, Olga Flint, BIOS Scientific Publishers ISBN 1-8872748-04-X.

3. Structure

Once a specimen is placed on the slide and observed it will be necessary to decide what type of material is present. It is therefore essential to build a knowledge base of the typical structures of the major classes of materials. These may be broken into the following sections:

3.1 Plants

roots	fruits	wood
stems	seeds	algae
leaves	starches	moulds
flowers	pollens	

3.2 Animals

muscle	hair	skin
fatty tissue	insects	connective tissue
offal	spiders	blood
bone	mites	molluscs
nail	fish scales	crustacea

3.3 Minerals and Man-made Materials

asbestos	dusts	paper
mica	fly ash	textiles

The basic components of structure should be studied with the assistance of a text book such as Maud Jepson's *Biological Drawings*³, Winton⁴, McCrone's *Particle Atlas*⁵.

The list of materials in Appendix A gives examples of the above categories that may be useful to observe.

4. Practice

Once the basic structure of materials has been studied it is then possible to start to differentiate between different forms of the same structural type, by identifying characterising features. Reference textbooks such as Jackson & Snowdon⁶, and Greenish & Collin⁷ contain excellent diagrams showing the characterising features of many plant materials. It is however, most important that you form your own personal interpretation of these features and characteristics as the recognition process is based on mental recall of structures observed previously.

Simple sketch diagrams assist greatly in forming a record for future reference.

³ *Biological Drawings with notes Parts 1 & 2*, 28th impression 1982, Maud Jepson, John Murray (Publishers) Ltd ISBN 0 7195 0726 X.

⁴ *The Structure and Composition of Foods, Vol I to IV*, Winton A.L., Winton K.B., Wiley 1939.

⁵ *The Particle Atlas, Edition 2, Vol II*, Walter C. McCrone & John G. Delly, Ann Arbor Science Publishers Inc. ISBN 0-250-40008-1.

⁶ *Powdered Vegetable Drugs*, Jackson, B.P. and Snowdon, D.W., Stanley Thornes, London 1974 ISBN 859500055.

⁷ *Anatomical Atlas of Vegetable Powders*, Greenish, H.G., and Collin, E., Churchill, London 1904.

5. Practising for the MChemA Examination

5.1 Comparison Specimens and Unknowns

It is unlikely, particularly in the early stages of learning microscopy, that many unknowns will be identified without consulting comparison specimens. It is recommended that a comparison specimen is examined if one is available. There is no APA reference collection of microscopy specimens but the secretary to the Training Committee will know who holds the collection of specimens used for the Reading MChemA training course.

It is understood that, during the MChemA practical microscopy examination, a candidate can ask for specified reference specimens to confirm their identification.

When practising microscopy it is a good idea to get a senior member of staff (preferably with the MChemA) to set up a series of unknowns, contaminated specimens, etc. as a test. This can be done even from the early stages e.g. when examining starches or herbs.

Experience of teaching microscopy has shown that most people respond well to the challenge that is created by such 'unknowns'.

5.2 Diagrams

If evaluating unknown materials for the MChemA exam, it is important always to endeavour to draw diagrams of what you observe, together with brief notes of your observations. This allows the examiners to give some credit for the work done even if the specimen has not been positively identified.

Even in everyday work, such detailed information can be extremely useful for future reference.

The following procedure has been developed by several lecturers at the Reading MChemA training course to provide a structured approach to the identification of unknowns. Although quite detailed in parts it is not intended to be a definitive guide, but simply a useful tool.

6. General Procedure For Evaluating Unknown Powdered Materials And Substances

6.1 Note the appearance, colour and texture of the specimen.

With care, note the odour. Do not taste unless you have good knowledge of the source of the specimen. (Be aware of the laboratory policy on the tasting of specimens). Always write notes and record simple diagrams of what you observe.

6.2 With the whole specimen spread out onto a sheet of white paper, inspect with a hand-lens.

Pick out anything which looks unusual or different from the rest of the specimen for separate examination. Look out, in particular, for insects, insect parts, mould contamination, stalk material, stones, seed coating etc. It might be possible to achieve a partial separation of mixtures at this stage, for example with 'mixed herbs', or a mixture of vegetable fragments with a starch. It is best to move the specimen systematically from one side of the paper to another, in a thin layer with a spatula, to facilitate examination for contaminants.

6.3 Prepare an aqueous mount of the specimen, or the separated components.

Place a small quantity of the material (the amount to use comes with practice) onto a clean slide. Wet the material with a drop of water, alcohol/water, or glycerine/water.

Note: bottles of glycerine water are prone to develop mould growth. A small globule of mercury in the bottom of the bottle will inhibit this mould growth.

Disperse with a spatula/glass rod and carefully place a clean cover-slip over the wetted material, taking care to prevent or eliminate air bubbles as far as possible.

6.4 View the material under the lowest power of the microscope

For example use a 4x objective and a 10x eye piece, and scan the whole of the slide. This is important - do not start on a high power and do not examine just part of the slide.

If any characteristic features are seen, then higher magnifications (100x or 400x) can be used - but always revert to the lowest power for scanning or searching.

6.5 Try the application of crossed polars.

A piece of Polaroid film is placed on top of the condenser, or at some other place below the stage. This is the polariser. A further piece of Polaroid film (the analyser) is placed over the eyepiece and rotated until a dark background is produced. Birefringent material appears bright on a dark background. Isotropic material e.g. glass remains dark. Starch grains generally show a characteristic 'cross' pattern.

6.6 If the specimen is mostly or completely opaque, remove the cover-slip and macerate the specimen with a spatula. This may break up the material sufficiently for some structures to be seen. It may also break up superimposed layers of cells and transverse sections of, for example, leaf specimens, may be visible. At this stage oil globules may also be observed. If these are sufficient to cause problems, it may be advisable to defat the specimen and re-examine.

6.7 Add a drop of iodine solution to the side of the cover-slip so that it seeps underneath and mixes with the specimen. View the reaction, if any.

Starch----Blue/Violet----Black

Protein----Yellow

Note the structures which stain (grains, conglomerates, amorphous material etc.). If

necessary, view several slides - some of the 'characteristic' features shown in the textbooks are not as common as might be expected !

Cooked starch loses the characteristic grain structure and stains pink / red with iodine.

Note: No matter how good the microscopist, it is unlikely that everything visible on a slide can be identified. Learn to recognise what is characteristic, and it is only practice which will give the confidence to do this.

The general nature of the specimen should now be suspected. If it is of vegetable origin the action of 'clearing agents' should be examined.

6.8 Clearing of Specimens

This is the procedure whereby opaque vegetable tissue is rendered transparent, or partially transparent, to light such that characteristic microscopical features might be seen. These clearing procedures dissolve any starch or protein present and, therefore, some features are lost. Do not omit the examination of the aqueous mount, as a preliminary, or the starch and protein characteristics may be missed.

The material left behind after clearing is mostly the cellulose cell walls, lignified tissue and mineral material. Some specimens clear easily, e.g. parsley, others are more difficult, e.g. castor seed, and conditions and procedures must be adapted to suit the specimen in hand.

Two procedures commonly used are chloral hydrate and acid/alkali (fibre preparation).

6.8.1 Chloral Hydrate

The reagent is prepared by dissolving 50 grams of chloral hydrate in 20 ml of water. (Caution chloral hydrate is hazardous to health).

There are two ways of proceeding, both of which have advantages and disadvantages -- 'slide' and 'tube/beaker'.

Slide: A small quantity of the specimen is placed on a clean slide and mixed with 2-3 drops of the chloral solution. The use of a cover-slip is optional at this stage. The slide is warmed, either over a Bunsen flame, or on a hotplate, or on a boiling waterbath until the specimen takes on a 'transparency'.

Replenish with chloral if necessary (do not allow it to evaporate completely to dryness). Finally, allow to cool slightly and place a cover-slip over the specimen, adding sufficient chloral solution or glycerine.

Do not add water to the slide since it will often cause precipitation of materials dissolved by the chloral solution.

There are two major potential drawbacks of clearing on a slide - only a limited quantity of specimen can be placed on the slide, and the small quantity of chloral will evaporate if strong heat is used to clear difficult subjects. Therefore, if there is plenty of specimen, or if the substance is difficult to clear then the following procedure is recommended.

Tube / Beaker : Take a small spatula load of the specimen in a test tube or a small

beaker, add 1-2 ml of chloral solution and boil gently over a Bunsen flame until clear. The specimen, or part of it at least, may then be transferred to a clean slide by means of a spatula or a piece of glass tubing. Using a piece of glass tubing or a spatula pick out parts of the seed coats of ground seeds, - in other words, select 'characteristic' parts of the unknown.

The disadvantages of this technique are that it uses a comparatively large amount of specimen (which might not always be advisable) and, furthermore, any mineral matter present e.g. sand will sink to the bottom of the tube or beaker and might be missed when part of the material is taken out with a spatula or glass tubing.

However, one advantage of this technique is that several slides can be prepared from the one 'clearing'. Furthermore, it is possible advantageously to concentrate uncommon but characteristic features of a specimen.

The action of chloral often liberates oil globules, and if there are so many that they interfere with viewing of the structures of the specimen, then the specimen should be re-examined after defatting. Alternatively, a specimen which has been defatted; e.g. a defatted pulverised nut kernel, may be encountered. If this occurs, remember to mention the 'defatted state' when reporting on the specimen. Again, do not be tempted to add water to the chloral hydrate after clearing.

6.8.2 Acid/Alkali

The 'preparation of a crude fibre' is much more vigorous than a chloral hydrate clearing and tends to remove more colouring matter from coloured specimens.

Procedure

- i) Defat a portion of specimen, if necessary, with a small quantity of diethyl ether. TAKE CARE HIGHLY INFLAMMABLE
- ii) Boil in a test tube with some 0.1M hydrochloric acid
- iii) Filter and wash with water
- iv) Transfer back to a test tube and boil with 0.1M caustic soda
- v) Filter, wash with water and then alcohol
- vi) Transfer to a beaker or tube and examine slides prepared from the residue

A fibre preparation is advantageous when dealing with a particularly starchy material, e.g. cereals, since chloral can become 'overloaded' and exhausted with the starch forming a gel-mass.

If the specimen remains deeply coloured or opaque after a clearing process it may be due to a massive amount of pigmentation. This can be eliminated by bleaching with 'Domestos' or a similar chlorine preparation.

Castor seed is a particular substance which causes problems of pigmentation, and it is useful to examine some castor seed powder by this technique as an exercise.

Very hard woody substances such as nut shells or fruit kernels might require even more drastic treatment to reveal their characteristic feature, using a mixture known as Schultzes Maceration Fluid, (50% nitric acid plus potassium chlorate), which destroys lignin and breaks up such hard tissue.

6.9 Staining of Specimens

The action of iodine solution is one commonly used staining reaction, and there are many other biological stains which can be employed to do a myriad of different jobs. Two particularly useful ones are described below :

6.9.1 Phloroglucinol Staining.

A stain which has been found to be of general use and application in plant tissue identification is phloroglucinol, which stains lignified tissue pink-red.

Procedure

To a part of the specimen cleared with chloral hydrate solution, add 2-3 drops of phloroglucinol solution (1% in alcohol) and evaporate almost to dryness. Add 2-3 drops of concentrated hydrochloric acid and examine. The procedure can be carried out on a slide, or in a small beaker. Lignified tissue is stained pink or red. It is useful when characterising seed/fruits, as well as the more obvious woods, barks, roots and also for picking up 'sawdust', nut shell or stone cells, when present as a contaminant.

6.9.2 Toluidine blue

This is a basic dye widely used for staining because it has metachromatic properties which can be used to identify the many food constituents which contain anionic groups. Details of how the dye works can be found in Food Microscopy by Olga Flint².

Preparation of toluidine blue stain mountant

Dissolve sufficient toluidine blue (as certified by the American Biological Stain Commission, dye content 85 – 94%, available from Sigma or Aldrich) in distilled water to give the equivalent of 70 mg of pure dye per 100ml. Add 37.8 g of glycerol to 70 ml of aqueous toluidine blue, mix and add 1.01 g of phenol. Allow to stand overnight.

Procedure

Using a dropper place one or two drops of the stain on a section, smear or squash of the specimen so that it is completely covered. Leave for 1 minute, apply a coverglass and leave for another minute before inverting and pressing very gently on blotting paper to remove excess stain. The results seen with brightfield illumination are summarised below:

Component	Toluidine blue stain effect
muscle fibres	pale blue with fresh meat products, more purple if phosphate present, darker blue if heat treated
muscle fibre nuclei	red-violet
raw collagen	pale pink
cooked collagen	pale lilac
fibroblasts	blue-violet
elastin fibres	Turquoise
soya protein (grits, isolate, TVP)	dark purple-blue
soya and other plant cell walls	Magenta
wheat gluten	pale blue-green
lignified cellulose from onion, spices, bran etc.	dark blue or blue-green
rusk	Unstained
fat	unstained (unless acidic)
fatty acids	pale blue
food gums (not starch based)	pink, purple, magenta

6.10 Examination of Inorganic Materials and Atmospheric Deposits

The structure and characteristics of inorganic powders and atmospheric deposits are best observed without a cover-slip and using 'top oblique' illumination. The best procedure to adopt is to moisten a small part of the specimen on a slide with water, then allow to evaporate to dryness on a boiling water bath. This generally 'fixes' the material to the slide surface. The top oblique illumination is arranged so that it does not pass through the condenser system of the microscope, but illuminates the object from the top and from the side. The surface details of the (naturally) opaque particles can then be seen more easily than when transmitted illumination is used. A white background with oblique illumination is often an advantage.

6.11 Contaminants

One of the problems encountered by Public Analysts in their work is of the detection and identification of contaminants. The first step in this process is to examine the specimen under a binocular microscope using a fairly low power zoom lens. In any problems set for microscopy, the following potential contaminants should be searched for :-

Insects and/or insect parts. These can be very small and any dark-coloured 'specks' noted during the initial visual examination should be separated and examined.

Insect excreta and webbing, also referred to as "frass". This may be lost completely on clearing but should be obvious on close visual examination.

Sand/mineral matter. Detected by the 'gritty' texture under a coverslip, and by its birefringence under crossed polars. Beware of being misled by calcium oxalate crystals in some powdered plant materials.

Glass is isotropic, and does not exhibit birefringence under crossed polars.

Sawdust is detected easily by phloroglucinol staining. Examine both hardwood and

softwood sawdusts as 'practice standards'.

Nutshells/fruit stones. These contain much lignified tissue, which stains well with phloroglucinol. If very dense, it may require more vigorous clearing (see above, with Schultz's Maceration Fluid) for positive identification of the species or the source. However, the simple identification of "nutshell/fruit stone" may be adequate for most purposes.

Rodent Contamination. Rodent faeces are a characteristic shape, usually contain rodent hairs and partially digested food or packaging material.

Bat excreta. These are often characterised by a large number of insect fragments.

Owl pellets. These usually contain rodent fur and bone fragments as well as partially digested food.

Hairs. The hairs of various animals should be studied under the microscope - mouse, rat, cat, rabbit, dog, squirrel, cow, sheep etc. using size, shape and medulla features to characterise them. This may be facilitated by examining the scale patterns (see "Preparation of Replicas", below).

Other contaminants. Learn to recognise textile fibres (natural and man-made); asbestos; glass wool and rock wool (note isotropic behaviour); metal powder, rust particles; paint particles; fly ash spheres, brick dust, in atmospheric deposits as well as in bulk (as pulverised fuel ash - PFA); mould contamination; iron bacteria (in rust-contaminated water); algae, yeasts; etc.

6.12 Preparation of Replica - to examine an opaque item, or a large object for surface characteristics and features.

Top illumination (and/or the use of a metallurgical microscope may be possible, but is not always available) may not always give a particularly good result because of the colour/opacity of the item. This is where the preparation of a replica can be of use. Here, the surface characteristics are 'moulded' into the surface of a transparent material. The transparent material used can be gelatine but certain brands of clear nail varnish are better. One which sets slightly 'tacky' and does not harden rapidly is ideal. Also suitable is a solution of PVA in water which can be prepared and spread on several slides, allowed to dry and set and which becomes quite tacky on warming gently. The technique applied to hairs/fibres is as follows:-

6.12.1 A small smear of nail varnish or PVA solution is placed on a slide and allowed to become 'tacky' This will take quite a short time, but depends on a variety of factors and requires experimentation.

6.12.2 Lay the hair or fibres onto the tacky surface. Press gently with another glass slide if necessary to make an impression. Leave for a few minutes and then carefully remove the other slide and the hair, leaving behind an impression of the scale pattern on the applied surface. This can then be examined using the microscope. Darkfield illumination if available (often found on a phase contrast microscope) gives excellent results.

6.12.3 Problems which may be experienced are as follows:-

i) If the nail varnish was too fluid, the hair/fibre may sink below the surface and a distorted replica is produced when the hair/fibre is removed.

ii) If the nail varnish is too hard when the hair/fibre is laid down, a poor, incomplete pattern is produced.

iii) If the nail varnish is a 'quick hardener' or if the hair/ fibre is left on for too long, the hair/fibre will be stuck firmly, and will either break when removed, or will remove part of the nail varnish when removed.

When examining the surface of a larger item, a generous layer of nail varnish is painted onto the surface, and then removed by 'peeling off' when it has almost hardened.

It is usually advisable to ensure that the surface to be examined is slightly 'greasy' to prevent the nail varnish from sticking too firmly.

Appendix A: Suggested Range of Materials for Study

Class	Materials
Starches	Arrowroot Bean Starch Cassava Lentil Starch Maize Starch Oat Starch Pea Starch Potato Starch Rice Starch Rye Starch Sago Soluble Starch Starch (Cooked) Tapioca Tous Les Mois Starch Wheat Starch
Cereals, Flours	Barley Flour, Ground & Husk Bean Flour Buckwheat Castor Seed, defatted & Meal Gram Flour Lentil Flour Maize Cob, Maize Meal Millet Seed Oatmeal Pea Flour, Pea Ground Rice Starch with Husk, Rice Bran Rye Flour Soya Flour Wheat Flour, Wheat Bran, Wheat Gluten
Leaves & Grasses	Alfalfa Basil Leaf Bay Leaf Belladonna Leaf Buchu Leaves Coca Leaf Dandelion Herb Digitalis Leaf Fenugreek Leaf Grasses(Various) Hamamelis Leaf Hay Hyoscyamus Leaf Lucerne Marjoram herb Mint, peppermint Mint, spearmint Oregano Parsley Rosemary Herb Sage Leaf Senna Leaf Silage Stramonium Leaf Tarragon Tea, Tea Dust Thyme Tobacco & Snuff
Flowers & Flowering Tops	Cannabis & Cannabis Resin Chamomile Flowers Cloves, Cloves Stems Hops Opium Pyrethrum Saffron
Fruits, Seeds & Nuts	Acorn Almond Aniseed Apple Banana Brazil Nut Capsicum Caraway Cardamom Cashew Cayenne Celery Seed Chestnut Juniper Berry Lemon Peel Linseed, Linseed Cake Locust Bean Mace Mustard Black, Brown & white Nutmeg Nux Vomica Olive Stones, Olive Pomace Orange Peanut Pear Pepper Black

Class	Materials	
	Chilli Pepper	Pepper White
	Cocoa Bean, Nib & Shell	Pimento
	Coconut	Poppy Seed
	Coffee Beans &	Rape seed, Rape Bran, Rape Meal
	Parchment	
	Coffee (Roast, Extracted)	Safflower, Safflower Meal
	Colchicum	Senna Pods
	Coriander	Sesame Seed, Sesame Cake
	Cotton Seeds, Cotton Cake	Soya
	Cubebs	Star Anise Fruit
	Cumin Seed	Stramonium Seed
	Dill	Sun Flower Seeds
	Fennel	Tomato
	Fenugreek Seed	Vanilla
	Fig Fruit	Walnut
	Hazelnut	
Woods & Barks & Stems	Aloes	Pine Needles
	Angelica	Pine Wood
	Cascara	Quassia Wood
	Cassia Bark	Quillaia Bark
	Cinchona Bark	Sawdust
	Cinnamon Bark	Slippery Elm Bark
	Cork Powder	Wild Cherry Bark
Roots & Rhizomes etc.	Belladonna Root	Liquorice Root, Liquorice Root (Decort)
	Chicory Root	Male Fern
	Colchicum	Marshmallow
	Dandelion Root	Onion Powder
	Garlic Powder	Orris
	Gentian Root	Rhubarb Root
	Ginger Root	Sarsaparilla
	Ginseng	Scammony
	Horseradish	Senega Root
	Ipecacuanha Root	Squill
	Jalap Root	Turmeric
Fibres & Hairs	Alpaca	Kapok
	Cat Hairs	Linen
	Cattle Hairs	Man-made Fibres, Various
	Cotton	Mouse Hairs
	Dog Hairs	Mohair
	Duck Down	Rodent Hairs
	Feathers (Various)	Silk
	Flax	Sisal
	Hemp	Spiders Web
	Hessian	Wool
	Horse Hair	Hogs hair
	Human Hair	
	Jute	
Gums	Guaiacum Resin	
	Guar	

Appendix B : Materials Set for Examination 1970 - 1997.

Year	Materials set for MChemA practical examination
70	Cannabis dandelion and chicory ground barley and insects hyoscyamus dried grass and sawdust flyash
71	chopped human hair dried grass black pepper castor meal maize and insect 'frass' and insects with some barley contamination nux vomica
72	feathers cockroach excreta powdered tobacco cassia barley infested with weevils burnt bread containing iron compounds and minerals
73	No information available
74	Tobacco jute flour with mites and rodent excrement castor meal mouldy fruit tarts powdered ginger
75	mint wheat flour with bird droppings belladonna mushroom powder fly ash gentian root
76	soya flour powdered ginger powdered cinnamon mica sphagnum moss mixture of fibres from filter of a clothes washing machine (wool, cotton, human hairs & viscose)
77	arrowroot ground rosemary ground ginger ground coriander human hairs, chopped cannabis resin
78	amosite asbestos fibre

Year	Materials set for MChemA practical examination oil fired boiler ash ground coriander barley flour ground sage hessian fibre
79	ground cocoa shell dried pea flour wheatflour with mouse hairs ground rhubarb root tea dust fibre mixture mohair 70% : wool 25% : 5% nylon
80	coffee substitute : roasted barley, chicory, fig and soya beans digitalis leaf gentian root arrowroot ground cassia bark crushed oats with 2% of black mustard added
81	belladonna root wheat and oat flour with rodent hairs anise fruit stramonium leaf cotton and polyester fibre mixture colchicum corm
82	soya flour nux vomica ginger root fennel dried potato raw cocoa dust
83	cotton polyester fabric sago and maize starches rhubarb root stramonium leaf caraway seed chinchona bark
84	senna leaf gentian root rice flour tobacco jute cassia
85	ginger root Indian hemp barley flour jute fibre capsicum fruit quillaia bark

Year	Materials set for MChemA practical examination
86	digitalis leaf mohair : wool : nylon mixture rice flour ginseng root oil fired boiler ash star anise fruit
87	tea dust chrysotile asbestos fibres / liquorice root hessian bean flour oatmeal containing rodent hairs cocoa shell
88	cigarette ash powdered vanilla foxglove leaf powdered uncorticated groundnut extraction asbestos fibres ginger root
89	defatted soya flour dried sage rice flour powdered horseradish root real silk cinchona bark
90	animal feed with mites and copper II sulphate crystals dust from rural area (cereals dust, feathers and pollen) coriander tobacco sweepings from packaging room (concrete dust and cardboard) ground defatted peanut
91	& No part C examination set
92	
93	turmeric wheatflour contaminated with rodent droppings loaf of bread with baked in paper fibres pollen and dust from car paintwork mixture of cotton and polyester described as 100% cotton dried ginger
94	No part C Examination set
95	ground almonds adulterated with another nut textured soya protein mouldy bread flour with gritty texture ground coffee adulterated with chicory honey sold as 'heather honey'

Year	Materials set for MChemA practical examination
96	<ul style="list-style-type: none"> ground paprika rodent droppings in a bread roll dust from window sill ground ginger adulterated with wheat flour instant mashed potato fabric described as 'silk' – a blend of silk and synthetic fibres
97	<ul style="list-style-type: none"> arrowroot with maize starch fibrous matter in sandwiches – jute fibres ground caraway seed – actually fennel flour with live psocids ground cassia with sandy matter milk with fibrous matter – tobacco fibres

Appendix C : Bibliography

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Note : Apart from a few of the more recent publications most of the above books are

no longer in print. Second hand book shops and reference libraries may have copies.