

USPIO encapsulating nanoliposomes with high entrapping efficiency, stability and magnetic properties

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Introduction

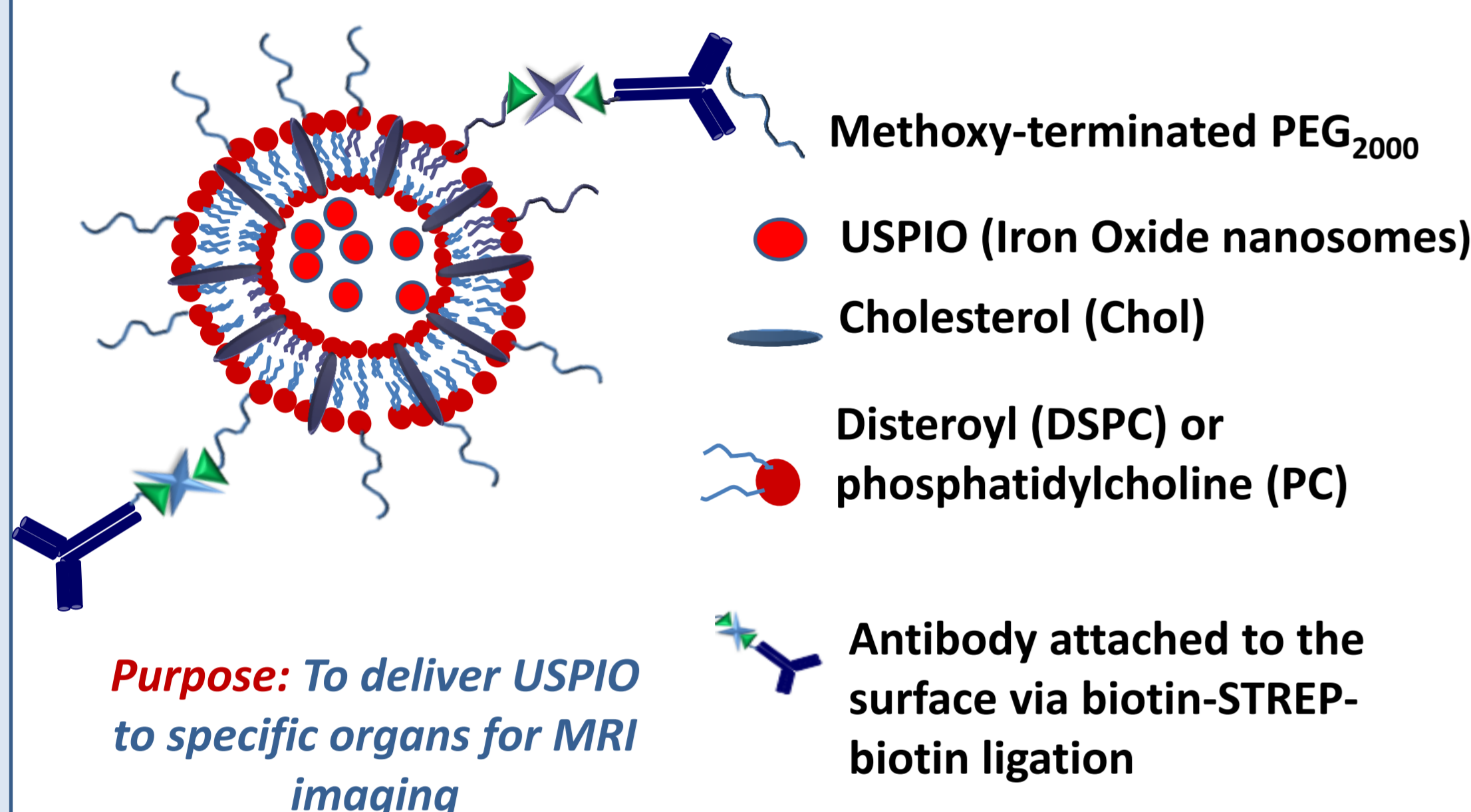
Magnetic colloidal suspensions or nanoparticles (ultra-small super paramagnetic iron oxide cores [USPIOs]) have widespread applications as magnetic resonance imaging (MRI) T₂ contrast agents.

If used in combination with nanosized drug carriers they may serve as theranostic systems (i.e. delivery systems with combinatory therapeutic-diagnostic modalities).

USPIO-entrapping nanosized liposomes may be considered as alternative formulations (magnetoliposomes [MLs]), with high magnetic properties (entrapment of many USPIOs per vesicle) and ability to co-load high amounts of drugs.

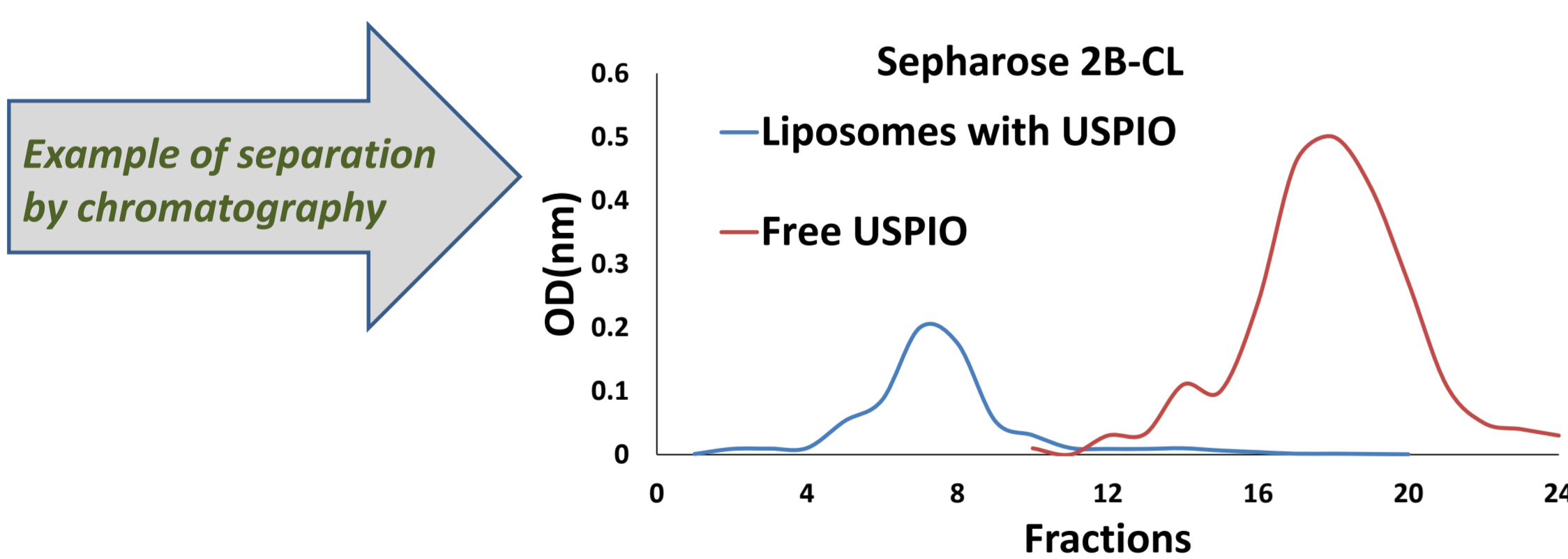
Herein, for the first time, the dried-rehydrated vesicle (DRV) technique [1] was utilized to prepare USPIO (P904, Guerbet, FR) entrapping MLs, with high loading. Targeted MLs were additionally formed by decorating pre-formed MLs with OX-26 anti-transferrin receptor MAb by biotin-streptavidin ligation, as recently reported [2]

Model of Targeted MLs



Preparation of MLs

- Empty SUV (Small Unilamellar Vesicle) liposomes are mixed with USPIO (Guerbet, FR).
- Mixture is Freeze dried & Rehydrated [1]
- Vesicles are extruded through 400 nm and then 100 nm pore membranes.
- Purification: Size exclusion chromatography (Sephacrose2B-CL)
- Mab decoration measured by Elisa and observed by TEM.



Liposome type and encapsulation efficiency

Type of liposome	Encapsulation (EE) (Fe / lipid) - (mM / mg)	Size (nm)
SUV	0,01354	125.3
MLV	0,02792	5412
DRV	0,60564	8156
DRV(extruded)	0,12417	117.8

PC/Chol 4:1+ 4 mol% PEG-lipid (concentration 20mg/ml)

- Extruded-DRV technique best for nanosized MLs with high EE

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Effect of DRV preparation conditions on USPIO encapsulation efficiency (EE)

Lipid composition	Initial volume(USPIO solution) (λ) -hydration buffer	Initial ratio (USPIO/lipid) mM/mg	EE mM/mg	
			DRV	Extruded-DRV
PC/Chol (2:1)	100-10%PBS	2,57	0,87	0,21
	100-PBS		0,485	0,066

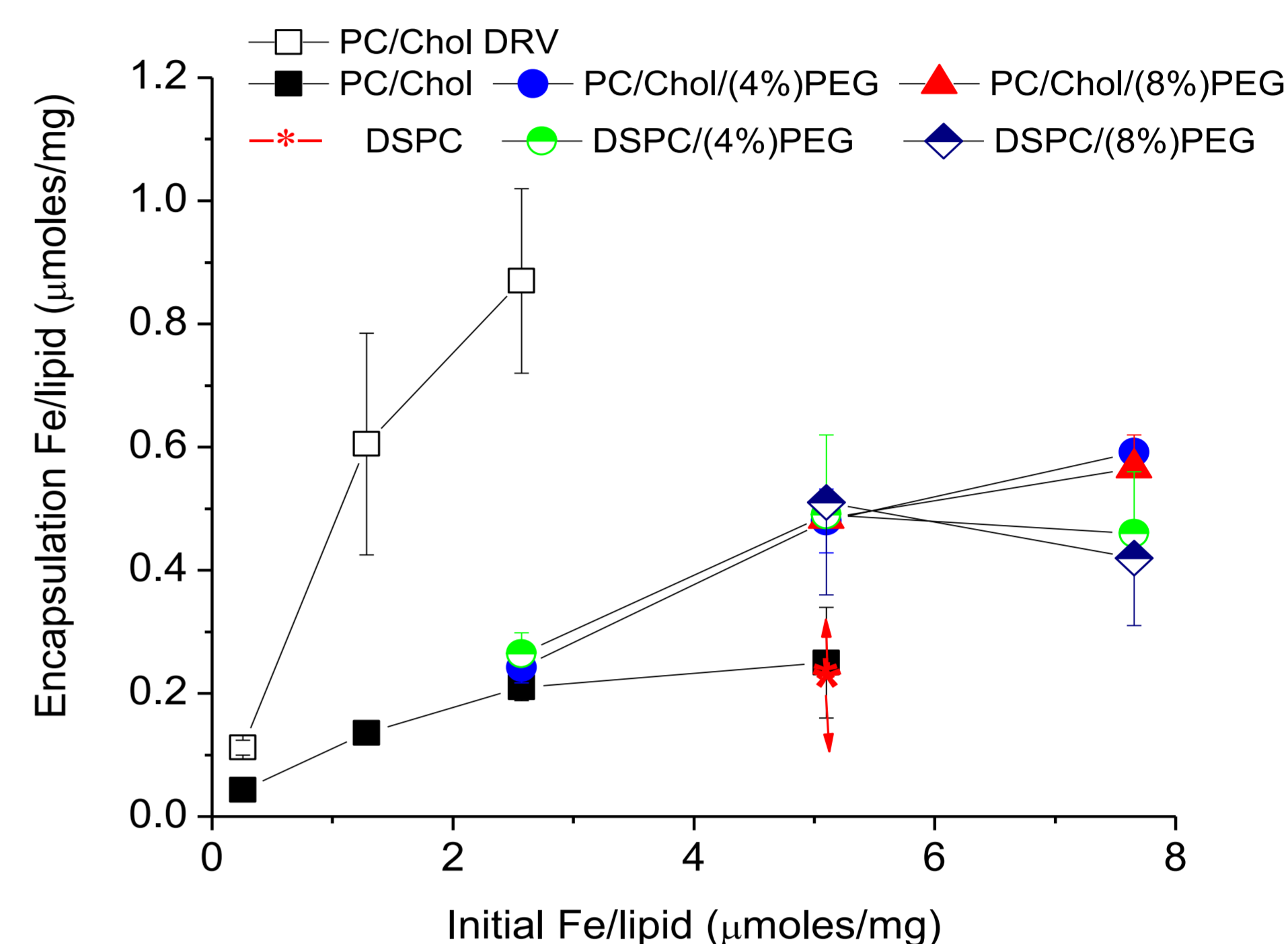
- Concentration of buffer (used for SUV formation) and USPIO/lipid ratio affect EE

Effect of ML lipid membrane composition and PEGylation on USPIO EE

- PEG-coating affects USPIO EE:

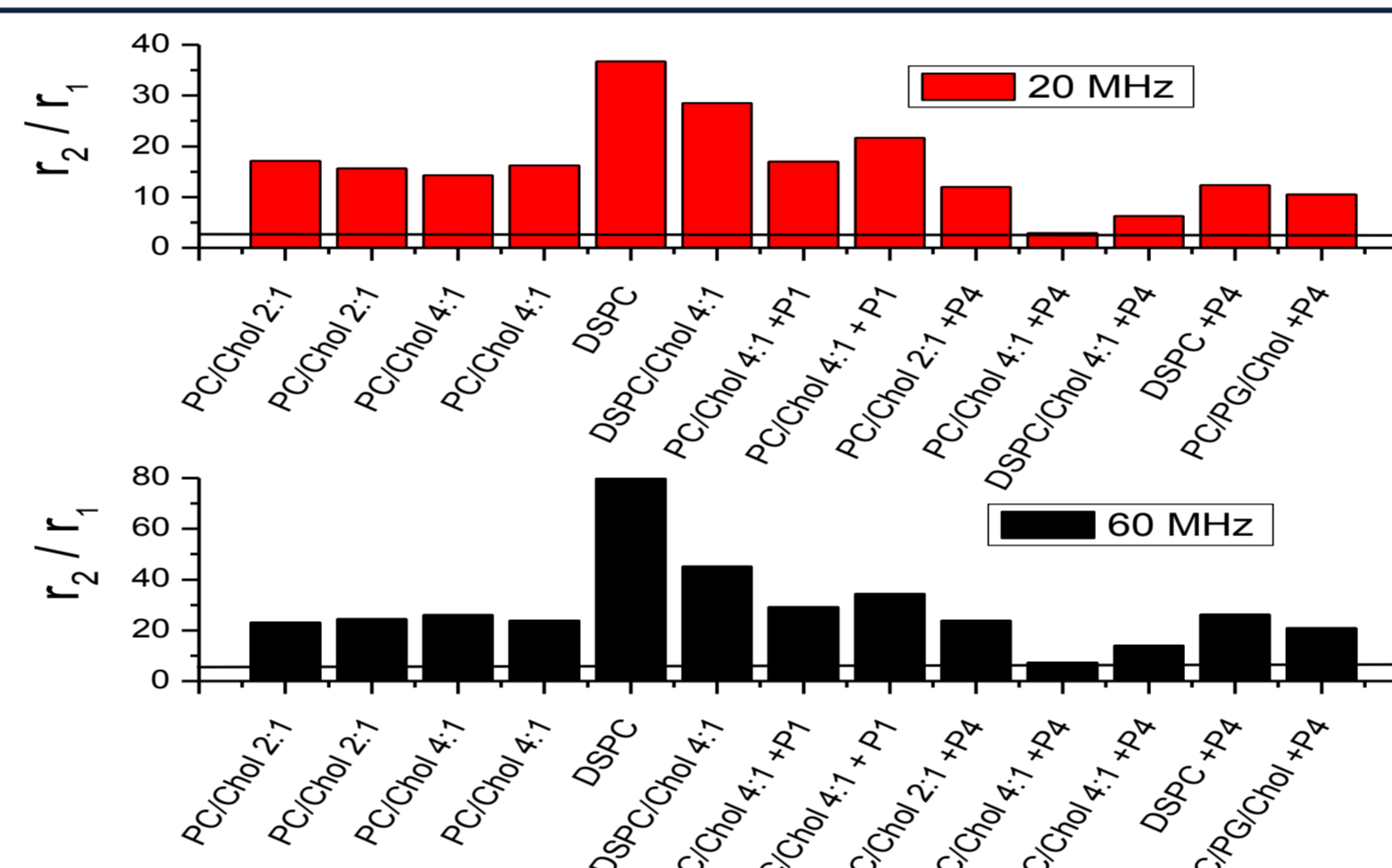
ML coating with 4mol% PEG-lipid increases EE.

Higher PEG content (up to 8 mol%) has no further effect.



Characterization of MLs and targeted MLs

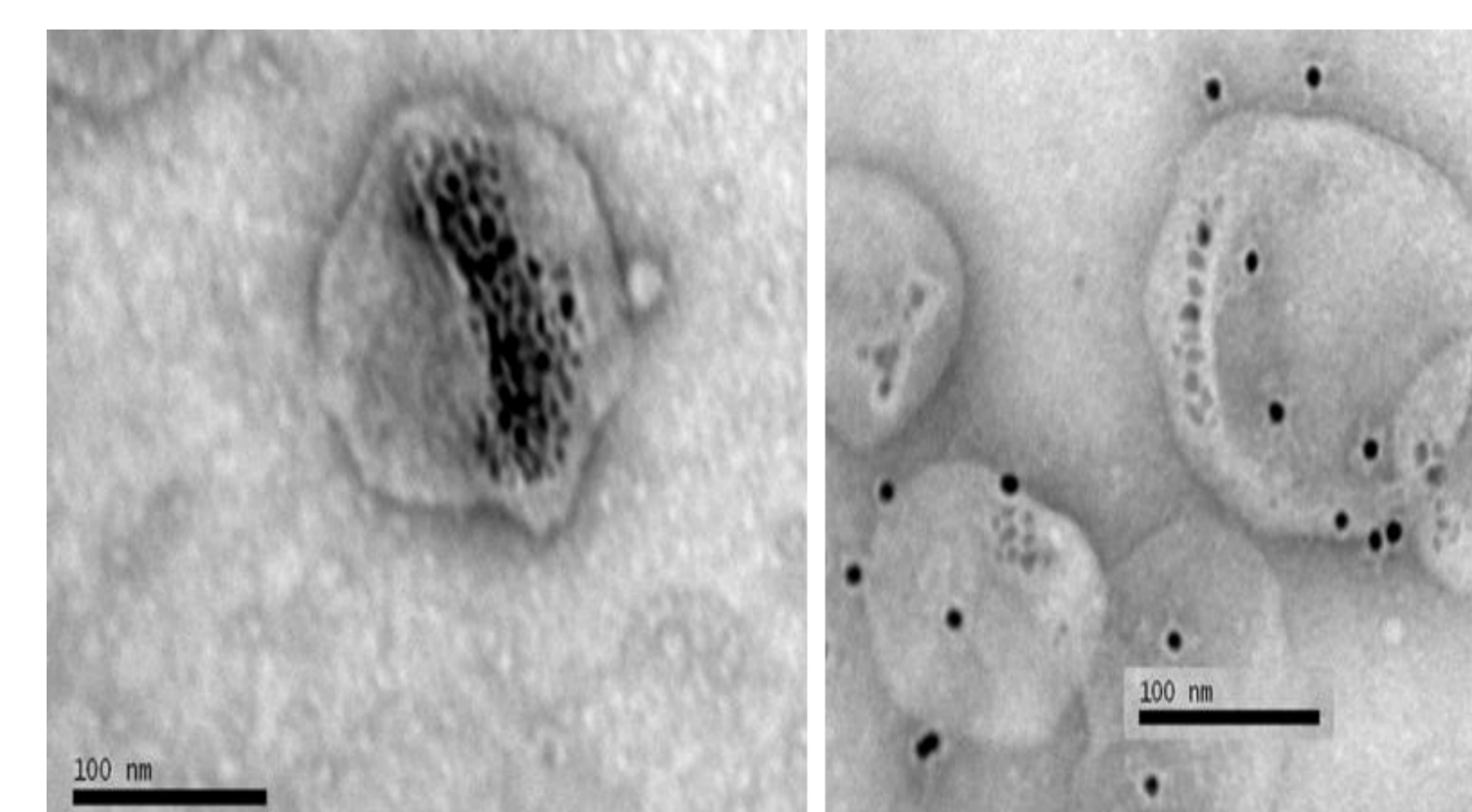
Magnetic properties of MLs



r₂/r₁ values represent ML ability to enhance MRI contrast. LINE, parallel to the axis-x represents value for free USPIOs

- MLs are efficient T₂ contrast agents; PEG-coating decreases magnetic properties; Mab decoration (80 % yield [Elisa]) does not decrease EE

Targeted MLs (TEM)

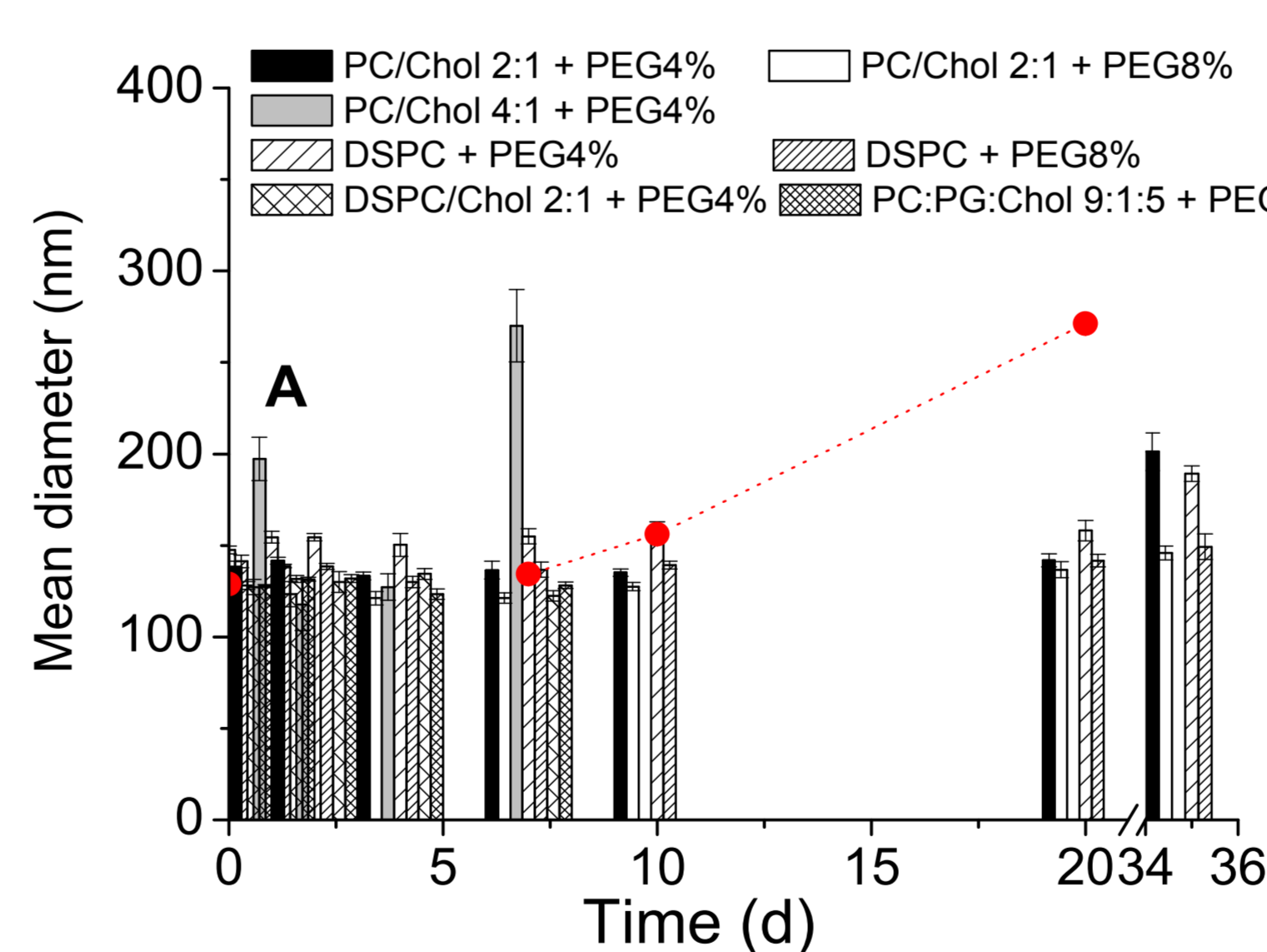


Control MLs (left) and Targeted-MLs (right), after reaction with gold immunoparticles

Stability of MLs and targeted MLs

Size stability

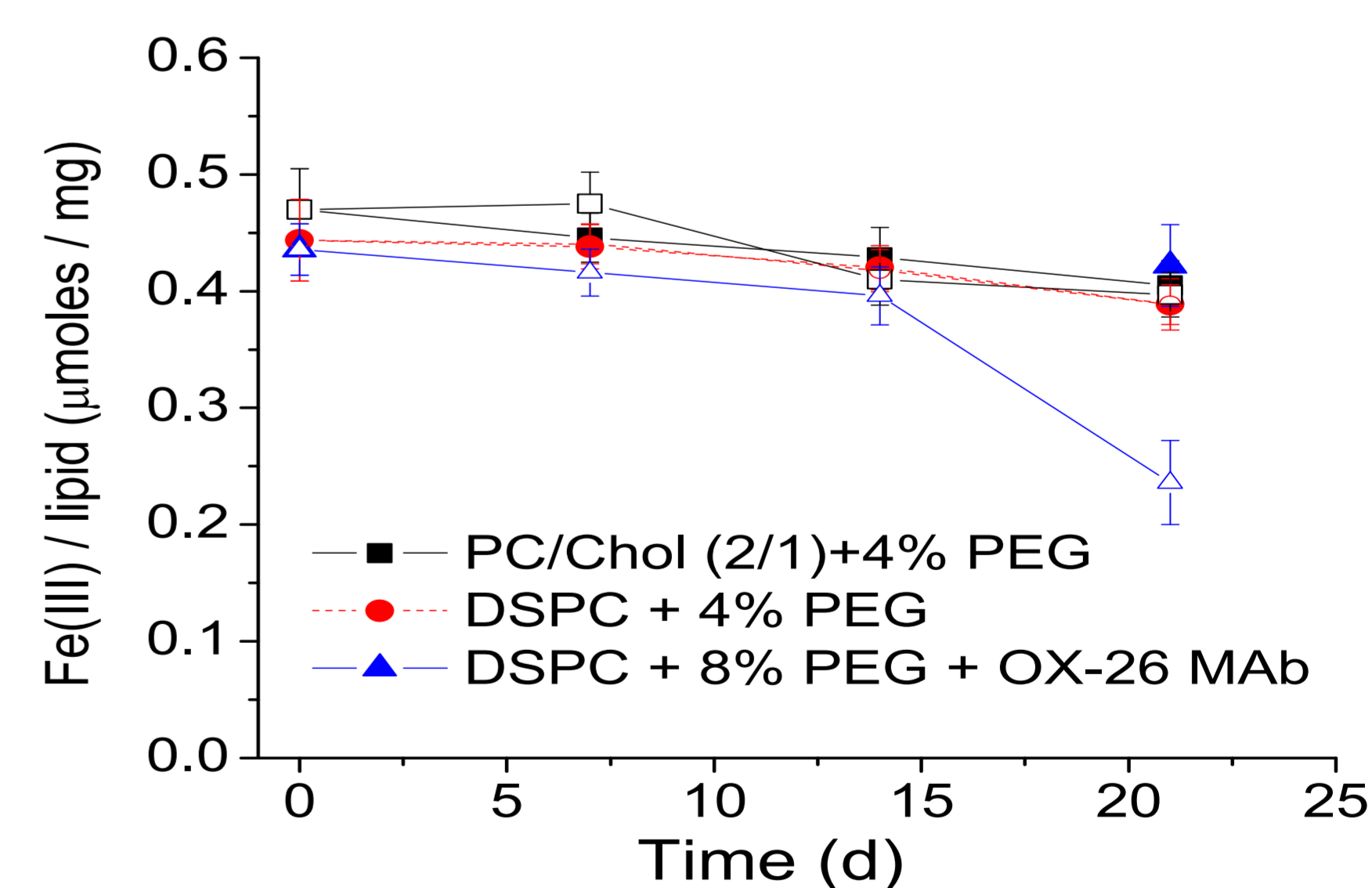
Size of MLs during storage at 37°C. Red dots = targeted MLs



- MLs & targeted MLs demonstrate colloidal stability and retain USPIO for prolonged periods

Membrane integrity studies

USPIO Retention in MLs at 37°C



Conclusions

- Extruded-DRV technique gives nanosized MLs with very high USPIO EE (up to 12 %).
- EE is influenced by: DRV technique preparation aspects and PEG-coating.
- Most ML-types are efficient T₂ contrast agents (since r₂/r₁ ratios are higher than free USPIOs).
- PEG-coating increases EE and stability; however r₂/r₁ ratios decrease (compared to non-PEGylated MLs)
- Targeted MLs were formed without significant loss of encapsulated USPIOs; they retain nanosize and integrity during storage for 1 month at 4°C (not shown) and up to two weeks at 37°C.

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