# DOWICIL '100' A FIXATIVE-CUM-PRESERVATIVE FOR ZOOPLANKTON


ABSTRACT

Results of a set of experiments using a quaternary ammonium-type chemical with a formaldehyde base, Dowicil '100' 1-(3-chloroallyl) -3, 5, 7 - triaza-1-azoniaadamantane chloride, as an alternative to formaldehyde for fixation and preservation of plankton are reported. The anti-microbial properties of this chemical are independent of the pH of the substrate. Experiments using Dowicil '100' in 10, 20 and 30% strength in distilled water and sea water with and without additives were conducted. Colour and pigments were not preserved adequately and calcareous material was found to undergo slow dissolution. Cytoplasmic preservation was found to be good with least plasmolysis and plankton with chitinous exoskeleton were also well preserved. A strength of 30% for fixation and 10% for preservation is recommended.


With a view to evolve an alternative fixative-cum-preservative to formaldehyde for zooplankton, a number of chemicals were used in experiments to compare their suitability. These experiments were carried out at the Indian Ocean Biological Centre during 1968-1972 in accordance with SCOR/UNESCO/WG - 23 recommendations. Of these chemicals, one was a quaternary ammonium-type product known as Dowicil '100', which has a formaldehyde base (Dow Chemical Company 1965, 68). Chemically it is 1-(3-chloroallyl) - 3, 5, 7 - triaza - 1 - azoniaadamantane chloride. The mechanism of action of Dowicil '100' depends upon the controlled release of formaldehyde in aqueous substrates. However, the antimicrobial properties of Dowicil '100', in contrast to those of formaldehyde, are independent of the pH of the substrate. Consequently, Dowicil '100' preservative can be utilized in aqueous systems without the need of pH adjustment - an important factor when the substrate is pH sensitive.

Dowicil '100' with strengths of 10, 20 and 30% were prepared in distilled water and sea water. Solubility of Dowicil '100' in water is very high, at the rate of 230 gm / 100 gm of water at 28°C. The pH of the solutions when prepared were from 5.7 to 4.3 for 10 to 30% solution in sea water and 5.1 to 4.5 for 10 to 30% solution in distilled water. However, on ageing a pH $\simeq$ 5.5 was maintained at an almost constant level for the last four years whether it be sea water or distilled water preparation. Yellow colour of the aqueous solutions was found varying in intensity, depending on the

concentrations. Freshly prepared solutions were taken on board the vessel 'Blue Fin' in 1968 and fresh undamaged plankton collected in excellent condition (Balachandran, 1974) was added to the test fixatives in the ratio of 1 : 9. These series were repeated with Dowicil '100' solutions to which additives such as borax, hexamine, sodium acetate, calcium carbonate, potassium oxalate, sodium benzoate and propylene glycol were added in varying concentrations of 0.5 to 15%. Based on those characters of taxonomic importance, the preservative qualities of jelly-like, calcareous and cuticular plankters; fish eggs and larvae etc. were evaluated. Changes in pH were recorded along with changes in light and temperature in the laboratory. Observations were carried out at the time of addition of zooplankton and at the intervals of 1, 7, 15 and 30 days; 2, 3, 6 and 12 months; 2, 3 and 4 years. The results are interpreted by visual examinations of the preserved material.

Though the colour of the eye of the organisms and their pigmentation particularly in amphipods, euphausiids and decapods, were not retained adequately, the natural colour of Dowicil '100' seemed to have imparted to them some yellow colouration. Dowicil '100' solution containing 1% sodium acetate seems to improve colour preservation. Dinoflagellates were found in moderately good condition. Zooplankton with calcareous shells, where skeletal taxonomy is important, were found to undergo slow physical dissolution and deterioration probably due to the acidity developed at pH around 5.5. Hence it is not good for plankton with calcareous skeleton. However, in samples containing 3 – 5% potassium oxalate or 1% calcium carbonate, the shelled forms were found in better condition as the preservative could maintain a pH above 6.0. Cyphonautes and echinoderm larvae were also fairly well preserved. Among the jelly like plankters, where cytoplasmic preservation is important, medusae, chaetognaths, salps, doliolids appendicularians etc. remained in good condition with least plasmolysis. However, ctenophores, on agitation disintegrated easily. Conditions of plankters with a chitinous exoskeleton was satisfactory. Preservation and storage in darkness and in an airconditioned room was found to be better than when the material was exposed to light and at varying temperatures. In conclusion it may be stated, the advantage in the use of Dowicil '100' is that its odour is neither disagreeable nor irritating as with formaldehyde, osmic acid etc. The ability of Dowicil '100' to protect zooplankton from microbial spoilage, thus prolonging their shelf life at comparatively low concentrations is remarkable. Dowicil '100' may be preferred in situations, where the economy in weight of fixatives and the bulk are important. Also preparation of the *in situ* Dowicil '100' solutions is easier. In view of the controlled release of formaldehyde in solution, 30% strength can be preferred for fixation and 10% for preservation. Whether Dowicil '100' liberates sufficient active formaldehyde at room temperature to keep the fixed tissues in sound condition for a duration of several years, is still to be ascertained.

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REFERENCES

Balachandran, T. 1974. On methods of collection, handling and storage of zooplankton in tropics. *Curr. Sci.*, **43** (5): 154-155.

The Dow Chemical Company Pamphlet. 1965, 1968.