Oxonol-Kation (**6**) anzeigt. Durch Behandlung mit Natronlauge (0,1 M) entwickelt sich ein tief blau gefärbter Fleck, dessen Farbe allmählich nach gelb umschlägt und dabei im langwelligen UV-Licht (365 nm) intensiv fluoresziert. Dabei bildet sich zunächst das Phenolat, das sofort zum blau gefärbten Oxonol-Anion **7** oxidiert wird, welches durch Öffnung des Lactonringes in das gelb gefärbte, fluoreszierende Carboxylat-Anion **8** übergeht (Schema).

Zur Abschätzung der unter den Bedingungen des EuAB entstehenden Glyoxylsäuremengen wurde die entstehende Färbung photometrisch vermessen und gegen Lösungen verglichen, die durch analoge Umsetzung von Glyoxylsäure und Resorcin erhalten worden waren. Im beschriebenen Fall wurden etwa 7% des Glycins zu Glyoxylsäure abgebaut. Erwartungsgemäß war die Glyoxylsäureausbeute bei Durchführung der Reaktion mit einer Lösung des Glycins in Natronlauge (0,2 M) anstelle Wasser höher: Die Färbung war etwa vier Mal intensiver (und möglicherweise durch die Resorcinmenge (0,18 mmol) limitiert). Bei stärker alkalischen Reaktionsbedingungen muss jedoch mit einem Abfall der Ausbeute an Glyoxylsäure gerechnet werden, die dann in einer Cannizarro-Reaktion zu Oxalsäure und Glykolsäure disproportioniert wird (Debus 1856).

Experimenteller Teil

50 mg Glycin, in 5 ml Wasser (bzw. 5 ml Natronlauge, 2M) gelöst, werden mit 1 ml Natriumhypochlorit-Lösung (2,5 bis 3% aktives Chlor) 2 min und nach Zugabe von 1 ml Salzsäure (conc.) weitere 4 min zum Sieden erhitzt. Nach dem Abkühlen werden 1 ml Resorcin-Lsg. (2%) und 2 ml Salzsäure (conc.) zugegeben. Die Lösung wird zu insgesamt 10 g ergänzt (Reaktionslösung).

Die Lösung wird 5 min im siedenden Wasserbad erhitzt und mit 10 ml Wasser verdünnt (Produktlösung). Nach dem Abkühlen werden 5 ml Produktlösung mit 6 ml Natronlauge (2 M) versetzt. Die Extinktion der Lösung wird unverzüglich bei einer Wellenlänge von 540 nm gegen den Blindwert gemessen (Schichtdicke: 2 mm).

Blindwert: 1 ml Resorcin-Lsg. werden mit 3 ml Salzsäure (conc.) versetzt und mit Wasser zu insgesamt 10 g ergänzt. Die Mischung wird wie die Reaktionslösung behandelt.

Referenzlösung: 2,5 mg, 5 mg bzw. 7,5 mg Glyoxylsäure-Hydrat (Aldrich; Deisenhofen/Germany) werden in 5 ml Wasser gelöst, mit 3 ml Salzsäure (conc.) sowie 1 ml Resorcin-Lsg. (2%) versetzt und mit Wasser zu insgesamt 10 g ergänzt. Die Mischung wird wie die Reaktionslösung behandelt.

Dünnschichtchromatographie: 10 ml Produktlösung werden mit 5 ml Ethylacetat extrahiert. Der Extrakt (1 µl) wird auf einer Kieselgel-GF_{254}-Schicht chromatographiert.

Fließmittel 1: Aceton/Methanol (60 + 40); Rf = 0,73 (Resorcin 0,65) [Auterhoff et al. 1976],

Fließmittel 2: Ethylacetat; Rf = 0,46 (Resorcin 0,58).

Detektion: 1) UV-Licht 254 nm
2) durch kurzes Eintauchen in ethanolische Schwefelsäure (5%, v/v)
3) durch kurzes Eintauchen in Natronlauge (0,1M)

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Use of electrolyte induced flocculation technique for an *in vitro* steric stability study of steric stabilized liposome formulations

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The aim of the present study was to investigate the electrolyte induced flocculation as a tool to evaluate the steric stability of the prepared liposomes. Various liposomal formulations containing methotrexate were formulated using the lipid film hydration technique with different ratios of drug, lipids (Phosphotidyl choline & Cholesterol) and surface coating agents (Methoxy poly ethylene glycol 5000, Methoxy polyethylene glycol 2000, Pluronic F-68, Pluronic F-127, Tween 20 and Tween 80). The formulations have been optimized for their entrapment efficiency, particle size and steric stabilization effect. The electrolyte induced flocculation test was carried out with different concentrations of sodium sulphate solutions. The results suggested that out of all the polymers used, the poly ethylene glycols proved to provide better steric stabilization to the liposomes even at higher concentrations of electrolyte.

Sterically stabilized liposomes were developed with the primary goal of evading the rapid clearance by the reticuloendothelial system, after i.v. injection thus allowing them to remain longer in the circulation (Papahadjopoulos et al. 1991). The coating of liposomes with hydrophilic polymers renders steric stability to the liposomes by providing a hydrophilic surface, which is thought to limit the binding of serum opsonins as well as direct interactions with cells, most importantly, of the reticuloendothelial system (Huang et al 1993). This results in enhanced circulation times and increased localization in the tumor (Hobbs et al. 1998; Huang et al. 1992; Stewart et al. 1998). In the earlier studies, the steric stabilization effect of the long circulating liposomes was determined mainly by *in vivo* circulation time and by *in vivo* drug release studies (Allen et al. 1989; Gabison et al. 1994; Working et al. 1994; Yuan et al. 1994). Hence the present work was focused on the *in vitro* steric stability of stabilized liposomes using the electrolyte induced flocculation technique.

The liposomes were prepared using methotrexate as a model drug by the thin film hydration technique. The conventional liposomes were prepared using 83 mg of egg phosphatidyl choline, 17 mg of cholesterol and 5 mg of methotrexate (molar ratio 1 : 0.4 : 0.1). The sterically stabilized liposomes were prepared using one of the following hydrophilic polymers, Tween® 20 (T20)-4% wt of lipids, Tween 80 (T80)-4% wt of lipids, Pluronic F-68 (PF-68)-4% wt of lipids, Pluronic® F-127 (PF-127)-4% wt of lipids, methoxy polyethylene glycol 5000-phosphatidyl ethanolamine (mPEG5000-PE)-5 mol% of lipids, and methoxy polyethylene glycol 2000-phosphatidyl ethanol-

amine (mPEG 2000-PE)-7 mol% of lipids, to provide a hydrophilic coating over the surface of the liposomes providing steric stability to the liposomes. The electrolyte induced flocculation test (Lin et al. 1994) was used to estimate the extent of steric barrier present around the liposomes. The physical stability of a dispersed system is mainly dependent upon the competitive forces of attraction (van der Waal's forces) and repulsion (either electrostatic repulsive forces or steric stabilizing barrier or both). In addition to the electrostatic and van der Waals forces, a number of other interactions (depletion and steric interactions) could play an important role in colloid stability (Tadros and Vincent 1983). Steric stabilization occurs due to the presence of steric barriers from the adsorbed non ionic molecules on particles that prevent the particles from coming close enough to allow van der Waals attractive forces between the particles to dominate (Tadros 1986). The conventional liposomes are predominantly electrostatically stabilized. Addition of electrolyte will compress the electrostatic double layer surrounding the liposomes and results in aggregation followed by flocculation with a corresponding increase in optical turbidity. But if the steric stabilized liposomes are mainly stabilized by hydrated steric stabilizing barriers produced by the surface modification due to the polymer incorporated, the system should be stable even if the electrostatic double layers have been compressed. Flocculation might occur even in steric stabilized liposomes after addition of a certain amount of a electrolyte, due to dehydration of the hydrated steric stabilized barriers. Thus if the optical turbidity of the liposomal dispersion is measured at 400 nm after adding different concentrations of electrolyte, the change in optical turbidity can be used to determine whether the liposomes are sterically stabilized or not. The scattering of the samples increase by the inverse 4^{th} power of the wavelength of the incident light, hence a lower wavelength (400 nm) was used for measurements (Betagiri et al. 1993).

The prepared liposomes were analyzed for drug content. The percent drug entrapment of various liposome formulations prepared, ranged from 86.6 to 95.8% (Table). The slight reduction in the percent drug entrapment in sterically stabilized liposomes compared to conventional liposomes might be due to the competitive accumulation of polymer with respect to methotrexate which is entrapped in the lipid bilayer. Spherical multi lamellar liposomes were obtained with particle size ranging from 812 to 1124 nm (Table).

The results of the electrolyte induced flocculation test suggested that the incorporation of various polymers increases the steric stabilization of the liposomes (Fig.). Out of all the polymers used, the polyethylene glycols proved to provide better steric stabilization to the liposomes, even in the presence of higher concentrations of the electrolyte. The conventional liposomes (ML-PL) showed a gradual increase in flocculation as the concentration of the sodium sulphate increases from 0 M to 2 M. The liposomes with

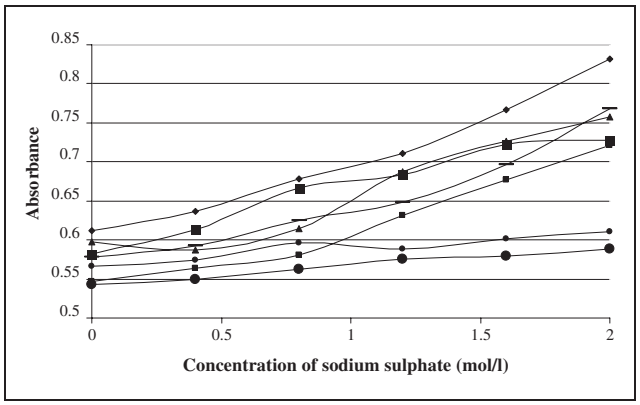

Fig: Results of electrolyte induced flocculation test of various liposome formulations. ML-PL; ML-T20; ML-T80; ML-PF68; ML-PF127; ML-mPEG2000; ML-mPEG2000

Tween and Pluronic initially showed steric stability, but as the concentration of sodium sulphate exceeded 0.8 M, flocculation started to occur.

Thus, the electrolyte induced flocculation technique can be used as a tool to optimize the type and amount of hydrophilic polymer necessary to provide a steric stabilization effect to the liposomes. Thus, the utilization of animals for preliminary investigations on the steric stability of the liposomes can be significantly reduced.

Experimental

1. Preparation of liposomes

Multi lamellar vesicles (MLVs) of methotrexate were prepared by the lipid film hydration technique (New 1990). Briefly, methotrexate and the lipids (egg phosphatidyl choline and cholesterol) were dissolved in a mixture of chloroform and methanol (ratio 2 : 1 by volume) in a 250 ml round bottom flask. In case of the preparation of sterically stabilized liposomes, one of the hydrophilic polymers mentioned earlier was also added along with the lipids in organic layer. The flask was rotated in the rotary flash evaporator at 100 rpm for 20 min in a thermostatically controlled water bath at 37 °C under vacuum (600 mm of mercury). The thin dry lipid film formed was hydrated using 2 ml of distilled water at 100 rpm at room temperature for 30 min. The liposomal suspension so formed was then transferred to a suitable glass container and sonicated for 30 min using a probe sonicator (model – RR-120, Ralsonics, Mumbai) in an ice bath for heat dissipation. The sonicated dispersion was then allowed to stand undisturbed for about 2 h at room temperature for swelling. The unentrapped drug was removed from the liposomal suspension by centrifugation at 10,000 rpm for 30 min. The percent drug entrapment of the prepared liposome formulations was determined by breaking the liposomes using methanol and measuring the absorbance at 303 nm (Arthur et al. 1976) on Shimadzu 1601 UV-Visible Spectrophotometer using methanol as a blank. The particle size analysis of the liposomes was carried out with a laser diffraction particle size analyzer (Malvern Master sizer 2000 SM, U.K.) which follows Mie's theory of light scattering.

2. Electrolyte induced flocculation test

Sodium sulphate solutions ranging from 0 M to 2.0 M were prepared in 16.7% sucrose solution. An appropriate volume of liposome formulation which gives a final concentration of 1 mg/ml of lipid was taken and the

Table: The percent drug entrapment and particle size determination of the liposome formulations

Liposomal batch	Mean drug entrapment (% ± S.E)*	Mean particle size (nm)
Conventional methotrexate liposomes (ML-PL)	95.8 ± 1.42	782
Methotrexate liposomes-T20 (ML-T20)	92.3 ± 2.35	845
Methotrexate liposomes-T80 (ML-T80)	89.4 ± 1.65	1124
Methotrexate liposomes-PF-68 (ML-PF68)	86.6 ± 2.36	1024
Methotrexate liposomes-PF-127 (ML-PF127)	90.1 ± 3.65	1048
Methotrexate liposomes-mPEG 5000-PE (ML-mPEG5000)	89.5 ± 2.66	812
Methotrexate liposomes-mPEG 2000-PE (ML-mPEG2000)	88.6 ± 1.79	875

* n= 6

volume was made up to 5 ml using the sodium sulphate solutions of various concentrations. The resulting dispersions were mixed and the absorbances were measured within 5 min at 400 nm on a Shimadzu 1601 UV-Visible Spectrophotometer against respective blank.

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Onset and offset pharmacodynamics of propofol

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Propofol whole blood and plasma concentrations at offset of hypnosis in eighteen patients were inversely related to patient age and body fat. The relationship between propofol concentrations and body fat is derived from the relationship between age and body fat and age was the single independent predictor of concentrations at offset of propofol hypnosis.

Patient age and body fat may influence propofol pharmacodynamics. Concentrations at which 50% of volunteers fell asleep following a 2 mg/kg bolus dose were higher in younger than in elderly subjects (Schnider et al. 1999) and lower hypnotic doses, shorter times to hypnosis and higher propofol plasma concentrations were also noted as patients aged (Adachi et al. 2001). Total body weight also influenced propofol pharmacodynamics, after adjustment of propofol infusion rate to patient body weight. Heavier patients needed higher infusion doses and displayed shorter times to hypnosis than lighter patients (Adachi et al. 2001) and propofol doses were inversely related to lean body mass and not body weight (Adachi et al. 2001; Servin et al. 1993; Leslie and Crankshaw 1991; Chassard et al. 1999). Obese patients were at risk of overdose when weight-normalised propofol infusions were used (Gepts et al. 1987). The current study specifically examined the influence of patient age and body fat on propofol whole blood and plasma propofol concentrations at onset and recovery.

As age increased there was a significant decrease in propofol whole blood and plasma offset concentrations (Fig.). At onset no such relationship was observed between age and propofol concentrations in either whole blood or plasma. When patients were divided into two age groups ($<$ or $>$65 years), similar to the age cut-off used previously (Adachi et al. 2001), patients over 65 years required lower whole blood propofol concentrations (4.1 ± 1.7 μg/mL) at onset compared to patients less than 65 years of age (5.3 ± 1.3 μg/mL, $P < 0.05$). Similarly, patients over 65 years recovered from propofol at lower concentrations (1.3 ± 0.4 μg/mL) compared to patients under 65 years (2.3 ± 0.9 μg/mL, $P < 0.01$). An inverse correlation was noted between body fat and propofol whole blood (or plasma) onset ($r^2 = 0.382$, $P < 0.01$) and offset ($r^2 = 0.353$, $P < 0.05$) concentrations; as body fat increased whole blood and plasma concentrations at onset and offset decreased. Hypothesising that body fat increases with age, indeed it was found that body fat correlated positively