International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1116-1124 www.ijprajournal.com ISSN: 2249-7781

An Overview of PEGylation's History, Benefits, and Methods for Resolving the PEG Dilemma.

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Submitted: 20-11-2023 Accepted: 30-11-2023

ABSTRACT: Polyethylene glycol (PEG) conjugation is a fast-developing approach that supports conventional drug delivery techniques to overcome obstacles in therapeutic delivery. PEGylation is the term used to describe the process of joining one or more polyethylene glycol (PEG) chains to modify a protein, peptide, or non-peptide molecule.Many proteins, including cytokines, enzymes, hormones, growth factors, lactoferrin, antibodies, and antibody fragments, have shown increased activity as a result of PEGylation. PEGylation increases the plasma half-lives of these compounds by more than 1.5 to several hundred-fold, reducing their antigenicity and the consequent antibody production against them. According to research, PEGylated liposomes can also have certain unfavourable effects, such as high cell uptake, poor "endosomal escape" of pH-sensitive liposomes (PSL), and the accelerated blood clearance (ABC) phenomena.These issues have led to the term "PEG dilemma". This review discusses some strategies for resolving the PEG dilemma.

KEYWORDS: PEGylation, Polyethylene glycol, PEG, PEG dilemma

I. INTRODUCTION

Polyethylene glycol (PEG) conjugation is a fast-developing approach that supports conventional drug delivery techniques to overcome obstacles in therapeutic delivery[1]. PEGylation is the term used to describe the process of joining one or more polyethylene glycol (PEG) chains to modify a protein, peptide, or non-peptide molecule. This polymer has FDA approval and is highly soluble in water, non-immunogenic, non-antigenic, and nontoxic[2].PEGylation changes the biomolecule's conformation, hydrophobicity, and electrostatic binding, among other physical and chemical characteristics, and improves the drug's pharmacokinetic behaviour. PEGylation generally reduces immunogenicity and increases medication solubility [2].PEGylation polypeptide drugs protect them and improve their pharmacodynamic and pharmacokinetic profiles. The biotechnology sector is finding polypeptides that have considerable potential as novel therapeutic candidates to address particular symptoms of disease[3]. However, polypeptide drugs are rapidly degraded by proteolytic enzymes and neutralized by antibodies, among other shortcomings. This reduces their half-life and circulation time, thereby limiting their therapeutic effectiveness [3].

Many proteins, including cytokines, enzymes, hormones, growth factors, lactoferrin, antibodies, and antibody fragments, have shown increased activity as a result of PEGylation. Large-scale protein manufacturing has been made possible by recombinant DNA technology; however, the antigenicity of these proteins has hampered their therapeutic applicability. PEGylation increases the plasma half-lives of these compounds by more than 1.5 to several hundred-fold, reducing their antigenicity and the consequent antibody production against them[1].

A polymer-protein combination By creating steric hindrance, the polymer PEG protects the protein surface from chemicals that degrade it. Additionally, the reduced renal clearance of the PEGylated protein is mostly caused by the larger conjugate. Activated PEGs, or commercially accessible PEGylation reagents, typically belong to one of three classes. acylating reagents, alkylating reagents, and thiol-reactive reagents. Most of the Marketed PEGylated Biopharmaceuticals are Modified with Acylating PEG Reagent.

II. HISTORY OF PEGYLATION.

Frank F. Daviesinitially identified PEGylation in two groundbreaking research investigations on albumin and catalase modification in the 1970s[2]. He attached

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1116-1124 www.ijprajournal.com ISSN: 2249-7781

polyethylene glycol (PEG) to a protein to help it escape the body's immune response—and died on May 19. When he was 100 years old. First-generation PEG reagents were usually no larger than 5 kDa. Earlier PEG reagents contained degradable linkages and were not lysine-specific. One of the most widely distributed amino acids, lysine can account for up to 10% of the amino acid sequence in an average protein. Davis initially used cyanuric chloride (2,4,6-trichloro's-triazine) to prepare activated PEG that was reacted with catalase and bovine serum albumin yielding conjugates with extended circulation time and less immunogenicity than the parent proteins[4]. This was an important milestone because at that time it was not conceivable to modify an enzyme so extensively and still maintain its activity[2].

Most non-human proteins cause adverse immune reactions when injected into people, limiting their therapeutic value. Davis thought a polymer might give non-human proteins a protective cloak. He read in a medical journal that doctors had safely injected a copolymer that included PEG into patients undergoing major blood-vessel surgery to prevent lipid embolisms. So he decided to covalently link PEG to the enzyme bovine liver catalase.

The majority of non-human proteins have limited therapeutic value because they induce adverse immune reactions in humans when injected. Davis believed that non-human proteins might have a shielding polymer. He discovered via reading in a medical journal that physicians were able to prevent lipid embolisms in patients undergoing major blood-vessel surgery by injecting a copolymer containing PEG. So he decided to covalently link PEG to the enzyme bovine liver catalase. In the late 1960s when he became interested in developing a procedure whereby selected bioactive proteins could be utilized for human therapy," Davis wrote in a commentary about the discovery (Adv. Drug Deliv. Rev. 2002, DOI: 10.1016/s0169-409x(02)00021-2).

The pegylated enzyme lasted longer before being biologically degraded than the naked enzyme when Frank F. Davis injected it into mice, and it also did not cause the same immunogenic response. Davis patented the discovery (US Patent No. 4,179,337) and, in 1986, cofounded Enzon Pharmaceuticals with Abraham Abuchowski to commercialize it.

Adagen, a pegylated form of the enzyme adenosine deaminase, was Enzon's first medication and was authorized by the US Food and Drug Administration in 1990 for the treatment of bubble boy disease, a severe form of combined immunodeficiency illness. Since then, the FDA has approved more than 15 pegylated medications, such as Neulasta, which increases white blood cell counts following chemotherapy for cancer, and Mircera, which treats anemia in patients with chronic kidney disease.

Later on, Matsumura and Maeda found that high molecular weight(≥40 kDa), enhances the permeability and retention(EPR)effect. Liposomes are now well-recognized drug delivery vehicles that can be used in cancer therapy[2]. From the viewpoint of practical use, systemic administration is desirable because it can be performed easily. After intravenous administration, a liposome is adsorbed by biological components such as serum proteins (opsonins) in the systemic circulation. The reticuloendothelial system (RES), formerly known as the mononuclear phagocytic system (MPS) of the liver and spleen, is a crucial host defense mechanism that recognizes an opsonized liposome[5].

It was first reported in the early 1990s that liposomes modified with polyethylene glycol (PEG), or PEGylation, were able to circulate in the bloodstream following intravenous administration for unusually long periods of time. The PEG moiety creates an aqueous layer on the liposome surface, stabilizing the lipid bilayer and causing steric hindrance, which inhibits protein adsorption and reduces macrophage recognition[5].

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1116-1124 www.ijprajournal.com ISSN: 2249-7781

In the early 1990s, the FDA approved PEGaspargase for leukaemia and PEGademase for severe combined immunodeficiency disorder, which was two of the first pegylated medications. More recently, pegylated medications have been approved or are undergoing clinical trials to treat a variety of diseases, including wound healing, acromegaly, rheumatoid arthritis, neutropenia, hepatitis C, and various malignancies. A polypeptide medication is attached to repeating units of polyethylene glycol (PEG) through the PEGylation process. Scientists have developed new chemistries over the last 30 years to create PEG polymers and link them to desired polypeptide drugs. The chemistries used in PEGylation will be further refined by researchers in order to produce more therapeutic polypeptides[3].

III. WHY PEGYLATION IS REQUIRED.

- PEGylation gives the surfaces of nanocarriers "stealth" qualities that prevent the cellular immune system from recognizing or absorbing them. This results in a longer half-life, lower dosage and frequency, and better site-specific drug delivery. PEG's molecular weight and structure influence the degree of resistance; branched PEG offers more resistance than linear PEG[1].
- Reduced dosage frequency is made possible by PEGylation, which also lengthens the duration of the conjugates' blood retention and renal excretion. Small molecule medications, therapeutic proteins, peptides, and antibody fragments have all been PEGylated to take advantage of these favorable pharmacokinetic effects[2].
- PEGylation significantly increases the apparent size hydrodynamic radius 20nm of conjugated drug compound where the renal glomerular capillary diameter is 6-12 nm.
- Additionally capable of protecting therapeutic proteins from the body's digestive proteolytic enzymes are PEG's barrier qualities. Asparaginase PEGylated with branching PEG has been shown to withstand trypsin degradation[1].
- PEGylation helps nanocarriers in improving tumour-specific target ability.

PEGylation leads to various physicochemical changes that provide desired in vivo pharmacokinetic profile to therapeutic moiety. Additionally, possibility for modifications with customized linkers and targeting ligands allows us to achieve specific pharma codynamic outcomes.(Figure: 1)

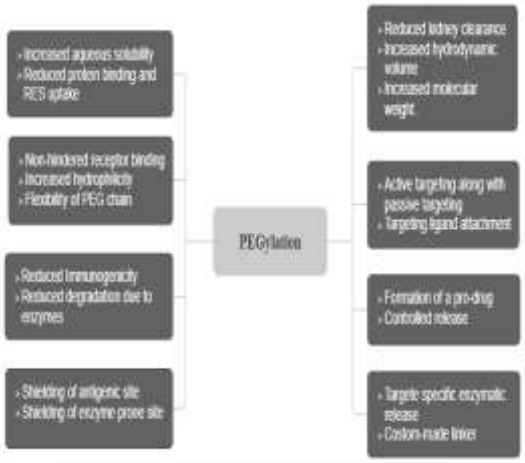

Figure 1: Importance of PEGylation.

IV. PEGYLATION DILEMMA.

Polyethylene glycol (PEG) is extensively used to increase the in vivo and in vitro stability of liposomes. Further investigation has revealed that PEGylated liposomes can also have certain negative impacts, including poor "endosomal escape" of pH-sensitive liposomes (PSL), low cellular uptake, and the accelerated blood clearance(ABC) phenomenon. These issues have led to the term "PEG dilemma"[6].

4.1 Strategies forover coming the pegdilemma

1) Enhancement of CellularUptake

The positively charged surface of the lipoplexes ensures a successful binding to the cell surface. Anionic glycoproteins, such as heparan sulphate proteoglycans, have been proposed as the mediators of lipoplexes' interactions with the cell surface. The first strategy to address this problem is to display ligands for receptors on the surface of targeted cells using PEGylated carriers. "Active targeting" refers to this process, which is anticipated to improve the carriers' selectivity, binding, and uptake by the targeted cells. Target molecules are carefully selected based on their specificity, level of expression on tumor cells, and ability to internalize PEGylated nanoparticles that have been ligand-modified[7].

Target ligands such as antibodies and glycoprotein transferrin (Tf), which binds iron, are widely used to deliver drugs and nucleic acids to Tf receptors over expressed in tumor cells. While folate receptors are not widely distributed in normal tissues, many types of tumor cells

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1116-1124 www.ijprajournal.com ISSN: 2249-7781

overexpress them[8,9] . Folate-modified liposomes enhanced the antitumor efficaciousness of encapsulated drugs and nucleic acids as well as their cellular uptake[10].

An attempt was made to use a PEGylated liposome modified with hyaluronan to target melanoma overexpressing CD44, a surface receptor that binds to hyaluronan[11,12]. Anisamide-modified PEGylated liposome-polycation-DNA nanoparticle (LPD) effectively delivered siRNA to lung carcinomas with high levels of sigma receptor expression, according to Huang and colleagues' study, when compared to nontargeted PEGylated LPD. The result was the silencing of the luciferase gene in metastatic tumors.

Antibodies have been thoroughly investigated as ligands for the tumor-targeting of liposomes due to their great specificity and affinity for target molecules. Immunoliposomes, or PEGylated liposomes modified with an antibody, are made by covering the PEG chain with a specific monoclonal immunoglobulin G (IgG) antibody, Fab′ fragment, and recombinant single-chain variable fragment. It has been demonstrated that immune liposomes specifically target the type 2 human epidermal growth factor receptor (EGF-R) (HER2)[13,14] EGF-R, which is upregulated in tumors like glioblastoma, hepatocellular carcinoma, and epithelial tumors, is another well-researched immune liposome target[15,16] By adding EGF to PEGylated nanoparticles, it was also possible to target the EGF-R[17,18].

Since arginine-glycine-aspartic acid (RGD) can recognize integrins expressed on both tumor cells and neovascular endothelial cells, it is the most frequently used motif. RGD has been used to deliver drugs and genes to specific targets [19]. It has become more common to target tumor endothelial cells via the RGD motif [20]20. Another peptide ligand that has been discovered to bind to CD13 or aminopeptidase N, which is over expressed on tumor endothelial cells and tumor cells, is the asparagine-glycine-arginine (NGR) motif peptide[21]. NGR motif peptides have been used to successfully deliver drugs and liposomes to the tumor vasculature. The value of APRPG-modified PEG liposomes was demonstrated by Oku and colleagues [21,22], who also found that the alanineplorine-arginine-plorine-glycine (APRPG) motif peptide attaches to tumor angiogenic vessels in a particular way[23,24].

2) De-PEGylation

Endocytosis or processes similar to endocytosis take place to internalize glycoproteins once lipoplexes have identified them. Endosomes should be protected from enzymatic degradation because they are fused with lysosomes, lipoplexes, and digestive compartments. PEGylation prevents fusion with the endosomal membrane and gives complexes steric stability.

- Endosomes and lysosomes, which have low pH intracellular environments, are used as PEG cleavage triggers [25]. In contrast to stable PEGylated lipoplexes, pH-sensitive lipoplexes with an orthoester linkage exhibited higher luciferase expression in a transfection experiment conducted by Szoka et al.[25]. Therefore, a cleavable PEG-lipid triggered in a tumor-specific manner would be beneficial for tumor gene delivery. Such cleavable bonds are like a) Hydrogen bond, b) Peptide bond, c) Disulfide bond, d) Ester bond, and e) Vinyl ether bond

a) Hydrogen bond :-DOX was covalently conjugated onto the hydrophobic segments of the amphiphilic block copolymer via a hydra pH-sensitive hydrazone bond. The DOX release profiles from the micelles in vitro demonstrated a strong reliance on the pH levels of the surrounding environment. The acid-cleavable hydrazone linkage between the DOX and micelles is responsible for the increased drug release rate in the acidic medium. Because pH-dependent hydrazone bonds are prone to hydrolysis and cleavage in acidic environments (particularly pH 5.3), an efficient concentration of DOX in a short period of time[26]. In blood, PH is basic (7.35-7.45) in nature, in normal cells PH is neutral (7.0-7.4) and Cancer cell PH is acidic (6.3-7.0) in nature.

b) Peptide bond :-MMPs (matrix metalloproteinases) are expressed at low levels in normal cells, but they are widely distributed in the extracellular space and highly expressed in tumor cells. MMP helps peptide bond dissolution.

c) Disulfide bond :-Disulfide-bridged cleavable bonds can be attached to PEG to overcome issues such as steric hindrance. The substantial concentration gradient of glutathione (GSH), particularly in the intracellular region, can selectively cleave the disulfide bond in the tumor environment because the concentration

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1116-1124 www.ijprajournal.com ISSN: 2249-7781

of GSH inside cells is almost three orders of magnitude higher than that outside of them. Furthermore, the disulfide-linked nanocarriers are highly stable prior to internalization in the target cells due to low extracellular concentrations of GSH.

d) **Ester bond :-**In view of the fact that ester bonds are susceptible to hydrolysis by esterases that are widespread in the plasma and tissues.Because of their narrow therapeutic window, the rapid uptake of anticancer agents may induce liver damage. In order to better control the release of the contents, two PEG-lipid derivatives (mPEG-CHEMS and mPEG-CHMC) are linked to the carriers via the ester bonds.

e) **Vinyl ether bond :-**Because the vinyl ether bond must be non-oxidative and either neutral or basic, the bond is labile under acidic or oxidative conditions. Four structurally similar acid-labile PEG-lipids were synthesized and connected by vinyl ether bonds. By removing the sterically stabilizing PEG layer, acid-catalyzed hydrolysis of the vinyl ether bond destabilized liposomes and promoted contents release on an hourly timescale at pH <5.

3) Acceleration of Endosomal Escape via MembraneFusion

Altering the endosomal membrane or accelerating membrane fusion are two other strategies to boost PEGylated carriers' endosomal escape. In order to speed up endosomal escape during gene delivery, the endosomal disruption mechanism has been widely used. Poly(ethyleneimine) (PEI) has a high transfection efficiency that can be attributed to its secondary and tertiary amines, which have a buffering effect or the "proton sponge effect."128 Melittin is a pH-responsive endosmotic peptide that Wagner and colleagues conjugated with PEGylated PEI using an acid-labile dimethylmaleic anhydride (DMMAn) linker[5].

4) pH-sensitive Cationic Lipids.

The use of pH-sensitive cationic devices provides an additional method of solving the PEG problem. In the 1980s, reports of pH-sensitive liposomes began to appear. The release of cargo into the cytoplasm was stimulated by protonating pH-sensitive liposomes made of phosphatidylethanolamine in endosomes and lysosomes [27,28].

Ionizable liposomes are useful for delivering nucleic acids, and Bailey and Cullis reported on the synthesis of an ionizable amino lipid called 1,2-dioleoyl3-dimethylammonium propane (DODAP)[29,30]. A tertiary amine that makes up the head group of DODAP is in charge of cationization at acidic pH values. The systemic circulation contains fewer liposomes with a net cationic lipid content because liposomes containing DODAP become neutral at physiological pH. Liposomes with only a small amount of PEG modification (approximately 5mol%) are able to circulate steadily in the blood due to their sensitivity to pH.

It is commonly known that traditional nanocarriers that have yet to have their surfaces modified are incredibly unstable in plasma and are unable to transport their contents to the desired tissues or cells successfully. Enhancing their stability has thus emerged as an essential concern. PEG derivatives are used as the coating layer of nanomaterials to increase their surface hydrophilicity and steric hindrance, thereby extending their circulation time in vivo and achieving a longer cycle time. Numerous researchers have taken an interest in it. Conventional PEG-lipids are usually linked by amide or ether bonds, which have a high degree of chemical stability and are therefore challenging to separate from the carriers. The steric effect of PEG-lipids would be resistant to degradation of the PEGylated nanocarriers in vivo, preventing interactions between nanocarriers and target cells. As a result, the conventional PEG-lipid materials may prevent the medication from being absorbed by cells and from escaping via endosomes. This article offers several options for the conventional PEG-lipids in order to solve the PEG dilemma.

V. CHALLENGES IN THE DEVELOPMENT OF PEGYLATED NANOCARRIERS.

The use of PEG-lipids in nanocarriers and the PEGylation dilemma have presented a significant obstacle to the creation of PEGylated nanocarriers. Following PEGylation, clathrin-coated pits on the plasma membrane of carcinoma tissue cancer cells are inaccessible to nanocarriers or macromolecules for clathrin-mediated endocytosis. The proteins caveolin-1 and clathrin are important for receptor-mediated endocytosis (CME)[31]. The following issues are inevitably brought about by pegylation.

a. First, Target cells become restricted by the steric hindrance of PEG chains through a

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 6 Nov-Dec 2023, pp: 1116-1124 www.ijprajournal.com ISSN: 2249-7781

ligand receptor-mediated mechanism called CME [32].A thick hydrophilic PEG coating is frequently required to prevent opsonization by obstructing the recognition of opsonins and the reticuloendothelial system's (RES) subsequent phagocytosis[33]. However, the targeting ligands of nanocarriers may be prevented from attaching to the appropriate receptors on the surface of cancer cells by these long polymer chains. In addition, the PEG layer might potentially hamper the medication's ability to release itself from the vehicles[34].

b. Second, PEGylation strongly hinders the endosomal escape of nano-vehicles, resulting in a notable decline in the delivery system's activity. It is commonly known that the primary pathway for the cellular transportation of nanomedicines is clathrin-mediated endocytosis. The first effect the endocytosed cargos are delivered after clathrin-mediated internalization at the plasma membrane is into early endosomes, which have an internal pH of 5.0–6.5. Subsequently, the early endosomes develop into late endosomes, which merge with lysosomes, which are intracellular organelles with a pH lumen that is ideal for the hydrolysis-related enzymes (4.5–5.0). Facilitating endosomal escape and guaranteeing cytosolic delivery of the drugs is a necessary step toward attaining an effective delivery[26].

c. Third, accelerated blood clearance (ABC) phenomenon This phenomenon, which results in increased clearance and decreased efficacy of PEG-conjugated substances/PEGylated nanocarriers, has been widely observed during the repeated administration of PEG-conjugated substances and PEGylated nanocarriers, including PEGylated liposomes, PEGylated nanoparticles, PEGylated micelles, etc[35]. When PEGylated liposomes are administered for the first time, an excessive amount of anti-PEG IgM is secreted, which causes the ABC phenomenon[36]. A second dosage of regular PEGylated liposomes caused an unexpected pharmacokinetic change. Additionally, after receiving the second dose, other nanocarriers that had undergone PEGylation were also discovered to exhibit this phenomenon. Due to the accelerated accumulation in the macrophage system, PEGylated nanocarriers that were administered repeatedly would be quickly removed from the systemic circulation. PEG inhibited the fusion of liposomes with the cellular and endosomal membranes. The "PEG challenges" or "PEG dilemma" is the term used to describe this set of negative consequences [26].

The use of PEG-lipid-modified nanocarriers in drug delivery systems has advanced significantly. PEG-lipids have been integrated into a variety of nanocarriers, such as solid lipid nanoparticles, polymer micelles, liposomes, and other types.

CONCLUSION

Many proteins, including cytokines, enzymes, hormones, growth factors, lactoferrin, antibodies, and antibody fragments, have shown increased activity as a result of PEGylation. According to research, PEGylated liposomes can also have certain unfavourable effects, such as high cell uptake, poor "endosomal escape" of pH-sensitive liposomes (PSL), and the accelerated blood clearance (ABC) phenomena.The “PEG Dilemma” is one of the biggest problems currently plaguing the development of drug delivery systems. At present, there are many ways to solve this problem from various angles.

wire. If we make a coil of many turns of wire, this magnetic field becomes many times stronger, flowing around the coil and through its center in a doughnut shape. When the coil of the solenoid is energized with current, the core moves to increase the flux linkage by closing the air gap between the cores. The movable core is usually spring-loaded to allow the core to retract when the current is switched off. The force generated is approximately proportional to the square of the current and inversely proportional to the square of the length of the air gap.

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