Liposzómák előállítási módjai

Dr. Voszka István

A felépítő lipidek

a) foszfolipidek

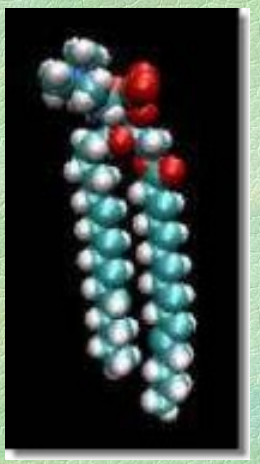
- *a zsírsavlánc* tulajdonságai befolyásolják a fázisátalakulást:

hosszabb zsírsavlánc – magasabb T_m

pl: DMPC (14:0) – 24 ºC

DPPC (16:0) – 41,5 ºC

DSPC (18:0) – 56 ºC



Kettős kötés a zsírsavláncban – alacsonyabb T_m.

pl: DOPC (18:1) – -27 ºC

Keverékek esetén a keverési aránytól függő köztes érték: pl:

DPPC: DOPC (80:20) – -2 ºC

DPPC: DOPC (70:30) – -7 ºC

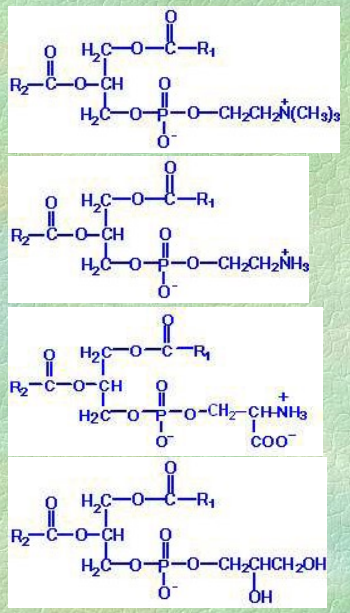
- ***fejcsoportok***
- nettó töltés nélkül (PC, PE)

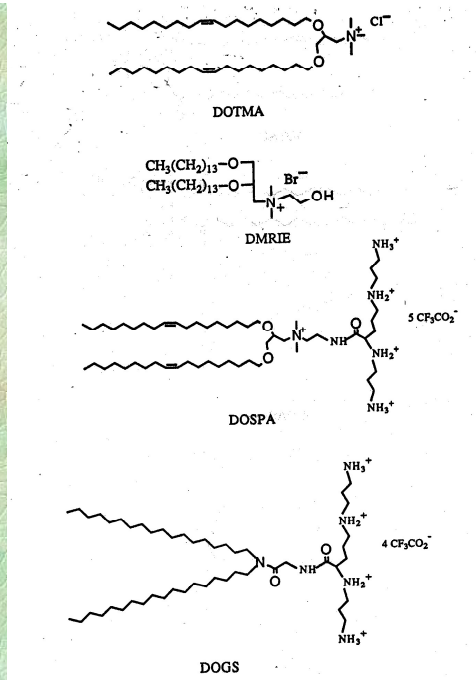
- negatív (PS, PG)
- pozitív (gangliozid, mesterséges lipidek)

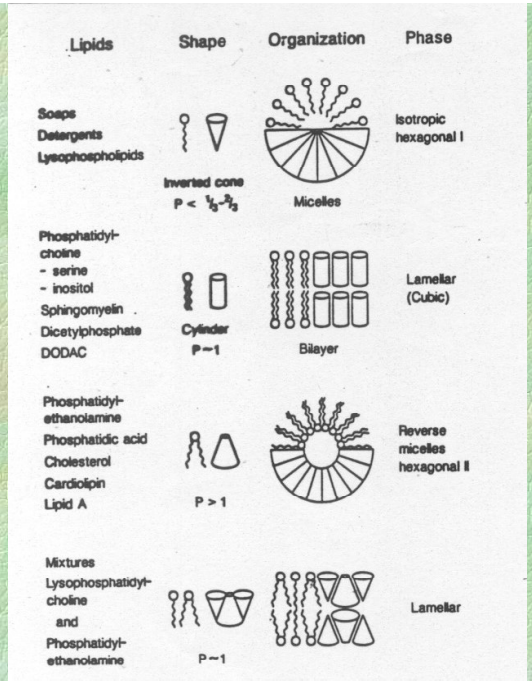
↓

10-30 % arányban

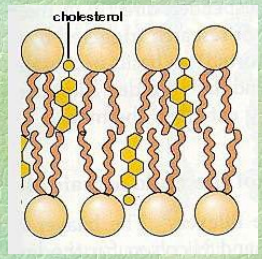
- hidrofil molekulák bezárási hatásfoka nő
- sejtekbe való felvétel hatásfoka nő
- élettartam csökken

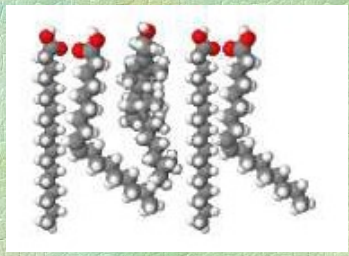






koleszterin (30-50 mol %)



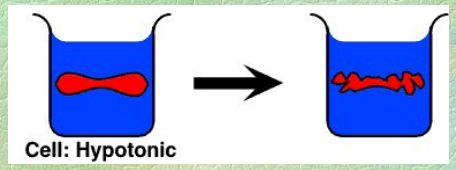


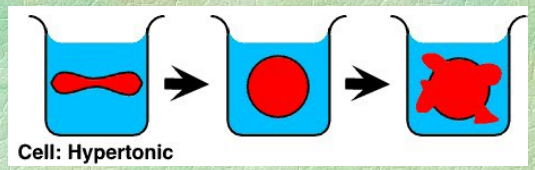
destabilizál

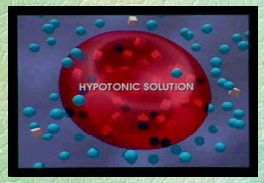
stabilizál

Vizes fázis

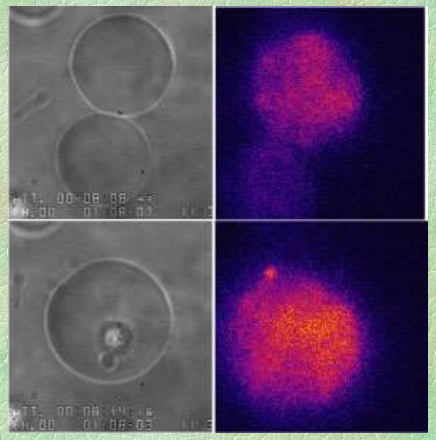
- ozmolaritás változása – méretváltozás







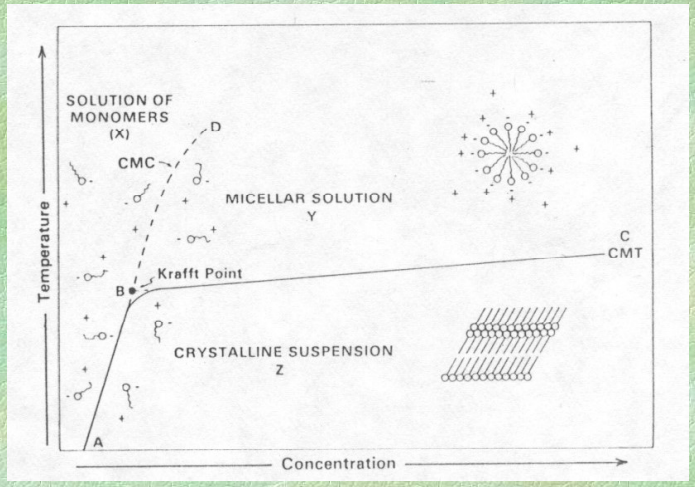
- pH → 6,5 alatt ill. 8,5 felett destabilizál
- kétértékű kationok → aggregálódás, fúzió



Lipid – víz arány

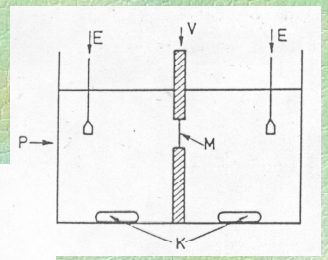
CMC (critical micellar concentration)

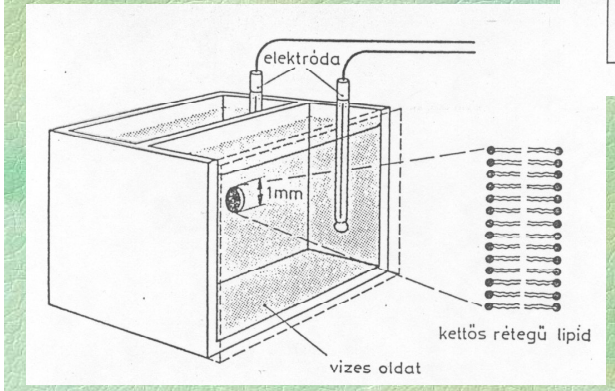
CMT (critical micellar temperature)

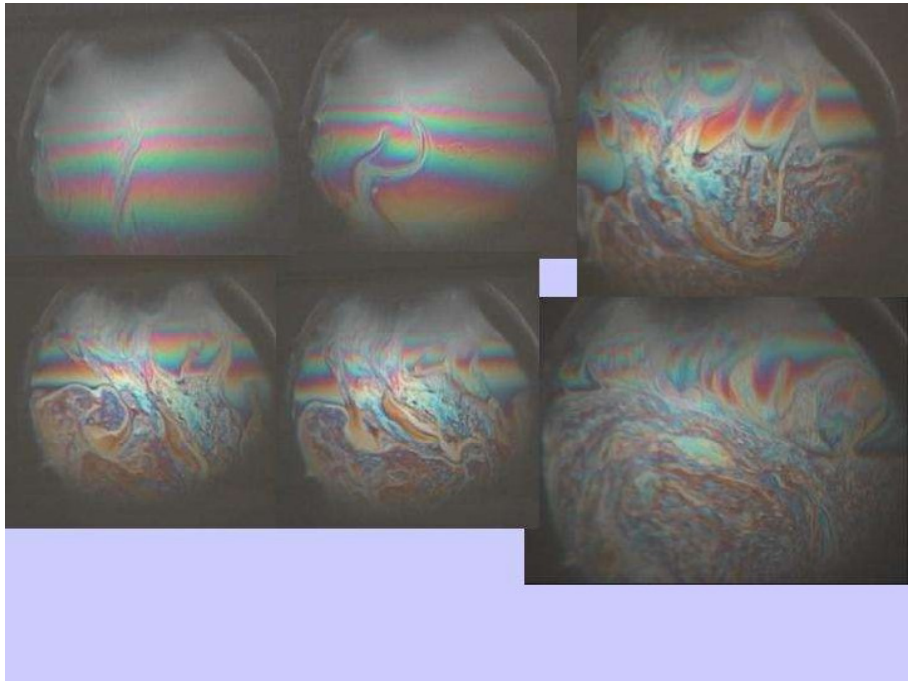


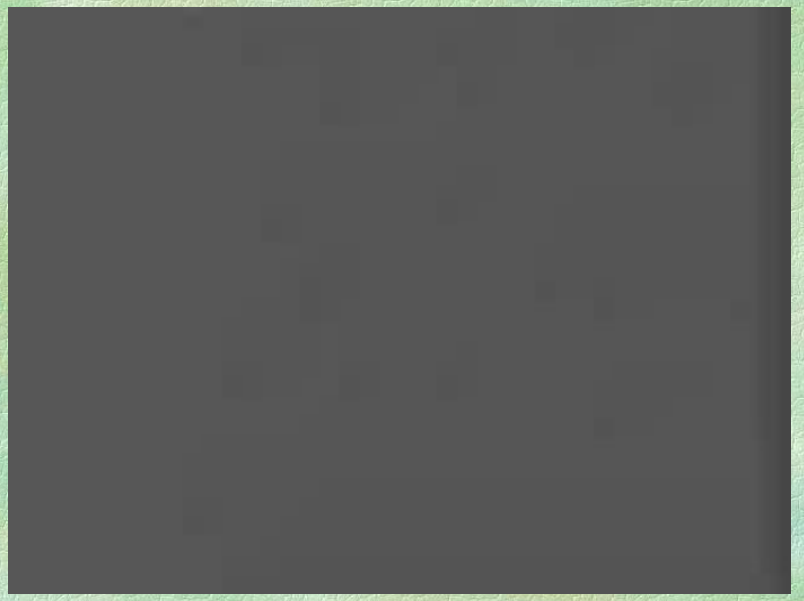
BLM (bilayer lipid membrane, black lipid membrane)

- a transzport vizsgálatára









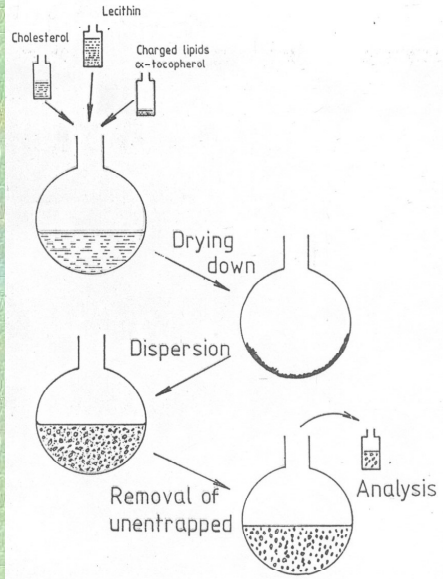
Liposzómák

- ***MLV*** (multilamellar vesicle)
  - a kettősrétegek száma változó
  - széles mérettartomány
  - kis bezárási hatásfok

MLV

- előállítása a legegyszerűbb (lipidfilm készítés + vizes fázis hozzáadása)
- fagyasztás (cseppfolyós N_2-ben
- T~ 80 K) – felolvasztás (T_m fölé)

Szűrés: az átmérő és a rétegek száma csökkenthető



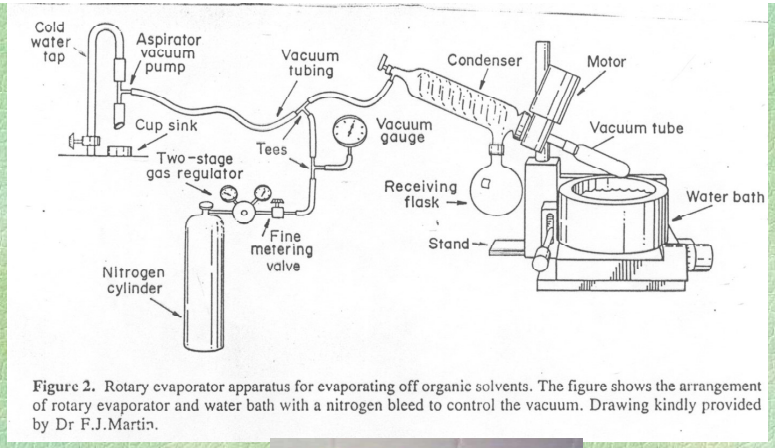
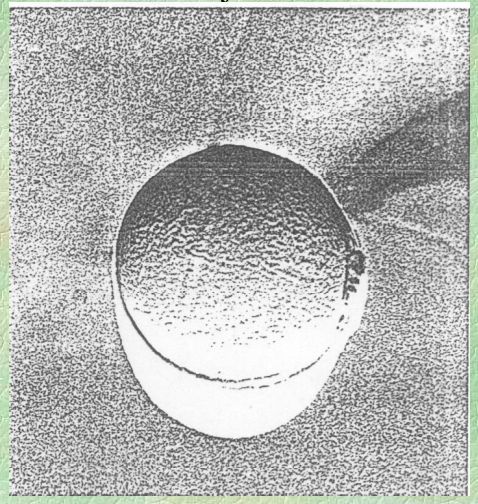
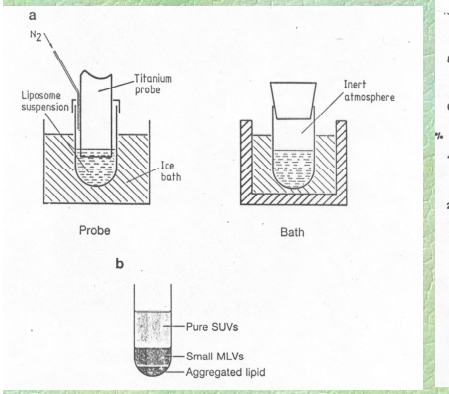


Figure 2. Rotary evaporator apparatus for evaporating off organic solvents. The figure shows the arrangement of rotary evaporator and water bath with a nitrogen bleed to control the vacuum. Drawing kindly provided by Dr F.J.Martin.

- ***SUV*** (small unilamellar vesicle)
  (átmérő: 100 nm alatt, minimum 20-25 nm)
  - kis bezárt térfogat
  - állás során fúzióra hajlamos



SUV előállítása:
- MLV-ből ultrahangozással



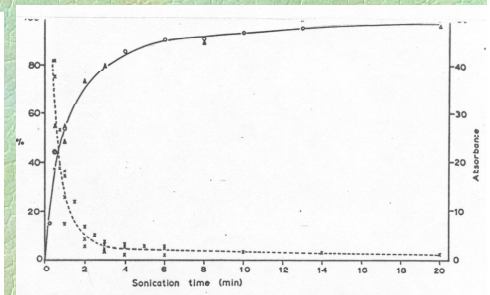
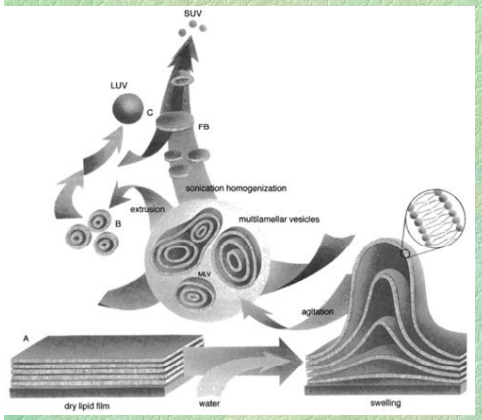
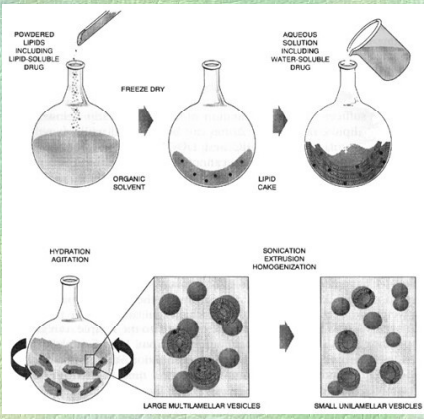


Fig. 4. Time course of sonication. Percent lecithin molecules in fraction II, Fig. 3, (O———O), percent $N(CH_3)_3$ groups giving high-resolution NMR integral (Δ———Δ), and absorbance at 300 nm (×———×) as a function of sonication time of a 1% (w/v) dispersion. The solid curve through the experimental points for percent molecules in fraction II and giving a high-resolution spectrum is calculated according to the theory of Finer *et al.* (1972) described in the text.





SUV előállítása:
- MLV-ből French press alkalmazásával

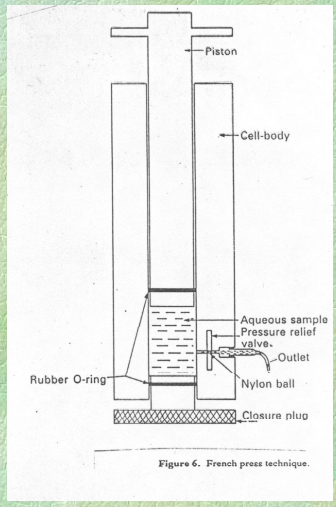
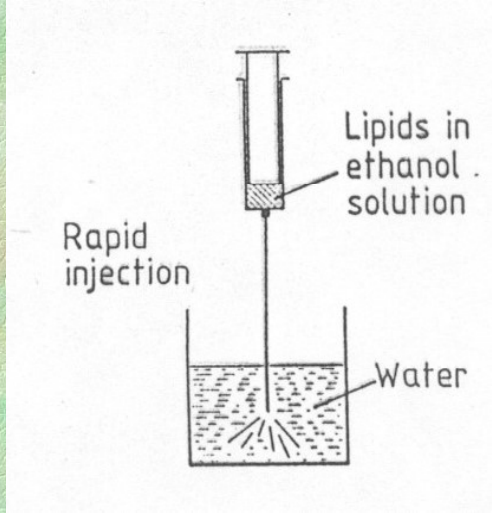
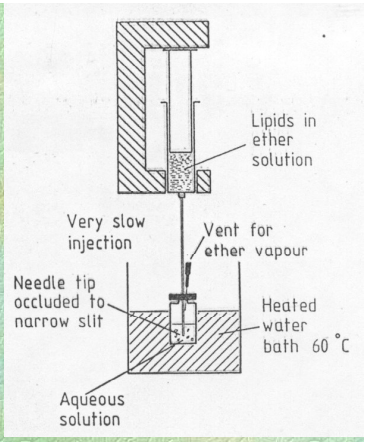


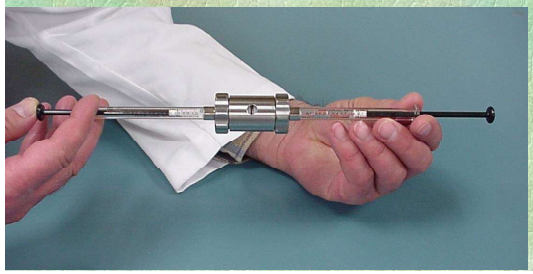
Figure 6. French press technique.

SUV előállítása:
gyors injektálással



- ***LUV*** (large unilamellar vesicle)
(átmérő 100 nm – 10 μm)
Előállítása:
  - MLV-ből filtrációval
  - SUV-ból fúzióval
  - lassú injektálással





LUV **előállítása:**
- fordított fázisú párologtatással

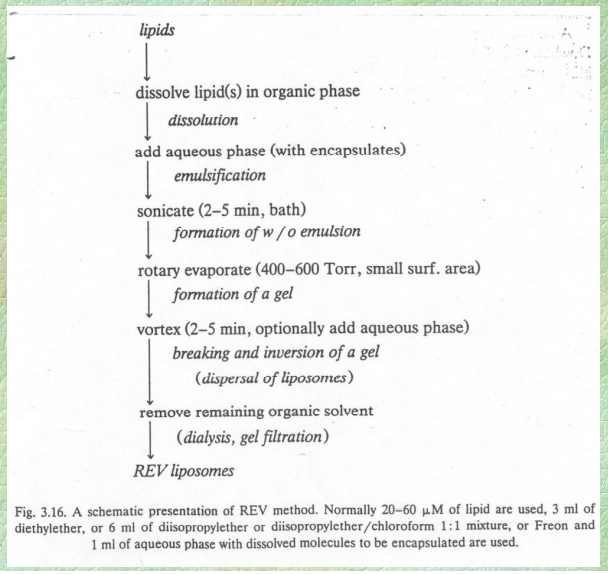
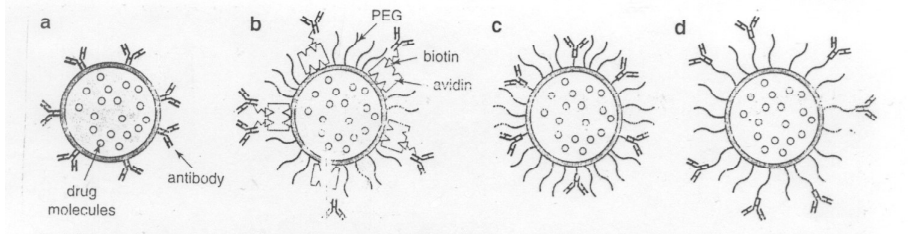


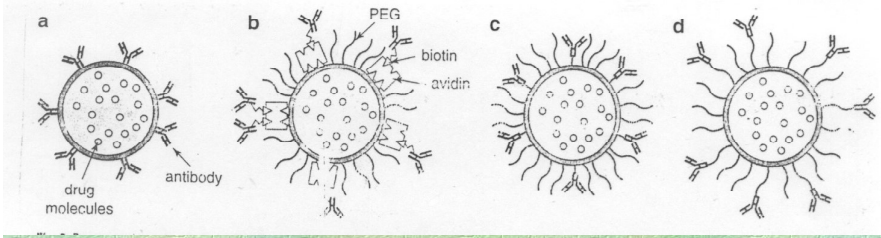
Fig. 3.16. A schematic presentation of REV method. Normally 20–60 μM of lipid are used, 3 ml of diethylether, or 6 ml of diisopropylether or diisopropylether/chloroform 1:1 mixture, or Freon and 1 ml of aqueous phase with dissolved molecules to be encapsulated are used.

Speciális liposzómák

a) Stabilizált („stealth”-S), sokáig keringő liposzómák
A felszínre kötött molekulák (pl. monoszialogangliozid-GM1, polietilénglikol -PEG, glukuronid származékok) elrejtik az immunrendszer elől.
- Telítetlen ill. töltött fejcsoportú lipidek beépítése a liposzómába csökkenti a cirkulációs időt.



Célsejt –szenzitív vagy immunliposzómák – antitestek a liposzóma felszínén → specifikus kötődés a megfelelő antitest-receptort hordozó sejttel. A célsejthez való kötődés destabilizálja a membránt → kiürülés.



(http://www.encapsula.com)

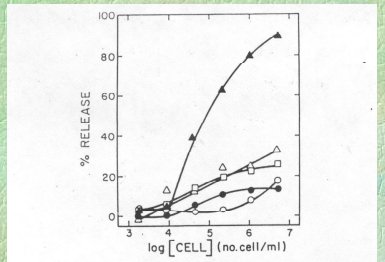


FIGURE 4: Cell-induced lysis of immunoliposomes. To uninfected (○, ●), HSV-infected (△, ▲, ○), or Sendai virus infected (□) L cells in suspension was added 0.58 nmol of calcein-encapsulted PE (△, ▲, ○, □) or PC (●) immunoliposome. To inhibit the lysis of PE immunoliposomes by HSV-infected L cells, 1.8 nmol of PC immunoliposomes containing no calcein was also added (△).

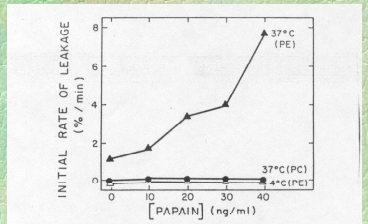


FIGURE 5: Papain-induced lysis of immunoliposomes. Various concentrations of papain were added to PE (△, ▲) and PC (●) immunoliposomes at 37 °C (▲, ●) and 4 °C (△), and the initial rate of calcein leakage was measured.

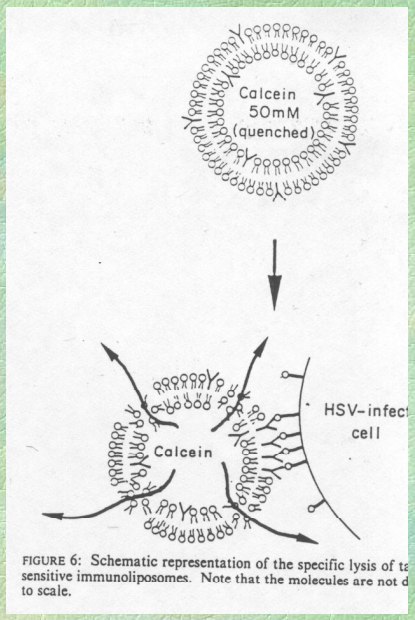
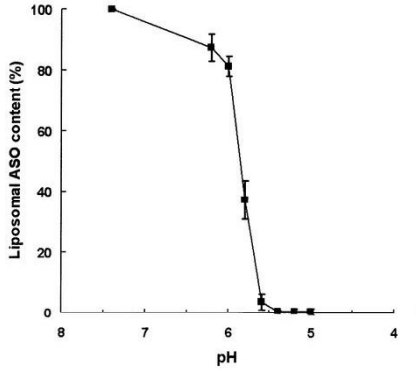


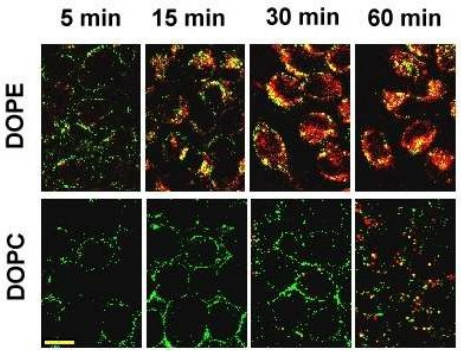
FIGURE 6: Schematic representation of the specific lysis of ta
sensitive immunoliposomes. Note that the molecules are not d
to scale.

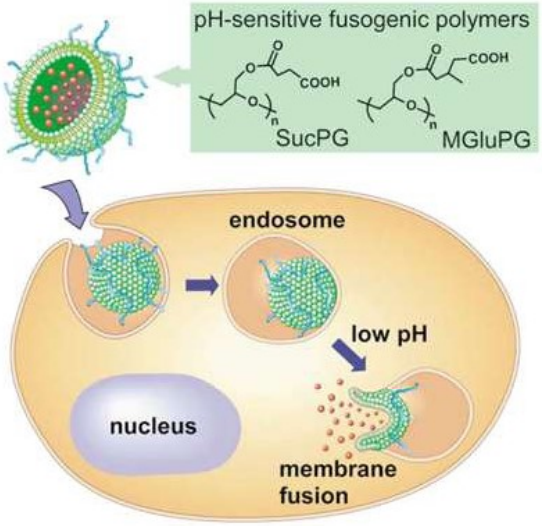
pH-szenzitív liposzómák

Savas közegben (pH 5-6,5, pl. gyulladásos környezetben) könnyen fuzionál, tartalmát endocitózissal üríti a célsejtbe

Jellemző lipidkomponens: DOPE





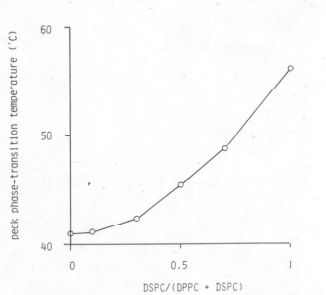


Cytoplasmic delivery mediated by pH-sensitive polymer-modified liposomes

Termoszenzitív liposzómák

T_m kicsivel a testhőmérséklet fölött – lokális hipertermia esetén üríti ki a tartalmát

Pl: DPPC/DSPC keverékből készült LUV (d ~ 200 nm)



ig. 1. Peak phase-transition temperatures of DPPC/DSPC mixtures measured by differential scanning calorimetry.

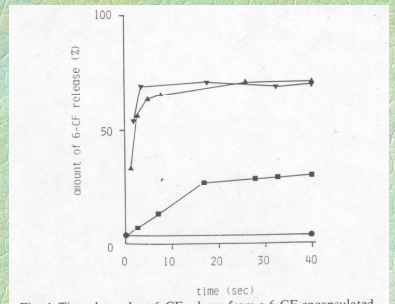


Fig. 4. Time-dependent 6-CF release from a 6-CF encapsulated thermosensitive LUV liposome (DPPC/DSPC = 9/1, w/w) when the liposome passed through a tube heated at different temperatures. The release rate was plotted against time for the liposome to pass through the heated tube. (●), 38° C; (■), 40° C; (▲), 41° C; (▼), 42° C.

Termoszenzitív liposzómák

TABLE 2

Stabilities of a CDDP-encapsulated SUV and a CDDP-encapsulated LUV liposome (DPPC/DSPC = 9/1, w/w) when stored at 4° C and room temperature (RT)

Liposome	Month	4° C	RT
SUV	0	97.5 [a]	–
	1	91.8 [b]	9.2 [b]
LUV	0	98.2	–
	1.5	98.2	95.1
	3	98.2	99.9
	6	97.1	96.3

[a] The latencies (%) of the liposomes were used as a measure for liposomal stability. [b] Remarkable coalescence was observed.

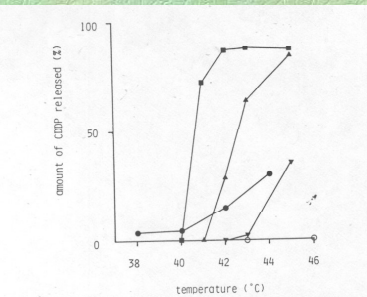


Fig. 5. Temperature-dependent release of CDDP from a CDDP encapsulated SUV liposome composed of DPPC/DSPC (9/1, w/w) and CDDP encapsulated LUV liposomes composed of DPPC/DSPC (9/1, 7/3, 5/5 and 0/10, w/w). The liposomes were diluted with saline by 10 times and incubated in a water bath maintained at constant temperatures for 15 min. The release rate was plotted against incubation temperature. (●), SUV, DPPC/DSPC = 9/1; (■), LUV, DPPC/DSPC = 9/1; (▲), LUV, DPPC/DSPC = 7/3; (▼), LUV, DPPC/DSPC = 5/5; (○), LUV, DSPC alone.

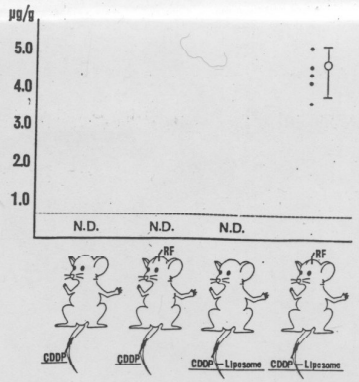


Figure 3. CDDP concentrations in the brain. Broken line shows the detectable limit of CDDP. N.D. is below the minimum level of detectability. Brain CDDP levels were significantly higher only when the CDDP-liposome was used with hyperthermia, while CDDP levels in the brains of other groups were undetectable.

Termoszenzitív liposzómák

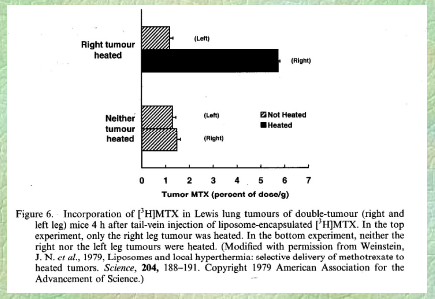


Figure 6. Incorporation of [^{3}H]MTX in Lewis lung tumours of double-tumour (right and left leg) mice 4 h after tail-vein injection of liposome-encapsulated [^{3}H]MTX. In the top experiment, only the right leg tumour was heated. In the bottom experiment, neither the right nor the left leg tumours were heated. (Modified with permission from Weinstein, J. N. *et al.*, 1979, Liposomes and local hyperthermia: selective delivery of methotrexate to heated tumors. *Science*, **204**, 188–191. Copyright 1979 American Association for the Advancement of Science.)

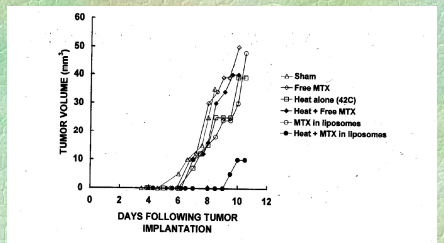


Figure 7. L1210 tumour growth in mouse feet after treatment with free or liposome-encapsulated MTX with or without heating to 42°C. Shams were given anaesthesia only. (Modified from Weinstein *et al.* 1980 with permission.)

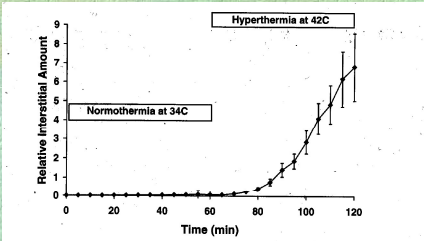
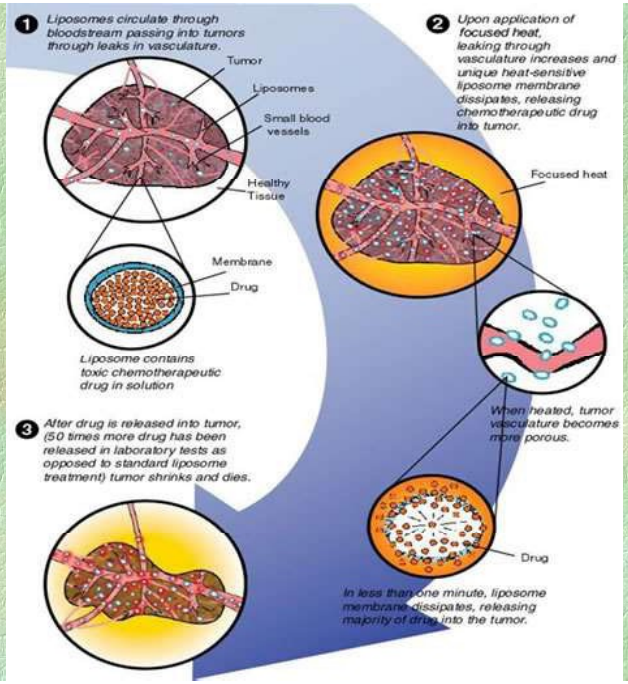
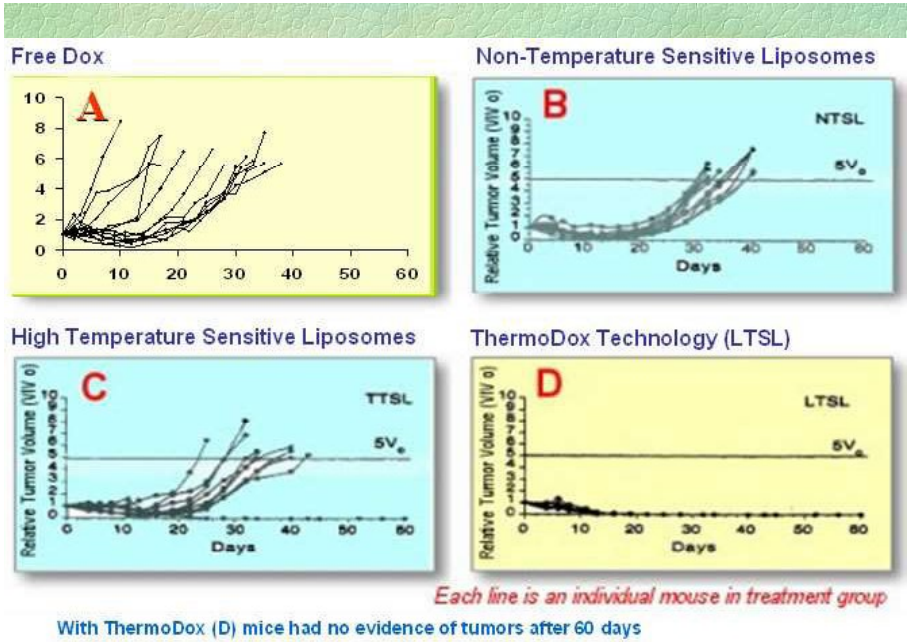


Figure 2. Extravasation of liposomes at 42°C in the tumour interstitium. The tumour was maintained at 34°C for 1 h and then heated at 42°C for another hour. Relative interstitial amount is the amount of liposomes in the tumour interstitium normalized to an initial vascular concentration of liposomes. (Modified from *International Journal of Radiation Oncology, Biology, Physics*, **36**, M. H. Gaber, N. Z. Wu, K. Hong, K. H. Shi, M. W. Dewhirst, D. Papahadjopoulos, Thermosensitive liposomes: extravasation and release of contents in tumor microvascular networks, pp. 1177–1187, Copyright 1996, with permission from Elsevier Science).





Fotoszenzitív liposzómák

Speciális fotoszenzitív lipidet tartalmaz, mely fény hatására polimerizálódik → a membrán permeabilitása és a bezárt hatóanyag kiáramlása megnövekszik

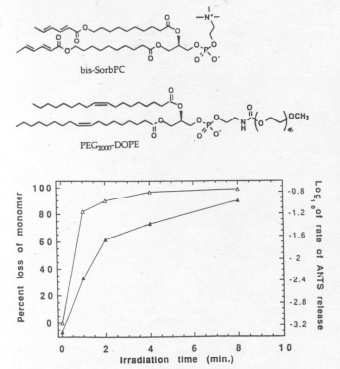
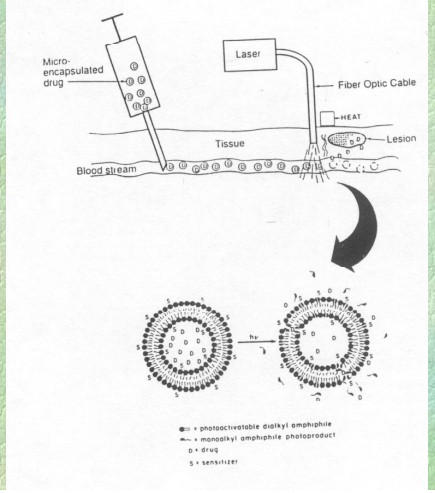


Figure 1. Effect of the photolysis of PEG-liposomes (pH 7 buffer) composed of PEG2000-dioleoylPE, cholesterol, dioleoylPC, and bis-SorbPC (molar ratio: 15/40/15/30). Both the percent loss of monomeric bis-SorbPC (△, left axis) and the log of the percent ANTS released per sec from the liposomes (▲, right axis) are shown as a function of the sample exposure time at 37 °C.



Fotoszenzitív liposzómák

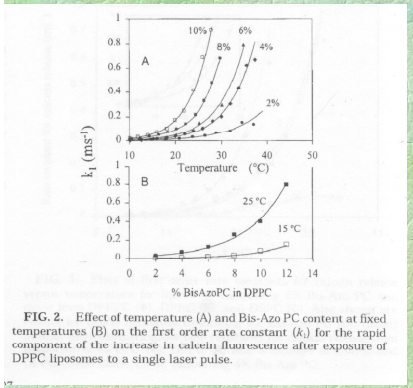


FIG. 2. Effect of temperature (A) and Bis-Azo PC content at fixed temperatures (B) on the first order rate constant (k_1) for the rapid component of the increase in calcein fluorescence after exposure of DPPC liposomes to a single laser pulse.

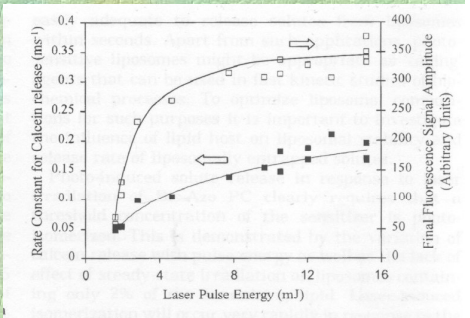
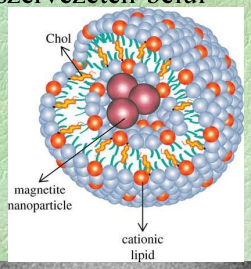


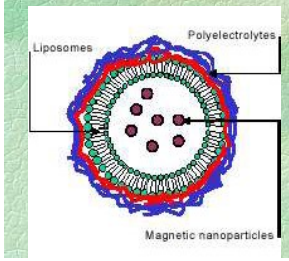
FIG. 4. Effect of energy in a single laser pulse on the first order rate constant for calcein release (■) and the final amplitude of the fluorescence increase (□) for DPPC liposomes containing 6% Bis-Azo PC.

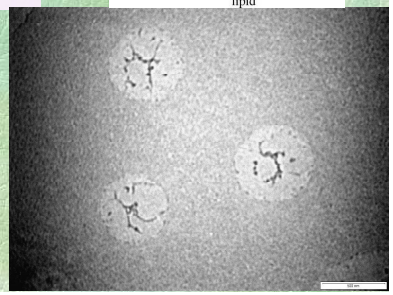
Mágneses liposzómák

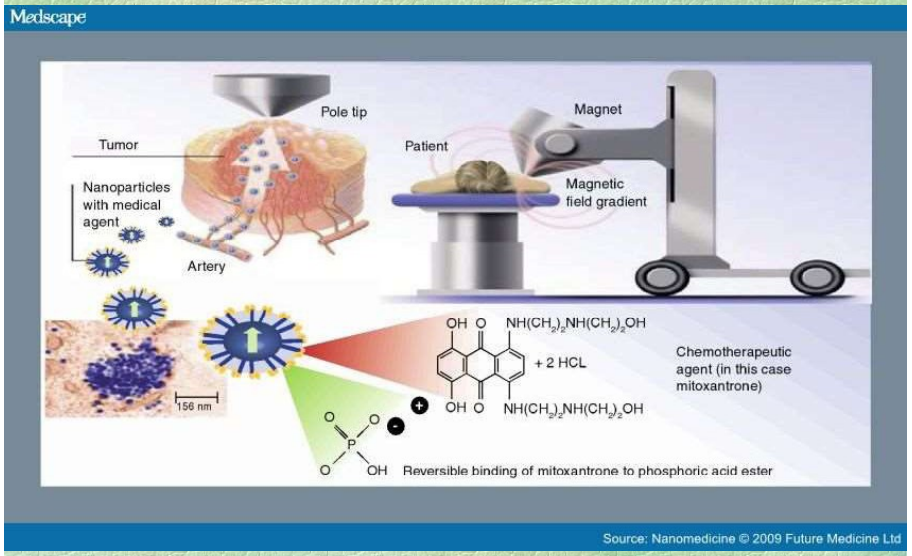
A liposzómába paramágneses anyagot építenek be (pl. vas-oxid)

Külső mágneses tér segítségével irányítható a szervezeten belül









Mágneses liposzómák

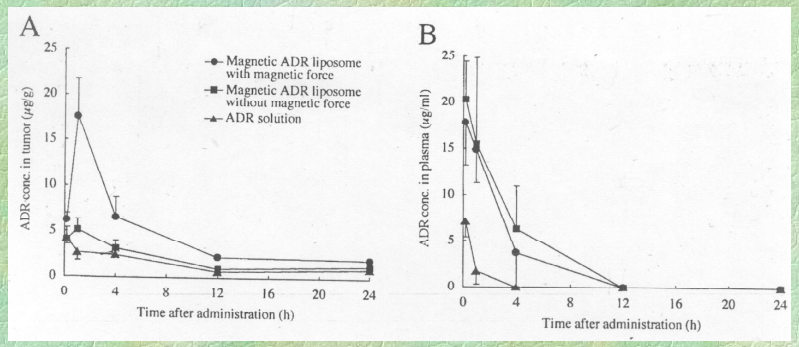
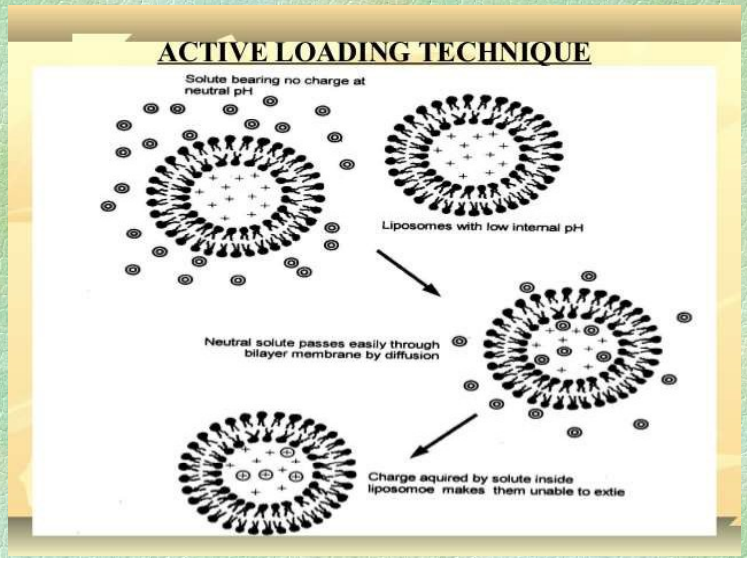


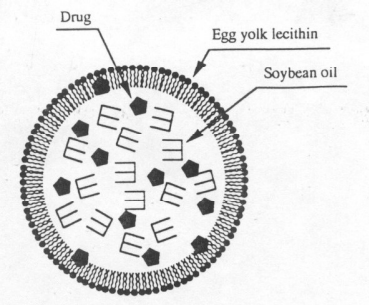
Figure 4. Time courses of ADR concentrations in A, tumor; B, plasma; C, liver; D, lung; E, heart; F, kidney following intravenous administration of ADR preparations via different administration modalities in osteosarcoma-bearing hamsters. Hamsters were studied 7 days after inoculation of osteosarcoma (tumor size was approximately 10 mm in diameter). The dose of ADR was fixed at 5 mg/kg body weight. One day prior to the animal study, a magnet with a magnetic field strength of 0.4 tesla was implanted in tumors in the magnetic ADR liposome group with magnetic force and in the ADR solution group. A non-magnetic neodymium alloy was also implanted in tumors in the magnetic ADR liposome group without magnetic force. Each value represents the mean ± SD of 4 trials.

Speciális módszer a hatóanyag nagy arányban történő liposzómába zárására: aktív töltés (active loading)



Lipid mikroszférák

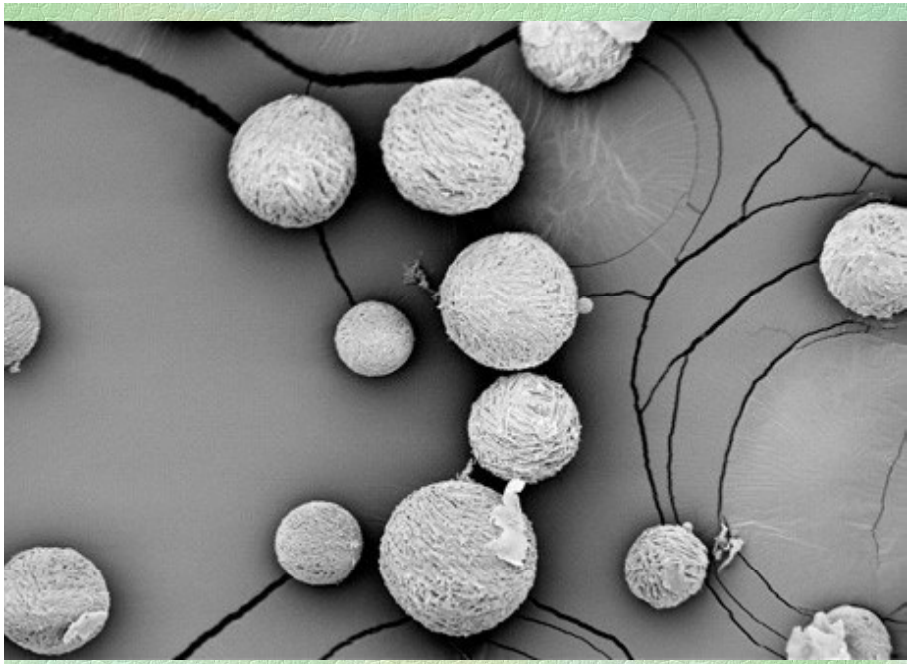
- lipid monolayer (d = 200 – 300 nm)
- lipofil molekulák zárhatók be
- vízben és zsírban is rosszul oldódó molekulák a lecitin rétegben tartózkodhatnak
- nem alkalmas hidrofil molekulák szállítására



Typical Components for LM

Soybean oil	50 ~ 200 mg
Egg yolk lecithin	12 ~ 18 mg
Glycerol	22 ~ 25 mg
Cosurfactant[a]	
NaOH	Adjusted to 5 ~ 7 of pH
Water for injection	Adjusted to 1 ml

[a] Fatty acid such an oleic acid.



Lipid mikroszférák

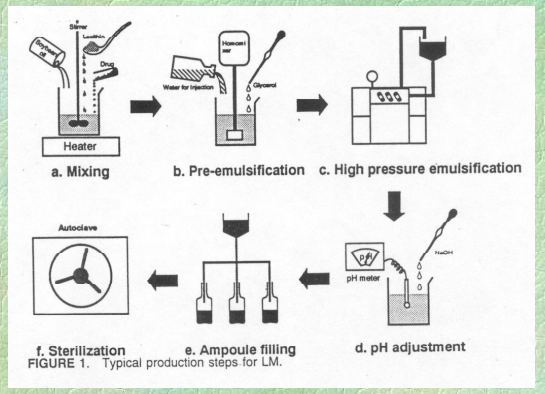


FIGURE 1. Typical production steps for LM.

Commercially Available LM for DDS

LM	Drug	Content	Company
Limethason	Dexamethasone palmitate	4.0 mg	Green Cross
Liple	Prostaglandin E_1	10 μg	Green Cross
Palux	Prostaglandin E_1	10 μg	Taisho
Lipfen	Flurbiprofen axetil	50 mg	Green Cross
Ropion	Flurbiprofen axetil	50 mg	Kaken