

## P40 Brain Slices Plastination Using Methanol for Dehydration

Mircea-Constantin Sora and Peter Brugger

*Department of Anatomy 2, Anatomical Institute, Vienna University, Vienna, Austria*

(received February 22, accepted March 29, 2000) **Key**

*Words: Brain slices, Methanol dehydration, Polymer P40*

---

### Abstract

Human brain slices, 4mm thick, were plastinated using methanol (methyl alcohol) as the dehydration medium instead of acetone. The slices were processed using the standard P40 technique and it was established that the use of methanol as a dehydration medium would not generate problems in plastination of brain slices. Some particularities are to be regarded only in the impregnation phase of the plastination process.

---

### Introduction

Brain slices can be plastinated by the conventional P40 technique (von Hagens, 1994; Barnett, 1997). One major problem for plastinators is that of using acetone for dehydration of specimens. Most laboratories have problems with handling acetone because of its explosion hazard. Methanol has been previously reported to serve as intermediary solvent before impregnation with Biodur S15 (von Hagens, 1992). With regard to these facts, another dehydration medium was sought for brain slices plastination. After studying the physical/chemical properties of solvents used generally for dehydration of human tissue it was decided to try methanol.

### Materials and Methods

#### Fixation and Slicing

Plastination of brain slices in accordance with the P40 procedure requires proper fixation of the brain. Human brains were obtained at post-mortem from cadavers. The brains were fixed for two months in 5% formalin. One brain was used for this study. The brain was washed in running tap water for two days and was then sliced at 4 mm on a rotary (meat) slicer. The wet brain slices were put between stainless steel grids which were assembled in a stainless steel grid basket for further processing.

#### Rinsing in running water

Address correspondence to: Mircea-Constantin Sora, Department of Anatomy 2, Anatomical Institute, Vienna University, Wahringerstr. 13/ 3, A-1090 Wien, Austria. Telephone: 43 1 4277 611 50/Fax.: 43 1 4277 611 70. E-mail: mircea-constantin.sora@univie.ac.at

The assembled basket was placed in a rectangular 20L bucket filled with tap water and rinsed overnight. It was then precooled at +5°C in order to avoid the formation of ice crystals during later processing (von Hagens, 1994).

#### Dehydration

The methanol used for dehydration of the brain slices was cooled down between -20°C and -25°C. To dehydrate 12 brain slices, 20 L of technical quality methanol was used. The dehydration time for 4mm thick brain slices was 6 days; the methanol was changed twice, first after 4 days and second after 2 days for a total of 3 baths. The final concentration of the dehydration bath was 98.5%. The density of methanol was measured by using a hydrometer (Merck Eurolab GesmbH, Austria).

#### Impregnation with P40

Dehydration being completed, slices were impregnated with Biodur P40 (Biodur, Rathausstr.18,69126 Heidelberg, Germany). The resin was precooled to +5°C. After placing the brain slices in P40 (the resin level should be 4 cm above the slices), the container was placed in a refrigerator at +5°C for two days. Forced impregnation was started in the morning at room temperature (Cook and Barnett, 1996). Vacuum adjustment began at 100 mmHg (130 mbar) and ran the whole day until a pressure of 10 mmHg (13 mbar) was reached. At room temperature the impregnation took about 24 hours.

### Casting and Curing

Each slice was cast in an individual flat chamber consisting of two glass plates with a 6mm silicone tube sandwiched between the two glass plates. The flat chambers were cured in horizontal position using UVA tubes of 200 Watts positioned above and below. The distance from the UV lamps to the flat chamber was 200mm. Jellification of P40, visible through the loss of transparency of the slice, happened after 30 minutes. Curing time was about 3 hours.

### **Results**

The results were good in that plastinated brain slices after methanol dehydration (figure 1) appeared identical in color and shape to those plastinated after dehydration with acetone (figure 2).

### **Discussion**

The aim of the present study was to present another dehydration possibility for P40 plastination. We do not suggest a replacement of acetone dehydration but we wanted to demonstrate the possibility of other ways to dehydrate.

An ideal dehydration solvent should have a high vapor pressure and should be soluble in water. This is the main reason for using acetone for dehydration. Its vapor pressure is 180 mmHg (233 mbar) (Material Safety Data Sheet, Merck Chemicals, Austria). Vacuum adjustment during impregnation starts at this point and impregnation is considered to be finished at 10 mmHg (13 mbar) at room temperature or at 5 mmHg (6.5 mbar) at +5°C (von Hagens, 1994). That means that there should be no more acetone in the brain slices. Regarding this fact we decided to try a similar solvent. By studying different chemical and physical data we decided to use methanol for dehydration (synonyms: methyl alcohol, methyl hydroxide, carbinol). Methanol is water soluble and has a high vapor pressure. See table 1 for comparison between the chemical and physical datas of acetone and methanol.

Its higher boiling point and lower vapor pressure make methanol less volatile than acetone. The flash point of acetone is significantly lower than the flash point of methanol. A fact that makes acetone more dangerous than methanol.

During impregnation the evacuation of methanol was observed by the formation of small bubbles on the surface of the polymer at 92 mmHg (120 mbar). At this point the vacuum was adjusted until it reached 10 mmHg (13 mbar). Bubbles [formation on the surface of the polymer was observed only veen 92 mmHg (120 mbar) and 61.5 mmHg (80 mbar).

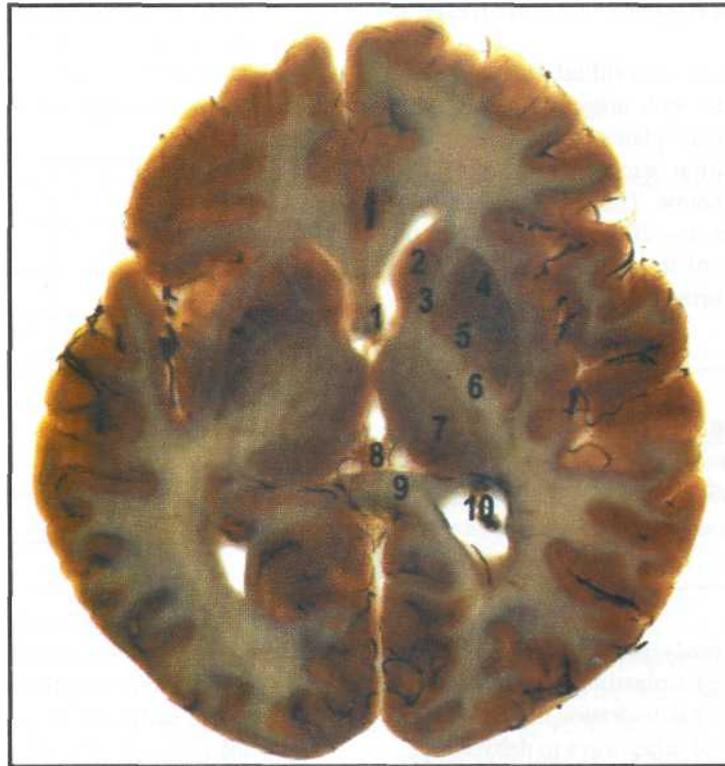
**Table.** Comparison between Acetone and Methanol (physical and chemical datas taken from Material Safety Data Sheets, Merck Chemicals, Austria; prices calculated from the purchase of 180L of each solvent from Merck Chemicals Austria).

	ACETONE	METHANOL
water-soluble	yes	yes
vapor pressure	233 mbar	123 mbar
lower explosion limit	2.5%	6%
upper explosion limit	13%	32%
boiling point	55°C	65°C
flash point	-18°C	+11°C
price of 1 liter	2.79\$	2.15\$

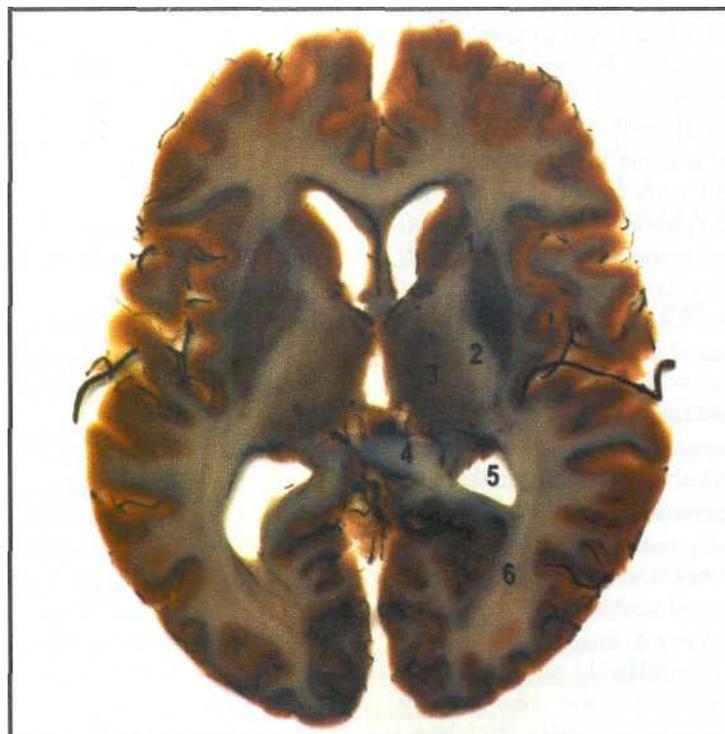
This was the major problem, because we did not know if we extracted all the methanol. The dehydrated brain slices (305.2 grams) were weighed when they were transferred from methanol to polymer. In order to determine if all of the methanol was extracted we weighed our impregnation bath containing the polymer and the brain slices before (10,841.3 grams) and after (10,561.6 grams) the impregnation. The lost of weight observed (279.7 grams) permitted us to conclude that all the methanol has been extracted during the impregnation step. Since the methanol extraction appears to be very fast (only between 92 mmHg and 61.5 mmHg) we think that only experienced plastinators should use methanol for dehydration.

### **Bibliography**

- Barnett RJ: Plastination of Coronal and Horizontal Brain Slices using the P40 technique. *J Int Soc Plastination* 12 (1): 33-36,1997. Cook P, Barnett R: Practical applications in plastination. Instructional videotape, Departments of Anatomy, University of Auckland and University of Otago, New Zealand, 1996. Merck Chemicals GesmbH: Material safety data sheet for acetone, Vienna, Austria. Merck Chemicals GesmbH: Material safety data sheet for methanol, Vienna, Austria, von Hagens G: Methanol and ethanol as last intermedium for the S15-technique. 6th Int Conf Plast, Kingston, Ontario, Canada, 1992. von Hagens G: Plastination of brain slices according to the P40 procedure. A step-by-step description, pp. 1-23, 1994.



**Figure 1.** P40 plastinated brain slice after dehydration with methanol. Column of fornix (1), head of caudate nucleus (2), internal capsule (anterior part) (3), putamen (4), globus pallidus (5), internal capsule (posterior part) (6), thalamus (7), pineal gland (8), splenium of corpus callosum (9) and choroid plexus (10).



**Figure 2:** P40 plastinated brain slice after dehydration with acetone. Internal capsule (anterior part) (1), internal capsule (posterior part) (2), thalamus (3), splenium of corpus callosum (4), lateral ventricle (5), optic radiations (6).