

## Comparison of the Solubilization of Human Platelet Protein, Cholesterol, Gamma Glutamyltransferase and Lactate Dehydrogenase by Triton X-100 and Brij-35

### İnsan Trombositlerinden Protein, Kolesterol, Gamma Glutamiltransferaz ve Laktat Dehidrogenazın Triton X-100 ve Brij-35 ile Çözünürlüğünün Karşılaştırılması

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#### Abstract

Triton X-100 and Brij 35 are nonionic polyoxyethylene based detergents. Compared with Brij 35, Triton X-100 is used more frequently for the solubilization of proteins and lipids. In this study, the solubilization of human platelet protein, cholesterol, gamma glutamyltransferase and lactate dehydrogenase in both detergents were compared. Platelets were treated with Triton X-100 and Brij 35 at various concentrations and various time periods. No significant differences were observed between the detergents at various concentrations and incubation times in terms of the solubilization of platelet protein, cholesterol, gamma glutamyltransferase or lactate dehydrogenase.

**Keywords:** Triton X-100, Brij 35, Platelets, Protein solubility.

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#### Introduction

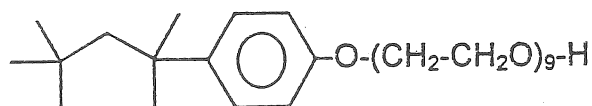
Detergents are useful for the solubilization of membrane proteins and lipids (Moss,1988; Banerjee *et al.*, 1983) and finding the best agent for a particular situation may depend on a process of trial and error. The choice of a suitable detergent is usually based on its ability to preserve enzymatic activity (Shiao *et al.*, 1989). The solubilization of a membrane by a detergent is based on the conversion of membrane bilayer to mixed micelles of detergent and membrane constituents (Helenius and Simons, 1975). Depending on the detergent concentration, the solubilization appears to occur in three steps; 1. Detergents bind to the membrane, 2. Membrane lysis occurs, 3. The membrane is solubilized in the form of detergent-lipid-protein complexes. The ionic change or nonionic nature of detergents, as well as the critical micellar concentration (CMC, the critical limit of detergent concentration at which micelles are formed) are among the factors that can modify the detergent enzyme interactions (Tanford and Reynolds,1976; Chang and Bock, 1980).

Triton X-100 (nano ethylene glycol octylphenol ether) and Brij 35 (polyoxyethylene lauryl ether) are nonionic polyoxyethylene based detergents (Fig. 1) (Ashani and Catrawas, 1980). Triton X-100 is most widely used for the solubilization of membranes (Banerjee *et al.*, 1983).

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Solubilization studies are investigated by analyzes of the cell lysis, marker enzymes and solubility of membrane components (Shiao *et al.*, 1989).



Triton X-100



Brij-35

Fig 1. Chemical structures of TritonX-100 and Brij 35.

Comparison of the solubilization of protein, cholesterol, gamma glutamyltransferase (GGT) (a membrane marker enzyme) (Yardımcı *et al.*, 1995) and lactate dehydrogenase (LDH) (a cytosolic marker enzyme) (Shiao *et al.*, 1989) derived from platelets at different incubation periods and various concentrations of Triton X-100 and Brij 35 were aimed in the present study.

## Materials and Methods

**Chemicals:** Gamma-glutamyl p-nitroanilide, glycyglycine, Triton X-100, Brij-35 and bovine serum albumin were from Sigma and other chemicals were of analytical grade.

**Isolation of platelets:** Venous blood from healthy donors was taken in to tubes containing 0.077 mol. L<sup>-1</sup> EDTA (9:1). The samples were centrifuged at 1500 rpm for 8 min to obtain platelet rich plasma (PRP). PRP was centrifuged for 10 min at 10000 rpm to precipitate platelet pellet. Pellet was washed three times with 0.03 mol./L<sup>-1</sup> Tris- HCl buffer.

**Solubilization of platelets:** Platelets were incubated within various detergent concentrations (0.1% -2%) at 4°C. After 16 h incubation, platelets were centrifuged at 10.000 rpm for 15 min. The supernatants were analysed for the content of various cellular markers. In the same time, protein, cholesterol, GGT and LDH levels were determined after solubilization with 1% Triton X-100, 1% Brij-35 at different incubation times.

**Analysis of protein, GGT, LDH and cholesterol:** Total protein was determined according to the Bradford method using bovine serum albumin as the standard (Bradford , 1976).

GGT activity was determined in the solubilized fraction according to the Szasz method (1969) using gamma glutamyl p-nitroanilide as the substrate and glycyglycine as the acceptor.

The activities of lactate dehydrogenase and cholesterol levels were determined using Hitachi 717 Autoanalyzer (Boehringer Mannheim).

## Results and Discussion

The solubilizations of protein, cholesterol , GGT and LDH at various concentrations of Triton X-100 and Brij 35 are shown in Figs 2 and 3. 83% of the proteins were solubilized by 1.5% Triton X-100, while 78% were solubilized by 1.5% Brij 35. This concentration dependent study showed no advantage of either detergent to the other.

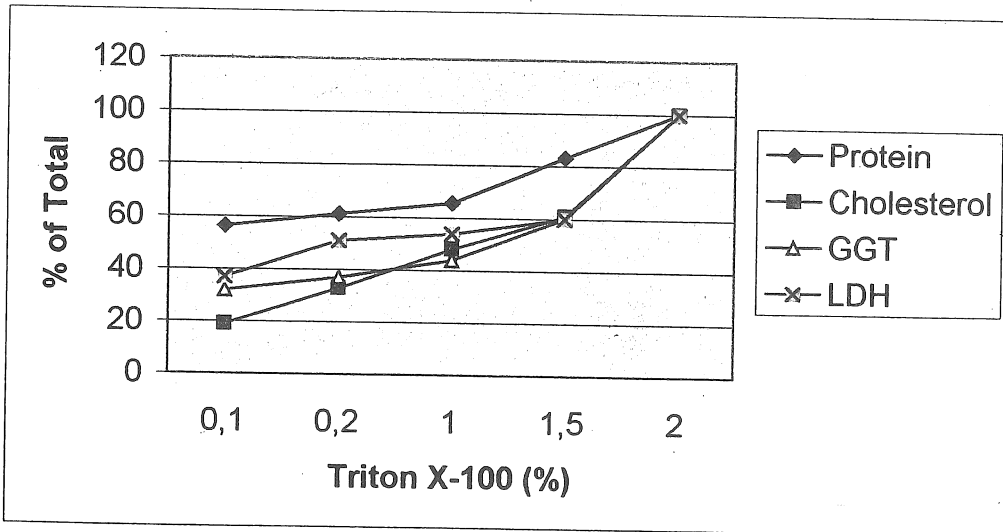


Fig. 2. Solubilization of protein, cholesterol, GGT and LDH from human platelet at various concentrations with Triton X-100.

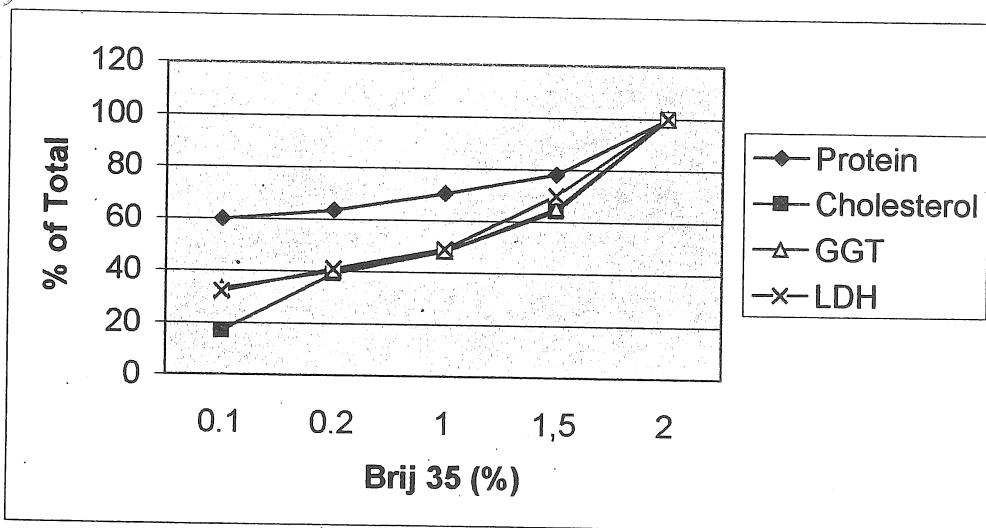


Fig. 3. Solubilization of protein, cholesterol, GGT and LDH from human platelet at various concentrations with Brij 35.

The results of solubilization studies on each detergent at different incubation times (30 min, 1, 2, 3, 6, 16 hrs) are shown in Figs 4 and 5. No significant difference was observed between Triton X-100 and Brij 35 in terms of the solubilization of platelet proteins, cholesterol, GGT or LDH at various incubation times.

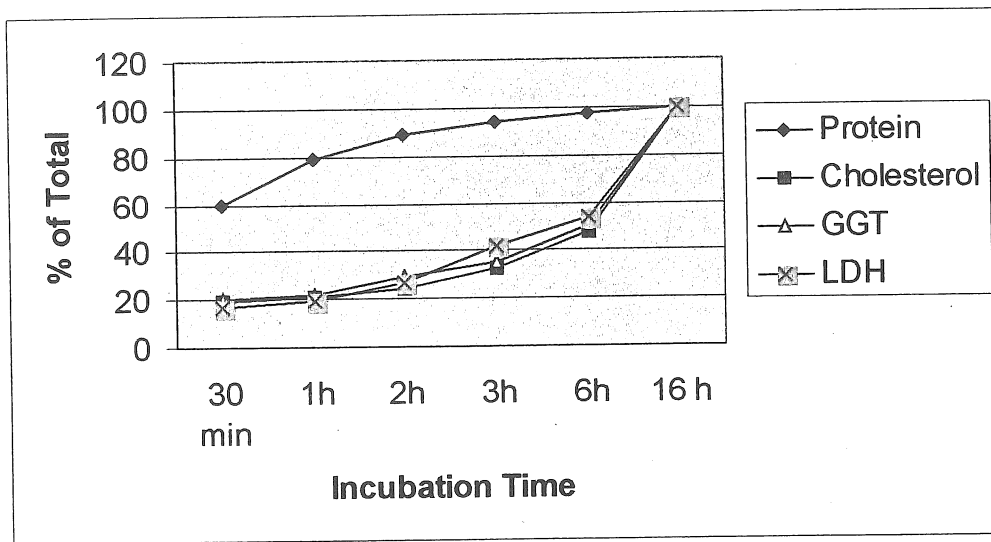


Fig. 4. Solubilization of protein, cholesterol, GGT and LDH from human platelet at various incubation times with Triton X-100.

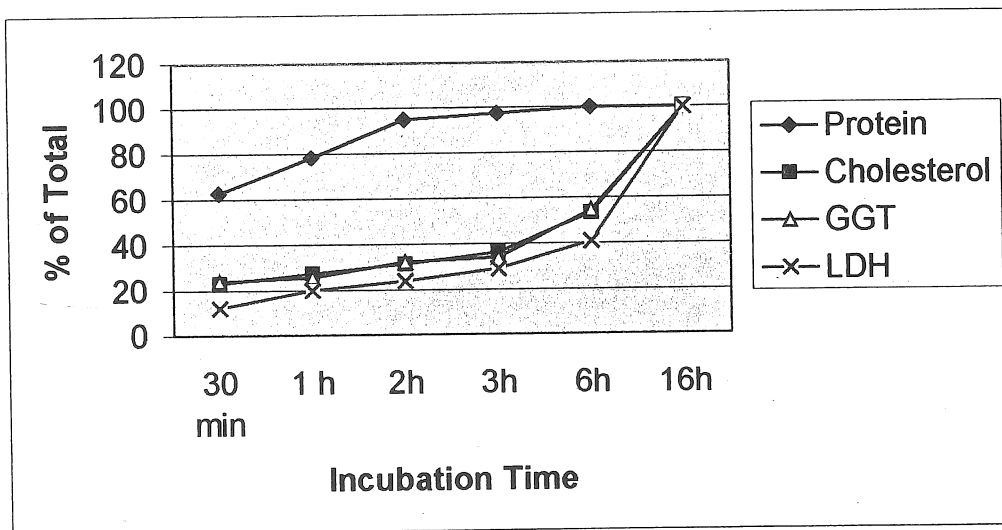


Fig. 5. Solubilization of protein, cholesterol, GGT and LDH from human platelet at various incubation times with Brij-35.

Detergents are widely used substances in biochemical research such as protein membrane isolation, electrophoresis techniques, liposome preparation and membrane solubilization (Satta, 1984; Mills and Freedman, 1983; Szoka and Papahadjopoulos, 1980). When detergents are used for solubilization, their concentrations and duration of application are very important (Banerjee *et al.*, 1983; Shiao *et al.*, 1989). At low detergent concentrations membrane proteins are engulfed in a coat of lipid and detergent molecules. At higher concentrations, more detergent molecules are expected to penetrate the membrane structure and finally dissociate the membrane completely so

that small units of detergents coated proteins and proteolipids are finally left (Banerjee *et al.*, 1983). The substance to be solubilized can be affected in the case of enzymes (Helenius and Simons, 1975). Nonionic polyoxyethylene based detergents, especially those produced industrially in bulk (e.g. Triton, Tween, Brij, Genapol, Thesit, Lubrol) are likely to contain peroxide and aldehyde impurities that may damage sensitive proteins (Ashani and Catrawas, 1980).

It is reported in the literature that the total mass of solubilized membrane lipids of sheep brain was always low at 0.5 % detergent concentration and attained a plateau between 1% and 2.5% for Chaps, Triton X-100 and Thesit except octyl glucoside (Banerjee *et al.*, 1983).

Shiao *et al.* (1989) showed that the protein solubilization and marker enzyme solubilization of platelets with octylglucoside, Triton X-100 and dodecylsulphate gave parallel graphics, and that detergents caused changes in platelet morphology.

This study compares the solubilization of human platelets by Triton X-100 and Brij 35. The results of our studies did not indicate any superiority of solubilization of platelet proteins, cholesterol, GGT or LDH between the detergents at various concentrations and incubation times so it is suggested that either can be used for such purposes.

## Özet

Triton X-100 ve Brij 35 noniyonik, polioksietilen bazlı deterjanlardır. Triton X-100, Brij 35'e oranla protein ve lipidlerin çözünürlüğünde daha fazla kullanılmaktadır. Bu çalışmada insan trombositlerinden protein, kolesterol, gamma glutamiltransferaz ve laktat dehidrogenazın her iki deterjanla çözünürlüğü karşılaştırıldı. Trombositler değişen konsantrasyonlarda ve zaman aralıklarında Triton X-100 ve Brij 35 ile muamele edildi. Protein, kolesterol, gamma glutamiltransferaz ve laktat dehidrogenazın trombositlerden çözünmesinde değişen konsantrasyonlar ve inkübasyon zamanlarında her iki deterjan arasında belirgin bir fark görülmedi.

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