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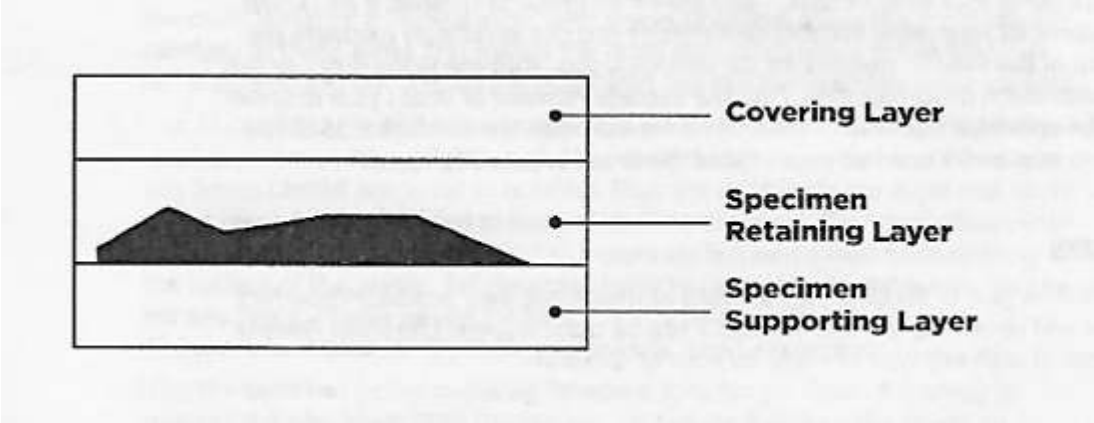
EQUIPMENT, SUPPLIES, AND SERVICES FOR INSTITUTIONS AND INDIVIDUALS

Embedding in Bio-Plastic



USING BIO-PLASTIC®

Bio-Plastic is a liquid synthetic resin that hardens with the addition of a catalyst. The reaction between the resin and the catalyst is indicated by a color change from blue to green. As the reaction progresses and the plastic gels (polymerizes), it becomes crystal clear.

MIXING BIO-PLASTIC® WITH CATALYST

See Reference Chart. The percentage of catalyst declines with increasing thickness of the block. This permits larger blocks to harden more slowly to avoid cracking. After adding catalyst to the plastic, stir gently with a clean, dry stirring rod to mix thoroughly. Avoid stirring in bubbles; if they occur, permit the plastic to stand for a short time, allowing bubbles to rise to surface. Mixing can be done in any sort of glass or metal containers or in unwaxed paper cups. Be sure the containers are clean and dry. The chart values are averages. Less catalyst may be used when embedding especially delicate specimens or objects likely to give trouble with air bubbles, Using a smaller amount of catalyst produces less heat and is less likely to harm the specimen. This also results in a greater amount of time that must elapse for hardening, giving any air bubbles time to escape. Larger proportions of catalyst result in a clearer and harder block, and the lines between successive layers are less apparent. However, using more catalyst increases the risk of cracking owing to the greater amount of heat released in the shorter period of time.

HELPFUL HINTS

Wear rubber gloves and eye protection while working with Bio-Plastic® materials. Use of disposable unwaxed paper cups for mixing Bio-Plastic® and catalyst eliminates cleaning of glassware. If glass or metal containers are used acetone may be used to clean them. When mixing small batches of plastic (less than 100cc) the “drop” makes a good unit measure. For example, in preparing a layer about 1/4" thick using 25cc of Bio-Plastic®, add 80/4 = 20 drops of catalyst. If you know the volume of your mold in cubic centimeters you can accurately calculate the amount of Bio-Plastic? needed for the various layers. Your medicine dropper can eliminate much drop-counting. Find the average number of drops your dropper will take up with a Squeeze of the bulb. If, for example, the average is 35 drops and you require 90, use two squeezes of the dropper plus 20 drops.

MOLDS

Glass dishes, jars, or tin cans may be used as molds but they produce imperfect shapes and removing the finished block can be troublesome. Use Mold Release Compound with any type of mold for ease of removal.

Bio-Plastic Reference Chart

How to Mix Bio-Plastic and Catalyst in Correct Proportion

Estimated Thickness of Bio-Plastic Layer to be Poured	% Catalyst by Volume	cc of Catalyst per 100 cc of Bio-Plastic	Drops of Catalyst per 100 cc of Bio-Plastic (Medicine or Eye Dropper)
Up to 1/4"	2%	2 cc	80 Drops
1/4" to 1"	1%	1 cc	40 Drops
1" to 2"	0.25–0.5%	0.25–0.5 cc	10–20 Drops

COMPLETING THE MOUNT

Curing and polishing the preparation and are described in Project 1.

PROJECT 1:

Refer to the appropriate Material Safety Data Sheet (SDS) before using any chemical.

Take a small dried starfish (the same procedure is used for any dry opaque specimen). Dry specimens give the best results when “wet” with Bio-Piastic®.

1. Soaking in uncatalyzed Bio-Plastic® removes air bubbles from the surface and air pockets from inside the specimen. Immerse the starfish, upside down, in uncatalyzed Bio-Plastic®, using a small disposable container such as a paper cup. Use 30-50 cubic centimeters (cc) of Bio-Plastic® to cover the specimen. If the specimen floats, it must be weighted down; a 1" diameter metal washer is excellent for this purpose. Note the minute the air bubbles rise to the surface as the specimen is impregnated. Cover the container with a sheet of paper to keep dust out, and leave overnight.

2. The next day select a mold. Select a mold that fully fits your specimen. Assemble according to directions.

3. With a camel-hair brush apply mold release compound to the inside surface of the mold. Mald release comes as a thin paste and dries quickly when applied to the mold surfaces. If using a mold with cracks or seams, apply the mold release carefully to these areas. This makes the mold leak-proof when liquid Bio-Plas- tic? is poured. Do not disturb the mold after the mold release has been applied.

4. Pour 15 cc of Bio-Plastic® into another disposable container. Add 12 drops of catalyst and stir with a 6" glass rod. Stir evenly and thoroughly for about a minute, being careful not to stir in bubbles. Pour the mixture in the mold and cover with a piece of paper folded to form a “tent” on the mold. The “tent” allows volatile elements from the Bio-Plastic® to evaporate but keeps dust from settling on the surface of the plastic. Set the mold aside to gel - at least two hours. May be left overnight. (These values are for a 2" x 2" x 34" deep mold with a capacity of 47.5 ccs. Adjust accordingly to fit your specific mold dimensions.)

5. After the layer has gelled to the point where it no longer flows, it is ready to support the specimen. With the forceps, lift the starfish from the plastic in which it has been soaking and drain off the excess. Place the specimen on a paper towel or blotter. After draining for 1 minute, move the starfish until most of the liquid plastic has been removed. Now position the starfish upside down in the supporting layer of the mold. In two hours the specimen will adhere firmly. This is very important where specimens have a tendency to float; you do not want to see the specimen rise to the surface when you pour the second layer.

6. After two hours put 30 cc of Bio-Plastic® into the same container in which you mixed the first layer. Add 12 drops of catalyst and stir thoroughly. Pour the mixture into the mold, flowing it over the specimen, to a height near the top of the mold. Do not let the plastic overflow the sides of the mold. Again, cover with the paper “tent” to protect from dust and set aside overnight. (These values are fora 2" x 2" x 34" deep mold with a capacity of 47.5 ccs. Adjust accordingly to fit your specific mold dimensions.)

7. The mold will now need to be heat cured to complete to polymerization and produce a hard, clear cast. Place the mold in close proximity (3-5") to a 40-60. watt light bulb in a goose-neck lamp. The temperature should be maintained at 120-140°F for 3-4 hours. Turn off the light and allow the mold to cool to room temperature. Remove the cast; it will come out easy, due to the mold release compound and some shrinkage of the Bio-Plastic® through hardening and cooling.

8. The cast is now ready to grind and polish. Lay a coarse sheet of emery paper (180 grit) on a smooth surface, Wet the back of the sheet to make it adhere to the flat surface. Grind the six surfaces of the Bio-Plastic® cast against this emery with a forward and backward motion. This should be done wet. Apply a puddle of water to the paper, and work in this puddle. Wet grinding eliminates dust, provides a more even cut, and allows you to work back and forth on the surface without tearing the emery paper. Next, move to an intermediate grade of emery paper and finally to a fine grade. Dip the block frequently in water to

observe the degree of finish. After grinding with the fine-grade paper, you will need to further refine the finish. Apply a small amount of liquid abrasive to a felt polishing board. Again, polish with a back and forth motion. The block should acquire a finer more transparent appearance. For the final polish, use another felt board, applying similar amounts of liquid polish. Always keep the two felt boards separate.

The more effort expended in grinding thoroughly through the various grits, abrasives and polishes the better the final appearance for your cast. If there are deep scratches on some of the surfaces of your block, it may be that at one stage of your grinding you did not spend enough time. Go back through the operations and resurface. Your efforts will be rewarded by the fine appearance of your Bio- Plastic? mount by careful attention to these rather simple operations of grinding and polishing.

PROJECT 2: WET OPAQUE SPECIMEN: THE GLYCERINE METHOD

Fresh plant or animal material cannot be directly embedded in Bio-Plastic®, Before embedding, the specimens must be “fixed” (their tissue preserved and hardened) in some preservative, All common preservatives (such as 5% formalin, 70% alcohol) contain water. Water and Bio-Plastic® do not mix and will cause the plastic to become cloudy. Replace the water with glycerine ; it prevents the Bio-Plastic® from penetrating the tissue. When Bio-Plastic® penetrates it makes the tissue transparent. Glycerine prevents this undesired effect. Be careful to remove traces of glycerine from the outside surface of the specimen. This is the reason for the acetone wash and careful drying of the specimen in a soft absorbent cloth. The glycerine method is used for preparing gross sections of preserved organs and animals, and for small entire organs and animals where a presentation of external form is desired. Proceed according to the following:

1. Selects as perfect a specimen as possible-well preserved, straight, and complete.

2. Transfer the specimen from the preservative to a 30% solution of glycerine in water for a period of 24 hours. Then transfer 50% and 75% glycerine solutions successively, leaving the specimen for 24 hours in each. Finally transfer to 100% glycerine, also for 24 hours.

3. Using forceps remove the specimen from the 100% glycerine, swish it around in acetone for a minute or so, then press the specimen gently into a clean dry cloth or facial tissue, This removes much of the glycerine from the specimen, Leave overnight.

4. Place the specimen in a small disposable container and cover in about 50cc of uncatalyzed Bio-Plastic®. Let it stand overnight. Remove the specimen and drain on a blotter or paper towel as for the starfish in Project 1. Discard the Bio-Pastic® as it is now contaminated with glycerine. Add fresh uncatalyzed Bio-Plastic® to cover the specimen. Leave overnight. Drain the Bio-Plastic® and repeat. (It is important to remove from the surface of the specimen as much glycerine as we can to prevent its “bleeding” into the Bio-Plastic® during the embedding; three such treatments are about right. With excessive soaking in Bio-Plastic® the plastic itself will begin to penetrate the specimen, and the specimen will begin to “clear”)

5. Prepare the supporting layer for the mold. In this project, a slightly larger mold will be required. The supporting layer should be about 1/3 the total capacity of the mold. Prepare mold with mold release compound as in Project 1. Dispense Bio-Plastic® into a disposable container. Add catalyst; stir well and carefully. Pour into the mold and allow to gel two hours.

6. Both the specimen and the mold with its supporting layer are now ready. Remove the specimen from it's third “bath” in uncatalyzed Bio-Plastic® and drain for 1 minute before positioning it on the supporting layer. Allow the specimen to “anchor” (adhere to the supporting layer) several hours. Add a second layer of Bio-Plastic.

7. Proceed with curing, grinding, and polishing, as in Project 1.

PROJECT 3: ALCOHOL-PRESERVED MATERIAL: TARANTULA

A large specimen such as a tarantula, offers a really challenging opportunity for a Preparation. Specimens are usually obtained and preserved in 70% alcohol. As we have learned in the previous project, water must be removed from the specimen before embedding. This is done during the alcohol-hardening process, described below.

1, Remove the tarantula from the bottle of preservatives in which it is supplied,

2. Use a piece of wallboard, 358" x 436" x 38" thick as a pinning slab. Position the specimen so that it is nicely positioned over the entire surface of the slab, yet contained within the four edges. Try for a natural, and symmetrical arrangement. This specimen is to be held in position with insect pins, but do not thrust the pins through the body or appendages of the tarantula, Using pins in pairs, angle them into the pinning board crossing one another above the legs of the specimen so that it is securely locked in place.

3. Select a suitable mold. However, we are not ready to apply mold release com- pound. Instead we are going to use the mold as a dish for hardening the tarantula in 99% alcohol. Take a piece of polyethylene plastic sheeting and lay it over the mold. Push it down all around to forma lining inside the mold, Take the tarantula, mounted on the pinning slab, and place it in the polyethylene-lined mold. Pour in sufficient 99% denatured alcohol to cover the specimen, then fold the loose edges of the polyethylene over the mold to reduce evaporation. Leave the specimen in the alcohol 2 to 4 days.

4. Next day pour off the alcohol (returning it to the supply bottle if you wish to save it). Take the pinning slab with the tarantula out of the mold. (Save the polyethylene sheet.) Gently remove the pins from the pinning slab and allow the specimen to air-dry for about half an hour.

5. Now the tarantula must be soaked in uncatalyzed Bio-Plastic® for three days. Again line the mold with a sheet of polyethylene as in the alcohol operation just completed, Using a small spatula or similar tool, gently lift the tarantula from the pinning slab with the spatula underneath the abdomen and thorax of the specimen. Carefully lower the tarantula into the mold and position it centrally. Pour in enough uncatalyzed Bio-Plastic® to cover the tarantula completely. The tarantula will tend to float in plastic but this can be corrected by placing, very carefully, a steel washer-weight on the abdomen and thorax of the specimen, Some of the appendages may tend to rise, but as long as they are covered by Bio-Plastic® the Operation will be successful. Fold the loose edges of the polyethylene over the plastic so that it is protected from evaporation and dust. Leave overnight.

6. Remove the washer-weight from the specimen’s back and lift the tarantula from the mold with a spatula. Place the tarantula on a paper towel or blotter to drain thoroughly. (The Bio-Plastic® that you have used for the soaking operation can be saved and used again, but only for similar plastic impregnation, not for embed- ding. Empty into a clear jar and label.)

7. Now prepare the supporting layer of Bio-Plastic®. Apply mold release com- pound to the inside of the mold. Pour the first layer, and allow to gel for two hours. Be sure to cover with paper “tent” so that dust cannot settle on the surface, but volatile elements are able to evaporate. When the surface is hard enough to support the tarantula, place the specimen on the supporting layer, carefully centered, and allow it to “anchor;’ preferably two hours. After anchoring, prepare more Bio-Plastic®. Stir, and pour covering the layer. The plastic should reach the brim of the mold at this time. When the mold is nearly full, check to make sure it is level. Use small slips of paper under a corner or two of the mold to level it if necessary. Allow the plastic to harden overnight.

8. Cure, grind and polish as per Project 1.

PROJECT 4: STAINING & COLORING: SEAHORSE

It is often desirable to stain biological specimens and/or color the background Bio-Plastic® so that the preparations may be more clearly seen and particular details of their structure demonstrated. By following the procedure here, you will obtain a very striking and interesting preparation.

1. Obtain a dried seahorse. Fill the jar with water, cap, and allow to stand over- night. (This softens the skeleton of the seahorse.)

2. The next day, transfer the specimen to a jar of 3% hydrogen peroxide for bleaching. This may take from one to two days or even longer. Check the specimen from time to time—it should be straw yellow to white in color.

3. Remove the specimen from the peroxide and return it to the first jar. (Recap the bleach solution.) Wash the specimen free of peroxide by putting the jar and the specimen under a faucet and letting the water run slowly for about an hour.

4. Pin the specimen out ona piece of wallboard type pinning slab 278" x178" x 3/8" thick. As in the case of the tarantula, arrange the specimen naturally within the edges of the slab, Again, do not thrust pins through the specimen, but across at angles, wedging the seahorse in the position in which you want it to dry. Dry overnight in this position on the slab.

5. The next day, remove the pins, pick the specimen up with a pair of forceps at the head end and dip into diluted 0.5% stock solution of Fast Green in 95% alcohol (recommended .0125% solution).“Dunk” up and down, and observe the intensity of the green dye colors of the specimen. The intensity will vary directly with the length of time it is left in the stain. When satisfied with the intensity of the color, remove the seahorse from the staining solution and rinse in 95% alcohol. Allow to air-dry on a blotter or paper towel overnight.

6. Transfer the stained and dry seahorse to uncatalyzed Bio-Plastic® in a small disposable container and soak overnight. Use the circular washer-weight hooked around the tail of the specimen to hold it under the surface of the plastic.

7. After the seahorse has soaked overnight, it is ready to embed. Pick a suitable mold for the size of your project.

8.To the Bio-Plastic® prepared for the supporting layer, add about 1/8 teaspoon of white Bio-Plastic® Opaque Color. Mix this thoroughly with the Bio-Plastic before adding catalyst. Then add the catalyst in the normal manner, mix, and pour the supporting layer as you have learned in previous projects. Leave this for two hours. Proceed to drain your seahorse, as you have done with previous specimens after soaking, and let the seahorse “anchor” to the white layer of Bio-Plastic®. The cover- ing layer, of course, has no color added to it. Cure, grind and polish. Note in the finished block how the white background adds contrast to the stained specimen.

PROJECT 5: STAINED & CLEARED ANIMAL SPECIMEN: PIG EMBRYO

Your experience in understanding the need for dehydration techniques, the importance of staining, and in general, familiarity with Bio-plastic® techniques is considerable at this stage. Try a small pig embryo that has been stored in 5% formalin. Here we demonstrate the method of dehydrating in alcohol, staining the specimen during the dehydration, and then mounting in Bio-Plastic®. Once again, it is important to point out that most mounting media, such as Bio-Plastic are not miscible with water. The water in this specimen must be replaced by alcohol.

Dehydration is accomplished by an ascending series of alcohol solutions. A series is used so that osmotic pressure created during the transfer of fluids through the tissue will be reduced and cause minimum shrinkage and distortion.

1. Wash the pig embryo in tap water for several hours.

2. Drop the specimen from the water-wash into 30% alcohol (a mixture of 30 parts of 99% isopropyl alcohol and 70 parts distilled water). Leave the embryo in the solution overnight.

3. Transfer the specimen from 30% to 50% alcohol; leave overnight.

4. Transfer the specimen from 50% to 70% alcohol. Again leave overnight.

5. Transfer from 70% alcohol to another jar of 70% alcohol to which has been added a sufficient amount of an alcohol borax carmine stock solution to make bright but dilute stain. (Recommended .0125% solution.) Leave in this solution until the pig embryo takes on a light pink color; overnight is recommended. Uniform staining is desired; but we find that whenever the specimen touches the side of the bottle the tissue does become stained. Make a little sling out of some gauze bandage, just about as wide as the opening in the bottle. The gauze should be about 6" long x 1" wide. Cradle the pig embryo in the center of the sling and lower it into the Borax Carmine stain bottle, looping both ends of the gauze over the edge of the jar so that the pig embryo is suspended in the stain solution.

6. When the pink color has been attained, transfer the specimen to 95% alcohol for a period of 24 hours.

7. Transfer to 100% alcohol overnight.

8. Repeat No. 7 using a fresh supply of 100% alcohal overnight.

9. Transfer now to a bottle containing a mixture of equal parts of 100% alcohol and xylene for 2 to 4 hours.

10. Transfer now to 100% xylene. The soft tissues will gradually become more transparent, showing some internal structure. If the specimen does not clear, there is still water in it; return it to 100% alcohol. The clearing process may take from two to eight hours, and should be watched regularly during this period.

11. Transfer the specimen to uncatalyzed Bio-Plastic® and leave there for a period of 2 to 7 days. Complete impregnation with uncatalyzed plastic is indicated when the specimen sinks to the bottom of the jar.

12. Again find a suitable mold to use for your sample. Finish the block in the usual seas.

Cleanup hints: Use acetone for cleaning liquid plastic from hands, containers, etc. Hardened plastic can be removed by soaking in acetone. Add a few drops of catalyst to contaminated liquid Bio-Plastic® for safe disposal.

EMBEDDING BOTANICAL MATERIALS

Plant materials can also be embedded in Bio-Plastic®. Dry, pressed botanical specimens may be prepared and embedded as outlined in Project 1. Specimens in preserving fluids or Evergreen solution, may be generally treated as follows:

1. Immerse specimen in 30% glycerine for 24 hours.

2. Transfer to fresh 30% glycerine for 24 hours.

3. Transfer to 50% glycerine for 24 hours,

4. Transfer to 75% glycerine for 24 hours.

5. Transfer to 100% glycerine for 24 hours.

6. Place specimens on clean cloths or toweling, cover with clean cloths or toweling, and allow to drain for 12 hours. If necessary, repeat by changing to fresh cloths or toweling.

7. Place specimens in uncatalyzed plastic for 12 to 24 hours. If plastic is discolored by the presence of excess glycerine at the end of this time, transfer to fresh uncataylzed plastic, Repeat until uncataylzed plastic shows no signs of glycerine contamination. The specimens can then be embedded.

It may be desirable to stain certain specimens that have been preserved in fluids that removed natural color. Such specimens must be dehydrated in a series of successively stronger alcohol solutions, as in Project 5. Specimens may be stained in a series of stronger alcohol solutions, as in Project 5. Specimens may be stained in 70% alcohol using the stain of your choice. For restoring green color, Fast Green or Light Green SF Yellowish work well. After staining, proceed as in Project 5 to complete the embedment.
For any special cases not covered by these instructions, please contact Ward's for further information, outlining the material you are working with and the preservations used.

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