LRA (Lipid Removal Agent) ..... Synthetic calcium silicate hydrate for the selective removal of lipids, endotoxins and other bio-organic molecules

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Picture .....
Packaging for LRA

Lipids are naturally-occurring hydrocarbons that are poorly soluble in water and soluble in non-polar solvents such as ether and chloroform. They serve many important functions in cells and other biological systems. For example, lipids serve as structural components in cellular membranes, provide energy reserves (e.g. triglycerides), and are vital in the role of metabolism as vitamins and coenzymes.

However important lipids are to living systems, they can interfere with analytical assays and biopharmaceutical production. For example, the presence of fatty acids in multi-residue pesticide analysis in food products can cause ion-enhancement during MS analysis. Phospholipids can lead to ion-suppression during pre-clinical and clinical studies during pharmaceutical development. Removal of endotoxins, lipopolysaccharides derived from the cell membrane of gram-negative bacteria (e.g. E. coli), is critical in biopharmaceutical applications involving gene therapy products and transfection studies.

In this report we discuss the use of a new synthetic calcium silicate hydrate called LRA (Lipid Removal Agent) for the selective removal of lipids, macromolecules containing lipid moieties and other biological macromolecules in both analytical and process scale applications.

Table 1 ..... LRA specifications

Moisture (% H_2O as shipped)	5.5
Water adsorption, wet endpoint (%)	500 – 650%
Screen analysis, +325 mesh (%)	6.0
Surface area (m^2/g)	120.0
pH of 10% slurry	8.4 – 10.2
SiO_2 (%)	45.0
CaO (%)	4.0
SiO_2 / CaO (%)	1.3 – 20.0
Lead (ppm)	10
Heavy metals (ppm)	20
Ignition loss (ppm)	20
Fluoride (ppm)	10

Table 2 ..... LRA lipid removal capacity

Lipid	Removal Capacity
Cholesterol	40 mg/g LRA
Triglyceride	82 mg/g LRA
Apolipoprotein A1	160 mg/g LRA
HDL	73 mg/g LRA
Phosphatidylcholine	265 mg/g LRA
Endototoxin (lipopolysaccharide)	796,000 EU/g LRA

What is LRA?

LRA (Lipid Removal Agent) media is a synthetic calcium silicate hydrate developed for the selective removal of lipids in the production of biopharmaceuticals. LRA media is highly specific for lipids, lipopolysaccharides (endotoxins) and lipoproteins in the presence of other biological macromolecules (e.g. albumin, immunoglobulins, and other proteins). LRA is often used for the removal of lipids in biotech process streams and analytical assays that require lipid removal during sample preparation. Please see **Table 1** for detailed specifications of LRA and **Table 2** for LRA's lipid removal capacity.

Targeted removal of lipoproteins

In this application, bulk LRA was incubated with cryo-reduced source plasma at increasing LRA concentration levels for a period of 3 hours at ambient temperature. Following incubation, the sample was centrifuged and the supernatant was analyzed via nephelometry for removal of protein. **Table 3** shows that proteins, for the most part, are not removed by LRA. Although some non-specific adsorption occurred for plasma ceruloplasmin, IgM and alpha-2 HS glycoprotein occurred at 80 g LRA / L plasma, adsorption to apolipoprotein A1 and B was specific relative to the majority of the other plasma proteins endogenous to the sample.

LRA effectively removes phospholipids

In this study we demonstrate LRA's high capacity for phospholipids. LRA (40 mg) was packed into a 3 mL SPE tube between two 20 µm PE frits. A 0.5 mL aliquot of a 0.1 mg/mL phosphatidylcholine in 80% acetonitrile was loaded onto the LRA SPE tube with the aid of an SPE vacuum manifold. The resulting eluate was analyzed for phosphatidylcholine breakthrough via LC-UV

Table 3 ..... Human plasma protein profile following three hour room temperature incubation with LRA media*

LRA Concentration in Plasma	0 g/L	20 g/L	40 g/L	80 g/L	Relative Adsorption by LRA
Proteins in Supernatant	mg/mL (% from starting plasma)				
Albumin	30.40 (100)	30.40 (100)	30.80 (101)	30.00 (99)	0
Immunoglobulin G	6.20 (100)	6.23 (100)	6.18 (100)	5.80 (94)	0
Immunoglobulin A	1.83 (100)	1.85 (101)	1.84 (101)	1.73 (95)	+
Immunoglobulin M	0.72 (100)	0.70 (97)	0.63 (88)	0.51 (71)	0
α-1 acid glycoprotein	0.62 (100)	0.61 (98)	0.61 (98)	0.61 (98)	0
Haptoglobin	0.93 (100)	0.95 (102)	0.97 (104)	0.97 (104)	0
Transferrin	2.27 (100)	2.30 (101)	2.38 (105)	2.40 (106)	0
α-1 Proteinase inhibitor	1.21 (100)	1.20 (99)	1.20 (99)	1.20 (99)	0
α-2 Macroglobulin	1.03 (100)	1.01 (98)	0.96 (93)	0.96 (93)	0
α-2 HS Glycoprotein	0.81 (100)	0.66 (81)	0.66 (81)	0.28 (34)	++
Ceruloplasmin	0.29 (100)	0.27 (93)	0.24 (83)	0.04 (14)	++
Apolipoprotein A1	1.11 (100)	0.65 (59)	<0.25 (<23)	<0.25 (<23)	+++
Apolipoprotein B	1.03 (100)	0.76 (74)	<0.35 (<34)	<0.35 (<34)	+++

* Data provided by Advanced Minerals Corporation

analysis. The LRA adsorbent offered 91.4% removal of the phosphatidylcholine with a single pass through the LRA SPE tube (data not shown).

Removal of endotoxins

Endotoxin removal is of critical concern in the development and manufacture of biopharmaceutical products. Zhang and coworkers (1) demonstrated that LRA media has an effective endotoxin removal capacity of 6 x 10^6 endotoxin units (EU) per gram LRA, and offered rapid adsorption kinetics as well. For example, over 99.9% endotoxin in a 5000 EU/mL sample was adsorbed onto the LRA media after mixing for only 2 minutes. They further demonstrated the performance of the LRA by comparing it against a number of other commercially-available endotoxin removal agents.

Plasmid DNA purification

Although LRA was developed to selectively remove lipids, researchers have exploited LRA's selectivity for other purification purposes. Winters and coworkers (2) characterized the adsorption mechanism of LRA and have developed a cost effective and simple method for removing key impurities from E. coli lysates in the purification of supercoiled plasmid products. In a single adsorptive contact step, they were able to remove genomic DNA and open-circular plasmid whereas supercoiled plasmid remains unbound. When combined with LRA's selective affinity towards detergents and endotoxins, LRA media offers a powerful purification tool in downstream polishing during bioprocess purification.

Conclusion

LRA media is a synthetic calcium hydrate developed for the selective removal of lipids in the presence of other biological macromolecules. Its high capacity and adsorptive properties are ideal for bioprocessing purification, but it can be scaled down for analytical sample prep purposes as well. In this report we showed LRA's capacity and adsorptive efficiency across a range of lipids including endotoxins, phospholipids and lipoproteins. LRA's unique selectively can also be extended to other applications as well including the purification of supercoiled plasmid DNA.

Although LRA is currently available in bulk packing, LRA media can also be supplied in common LPLC hardware such as SPE tubes, VersaFlash® cartridges, Büchner Funnels, etc.

Related information

Customers using LRA media in cGMP production of biopharmaceuticals can request an LRA Regulatory Support Package through Supelco Technical Service. The support package contains detailed information regarding LRA manufacturing and quality control procedures/specifications.

Table 4 ..... Ordering information

Cat. No.	Brand	Description	Package Size
13358-U	Supelco	LRA (Lipid Removal Agent)	100 g
13360-U	Supelco	LRA (Lipid Removal Agent)	500 g

References

[1] Zhang, J. P.; Wang, Q.; Smith, T. R.; Hurst, W. E.; Sulpizio, T. Endotoxin Removal Using a Synthetic Adsorbent of Crystalline Calcium Silicate Hydrate. Biotechnol. Prog. 2005, 21(4), 1220-1225.

[2] Winters, M. A.; Richter, J. D.; Sagar, S. L.; Lee, A. L.; Lander, R.J. Plasmid DNA Purification by Selective Calcium Silicate of Closely Related Impurities. Biotechnol. Prog. 2003, 19(2), 440-447.