White Paper

CURAPATH

Polysarcosine (pSar)–a safer, more effective alternative to poly-ethylene glycol (PEG)

ABSTRACT

Conjugation of polyethylene glycol (PEG) to therapeutic molecules can increase drug half-life, solubility, and therapeutic potency. However, an increasing number of healthy individuals develop anti-PEG antibodies. PEG immunogenicity can cause anaphylactic shock and dramatically reduce the efficacy of the treatment. Here we describe a potential solution to the problem. Polyaminoacid-based polymers such as polysarcosine (pSar), provide a non-immunogenic alternative to PEG. Non-toxic, biodegradable polymers are not recognized by the immune system and provide equal or better solubility and therapeutic potency compared to PEG. Polysarcosine, along with other polyaminoacid-based delivery systems, have been successfully developed & manufactured at Curapath cGMP manufacturing facility in Valencia, Spain.

Introduction

Polyethylene glycol is a hydrophilic polymer that has been frequently used in everyday products, including paints, cosmetics, food, and medicine. The PEG market reached 4.15 billion dollars in 2019 and is expected to grow at a CAGR of 10.8% from 2020 to 2026, primarily driven by strong demand from the pharmaceutical industry (https://www.gminsights.com/industry-analysis/polyethylene-glycol-market). In the pharmaceutical industry, polymers are widely used as a gold standard of bioconjugation to prolong biologics circulation in the blood, increase solubility and improve potency. Many PEGylated products, including peptides, proteins, small interfering RNAs, and even small molecules, were successfully tested in clinical trials over the past two decades. Sales of the two most successful products, Pegasys and Neulasta, exceeded $5 billion in 2011 (1, 2). PEG polymer use grew steadily in the pharmaceutical, cosmeceutical, and food industries making it one of the most abundantly manufactured polymers. With the successes observed for lipid-based nanoparticle delivery systems, it was not surprising that the two-leading vaccine-producing companies, Moderna and BioNTech/Pfizer, incorporated PEGylated lipids as part of the mRNA delivery of nanoparticles, in their race to stop COVID-19 pandemic. Both companies went on to manufacture and use hundreds of millions of doses to eradicate the coronavirus pandemic that claimed over 6 million human lives globally. That dramatic increase in the polymer use exposed a critical problem: PEG is immunogenic and should not be used for individuals with severe allergic reactions (3,4,5,6).

The Problem

PEG immunogenicity and potential toxicity due to the accumulation in the liver and other organs have been known for years. The presence of anti-PEG antibodies correlated with PEG-asparaginase clearance from blood (7). In 2011 a Phase III clinical trial in patients with severe chronic gout, treated with Pegloticase (Krystexxa), detected anti-PEG antibodies in 89% of the treated individuals (8). Consequently, 40% of the patients developed resistance to the treatment over time (9).

In 2017, a Nature paper published by Chia-Jung Chang and coauthors, identified a novel susceptibility locus for the immunogenicity of polyethylene, responsible for eliciting anti-IgM and anti-IgG antibodies (10). Further studies confirmed the finding, demonstrating that anti-PEG antibodies were detected in patients who have never been treated with PEGylated drugs, but might have consumed other products containing PEG (11). Anti-COVID vaccination with either BioNTech/Pfizer or Moderna PEGylated lipid nanoparticles delivering mRNA demonstrated that PEG could trigger an immune response, causing anaphylaxis–a potentially life-threatening reaction that can cause rashes, plummeting blood pressure, and shortness of breath (4,5,6).

Thus, an abundance of PEG-containing products in everyday life may have contributed to the growing frequency of immunoreactivity, use limitations, and rising safety concerns associated with treatments using PEG-polymer containing conjugates. In turn, this has prompted extensive research efforts in seeking non-immunogenic alternatives to PEG.

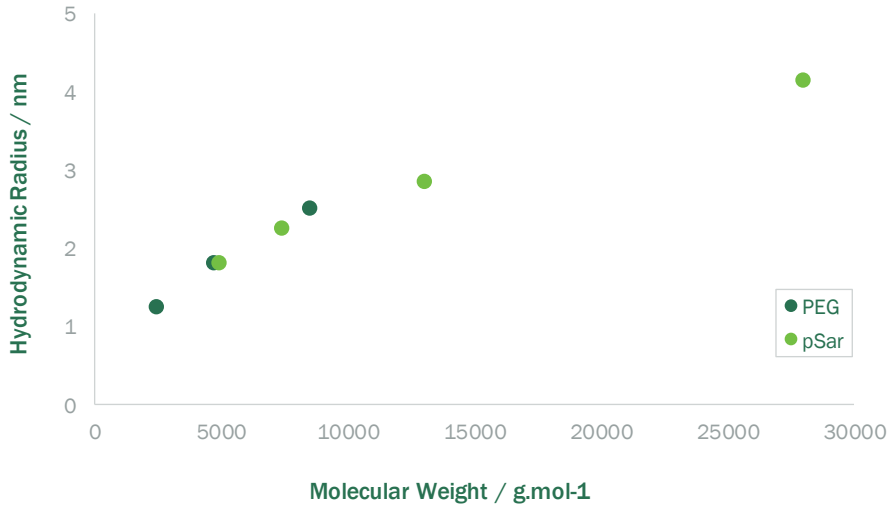
The Solution

Polysarcosine (pSar) is a nonionic hydrophilic polyamino acid formed from the endogenous amino acid sarcosine (N-methylated glycine). It was demonstrated that pSar has similar physico-chemical properties to PEG (Figure 1) including high water solubility, similar interaction with proteins, low toxicity (it degrades into natural amino acid), and low immunogenicity (12-15), making pSar an ideal candidate for PEG replacement.

FIGURE 1.

Hydrodynamic radius vs. Molecular weight correlation for PEG and pSar.

Adapted from Ref 14.



Direct comparison of the functional activity of PEGylated versus poly-sarcosinylated interferon demonstrated that the latter binds to the receptor with the same affinity as a pegylated conjugate but is much more potent in inhibiting tumor growth and also elicits considerably fewer anti-INF antibodies, in mouse models, compared to PEG-INF (Figure 2).

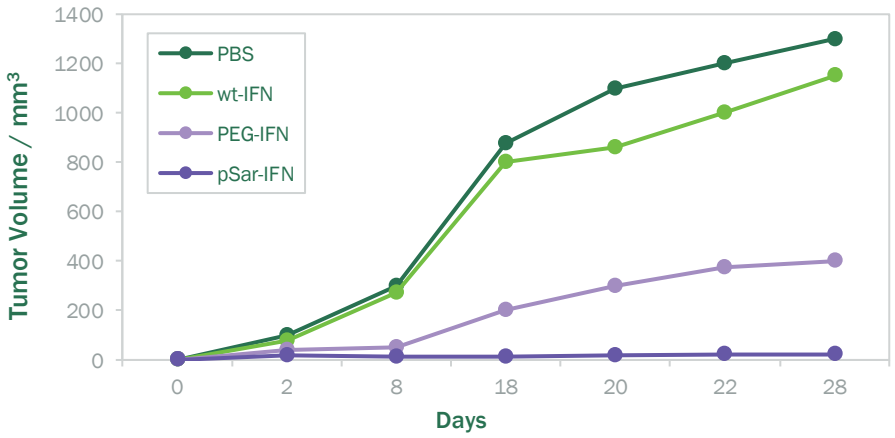


FIGURE 2.

Antitumor efficacy of the conjugates in the tumor-bearing mice. Tumor growth inhibition curve.

Adapted from Ref 16.

Furthermore, pSar-conjugation improves the stealth properties of the liposomes and avoids the Accelerated Blood Clearance (ABC) immune phenomenon. pSar liposomes demonstrated similar physicochemical properties compared to PEG-liposomes in terms of size, zeta potential, membrane polarity, and fluidity (14). However, a pharmacokinetic experiment with multiple injections showed that IgMs produced after the first dose of PEG-liposomes enhance conjugate uptake in the liver and reduce the blood half-life after the second dose. In contrast, pSar-liposomes do not elicit this antibody response and help to circumvent the ABC phenomenon (17) (Figure 3).

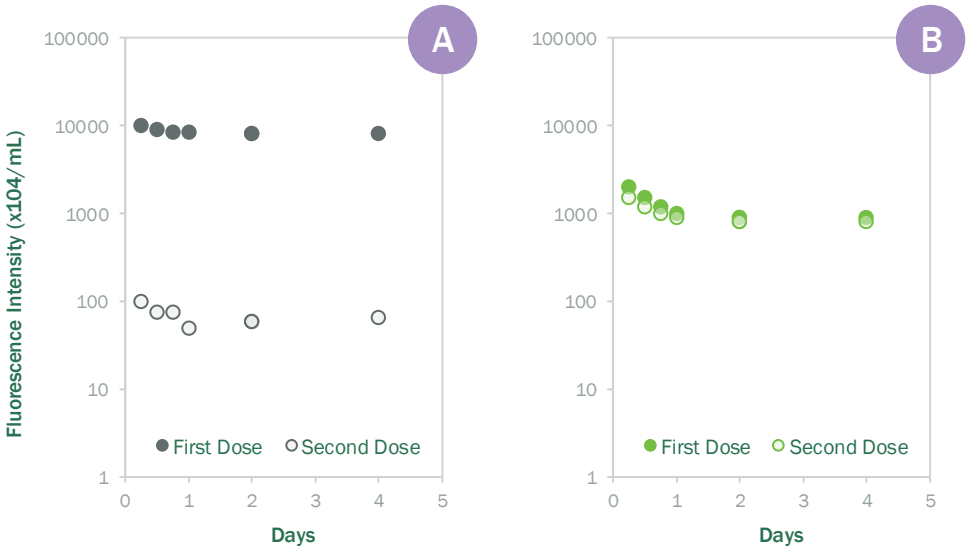


FIGURE 3.

Plasma concentration change for (A) PEG-liposome, and (B) pSar-liposome. Each bar represents the mean ± SD (n=4-5).

Adapted from Ref 17.

Polyamino Acid Development & GMP Manufacturing

Curapath has developed an extensive knowledge of polypeptide production, process development, and analytical methods (Figure 4) that have been paramount in consolidating robust Chemistry, Manufacturing, and Control packages and obtaining smooth regulatory approvals by EMA and FDA for our customers. Four of Curapath's collaborators are currently proceeding with Phase I and II clinical trials.

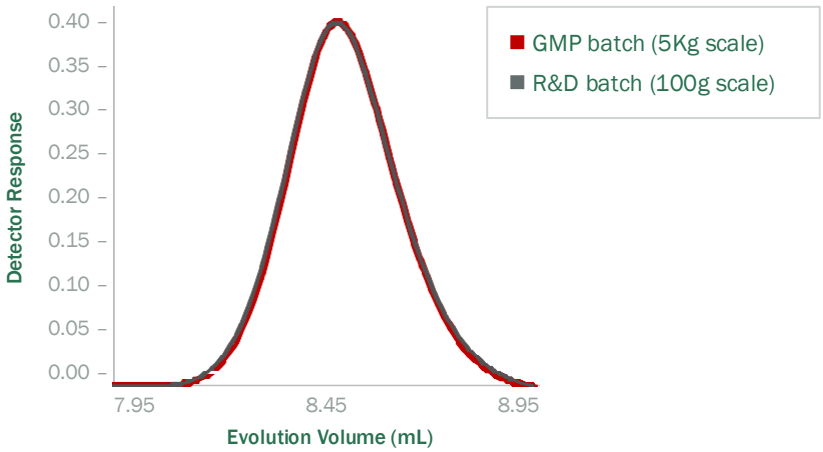


FIGURE 4.

Size Exclusion Chromatography profiles for a polyamino-acid produced at R&D lab-scale (Mw= 17.500Da, PDI=1,02) vs. GMP plant- scale (Mw=17.200Da, PDI=1,01).

Curapath internal data.

Conclusions

The development of biologics, including vaccines, and small molecule drugs require a non-toxic, biodegradable, non-immunogenic nanocarrier or excipient with a good safety profile. The dramatic increase in PEG use, both in the pharmaceutical industry and in household items, has affected the safety of the polymer. In contrast, pSar should be considered as a safe and effective alternative with an improved immunogenicity profile. Both GMP manufacturing and scale-up production have been developed at Curapath to support our clients.

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REFERENCES

1. Klauser A, Roche Group Media Relations, "Roche in 2011: Strong results and positive outlook," www.roche.com/med-cor-2012-02-01-e.pdf, 2012 Feb 1, p.7
2. Amgen, “Amgen 2011 Annual Report and Financial Summary," 2011 Annual Report.pdf, 2012 Feb 23, p. 71
3. Rose A, Pfizer Media Relations, https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-supply-united-states-100-million
4. de Vieze J, *Pfizer's vaccine raises allergy concerns*. Science 2021; 371 (6524), 10-11. DOI:10.1126/science.371.6524.10
5. McSweeney M, Mohan M, Commins S, Lai SK, *Anaphylaxis to Pfizer/BioNTech mRNA COVID-19 vaccine in a patient with clinically confirmed PEG allergy*, Frontiers in Allergy 2021 Sept 29 Article 715844.
6. Nilsson L, Csuth A, Storsaeter J, Garvey L, Jenmalm M, *Vaccine Allergy: evidence to consider for Covid-19 vaccines*, Current Opinion in Allergy and Clinical Immunology 2021 August; Vol 214 p401-409.
7. Armstrong JK, Hempel G, Koling S, et al. *Antibody against poly (ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients*. Cancer 2007;1 10:103-11.
8. Sundy JS, Baraf HSB, Yood RA, et al. *Efficacy and tolerability of pegloticase for the treatment of chronic gout in patients refractory to conventional treatment*. JAMA 2011; 306: 7 11-20.
9. Dalbeth, N, Merriman, TR; Stamp, LK Gout. Lancet. 2016; Oct 22; 388 (10055): 2039–2052.
10. Chang CJ, Chen CH, Chen BM, Su YC, Chen YT, Hershfield MS, Lee MM, Cheng TL, Chen YT, Roffler SR, Wu JY. *A genome-wide association study identifies a novel susceptibility locus for the immunogenicity of polyethylene glycol*. Nat. Commun. 2017 Sep 12;8(1):522.
11. Hoang Thi TT, Pilkington EH, Nguyen DH, Lee JS, Park KD, Truong NP. *The Importance of Poly (ethylene glycol) Alternatives for Overcoming PEG Immunogenicity*. Drug Delivery and Bioconjugation. Polymers (Basel). 2020; Feb 2;12(2):298.
12. Bleher S, Buck J, Muhl C, Suss, R et al. *Poly-Sarcosine Surface Modification Imparts Stealth-Like Properties to Liposomes*. Small (Weinheim an der Bergstrasse, Germany). 2019; Dec;15(50): e1904716. Doi:10.1002/smll201904716. Epub 2019 Nov 13.
13. Settanni G, Schäfer T, Muhl C, Barz M, Schmid F. *Poly-Sarcosine and Poly(ethylene-glycol) interactions with proteins investigated using molecular dynamics simulations*. Computational and Structural Biotechnology Journal. 2018; 16, 543–550.
14. Weber B, Birke A, Fischer K, Schmidt M, and Barz M. *Solution Properties of Polysarcosine: From Absolute and Relative Molar Mass Determinations to Complement Activation*. Macromolecules 2018; 51, 2653−2661.
15. Nogueira S, Schlegel S, Maxeiner K, Weber B, Barz M, Schroer M, Blanchet C, Svergun D, Ramishetti S, Peer D, Langguth P, Sahin U, Haas H. *Polysarcosine-Functionalized Lipid Nanoparticles for Therapeutic mRNA Delivery*. ACS Appl. Nano Mater 2020,3,10634-10645
16. Hu Y, Hou Y, Wang H, Lu H. *Polysarcosine as an Alternative to PEG for Therapeutic Protein Conjugation*. Bioconjug Chem. 2018; Jul 18; 29(7):2232-2238.
17. Son K, Ueda M, Taguchi K, Maruyama T, Takeoka, S, Ito, Y et al. *Evasion of the accelerated blood clearance phenomenon by polysarcosine coating of liposomes*. Journal of Controlled Release 2020; 322, 209–216.

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Curapath specializes in the custom design, development and GMP manufacturing of polyamino acids, and lipid and polymer nanoparticles accelerating the timeline of drug development for our clients. Curapath is a global organization, providing unique technical expertise in the composition, development and end-to-end manufacturing of drug delivery systems. Curapath utilizes a collaborative, hands-on approach when working with clients, functioning as an extension of their team. Curapath anticipates needs, and identifies and develops the best solutions for our clients' unique drug delivery challenges from preclinical development through commercial-scale GMP manufacturing.