Applications of Poly(ethylene oxide) in Drug Delivery Systems

Part II

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PHOTODISC

Poly(ethylene oxide) is gaining the attention of research and development organizations and its application is extending into a wide range of drug delivery systems.

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In a previous article, we reviewed the applications of high molecular weight poly(ethylene oxide)s (PEOs) in hydrogels and hydrophilic matrix systems (1). PEOs are nonionic, water soluble, and highly hydrophilic. They are characterized with flocculent, thickening, sustained-release, lubrication, dispersing, and water-retention properties. Grades of PEO differ according to their molecular weight, which range from 200 to 7×10^6. Products with molecular weights below ~25,000 are viscous liquids or waxy solids and are commonly referred to as poly(ethylene glycols) (PEGs).

PEO resins are made commercially by the catalytic polymerization of ethylene oxide in the presence of metallic catalyst systems. Uses of PEO include mucoadhesives, water-soluble films, rheology control agents and thickeners, and additives in pharmaceutical products. Pharmaceutical PEO grades have official status in *USP 23–NF 18*. The well-known properties of PEO and its regulatory acceptability have helped extend this polymer's application to various drug delivery systems.

Injectables

Although many biodegradable polymers are used for drug delivery, fabrication problems such as difficult processability, limited organic solvent, and irreproducible drug-release kinetics are not uncommon (2). Several studies have been conducted on the use of PEO in injectable drug delivery systems.

Jeong *et al.* synthesized a thermosensitive biodegradable hydrogel of PEO and poly(L-lactic acid). They observed that "an aqueous solution of these copolymers with a proper combination of molecular weights exhibited temperature-dependent reversible solution–gel transition. Desired molecular arrangements provided unique behavior that solution (at low temperature) formed gel (at body temperature). The use of these two biodegradable polymers has great advantage for sustained injectable drug delivery systems" (2).

In another study, Plotnikov *et al.* observed that intravenous injection of 0.1 mg/kg PEO (molecular weight = 5.8×10^6) to narcotized cats with experimental stenosis of the right carotid artery considerably improved blood flow and reduced blood pressure in stenosed vessel (3).

One strategy to control the drug release from a parenteral delivery systems is to modulate the biodegradation of a poly-

mer matrix. Witt *et al.* studied the degradation of ABA triblock copolymers—consisting of poly(lactide-co-glycolide) A-blocks and poly(oxyethylene) B-blocks—and poly(lactide-co-glycolide) with respect to swelling behavior, molecular weight loss, and polymer erosion. Implants were prepared by either compression moulding or extrusion. As they observed, "insertion of an elastoplastic B-block did not lower the processing temperature, but the entanglement of the polymer chains was significantly reduced. The swelling of the rods showed a volume extension of 130% for an ABA containing 50% PEO and 20% for an ABA containing 20% PEO. ABA triblock copolymers may widen the spectrum of parenteral drug delivery with regard to the release of pH-sensitive drugs as well as erosion-controlled release kinetics" (4).

Gutowska *et al.* reviewed the tissue-engineering approaches of *in situ* gel-forming systems of PEO. According to their work, "injectable polymer formulation can gel *in vivo* in response to temperature change (thermal gelation), pH change, ionic crosslinking, or solvent exchange. [The] kinetics of gelation was directly affected by its mechanism. Injectable formulations offer specific advantages over preformed scaffolds such as [the] possibility of a minimally invasive implantation, an ability to fill a desired shape, and [the] easy incorporation of various therapeutic agents" (5).

Li *et al.* reported a new class of injectable and bioabsorbable supramolecular hydrogels formed from PEO and α-cyclodextrin for controlled drug delivery. As they observed, "the hydrogel formation is based on physical crosslinking induced by supramolecular self-assembling with no chemical crosslinking reagent involved. The rheologic studies of the hydrogels showed that the hydrogels were thixotropic and reversible and that they could be injected through fine needles. The components of the supramolecular hydrogels potentially [were] biocompatible and nontoxic. Drugs [could] be encapsulated directly into the hydrogels *in situ* at room temperature without contact with organic solvents" (6).

Microparticulate systems

Liposomes. Scientists have explored the potential of PEO in targeted delivery systems using liposomes. A study by Anderson *et al.* found "the design of targeted oral liposomes is anticipated to improve the systemic delivery of poorly absorbed agents such as proteins and peptides." As they reported, "PEO–folic acid–cholesterol derivatives were synthesized and adsorbed at liposome surfaces encapsulating Texas Red–Dextran 3000 (TR-dex), a poorly absorbed neutral, hydrophilic, large molecular weight marker. Apparent permeabilities of Caco-2 cells to folic acid–PEO conjugates, TR-dex, uncoated TR-dex liposomes, and folic acid–coated TR-dex liposomes were compared at 2 h after administration. Intracellular delivery of TR-dex was detected by fluorescence microscopy. [There was] an increase in intracellular accumulation of TR-dex associated with folic acid–PEO–coated liposomes, [indicating] the potential of folic acid–targeted liposomes in the oral delivery of poorly absorbed large molecular weight agents" (7).

Nanoparticles. Calvo *et al.* observed that "hydrophilic nanoparticulate carriers have important potential applications for the administration of therapeutic molecules. The recently developed hydrophobic–hydrophilic carriers require organic solvents for their preparation and have a limited protein-loading capacity" (8). To address these limitations, the researchers prepared nanoparticles composed only of hydrophilic polymers. As they report, "The preparation technique, based on an ionic gelation process, is extremely mild and involves mixture of two aqueous phases at room temperature. One phase contained chitosan and a diblock copolymers of ethylene oxide and propylene oxide, and the other contained the polyanion sodium tripolyphosphate. Using bovine serum albumin as a model protein, it was shown that these new nanoparticles [had] a great protein-loading capacity (entrapment efficiency up to 80% of the protein) and provided a continuous release of the entrapped protein for up to one week" (8).

Suh *et al.* suggested that "drugs should be delivered to a vascular lesion at a high concentration over an extended period of time to control vascular smooth muscle cell proliferation" (9). They formulated paclitaxel, an antimicrotubule agent, into biodegradable PEO–poly(lactide-glycolide) nanospheres and studied its effect on vascular smooth muscle cell in culture. The paclitaxel-loaded nanospheres—prepared by an emulsion–solvent evaporation method—showed a sustained-release profile over four weeks and exhibited anti-proliferative effects comparable with those observed with free paclitaxel. Results suggested that "nanospheres loaded with paclitaxel may potentially be used as an endocytizable, local sustained drug delivery system for the prevention of restenosis" (9).

De Jaeghere *et al.* prepared nanoparticles with either physically adsorbed or covalently bound PEO coatings from various combinations of poly(lactic acid) and diblock or triblock copolymers of poly(lactic acid) and PEO (10). The nanoparticles were produced by a salting-out process and purified by a cross-flow filtration technique. The *in vitro* cellular uptake of the various types of nanoparticles was compared by flow cytometry after incubation with human monocytes in serum and in plasma.

De Jaeghere *et al.* also investigated "the formulation and process parameters [for] the successful production and long-term stability of freeze-dried PLA [poly(lactic acid)] nanoparticles with 'hairy-like' PEO surfaces. Nanoparticles with grafted, covalently bound PEO coatings were produced by the salting out method from blends of PLA and PLA–PEO diblock or triblock copolymers. Upon freeze drying, the presence of PEO at the nanoparticle surface could severely impair the redispersibility of the particles, especially in the PEO-grafted systems" (11).

Stolnik *et al.* studied the effect of surface coverage and conformation of PEO chains of poloxamer 407 on the biological fate of model colloidal drug carriers. They found that a long *in vivo* blood circulation time could be achieved for nanoparticles with a relatively low degree of surface coverage with PEO chains (12).

Potineni *et al.* developed and characterized a pH-sensitive biodegradable polymeric nanoparticulate system for tumor-selective paclitaxel delivery (13). They synthesized a representative hydrophobic poly(beta-amino ester) (poly-1) by conjugate addition of 4,4'-trimethyldipiperidine with 1,4- butanediol diacrylate. The researchers then prepared PEO-modified nanoparticles with an average size of 100–150 nm and a positive surface charge of 37.0 mV. According to the study, "the

nanoparticles were smooth, distinct, and spherical. The maximum paclitaxel loading efficiency was 97% at 1.0% (w/w) of the drug. Paclitaxel release studies showed that ~10% was released in the first 24 h, 80% after 3 days, and the entire content was released in approximately 5 days. Results of this study also demonstrate that PEO-modified poly-1 nanoparticles could provide therapeutic benefit by delivering the encapsulated drug to solid tumors" (13).

Microparticles. Microparticles are small in size and therefore, have large surface–volume ratios. They are widely used as drug carriers for controlled release. Microparticles containing ovalbumin were formulated from blends of poly(DL lactide-co-glycolide) and PEO–poly(propylene oxide) copolymers. The water–in oil–in oil (w/o/o) emulsion–solvent extraction technique was used to produce the microparticles. According to a study by Yeh *et al.*, "A protein loading level of more than 40% (w/w) was attained in microparticles having a mean diameter of ~5 μm. Linear protein release profiles more than 25 days *in vitro* were exhibited by certain blend formulations incorporating hydrophilic Pluronic F127. Thus, the w/o/o formulation approach in combination with poly(lactide-co-glycolide):Pluronic blends showed potential for improving the delivery of therapeutic proteins and peptides from microparticulate systems. Novel vaccine formulations also are feasible by incorporation of Pluronic L121 in the microparticles as co-adjuvant" (14).

Altaf *et al.* evaluated the effect of compression on beads having multiple layers of polymer and drug coats and the effect of cushioning excipients and compaction pressure on drug release from compressed-bead formulations. The multilayered beads consisted of several alternating layers of acetaminophen and polymer coats (Aquacoat) with an outer layer of mannitol as a cushioning excipient. As the researchers observed, "the compacted multilayered beads disintegrate in gastrointestinal fluids [with] a useful drug-release pattern. Percent drug release *versus* time profiles showed that the release of drug decreased from noncompacted beads as the amount and number of coatings increased, with only 43% of drug released in 24 h for coated beads with 10 layers. It was shown that beads of drug prepared by any method can be spray-layered with excipients such as Avicel and mannitol" (15).

Park *et al.* prepared biodegradable poly(ϵ-caprolactone)–PEO microcapsules (16). They observed the effects of emulsifier, emulsifier concentration, and stirring rate using an image analyzer and a scanning electron microscope. The microcapsules were made in spherical forms with mean particle sizes ranging from 170 nm to 68 μm. The adhesion between water and poly(ϵ-caprolactone)–PEO increased with an increased content of PEO, probably as a result of the increased hydrophilicity. In addition, the drug-release rate from the microcapsules significantly increased with an increase in PEO content, which also could be attributed to the increase of hydrophilic groups or the degree of adhesion force at interfaces.

Mucoadhesive systems

Needleman *et al.* examined the physical properties of gels and semisolid formulations that favor retention and bioadhesion *in situ*. Three candidate bioadhesives were selected: chitosan, xanthan gum, and PEO aqueous formulations, and the hydration rates and rheological properties of these formulations were determined. According to their work, "Hydration experiments indicated a direct relationship between the rate of hydration and bioadhesion or retention. Rheological studies suggested that a gel structure could be an important determinant of retention where shear-displacing forces are present *in vivo; e.g.,* the oral mucosa" (17).

Apicella *et al.* investigated water-soluble PEO of two different molecular weights (600,000 and 4,000,000) and their blends as potential mucoadhesive drug delivery systems (18). The mechanisms and rates of drug release were significantly affected by the polymer molecular weight characteristics, polymer swelling and dissolution rate, and drug diffusivity in the polymer gel surrounding the tablet. The adhesion capacity depended heavily on the average molecular weight (19). The *in vitro* test data indicated that maximum adhesion occurred at an average molecular weight of 400,000 and a further increase in molecular weight caused a decrease in adhesion. Nonetheless, the actual adhesion time measured for tablets placed in the buccal pouch of patients did not decrease once the molecular weight exceeded 400,000. The adhesion remained approximately constant once a critical molecular chain was reached.

An article by Park *et al.* describes their study of the effect of thermosensitive mucoadhesive vaginal vaccine delivery systems on the local and systemic antibody responses to HPV (human papillomavirus) 16 L1 virus-like particles: "HPV 16 L1 virus-like particles expressed from recombinant baculovirus-infected Sf21 insect cells were delivered in phosphate-buffered saline or thermosensitive mucoadhesive delivery systems composed of poloxamers and varying amounts of PEO. The mucoadhesiveness of poloxamers–PEO-based delivery systems increased with the percent of PEO, but formulations with PEO higher than 1.0% were too viscous to be administered into the vagina" (20).

As described by Varma *et al.*: "The mucoadhesion, swelling and drug release behavior of PEO and Carbopol matrices were studied using a water-soluble model drug diltiazem hydrochloride. The mucoadhesive strength of the matrices increased with an increase in polymer content. The results showed that PEO was more mucoadhesive than Carbopol. Mucoadhesion of the tablets was dependent on the swelling. There was a marked increase in the swelling index of matrices containing high polymer content of PEO as compared [with] Carbopol" (21).

Ocular drug delivery systems

Transcorneal transport (*i.e.,* drug penetration into the eye) is not an effective process. It is estimated that only one-tenth of a dose penetrates into the eye. The viscosity of the ophthalmic formulation plays an important role in the absorption of the drug. PEO has been used to improve the viscosity of formulations intended for the eye. Perez *et al.* studied a two-layer composite material composed of a thin-layer of corneal tissue and a synthetic PEO hydrogel (22). The gels were synthesized by electron irradiation–induced crosslinking of an aqueous solution of PEO onto a thin layer of collagenous tissue substrate. Light microscopic studies indicated that the interface between the corneal tissue and PEO gel appeared well adherent with no

gaps in the interface. SEM studies of the material surface showed an architecture similar to that of normal corneal tissue.

Di Colo *et al.* evaluated the application of high molecular weight (400 kDa) linear PEO in gel-forming erodible inserts for the ocular controlled delivery of ofloxacin *in vitro* and *in vivo.* Using powder compression, they prepared 0.3-mg ofloxacin inserts of 6-mm diameter and 20-mg weight. As they report, "The *in vitro* drug release from the inserts was controlled mainly by insert erosion. The erosion time scale was varied by compounding PEO with Eudragit L100 (EUD), 17% neutralized (EUDNa17), or 71% neutralized (EUDNa71). The insert erosion rate depended on the strength of interpolymer interactions in the compounds and on the hydrophilic–hydrophobic balance of the compounds. The gel residence time in the precorneal area was in the order PEO–EUDNa71 < PEO < PEO-EUDNa17. Compared with commercial ofloxacin eyedrops, drug absorption into the aqueous humor was retarded by the PEO–EUDNa71 inserts and both retarded and prolonged by the PEO–EUDNa17 inserts. Maximal concentration in the aqueous (C_{max}), AUC in the aqueous for concentrations (AUC_{eff}) > MIC [minimum inhibitory concentration], and t_{eff} (permanence time in the aqueous at concentrations > MIC) were strikingly increased by plain PEO inserts with respect to commercial eyedrops. Bioavailability increase may be ascribed to PEO mucoadhesion or increased tear-fluid viscosity" (23).

Di Colo *et al.* also investigated the effects of molecular weight (200–2000 kDa) of PEO on the ocular controlled delivery of ofloxacin. As they describe, "PEO 2000 was unsuitable as an insert material since the resulting gel spilled from the eye due to excessive swelling. The *in vitro* drug release from inserts based on PEO 200, PEO 400, and PEO 900 was mainly controlled by insert erosion, whereas PEO 2000, it was mainly diffusion-controlled in a first phase followed by an erosion-controlled phase. PEO 400 and PEO 900 inserts have shown potential for a topical treatment of endophthalmitis" (24).

Osmotically controlled drug delivery

The design and development of a sustained-release formulation that contains a drug of very low solubility is particularly difficult. The formulation should be a compromise between the enhancement of the drug-dissolution rate and the modulation of the delivery rate from the dosage form.

Liu *et al.* prepared a sandwiched osmotic tablet core for nifedipine, a water-insoluble drug (25). The tablet core was composed of a middle push layer and two attached drug layers. They observed that PEO and nifedipine were miscible, which supported the application of PEO in nifedipine dosage forms. Liu *et al.* also prepared a sandwiched osmotic tablet system composed of an osmotic tablet core surrounded by a cellulose acetate membrane with two orifices on both sides for delivering nifedipine (26). In this study, the polyethylene oxide amount in the drug layer had a marked positive effect on nifedipine release.

In another study, Liu *et al.* investigated the effect of molecular weight and amount of PEO on the monolithic osmotic tablet system of nifedipine (27). PEO with a molecular weight of 300,000 g/mol was a suitable thickening agent. The amount of KCl and PEO had "comparable and positive effects, and nifedipine loading had a negative influence on drug release. The monolithic osmotic tablet system can be used in a drug-controlled delivery system, especially for water-insoluble drugs" (27).

Razaghi and Schwartz developed osmotically rupturable systems of cyclobenzaprine hydrochloride (28). As they reported, "Systems were designed using mannitol (osmotic agent) and PEO surrounded by a semipermeable membrane. When placed in an aqueous environment, osmotic water imbibition into the systems distended and swelled the systems until the membrane ruptured and released the active compound to the outside environment. Tablets with increasing amount of PEO exhibited longer rupture times. This may be due to osmotic pressure-modulating properties of the polymer, changing the rate of water imbibition into the systems. There was a decrease in drug release rate with inclusion of PEO in the core. It was observed that the devices with thicker films produced longer rupture times" (28).

Pulsatile drug delivery

Pulsatile drug delivery is based on a chemical oscillator that can drive the pulsed release of an active drug formula. Pulsatile drug delivery can be an effective method of circumventing tolerance during long-term drug treatment. Krogel and Bodmeier prepared and evaluated a pulsatile drug delivery system composed of an impermeable capsule body filled with drug and an erodible plug placed in the opening of the capsule. As they reported, "The erodible plugs were prepared either by direct compression followed by placing the tablets in the capsule opening or by congealing a meltable plug material directly within the capsule opening. The erosion time of the compressed plugs increased with increasing molecular weight of the hydrophilic polymer (hydroxypropyl methylcellulose, PEO) and decreasing filler (lactose) content. The erosion time decreased with congealable lipidic plugs having a high hydrophilic-lipophilic balance and inclusion on surfactants" (29).

PEGylation

PEGylation is a technology enabling the chemical attachment of PEG chains to a broad range of drug substances such as peptides and proteins, including antibody fragments, small molecules, and other drugs. PEGylation increases drug circulation time in the bloodstream, improves drug solubility and stability, and reduces immunogenicity. Potential advantages of PEGylation include a decreased dosing frequency, improved drug efficacy and safety, improved stability, and simplified drug formulation (30–32).

Peginterferon alfa-2a (40 KD) is effective in patients infected with viral genotype 1 and those with liver cirrhosis. Viral RNA measurements taken at 12 weeks can predict the probability of achieving sustained virological response to peginterferon alfa-2a (40 KD) therapy. Peginterferon alfa-2a (40 KD) has comparable safety with interferon alfa-2a.

The addition of ribavirin to peginterferon alfa-2a (40 KD) further enhances the therapeutic benefit for patients with hepatitis C. Peginterferon alfa-2a and peginterferon alfa-2b have been approved for treating chronic hepatitis C virus (HCV) in-

fection in adults who have compensated liver disease and have not been previously treated with interferon alfa. Peginterferon alfa-2a and peginterferon alfa-2b also have been approved for use in combination with ribavirin as a therapy for these adults. According to a study by Baker, "Combining peginterferon alfa-2a or alfa-2b with ribavirin produces better activity against HCV than either drug alone. Interferon works by binding to specific receptors on the cell surface that initiate a complex cascade of protein–protein interactions, thereby leading to rapid activation of gene transcription. The effects of this interferon-stimulated gene modulation depend on the biological system and may inhibit viral replication in infected cells, inhibit cell proliferation, and result in immunomodulation. PEGylation of the interferon molecule increases its size. Absorption of the larger PEGylated molecule is slower, its half-life is longer, and its rate of clearance from the plasma is lower than that of the native interferon. Therefore, the PEGylated molecule increases the duration of biologic activity. Factors that appear to influence the success of PEGylated interferon therapy are HCV genotype, baseline viral load, presence of fibrosis or inflammation shown on the liver biopsy at baseline, and the patient's body weight or body surface area. Patients infected with HCV genotype 1 tend to have a lower response rate, require longer courses of therapy, and respond better when treated with a PEGylated interferon plus ribavirin. Patients infected with HCV genotypes 2 or 3 have comparable responses when treated with interferon plus ribavirin or PEGylated interferon plus ribavirin and can be treated with a lower dose of ribavirin and a shorter course of therapy (24 weeks *versus* 48 weeks for patients with genotype 1)" (32).

Arpicco *et al.* described the synthesis, characterization, and reactivity of new methoxypoly(ethylene glycol) (mPEG) derivatives containing a thioimidoester reactive group (33). These activated polymers reacted with the lysyl epsilon-amino groups of suitable proteins, thus generating an amidinated linkage and preserving the protein's positive charge. The researchers used mPEG derivatives of molecular weight 2000 and 5000 Da and prepared two spacer arms, introducing chains of various lengths between the hydroxyl group of the polymer and the thioimidate group. "These mPEG derivatives modified gelonin, a cytotoxic single-chain glycoprotein widely used to prepare antitumoral conjugates, whose biological activity is strongly influenced by charge modification. The reactivity of mPEG thioimidates toward lysil epsilon-amino groups of gelonin was evaluated, and the results showed an increased degree of derivatization in proportion to the molar excesses of the polymer and to the length of the alkyl spacer. Evaluation of the pharmacokinetic behavior of native and PEG-grafted gelonin showed a marked increase in plasma half-life after protein PEGylation; in particular, the circulating life of the conjugates increased with increased molecular weight of the polymer. A biodistribution test showed lower organ uptake after PEGylation, in particular by the liver and spleen"(33).

In a study on the uses and properties of PEG-linked proteins, Delgado *et al.* conclude, "Enzyme deficiencies for which therapy with the native enzyme was inefficient (due to rapid clearance and/or immunological reactions) can now be treated with equivalent PEG-enzymes. PEG-adenosine deaminase has already obtained FDA approval. PEG-modified cytokines have been constructed and, interestingly, one of the conjugates, PEG-modified granulocyte-macrophage colony-stimulating factor, showed dissociation of two biological properties. This novel observation may open new horizons to the application of PEGylation technology. The biotechnology industry has also found PEG-proteins very useful because PEG-enzymes can act as catalysts in organic solvents, thereby opening the possibility of producing desired stereoisomers, as opposed to the racemic mixture usually obtained in classical organic synthesis" (30).

In a study of the effect of PEGylation on proteins stability, Diwan and Park observe, "During encapsulation of proteins in biodegradable microspheres, a significant amount of the protein may undergo denaturation to form irreversible insoluble aggregates. Incomplete *in vitro* release of proteins from the microspheres is a common observation. An attempt was made to overcome this problem by PEGylation of the protein to be encapsulated. Lysozyme, a model protein, was conjugated with methoxy poly(ethylene glycol) (mPEG, molecular weight 5000). The conjugate was characterized by SDS–PAGE [sodium dodecyl sulfate–polyacrylamide gel electrophoresis], SE–HPLC [size exclusion–high performance liquid chromatography], and MALDI-TOF [matrix-assisted laser desorption ionization–time of flight] mass spectroscopy. The PEGylated lysozyme (Lys-mPEG) consisted of different isomers of mono-, di-, and tri-PEGylated with about 15% as native lysozyme. The specific activity of the protein was retained after PEGylation (101.3 ± 10.4%). The microsphere encapsulation process was simulated for PEGylated and native lysozyme. PEGylated lysozyme exhibited much better stability than native lysozyme against exposure to organic solvent (dichloromethane), homogenization, and showed reduced adsorption onto the surface of blank PLGA [poly(lactic-co-glycolic acid)] microspheres. Release profiles of the two proteins from microspheres were very different. Native lysozyme had a high initial release (about 50%) followed by nearly no release (about 10% in 50 days). In contrast, Lys-mPEG conjugate showed a triphasic and near-complete release over 83 days" (31).

Hinds and Kim studied whether the site-specific attachment of PEG to insulin could enhance the physical and pharmacological properties of insulin without negatively affecting its biological activity or immunological properties. As reported in their study, "Electrophilically activated derivatives of low molecular weight monomethoxy poly(ethylene glycol) were chemically coupled to insulin *via* its amino groups at positions phenylalanine-B1 or lysine-B29, with an amide bond formed between the polymer and protein. The site-specific attachment of monomethoxy poly(ethylene glycol) to insulin did not substantially alter insulin's secondary–tertiary structure, self-association behavior, or potency *in vivo.* The attachment did significantly enhance insulin's resistance to aggregation. In addition, the PEGylation of insulin almost completely eliminated the resultant conjugate's immunogenicity, allergenicity, and antigenicity. Finally, the conjugates were observed to remain in systemic circulation for longer periods of time than unmodified insulin after subcutaneous administration" (34).

Human rIL-2, expressed and purified from *Escherichia coli*, is currently being tested as an anticancer therapeutic agent. As Katre observes, "Some of the patients undergoing clinical trials with rIL-2 have developed antibodies to rIL-2" (35). Katre also described a chemical modification of rIL-2 that reduces its immunogenicity: "rIL-2 was chemically modified with a water-soluble polymer, monomethoxy poly(ethylene glycol). The covalent conjugate PEG-rIL-2 enhanced the solubility and extended *in vivo* circulation. Attachment of PEG to rIL-2 reduced its immunogenicity when tested in rabbits and mice. Antigen-specific IgG antibody titers were 100–1000-fold lower when PEG-rIL-2 was used as the antigen, compared with rIL-2. In a long-term study, 7 of 10 rabbits injected with PEG-rIL-2 had no antigen-specific IgG antibody response. In these 7 rabbits, the *in vivo* behavior of the injected PEG-rIL-2 remained essentially unchanged after repeated immunizations. PEG-rIL-2 injected before rIL-2 injections immunosuppressed the antibody response to rIL-2 in rabbits. Maintenance of the systemic exposure of PEG-rIL-2 after repetitive dosing is related to its decreased immunogenicity. Thus, the covalent attachment of PEG to rIL-2-enhances its potential as an anticancer therapeutic" (35).

Lee and Park isolated mono-PEGylated epidermal growth factor (EGF) isoforms (36). As they reported, "EGF was PEGylated with an N-hydroxy succinimide–PEG (NHS–PEG) derivative (molecular weight = 3400). Mono-PEGylated EGF fraction was separated by gel-filtration HPLC and three mono-PEGylated EGF isoforms were purified by reverse phase-HPLC (RP-HPLC). Tryptic digestion mapping of both EGF and mono-PEGylated EGF isoforms was performed to identify the PEGylation sites using RP-HPLC. The digested fragments were also analyzed by MALDI-TOF mass spectroscopy for further verification of the three PEG conjugation sites. The biologic activity of positional isoforms was evaluated by a cell proliferation assay and a receptor tyrosine kinase activity assay to determine the effect of PEGylation site on its activity. Mono-PEGylated EGF was composed of three positional isomers. Tryptic digestion mapping and MALDI-TOF analysis permitted the identification of the PEGylated site of the three isoforms at N-terminus. Lysine 28, and Lysine 48. PEG-N-terminus EGF, among the three positional isomers, showed the highest activity in a cell proliferation assay and in a receptor-binding assay" (36).

In another study, Lewanski and Stewart observe, "Anthracyclines such as adriamycin have a broad spectrum of activity in human tumours, but are limited, to an extent, by their nonselective delivery to a host of normal tissues and hence, subsequent toxicity. The development of liposomes has offered a drug delivery system with significant potential to target tumours whilst sparing normal tissues. A significant breakthrough has been achieved by coating the liposome with polyethylene glycol (PEGylation), and thus altering the pharmacokinetics of the drug considerably. In this review, the authors discuss the promising data now emerging with PEGylated liposomal adriamycin, and also describe possible future applications" (37).

Water-soluble films

A 1964 US Patent by Kelly reports "PEO can be combined with plasticizer additives for the thermoplastic processing of films, extrusions, and molded forms of the resins. These plasticizers perform two important functions: First, they lowered the melt viscosity of the resin, making it less susceptible to degradation by shearing action during thermoplastic processing. Second, they improve its resistance to 'stress cracking.' Films of these resins tend to crack when only minor stress is applied. This is accelerated by exposure to ultraviolet light. However, this undesirable characteristic can be inhibited by the addition of plasticizers in combination with ultraviolet radiation and oxidation inhibitors. Preferably, these plasticizers should contain between 13 and 20 moles of ethylene oxide in the polyglycol chain. They are used in concentration upto 20 wt% of the composition" (38).

Rodgers *et al.* prepared films of PEO and carboxymethylcellulose and tested them for strength and tissue adherence. According to their study, "mechanical tests indicated that tensile strength and elongation were inversely correlated. All films tested had excellent tissue-adherence properties"' (39).

Tsvetanov *et al.* described a film-formation process that included the dissolution of PEO with a water-soluble polymer (*e.g.,* polysaccharide) in a solvent consisting of water, an organic solvent, or a mixture in any proportion of water and organic solvent, in the presence of an effective quantity of an agent photoinitiator or a reticulating agent. As reported in their study, "The solution obtained in organic solvent was dried until the dry film absorbed 10–100% water. The water-containing film then was irradiated by UV light in the 200–400 nm wavelength range for enough time to allow reticulation. The resulting films are useful in the cosmetic or medical industry" (40).

Conclusion

The reported and established applications of poly(ethylene oxide) are numerous and diversified. For example, injectable drug delivery systems comprising PEO-poly(lactic acid) exhibited a reversible solution-gel transition. Researchers have used PEO to prepare liposomes, nanoparticles, and microparticles. The mucoadhesive properties of PEO, which depend on the molecular weight, also have been studied, and PEO has shown to be more mucoadhesive than Carbopol. PEO has been used in ophthalmic products in which it increases the duration of action of the therapeutic agent by increasing the bioadhesion. Bioavailability of ofloxacin was enhanced from the erodible ocular inserts of PEO. PEGylated proteins (*e.g.,* peginterferon alfa 2a, peginterferon alfa 2b, and pegfilgrastim) have been FDA approved. Finally, water-soluble films of PEO have been prepared using a suitable plasticizer.

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