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ION TRANSPORT ACROSS VESICLE BILAYERS MEDIATED BY AN ARTIFICIAL CHANNEL COMPOUND

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Summary: A polymer of an isocyanide $[R-N=C<]_n$ (\bar{R} = 4'-benzo-18-crown-6) whose pendant crown ether side chains form molecular channels, is able to transport cobalt ions across the bilayer membrane of positively charged vesicles.

Ion transport across the membrane of the living cell is mediated by carrier type (e.g. valinomycin) or channel type (e.g. gramicidin A, transport proteins) ionophores.¹ Models of the former category of compounds have been synthesized and extensively studied both in natural and model membrane systems.² Synthetic models of the latter category are unknown.³

Very recently, we reported the first synthesis of a cation channel.⁴ The compound is a polymer of an isocyanide $(R-N=C<)_n$, 1, which contains benzo-18-crown-6 side chains. The crown ether rings are on top of each other at a distance of 4 Å and form 4 channels which run parallel to the polymer axis (Figure 1).

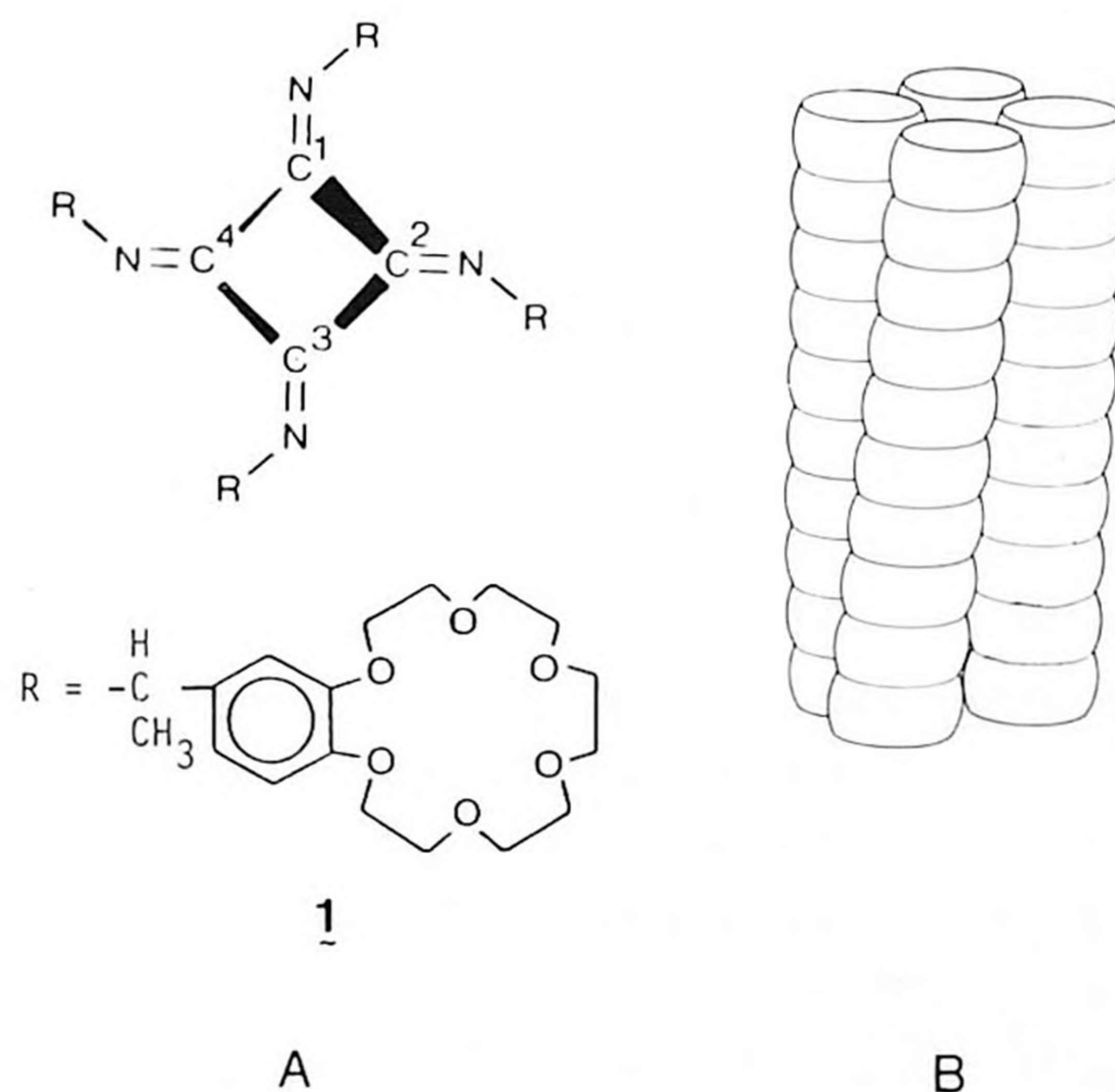
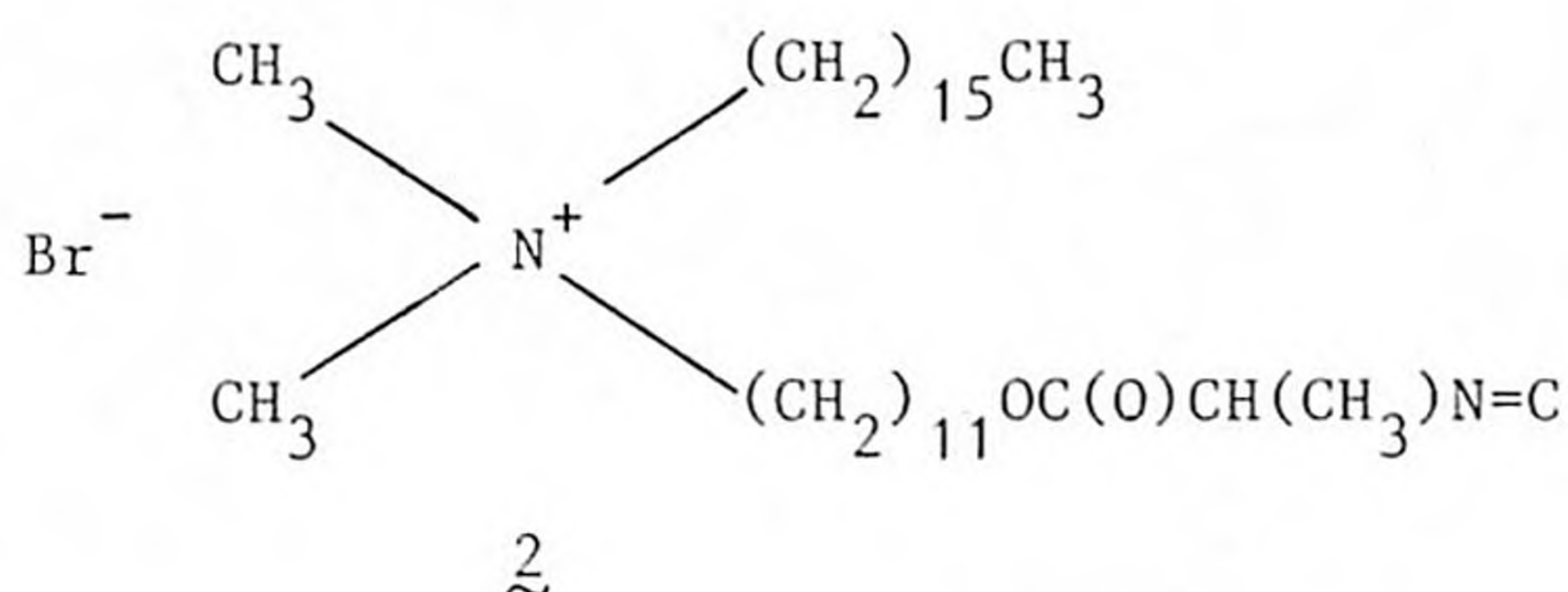


Figure 1. Projection of polymer 1 along its helix axis. Repeating unit C^5 is behind C^1 , etc. (A). Schematic picture of the four molecular channels (B).

Compound 1 displays the main features which are thought to be important for a channel mediated ion transport, *i.e.* a series of ion binding sites,⁵ pores with a polar interior and an apolar exterior, a hydrophilic top and bottom which may face the two aqueous sides of a membrane, and a chain of sufficient length⁶ to span the membrane. In this communication we present preliminary evidence that compound 1 can be incorporated into vesicles of a synthetic surfactant and that it is able to transport cobalt ions across the bilayer membrane.⁷

Experiments were performed with amphiphile 2. On dispersal in water this compound forms



relatively stable, closed vesicles.⁸ Compounds 1 (2.0 mg, 0.14 μmol) and 2 (20 mg, 32 μmol) were dispersed in water (1 ml) by vortexing for 2 min at 20°C. The following features indicate that channel compound 1 is incorporated in the bilayer of the vesicles. *i.* Electron micrographs taken by the freeze fracture technique reveal the presence of closed aggregates with average diameters of 200 Å. Electron micrographs of vesicles prepared under the same conditions without compound 1 show diameters of 700 Å. The decrease in vesicle size when 1 is added, suggests incorporation of this compound. A similar effect has been observed when proteins are built into liposomes.⁹ *ii.* Gel filtration (Sephadex G 100) of the dispersion and examination of the eluted fractions by UV and IR reveals that the vesicles and compound 1 are present in the same peak in the void volume of the column. Blank gel filtration experiments with dispersions of the separate components show peaks at different elution volumina, *i.e.* in the void volume (vesicles) and at a relative elution volume of about 4 (compound 1). *iii.* Vesicle dispersions containing compound 1 show a lower phase transition temperature than vesicle dispersions without this compound. According to ¹H NMR line width measurements these transitions occur at ≈ 24 and $\approx 30^\circ\text{C}$, respectively.

Cobalt ion transport across the vesicle bilayer membrane was measured in the following way.¹⁰ Vesicle dispersions incorporating the dye 4-(2-pyridylazo)resorcinol mono sodium salt (PAR) in its aqueous interior were obtained by vortexing surfactant 2 (10 mg, 16 μmol), channel compound 1 (1 mg, 0.07 μmol) and an aqueous solution containing PAR (1 ml, 8.2 μmol , pH 7.5). The exo vesicular dye was removed by dialysis (Amicon Diaflo UM2 or YM2 filter) with diluted aqueous HCl (160 ml, pH 3). The pH of the resulting dispersions was raised to 7.8 by addition of NaOH, and the volume was adjusted to 25 ml. The latter dispersions (0.5 ml) were mixed with an aqueous solution of $\text{Co}(\text{NO}_3)_2$ (20 μl , 1.19 M) and the rate of cobalt ion transport from the outside to the interior aqueous solution was measured by following the increase in absorption of the cobalt-PAR complex at 510 nm. Blank experiments were performed with vesicles without compound 1. Figure 2 shows the results of a typical experiment. When the channel compound is present ion transport occurs with a transport rate constant of about 10^{-4} s^{-1} . Without this compound no transport takes place. Increase of absorption at 510 nm was only observed after destruction of the vesicles with Triton X-100.

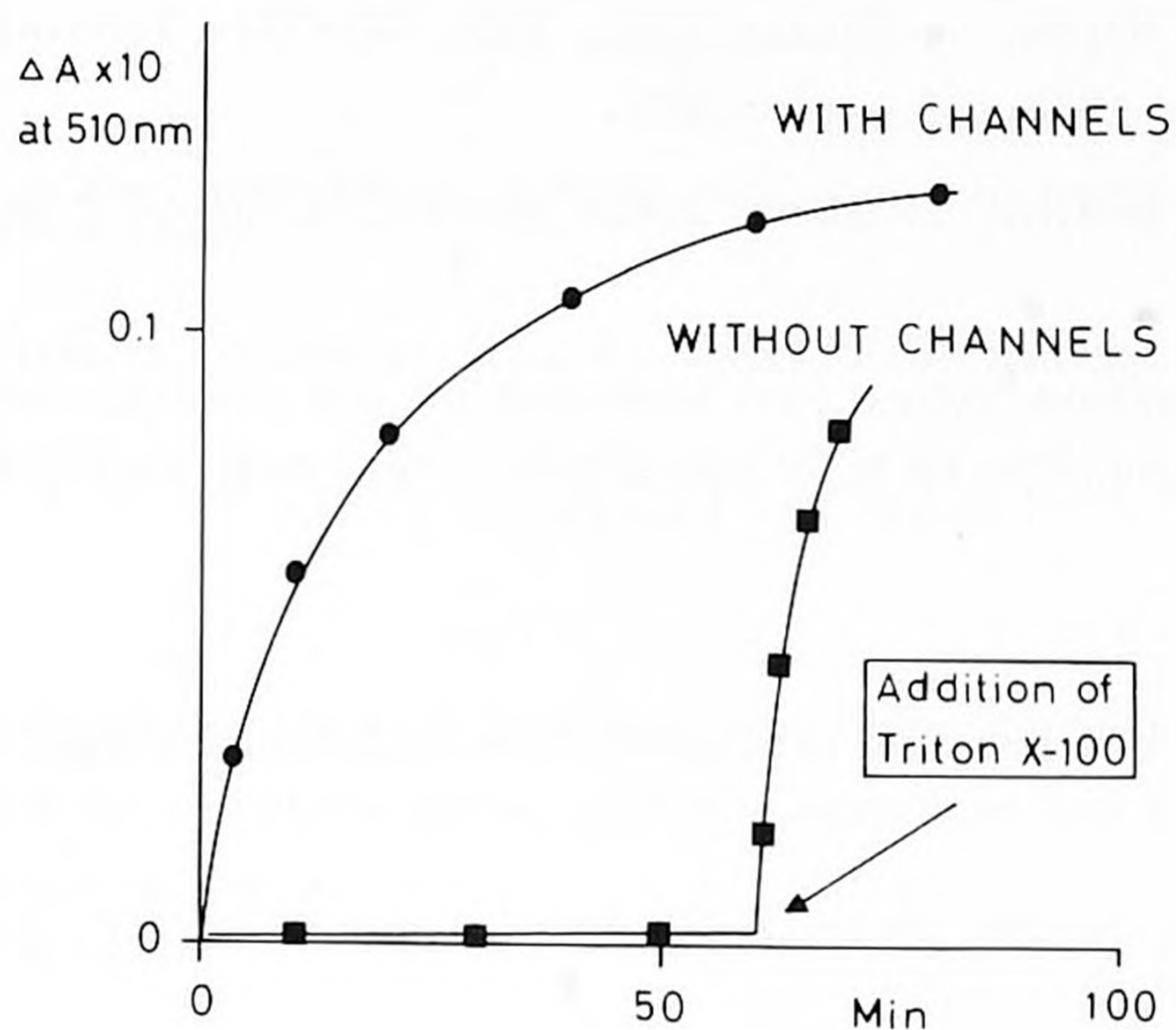


Figure 2. Cobalt ion transport across the bilayer membrane of vesicles derived from surfactant $\underline{2}$. Change in absorbance at 510 nm versus time for vesicle dispersions containing $\underline{1}$ (●) and without $\underline{1}$ (■)

The observed ion transport is not due to leakage of the vesicles caused by disordering of the bilayers upon incorporation of $\underline{1}$. If this would be the case the vesicle dispersions containing $\underline{1}$ would also display leakage of encapsulated PAR. Repeated dialysis of these dispersions, however, showed that no such leakage occurred.

The data presented here suggest that compound $\underline{1}$ may function as a channel type ionophore. Experiments which further support this idea are currently in progress.

Acknowledgement

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5. Association constants for various metal ions measured by the picrate extraction technique are (M^+ , $K_a \times 10^{-5}/l.mol^{-1}$): Li^+ , 17; Na^+ , 20; K^+ , 300; Rb^+ , 120; Cs^+ , 90; Co^{2+} < 0.05; c.f. Ref. 7.
6. Average chain length of the channels as estimated from molecular weight measurements is 40 Å, c.f. ref. 4.
7. More details in a full paper in the forthcoming special issue of Isr. J. Chem. devoted to ionophores.
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10. We have chosen cobalt ions for our experiments as ample water soluble dyes are available that form coloured complexes with this ion. Picrate extraction experiments show that the channels of 1 bind cobalt ions, although less strongly than alkali metal ions, c.f. ref. 5.

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