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Choosing A Microscope Slide Sealant: A Review Of Aging Characteristics And The Development Of A New Test, Using Low Oxygen Environments

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Abstract
Glyceel has been traditionally used to seal zoological mounts at the Natural History Museum (NHM), London. Production of this polymer has recently ceased and an alternative must be identified. A review of chemical composition and testimonies from the published literature have been combined with a new experimental method to achieve this goal. Of the 13 polymers tested, Canada balsam was found to combine mechanical stability, excellent barrier properties and good aging characteristics. It has also been found that Canada balsam does not react with glycerine, wax, saliva, phenol, or immersion oil. Canada balsam is therefore recommended as a ringing sealant for microscope slides. The new experimental method, developed for this investigation, utilises anoxic environments and has a wider application as a low-cost test of polymer mechanical stability and barrier properties.

Introduction
The prompting of this project was the discontinuation of the sealing solution Glyceel (a solution of linseed oils, alcohol, nitrocellulose, butyl acetate, and toluol). This was the most commonly used sealant (although certainly not the only one) in the nematode and copepod research groups in the Zoology Department at the Natural History Museum (NHM), London. The discontinuation of Glyceel has brought concern and heated discussion within the international nematode research community as a whole (Psmallion). An alternative needs to be found which would be at least as effective as Glyceel. Although it is commonly recognised that sealing permanent slides is very important, particularly those slides using mountants such as glycerine, there appears to have been very little work done to test the products available. The impact of this is that, when working with slides from the collection, it is often very hard to tell what products have been used. To have a universally accepted sealing medium for glycerine slides in the Zoology Department, would be highly advantageous for the curation of the collection. Additionally the mountants and sealants used for the slide making should be routinely entered into the database when accessioning new slides. This means that the longevity of different media could be assessed and, when breaking open slides in the future, we will know the ringing media used and therefore the best solvent or best method for opening the slide.

A vast array of different sealing products has been used by the scientific community as a whole. Overall this appears to be quite a controversial area, and the amount of actual research done appears to be scant. This paper aims to bring some firm answers to this subject.

This paper assesses the suitability of the available sealants through innovative experimentation. Oxygen sensing equipment is used to test mechanical stability and barrier properties of 13 polymers, used in conservation, entomological and zoological slide preparation. The results are compared with known aging characteristics of the sealants or their chemical constituents, through a review of published literature.

The polymer with the best barrier properties, aging characteristics and mechanical stability is identified. Before the appropriate polymer is recommended as a suitable alternative to Glyceel, it received more thorough testing, in simulated situations, to ensure that it does not react with other chemicals used in slide preparation and use, and also to establish best practice.

This paper will have relevance to all scientific workers who prepare or conserve slides, by identifying an alternative to Glyceel. It also contains a review of aging characteristics and a new method to test the mechanical stability and barrier properties of polymers. This has relevance to the use of adhesives, lacquers and consolidants in conservation.

1. Slide Storage at the NHM
Slides housed in the Zoology department at the NHM are normally stored horizontally. This is an important consideration when choosing mountants in particular but also sealants, as products such as Canada balsam will always be liable to move if stored vertically. The slides lie on wooden felt lined trays, or in hard cardboard folders within wooden cabinets in specially designated rooms. The recommended conditions for slides in collections are an ambient temperature of 18^{o}C and an ambient relative humidity of about 60% (MGC 1992b). This, however, is very difficult to maintain without special environmental controls. The Zoology Department at the NHM is air conditioned, but occasional malfunctions and power-cuts are unavoid-

able. Monitoring the conditions over a year in one of our slide rooms recorded temperature ranges of 18-27°C and humidity ranges between 37-67%RH.

The slides made for housing the free-living nematodes (round worms) are glass slides. A wax ring is made on the slide and within this the nematodes are mounted in glycerine, glass beads are added for support, a glass coverslip is placed over the wax ring and the slide is then placed for a few moments on a heat tray to melt the wax. It is after this process that the slide is sealed with a ring of the chosen polymer.

The importance of sealing the slides, particularly when using glycerine, has long been known: 'No matter how careful may have been the arrangement of the specimen, if the last act of sealing the cover is carelessly done, not only will all the previous labour be lost, but the specimen as well' (Pike 1890: 268). 'The troublesome leaking of glycerine cells is due in part to the penetrating character of the fluid and partly to its expansiveness in warm weather and therefore a very strong cement is required to hold them' (King 1889). Figs. 1-2 show some examples of failed slides, discovered within the zoology collections at the NHM.

Fig. 1 A - Sealant has become brittle and chipped away leaving the mountant exposed. B - A reaction has occurred within the slide. Possible cause oxidation, indicating the slides sealant has been breached.

Fig. 2 A - Sealant breakdown evident as air has entered the slide, also possible reaction between mountant and sealant evident. B - Wax sealant has broken down. A reaction in the bottom corner is also now evident, if this were to continue it could cover the slide obscuring the specimen.

2. Sealants within the Literature

The major fault with many of the products which have been used in the past is their propensity for cracking. Fluctuating temperatures is probably the largest strain contributing to this. According to Morse (1992) 'Most known mounting media and ringing materials have unsatisfactory characteristics'.

Brown (1997) produced a comprehensive paper reviewing microscope slide techniques. He also states the importance of sealing glycerine mounts. His suggested sealants were Euparol, Glyceel, Glyptal (stated as being only red alkyd enamel resin insulation paint produced by General Electric) and Murreyite. He also mentions the use of nail varnish. He later notes, however, that Murreyite reacts with some mountants and that 'Disney advocates Glyceel, Trycolac and 'ladies' nail varnish with or without colour'.

Disney (1983) mentions using Glyceel, but by 1994 had changed to using nail varnish. B.Georgiev (pers. comm. August 2005), a parasitic worm researcher at the NHM, believes that cheap nail varnish does not work particularly well, but expensive nail varnish is very good. Other entomological journals mention Euparol (Freeman, 1983) and Polyvinyl lactophenol (Gurr) or PVLP (Fink, 1987). Huys and Boxshall (1991) state that preparations have to be sealed and that many commercial sealants such as: Araldite, Murrayite, Bioseal and Glyceel are available (Araldite is Bisphenol A epoxy resin plus Beithylphthalate).[1] Although glues are highly accessible and cheap, it is unknown how they react to stress and age. Morse (1992) suggests that Paraloid (Acryloid) B72, a common conservation glue, may be a good mounting medium.

One of the earliest records of Glyceel is written by Thorner (1935). It was meant to be 'especially good for glycerine mounts'. Wagstaffe and Fiddler (1968) recommend nail varnish (used extensively by researchers for its cheapness and accessibility), or Glyceel for lactophenol mounts and Goldsize for glycerine jelly, glycerine and aqueous mounts. 'Nail varnish is suitable for ringing lactophenol, glycerine, gum, Canada balsam and most other types of mounts' (Wells 1978).

Much more analysis and discussion has been conducted about mountants than about sealants. At the NHM Canada balsam has been used for over 150 years as a mountant and, in some cases, a sealant as well, to great effect. Problems have been experienced at the NHM with some of the new synthetic mountants: they have a tendency to 'craze' over the years. Upton (1993) claims that 'Resin-based mountants such as Canada balsam and Euparol, which have stood the test of time, are probably the best option for permanent mounts, although it is possible that some modern synthetic mountants will prove to be of archival quality'.

Testing Sealants

1. The aim of developing a new test method

A suitable replacement must be found for Glyceel. Most bacteria require oxygen to decay organic matter so sealants must form impermeable barriers. A new, low-cost method has been devised to test the barrier properties and mechanical structural integrity of sealants on the market.

The new test was based around anoxic environments, recently incorporated into preventive conservation practices at the NHM. It was surmised that without anoxia the specimen on the slide cannot decay. For this reason a sealant should form an oxygen impermeable barrier. If the sealant does not form an initial impermeable barrier then it is not performing correctly. If the sealant subsequently becomes permeable, it can also be dismissed as a failure: oxygen and pollutants potentially flowing in, to damage the specimen, and glycerol, phenol or other mounting media being lost. To remain intact the sealant must not peel away from the glass substrate and it must not crack or lose integrity.

The new test attempts to simulate environmental stress over time by accelerated expansion and contraction of the media, using alternating temperature fluctuations. High humidity may encourage plasticity in the resin, and would not reflect realistic storage conditions, so water was not incorporated. Relative humidity will automatically have varied slightly during the test as temperatures fluctuated.

The results of this test are compared with known aging properties of sealants, and predictions based on chemical composition, to identify the most suitable sealant resin to be used to seal a cover glass over specimen sample slides.

2. Development of the new test method

Due to the large size of sachets of oxygen-scavenging chemicals (RP System™), relative to the size of slides, the microenvironments were created in reverse: oxygen sensors were placed within a small glass vessel, which was then sealed with a coverslip and the polymer being tested. The glass vessel was then placed within an oxygen-free microenvironment, constructed from a heat-sealed transparent barrier film (Escal ™) containing oxygen-scavenging chemicals.[2] If an impermeable barrier was formed by the polymer, the oxygen would remain within the glass vessel. If the seal was compromised, the oxygen would flow out of the vessel, to be scavenged by the chemicals. Two types of oxygen indicator were used: RP System ™ "Ageless Eyes" (which turn from purple to pink when oxygen falls below 0.1% and returning to purple at above 0.5%) and SensiSpots ™ (which can be read electronically to give more precise oxygen levels).[3]

Fig.3 Oxygen indicators: on the left is a SensiSpot™ and on the right an "ageless eye".

Various permutations were tested, before a final experimental design was developed. Problems to overcome included scratching of the barrier film by the cover glass, ease of reading sensors, and pressure being applied to the coverslips by the sealed bags. Experiments were also conducted using more than one layer of sealant, but this was not found to affect the results. Thermal expansion was induced by placing the vessels within an oven at 40 degrees Celsius. The barrier film bags were not compromised by the heat, but the "Ageless Eyes" ceased to change colour, due to thermal alteration of the reactive chemicals. SeniSpots ™ must therefore be used if microenvironments are heated. The combination of light, heat and oxygen often react with a polymer in an uncertain manner (Horie, 1987: 31). The additional problem with heating in accelerated aging tests is that different reactions are accelerated differentially. Deterioration may also be affected by more than one factor e.g. heat plus light or light plus oxygen (MGC, 1992a: 106). The heating only caused failure in one polymer, so was abandoned in favour of alternatively placing the environments in a freezer and then at room temperature, which was far more effective.

Attempts were made to assess the performance of sealants in more realistic circumstances: by sealing coverslips onto glass slides. The "Ageless Eyes" were too large for this experiment, and SensiSpots TM failed to give correct readings. In light of this, the most suitable vessel was found to be a flat-bottomed glass sample vial with a flat rim.

3. Recommended test method:

After a series of trials the following methodology was developed: inside a glass vial, place an "Ageless Eye" or a fluorescing oxygen indicator (Sensispot TM) on a small cube of inert foam to decrease the distance from the edge of the tube (measurements are most accurate at approx. 10mm). Seal a glass vial with a circular cover glass and the sealant to be tested. There will be oxygen in the test tube and the oxygen level will be approximately 20%.

Place each glass vial in a hole cut into inert foam. Place this in turn in an acid-free cardboard tray which is deeper than the height of the vials. This keeps the vials upright, prevents the outer bag being scratched by the cover glass, and also prevents pressure being applied to the cover glass.

Construct an oxygen impermeable plastic bag, large enough to enclose the tray, using a crossweld heat sealing machine to create double-seals. Place sufficient packets of oxygen-scavenging chemicals into the bag and seal it.

Leave the bag at room temperature for a few days until the first measurements are taken. The bag should then be placed into a freezer at –20 to –40 degrees Celsius for at least 24 hours. When the bag has returned to room temperature the oxygen levels should be recorded. In the case of the "Ageless Eyes", if the oxygen barrier is intact the "Eyes" will remain pink (<0.1% Oxygen present). This process will be repeated until all of the seals have been compromised, or do not appear to be affected. Fig. 4 shows the microenvironment containing vials with SensiSpots TM.

Fig. 4 Glass vials sealed with 13 sealants, plus one control, each with a SensiSpot on inert foam. The tray is in a microenvironment created with

Materials:

- Sealants to be tested – small amounts
- One glass vial per sealant plus one for control
- One cover glass per sealant plus one for control
- One SensiSpot or "Ageless Eye" per sealant plus one for control
- Inert foam
- Cardboard box with sides slightly greater than height of the vials
- Oxygen impermeable barrier film
- Freezer
- Oxygen sensing machine (if using Sensispots)

13 sealants were tested within this project: Canada balsam; Histomount; Euparal; Japan gold size; Clinique All-In-One nail varnish (aka "Clinique"); Sally Hansen® Hard-As-Nails TM nail varnish (aka "Sally"); DPX; Zaponlack; Loctite® Superglue; polyvinyl lactophenol Gurr (PVLP), Paraloid B72, Polyvinyl Acetate (PVAc), Glyceel.

Results

Fig. 5 shows typical failure patterns for the 13 sealants tested, Fig. 6 contains the results of 18 experiments. "Sally" nail varnish, DPX and Canada balsam retained a barrier to migrating oxygen for the longest periods and under the greatest thermally-induced mechanical stress, exhibiting the greatest mechanical stability and barrier properties. PVAc, Paraloid, Goldsize and Superglue did not generally form an oxygen barrier even at room temperature. A sealant could fail immediately because it is permeable or due to initial shrinkage as it cures. PVLP leaked slowly, independent of environmental conditions. A slow leak could be due to diffusion between the molecules of a polymer. The remaining sealants failed during periods of induced thermal stress.

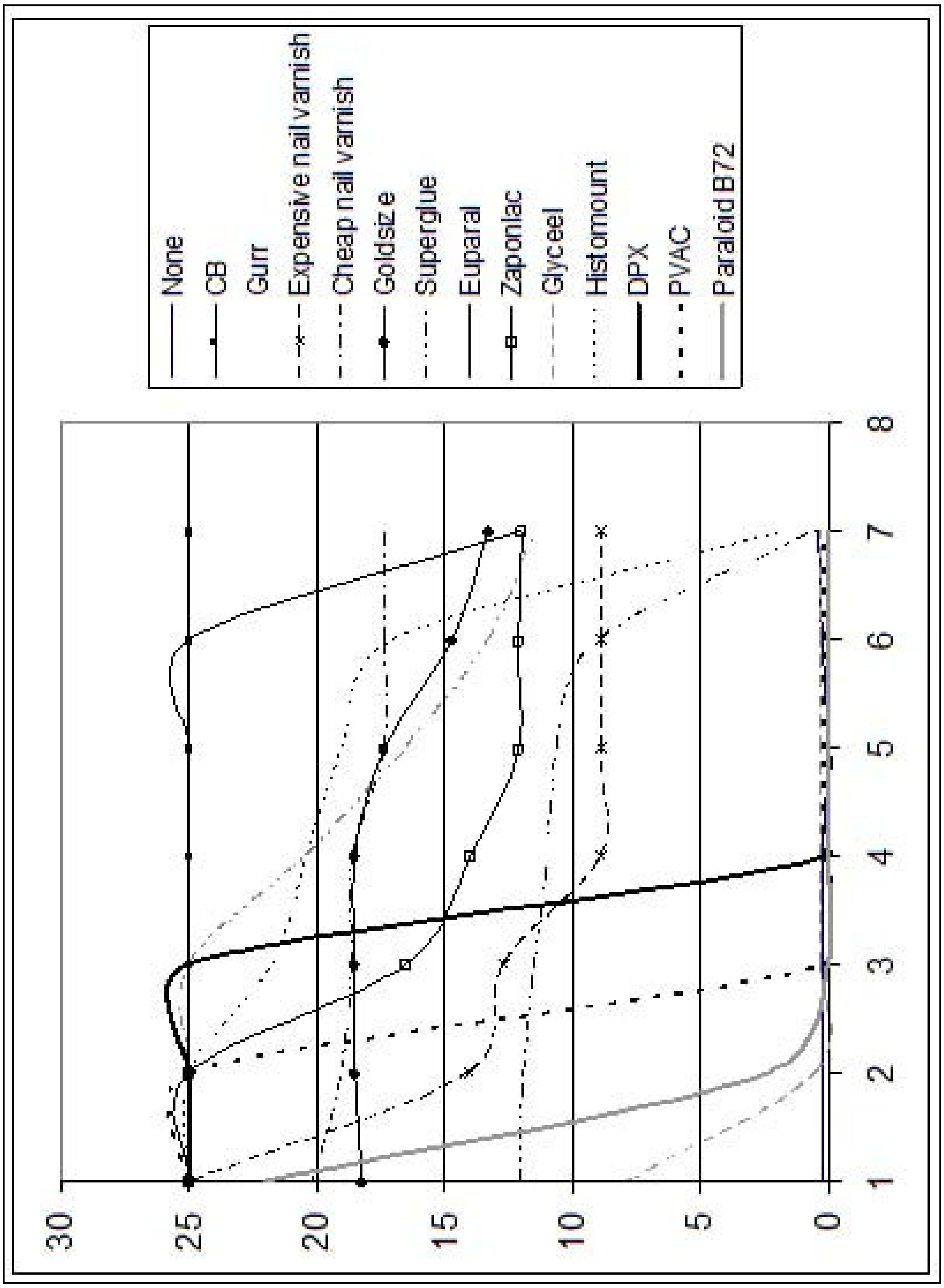
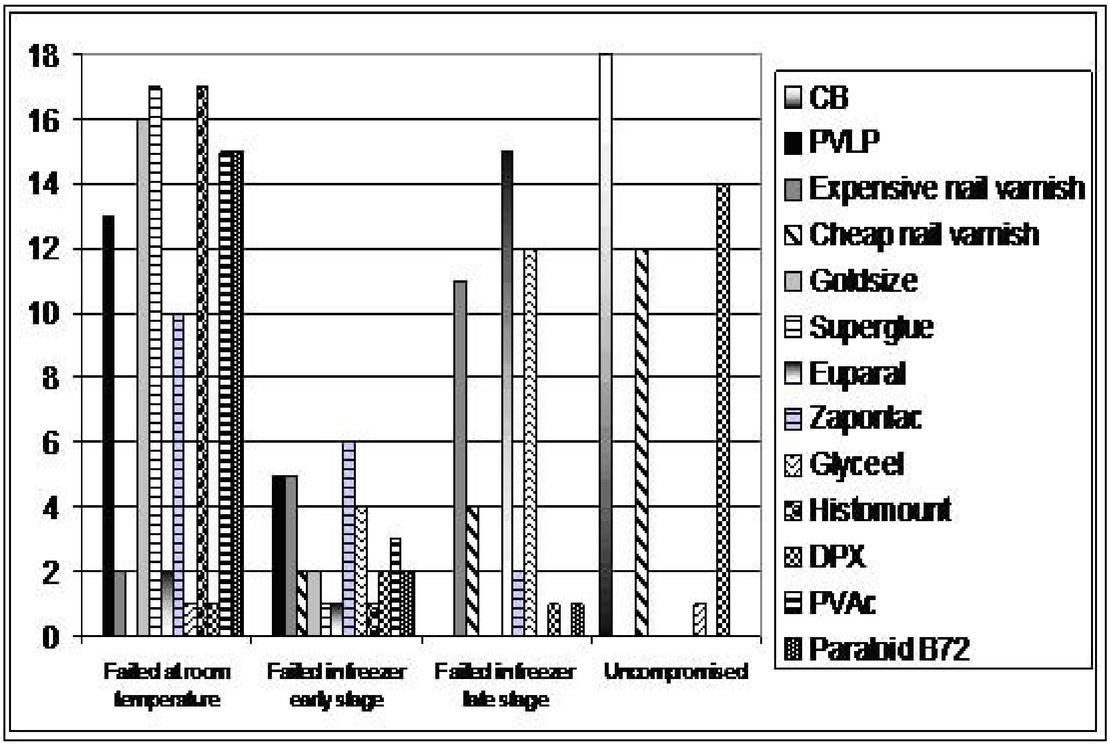


Fig. 5 Shows a typical graph of performance. In this example Canada balsam remained uncompromised whilst Paraloid failed at room temperature. Euparal failed in the last stage, cheap nail varnish and goldsize leaked slowly, expensive nail varnish, zaponlac, PVLP (gurr), histomount, DPX and PVAc failed in the early stages of freezing. (Stage 1 represents testing after 2 days at room temperature; subsequent stages represent 24 hours in the freezer at -40 degrees Celsius).



Chemical Evaluation of the Sealing Media Tested

Polymer degradation can manifest as crazing, chalking, discolouration, tackiness, embrittlement and loss of surface gloss. Polymers change when they age, as internal stresses relax or chemical reactions take place (Horie 1987: 23). Polymers can degrade due to high temperature, light radiation, hydrolysis, biodegradation (natural polymers), oxidation and the effects of sulphur dioxide and nitrogen dioxide in the atmosphere (Mc Neill 1992: 14; 18).

Adhesives used in preparation and conservation should be chemically and physically stable so that their initial mechanical properties and solubility are retained. They should have sufficient flexibility so that they do not crack. Resins with higher molecular weight are more flexible. Extensive hydrogen bonding between polar oxidation products, however, can cause embrittlement in natural and ketone resins with age (De la Rie 1992: 63). Additionally, if the solvent-free adhesive is weak and brittle then the loss of the solvents +/- plasticisers over time leads to embrittlement. Shrinkage can also create internal stresses (MGC 1992a: 104).

A polymer which is too soft will flow and pick up dirt. If it is too hard, it will crack when stressed and not be able to respond to movements within the object. Above the glass transition temperature (Tg) large sections of polymer chains are able to move cooperatively and adjust to stress. Internal movements can also be limited by intermolecular forces such as hydrogen bonds and the interlocking of bulky side groups (Horie 1987: 18).

A barrier to oxygen, water, hydrogen sulphide and sulphur dioxide must have closely-packed molecules and be below its Tg (MGC 1992a: 115). Impermeability increases with higher Tg and greater crystallinity (De la Rie 1992: 66) but flexibility decreases. Plasticisers can lower the Tg of more rigid, closely-packed polymers, but these decrease the purity and are lost over time.

1. Nail varnish

The resin dubbed "Sally" was Sally Hansen® Hard-As-Nails with nylon, clear. The ingredients are: Butyl Acetate – solvent; Ethyl Acetate – solvent; Nitrocellulose – carrier; Isopropanol – solvent; Adipic Acid/ Neopentyl Glycol/Trimellitic Anhydride – plasticizer/hardener/intermediary; Trimethyl Pentanyl Diisobutyrate – plasticizer; Triphenyl Phosphate – plasticizer; Nylon-66 – plasticizer; Acrylates Copolymer – dispersant; CI 60725 – violet colour.

The commercial advertisement claims that the Nylon formula, fortified with silk proteins, helps strengthen and protect nails against chipping, splitting and breaking, protecting against everyday hazards such as water, detergents and housework.[4]

The sealant dubbed 'Clinique' was Clinique All-in-one base and top coat. The ingredients of this are: butyl acetate – solvent; ethyl acetate – solvent; nitrocellulose – carrier; phthalic anhydride/trimetallic anhydride/ glycols copolymer – plasticizer/epoxy resin/solvent; isopropyl alcohol – solvent; trimethyl pentanyl – curing agent.

Both types of nail varnish are nitrocellulose-based and contain plasticizers. The main difference is that the "Sally" nail varnish includes Nylon-66 as one of these plasticizers.

2. Plasticisers

A plasticiser is a substance which, when added to a material, makes it flexible, resilient and easier to handle. Modern plasticisers are man-made organic chemicals; the majority of which are based on esters of polycarboxylic acids with linear or branched aliphatic alcohols of moderate chain length, such as adipates and phthalates.[5] Plasticisers are non-volatile liquids with low molecular weights (Horie 1987: 20). They increase the flow (plastic deformation) capability of polymers, and reduce stiffness and Tg (MGC 1992a: 100). Plasticisers separate long chains and facilitate relative movement, but they are incorporated between the molecules of individual polymers and so weaken the intermolecular bonds, causing aging problems (MGC 1992a: 43). Plasticisers are added to decrease shrinkage during the transition from liquid to solid state. They can, however, diffuse over time and therefore cause shrinkage and cracking later (Horie 1987: 38; MGC 1992a: 43).

3. Nitrocellulose

Nitrocellulose is a cellulose ester formed from the reaction of nitric acid with some of the hydroxl side-groups of the cellulose molecule (MGC 1992a). It has a high Tg (glass transition temperature) of 100^{o}C making it brittle at room temperature, so it usually contains plasticisers to reduce the effective Tg (MGC 1992a: 44). It rapidly releases solvents to form a dry, strong, film (Horie 1987: 132). Solvents include acetone, ethanol and butyl-acetate (MGC 1992a: 50).

Nitrocellulose was first used in conservation in the late 19th Century (Horie 1987: 133). It is still used in conservation but it is unstable, sensitive to photo-oxidation and acid catalysed hydrolysis. Initial degradation of nitrocellulose is seen as an acidic surface bloom, followed by crazing and complete degradation (Williamson 1992). The primary mechanisms for degradation are acid catalysed ester cleavage, homolytic scission of the nitrogen-oxygen bond and ring disintegration (Selwitz 1988). Nitrocellulose is unstable, decaying in a process that is exothermic, accelerated by increasing temperature, and auto catalyzed by its own acidic nitrate products (Johnson 1977; de la Rie 1992: 76). This causes a decrease in molecular weight (Mw) and the production of nitric oxides and acids (Miles 1955; Ferreira and Combs 1951).

Cellulose nitrates are stronger and more rigid than Paraloid B72, which is in turn stronger than PVAc (Horie 1987: 22). Cellulose nitrates, however, are considered less stable synthetic resins than PVAcs. They are especially unstable when exposed to light (de la Rie 1992: 76). Better chemical and mechanical stability is achieved from acrylic lacquers such as Paraloid B72 (MGC 1992a: 115).

4. Nylon

Nylon represents the generic name for all synthetic fiber-forming polyamides and is characterized by great toughness; strength and elasticity; high melt point; and good resistance to water and chemicals. Nylon is a simple, small, heterochain synthetic thermoplastic polymer (McNeill 1992: 16) which is used as a plasticiser because it has a Tg around room temperature (Horie 1987: 122).

Nylon-66 - poly[imino(1,6-dioxohexamethylene)iminohexamethylene] (Horie 1987: 122) is considered to have excellent barrier properties with respect to oxygen and organic solvents.[6] Other nylons are discouraged in conservation because they pick-up dirt and lose strength and solubility with aging (Horie 1987: 123). Nylon is also particularly susceptible to photo-oxidation. Acrylics are much more stable (MGC 1992a: 102; Horie 1987: 122). Adhesives containing Nylon can also swell as it absorbs atmospheric moisture, which causes internal stress (MGC 1992a: 93). Nylon is also sensitive to cross-linking in contact with mildly acidic water (Horie 1987: 49).

5. Paraloid B72

Paraloid B72 is a copolymer of methyl acrylate and ethyl methacrylate 30/70 (MGC 1992a: 30; Horie 1987: 111). It is a heat and water-proof adhesive for repairs to pottery, wood, metal, ivory, glass and all porous surfaces except rubber. It is virtually colourless and tends to resist yellowing for several years. It does not set instantly so fragments can be easily re-adjusted and surplus adhesive removed with acetone, thereby making any joint virtually invisible.[7] It is the adhesive/consolidant that is used the most in conservation (Borgia *et al*. 2001: 513; Cappitelli *et al*. 2004: 399). Paraloid B72 is an extremely stable polymer (Horie 1987 35). It does not become insoluble or degrade significantly in normal conditions of exposure (Feller 1978; MGC, 1992a:115; Horie 1987: 104) although slow oxidation does occur (Ciabach 1983) and it has been tested and recommended for conservation (De Witte 1983: 1.5; Blackshaw and Ward 1982: 2.11; Cruickshank *et al*. 1996: 877; Thickett *et al* 1995: 202). Paraloid B72, however, is brittle and prone to cracking under internal stress (MGC 1992a: 116) even though its Tg is 40^{o}C.

6. Canada balsam and Euparal
Canada balsam is a turpentine semi-fluid resin from the *Abies balsamea* (L.) P.Mill fir (Mills and White 1987: 89). This viscous, sticky, colourless (sometimes yellowish) liquid turns to a transparent yellowish mass when the essential oils have been allowed to evaporate. It is insoluble in water but soluble in a number of organic solvents (Coppen and Hone 1995: 62). Canada balsam was of importance in optics because its refractive index (1.53 for the sodium D lines) is close to that of glass (Liu 1971) and has been used as a mounting medium since 1830s. 150 year old entomology slides at the NHM have not crystallised or absorbed moisture (Brown 1997: 7). Canada balsam is considered by Mound and Pitkin to be the only mountant that can be kept in a variety of climates without deteriorating (1972: 122). It does yellow with age, but most researchers do not see this as a major problem (Brown 1997: 7).

Euparal is a mixture of eucalyptol, sandarac (a resin from the tree, *Tetraclinis articulata* (Vahl) grown in north west Africa), paraldehyde and camsal (camphor and phenyl salicylate). Contributors to an online discussion have differing opinions on the longevity of Euparal.[8] One contributor claims that properly prepared specimens mounted in Euparal “do not crystalize, fog, shrink, crack or do anything but last in good condition for a very very very long time”. Another contributor, however, stated that “Euparal slides of chigger mites were made in 1987, and they're starting to polymerize and craze around the edges.”

“Resin-based mountants such as Canada balsam and Euparal, which have stood the test of time, are probably the best option for permanent mounts” (Carter and Walker 1999: 45). This is echoed by other workers: Halliday (1994) found that most workers consider Canada balsam to be the most suitable mounting medium.[9] Galtier and Phillips (1999: 68) state that “natural balsam remains unaltered by oxidation over long periods of time”. After an extensive survey of the microscope slide mountants within the entomology collection at the NHM, Brown (1997: 10) also concludes that Euparal and Canada balsam are unsurpassed by modern materials. Euparal is the best alternative to Canada balsam since it does not need carcinogenic xylene and does not yellow with age (Brown 1997: 8). Euparal, however, can damage fine structures due to early development of a meniscus and can also craze if poorly prepared (Hood 1940: 57).

Crazing of Canada balsam seems to occur quite rarely, but has been noted by other workers (Green 1995: 162). Cedric Shute at the NHM believes that Canada balsam only crazes if it has been poorly prepared (personal communication, 12 November 2005). Early slides were made from melted resin. If the resin was heated slightly too much the balsam would crack within a few years of preparation. Canada balsam slides are now made using resin dissolved in xylene instead of heated resin. Clare Valentine at the NHM has identified Canada balsam slides from 1840 which have survived intact ((personal communication, 10 November 2006). This is supported by Brown and de Boise (2006) who state that “Canada balsam is known to be stable over 150 years.”

7. Glyceel
Glyceel consists of linseed oil, alcohol, nitrocellulose, butyl acetate and toluol. Some early recipes contained ADM (Sunflower oil esters with propylene glycol).[10] Slides created in 1960 have been found to have dried out, with air penetrating the medium.[11]

8. Goldsize
Goldsize is an oil copal varnish with turpentine and driers. Copal is a hard, natural resin obtained directly from trees such as *Trachylobium* species (Gartner) Oliver (Africa), *Hymenaea courbaril* (L.) (South America) and *Agathis australis* (D.Don) Steudel (New Zealand). Copals are also obtained as fossil resins from Zaire and Zanzibar. Copals are diterpenoid resins that contain communic acids, communol, resene and volatile oil.[12]

Ageing of copal resins may result in the condensation of the low molecular weight compounds to form larger molecules which could affect the solubility. In addition, resins added to drying oil may be incorporated into a polymeric structure. van den Berg and Horst (2006) discovered that the polymers were relatively unstable to air after isolation; in the drying and ageing process, the remaining characteristic diterpenes were found to disappear completely, probably as a result of oxidative degradation and cross-linking.

Ageing of triterpenoid varnishes leads to yellowed products which can craze, become very brittle, and show a change in solubility. These physical changes are the consequence of molecular changes in the varnish, and it is likely that oxidation and possibly isomerisation and polymerisation reactions occur during ageing (van der Doelen 2006).

9. Histomount
Histomount is a piccolyte polyterpene hydrocarbon resin with toluene, produced by the polymerisation of betapinene (a polyterpene consists of isoprene molecules linked into loosely-twisted chains).[13] It is a synthetic mounting medium which is manufactured to be a pH neutral, UV stabilized preparation with a refractive index matched to glass.[14] Polyterpene resin is considered more stable to oxidation than most natural resin-based products.[15] Permount is a similar mounting medium (Piccolyte B-pinene polymer plus Toluene). This has gone through several formulations, however, because the earlier formulas tended to polymerize and craze over time (>5 years). Contributors to an online discussion reveal that this medium crystallizes within 20 years.[16]

10. PVAc - Poly(vinyl acetate)
PVAc is a copolymer of vinyl acetate and esters of maleic acids such as dibutyl maleate (MGC 1992a: 115). It has high resistance to heat, light, water, dilute acids and alkalis. It is considered to be a thermoplastic (heat setting polymer) of good chemical stability (MGC 1992a: 51). It dissolves in ethanol and acetone but also comes as an aqueous emulsion. Emulsions have advantages over ordinary solvent coatings because the carrier is water. They are, therefore, less toxic and are non-flammable. They also retain a more uniform thickness when set (MGC 1992a: 116). In the experiment detailed earlier in this paper, however, the increased surface tension, created by shrinkage during drying, may actually be beneficial because it pulls the two pieces of glass together.

Of several solvents tested, Thomson (1963) found PVAc to be the most resistant to light aging. De la Rie specifies this is true for soft, low molecular weight (Mw) PVAcs, which pick up dirt easily, but high Mw PVAcs are prone to cross-linking and chain-scission due to the loss of acetate side groups and the formation of conjugated polyenes during photodegradation (de la Rie 1992: 76). PVAc contains stabilisers and sometimes plasticisers that increase its susceptibility to oxidation and cross-linking (which decreases solubility and flexibility). Volatilisation of these chemicals, accompanied by acetic acid production, can lead to corrosion of metal objects and also leads to embrittlement over time (MGC 1992a: 52, 105, 93; Oddy 1975; Feast 1982).

11. PVLP - Polyvinyl lactophenol (Gurr)
PVLP is made by the combination of phenol, lactic acid and glycerol. Williamson (1992: 10) states that phenol is affected by photodegradation – but only changes colour. One contributor to an online discussion found some favourable short-term results (ca. 4 yrs.) with a PVLP-based series of mountants. Another, however, revealed that PVLP mounts used in the 1950s (for *Collembola* or springtails) proved disastrous, having shrunk greatly after about 5 years.[17]

12. Superglue
Superglue is an alkyl cyanoacrylate. These adhesives form by chemical reactions *in situ*, so they do not shrink as much as solvent adhesives and give stronger bonds (MGC 1992a: 15, 103). Superglue is sold as a monomer mixed with an acid which inhibits polymerization. The hydroxl groups, found on most surfaces in the presence of water, neutralize the acid and curing begins on contact (MGC 1992a: 56). Cyanoacrylates require clean, well-fitting surfaces and they are irreversible (MGC 1992a: 55). Darmon (1975) states that, although initial bond strength of cyanoacrylates is high, over time there is deterioration of bond strength in the presence of moisture. Cyanoacrylates cross-link under exposure from ultra-violet light and can also lose strength (Horie 1987: 105). They also degrade severely in alkaline conditions by hydrolysis (Leonard et al 1966).

13. Zaponlac
Zapon is a cellulose nitrate/camphor (plasticiser) solution in amyl acetate. It was largely displaced by cellu-

lose acetate and PVAc in the 1920s and 1930s, but it is still used by some conservators (Horie 1987: 132). It has been found to be soluble even after long aging (Horie 1987: 133) but does discolour (Doerner 1934). Smith et al (1984: 102) discovered that it becomes embrittled and yellow, and detaches from its substrate as it ages.

Cellulose nitrate, as discussed elsewhere, is inherently unstable and slowly decomposes at room temperature. Ultraviolet light, heat, and/or high humidities hasten its decomposition.[18]

14. DPX

DPX is a colourless, synthetic resin mounting medium, introduced to replace Xylene-Canada balsam mountant. It is composed of Di-n-butyl phthalate (plasticiser) and Polystyrene.[19]

Polystyrene, or Poly(1-phenylethylene), has a Tg of 95^{o}C. This makes it very rigid at room temperature and will build up stress during curing. As the plasticiser is lost over time, DPX becomes very brittle. Polystyrene is resistant to water and acid but it is highly unstable (Horie 1992: 114-5). It degrades by photolytic oxidation, causing backbone homolysis, followed by various cross-linking reactions of the terminal macroradicals (McNeill 1992: 21).

Discussion

Euparal, Canada balsam and Histomount appear to be the most stable, and least likely to degrade, according to the chemical discussion and the testimonials of professionals working with slide collections.

Personal experience by the authors, regarding ease of use for ringing slides, revealed that PVLP and Zaponlac, due to their low viscosity, did not ring properly. PVAc emulsion proved very difficult to apply, Glyceel created a very thin layer and would need two coats. Histomount, Goldsize, Euparal, Superglue and the nail varnishes worked well but the most satisfactory result was achieved by Paraloid B72 due to its high surface tension. Canada balsam varied in performance depending on consistency of the mixture with xylene. A 1-part-xylene to 20-parts-Canada balsam mixture was more effective than most sealants but was not as effective as Paraloid B72.

"Sally" nail varnish, DPX and Canada balsam retained oxygen impermeability in the experiments to test mechanical stability. DPX, however, becomes brittle over time, as its plasticiser is lost, and nail varnish is based on unstable nitrocellulose, plus plasticisers which again can be lost with age.

As the most appropriate alternative to Glyceel, Canada balsam was subjected to further tests: following standard procedures, nematode and copepod (crustacean)-style slides were created using Canada balsam plus lactophenol or glycerol, with and without a wax ring. Reactions at room temperature were observed over 2 weeks. The slides were also subjected to the application of immersion oil (Olympus and Leica, but also produced by Merck and Gurr and available from VWR) plus removal of the oil using saliva or Histoclear II (available from VWR, Histoclear II is flammable and irritating to skin, but less hazardous than xylene). It was found that Canada balsam does not react with any of these products.

Conclusion

The polymer recommended for sealing coverslips onto microscope slides is Canada balsam (available from VWR International). It combines mechanical stability with chemical stability and it has stood the test of time.

Care should be taken when wiping immersion oil off with xylene, because Canada balsam could become tacky if prolonged contact occurs. It is preferable to use saliva or Histoclear. Xylene is flammable, harmful by inhalation and irritating to skin.[20] COSHH and risk assessments must be carried out before use.

1. Limitations

In an environment with normal atmospheric oxygen levels, the sensor read 22-23% from uncovered indicators, but 25% behind escal and through glass. The oxygen-free control registered 1.4% behind Escal and 0.4% behind glass. Clearly the readings are affected by transparent barrier media and must be adjusted accordingly, or considered relatively. A difference of 1-5% was observed depending whether the SensiSpot™ was held <1cm or 6cm away from machine. Repeat readings of the same SensiSpot™ also varied by a few tenths of a percent, and readings were also affected by temperature (lower values when still cold from the refrigerator or freezer). Some SensiSpots™ detached from the foam base, some curled (e.g. Zaponlac and Clinique nail varnish), some became dimpled (e.g. PVLP Gurr) and in others the silvery layer deteriorated and became transparent. It was notable that within the vials sealed with PVAc the SensiSpots™ always lost their silvery layer. This is probably due to devolatisation of the polymer and the production of acetic acid which can corrode metal. The "Ageless Eyes" were also adversely affected by the PVAc fumes, turning a dark pink colour. The SensiSpots™ in vials sealed with Superglue sometimes developed a white film, possibly due to a reaction with surface condensation. All these factors made the SensiSpots™ difficult to read and suggest that these particular sealants off-gas damaging volatiles. The SensiSpots™, although giving precise readings, were also more time consuming to read and more expensive than the "Ageless Eyes".

2. Other Aging Tests
The Oddy Test is designed to determine the off-gassing potential of display and storage materials (Green & Thickett, 1995), but would not test the stability of resins. International standard tests and scientific investigations are usually unconnected with conservation and require considerable time and expense (Horie 1987: 39). Less expensive tests involve accelerated aging using light, heat and water (Horie 1987: 44-9). Blackshaw & Daniels (1979) outline some useful tests.

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