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Cyclodextrins Lessen the Membrane Damaging Effect of Nonionic Tensides

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Abstract. Nonylphenyl-ethyleneoxide polymers containing 5, 9 and 30 ethyleneoxide groups per molecule build into the hydrophobic fatty acid chains of the cell membrane phospholipid dipalmitoyl-phosphatidyl-choline (DPPC) resulting in a decreased main transition temperature, a decreased enthalpy of the main transition and in enhanced potassium permeability of DPPC liposomes. The α-, β- and γ-cyclodextrins form inclusion complexes with the tenzides lowering their free concentration. The complex formation lessens or sometimes totally prevents the membrane damaging effect of tensides. The effectivity order of cyclodextrins is $\beta CD > \gamma CD > \alpha CD$.

Key words: Liposomes, permeability, tenside cyclodextrin complex.

1. Introduction

Pesticide formulations generally contain nonionic tensides that improve the technical parameters (lower drop volume, higher suspension or emulsion stability, better spreading on the leaf surface etc.) of formulations [1]. Besides these effects the non-ionic tensides can modify the biological efficiency of an active ingredient [2, 3] and even its selectivity [4]. Sometimes they themselves show marked microbicidal effect [5, 6] and enhance the phytotoxicity [7, 8, 9]. The mode of action of non-ionic tensides has been explained by the fact that they interact with the membrane phospholipids [10] and increase the membrane permeability [11]. This effect depends on the lipophilicity and on the structural characteristics of the hydrophobic part [12]. Due to their capacity to form inclusion complexes with a large number of organic compounds cyclodextrins are finding growing acceptance and application in human therapy and in agrochemistry [13, 14]. As they form complexes also with tensides [15, 16, 17] it was assumed that the interaction between nonionic tensides and cyclodextrins can be used to lessen or to prevent the membrane damaging effect of tensides. The non-methylated cyclodextrins themselves exert only a negligible effect on membranes, however, the methylated derivatives show marked membrane damaging activity [18].

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2. Materials and Methods

The nonylphenyl-polyethyleneoxide nonionic tensides (Hoechst FRG) contain 5, 9 and 30 ethyleneoxide groups per molecule on average (abbreviated to T_5, T_9 and T_{30} respectively). The cyclodextrins: alpha-(αCD), beta-(βCD) and gamma-cyclodextrin (γCD) are produced by Chinoin (Hungary). Dipalmitoyl-phosphatidylcholine (DPPC) was used as purchased from the Sigma Chemical Co.

Differential Scanning Calorimetry (DSC) studies and the determination of the permeability of DPPC liposomes were carried out as described in [18] and [19]. The molar ratio of samples varied in the range DPPC:tenside:cyclodextrin 100:0–1:0–5. The main transition temperature (T_m °C) and the enthalpy of the main transition (ΔH mJ/mg) was determined from the DSC data. The permeability time constant ($P_t s^{-1}$) was calculated according to [20].

3. Results and Discussion

The results of DSC measurements are compiled in Figures 1 and 2. Each tenside considerably lessens the main transition temperature of DPPC (Figure 1). This observation suggests that the tensides – after binding to the bilayer surface – penetrate among the hydrocarbon chains of lipid molecules. This intercalation depends on

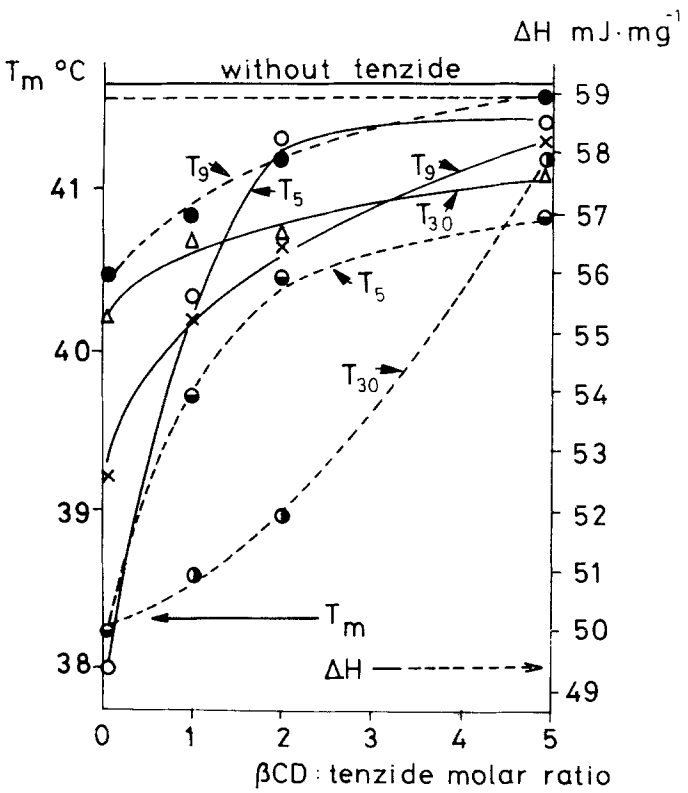

Fig. 1. Effect of tensides (T_5, T_9 and T_{30}) as a function of the βCD molar ratio on the main transition temperature (T_m) and enthalpy (ΔH) of DPPC. The DPPC:tenside molar ratio was 100:1.

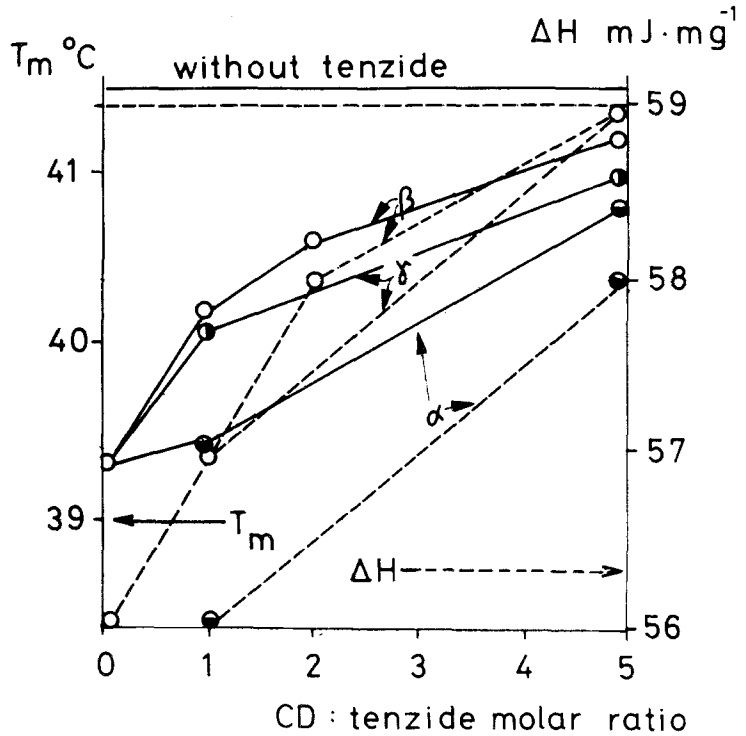

Fig. 2. Effect of T_9 in the presence of cyclodextrins on the main transition temperature (T_m) and enthalpy (ΔH) of DPPC. The DPPC : tenside molar ratio 100 : 1.

the ratio of hydrophobic and hydrophilic parts within the tenside molecule. The modifying molecules act as structural defects loosening the lipid packing density and lowering the transition temperature and enthalpy (Figure 1). βCD lessens or even annuls these effects in each case. The interaction between DPPC and the tensides decreases nonlinearly with the increasing molar ratio of βCD : tenside, indicating the formation of fairly stable inclusion complexes between tensides and βCD.

The fact that at higher βCD molar ratios the differences between the effects of the different tensides becomes rather small suggests that the bulky hydrophobic part of tensides (identical in each tenside) is responsible for the complex formation and the length of the hydrophilic ethyleneoxide chain (different in each tenside) has a negligible effect on the complex formation.

The various cyclodextrins exert different effects on the interaction between the T_9 tenside and DPPC (Figure 2). βCD exerts the highest and αCD the lowest preventive effect. This finding indicates that the dimensions of the βCD and γCD cavities are nearly equally adequate for the hydrophobic nonylphenyl moiety of the tensides. The lower effect of αCD is due to the smaller cavity.

The results of permeability determinations on liposomes support the conclusions formulated above (Figure 3). The intercalated tenside molecules disturb the organization of lipid bilayers. The efflux of potassium ions is higher through the partially disorganized, more loosely packed lipid layers. The cyclodextrins counteract the permeability increasing effect of T_9 and this effect increases with increasing CD : T_9

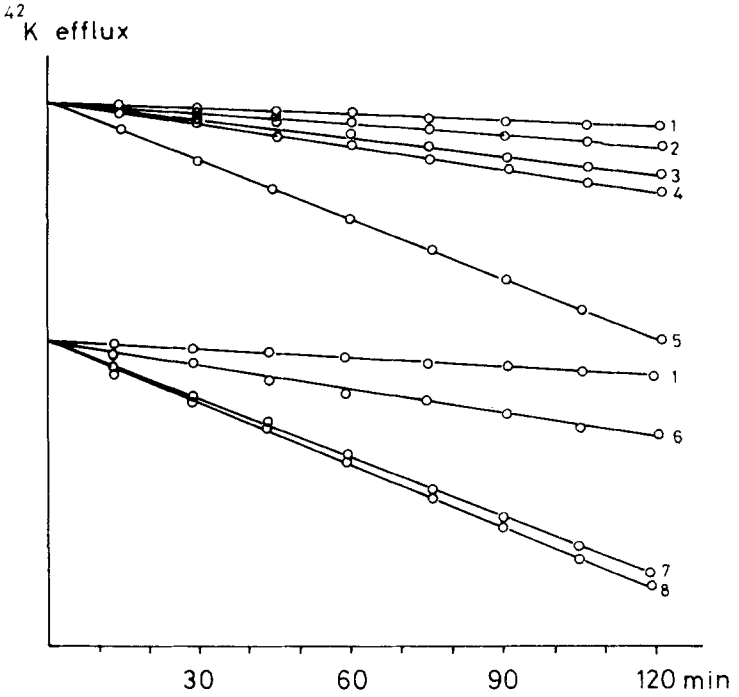

Fig. 3. Effect of T_9 in the presence of cyclodextrins on the ^{42}K efflux of DPPC liposomes. 1. DPPC; 2. DPPC:T_9:βCD 100:1:5; 3. DPPC:T_9:βCD 100:1:2; 4. DPPC:T_9:βCD 100:1:1; 5. DPPC:T_9 100:1; 6. DPPC:T_9:αCD 100:1:5; 7. DPPC:T_9:αCD 100:1:1; 8. DPPC:T_9 100:1.

molar ratio. αCD exhibits also in this case a lower preventive effect than βCD proving again the poorer stability of its inclusion complex with T_9. The permeability constant changes nonlinearly with the cyclodextrin:T_9 molar ratio (Figure 4). At higher cyclodextrin-tenside molar ratios the permeability increase caused by tenside is nearly completely supressed. The effectivity order of cyclodextrins is the same as in the DSC measurements: $\beta CD > \gamma CD > \alpha CD$.

Under such conditions in aqueous solutions, the majority of the tenside molecules are in the complexed form. The membrane damaging effect of free tenside at reduced concentration is of course lower.

Our data do not exclude the possibility that the cyclodextrin-tenside complexes may interact with the DPPC. However, the facts that the cyclodextrins do not show any membrane damaging effect and the tenside-cyclodextrin complex is highly hydrophilic and has considerable dimensions contradict this supposition.

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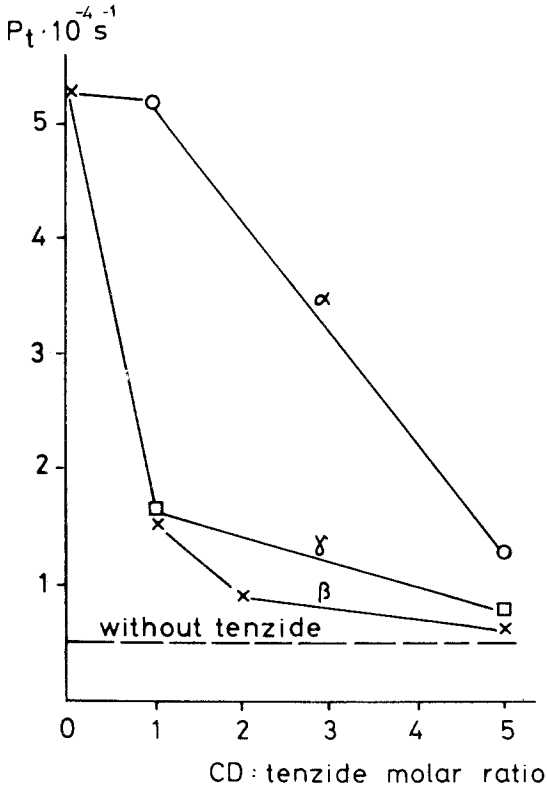

Fig. 4. Effect of T_9 in the presence of cyclodextrins on the permeability time constant of DPPC liposomes.

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