Drug Delivery

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Poly(ethylene glycol) in Drug Delivery: Pros and Cons as Well as Potential Alternatives

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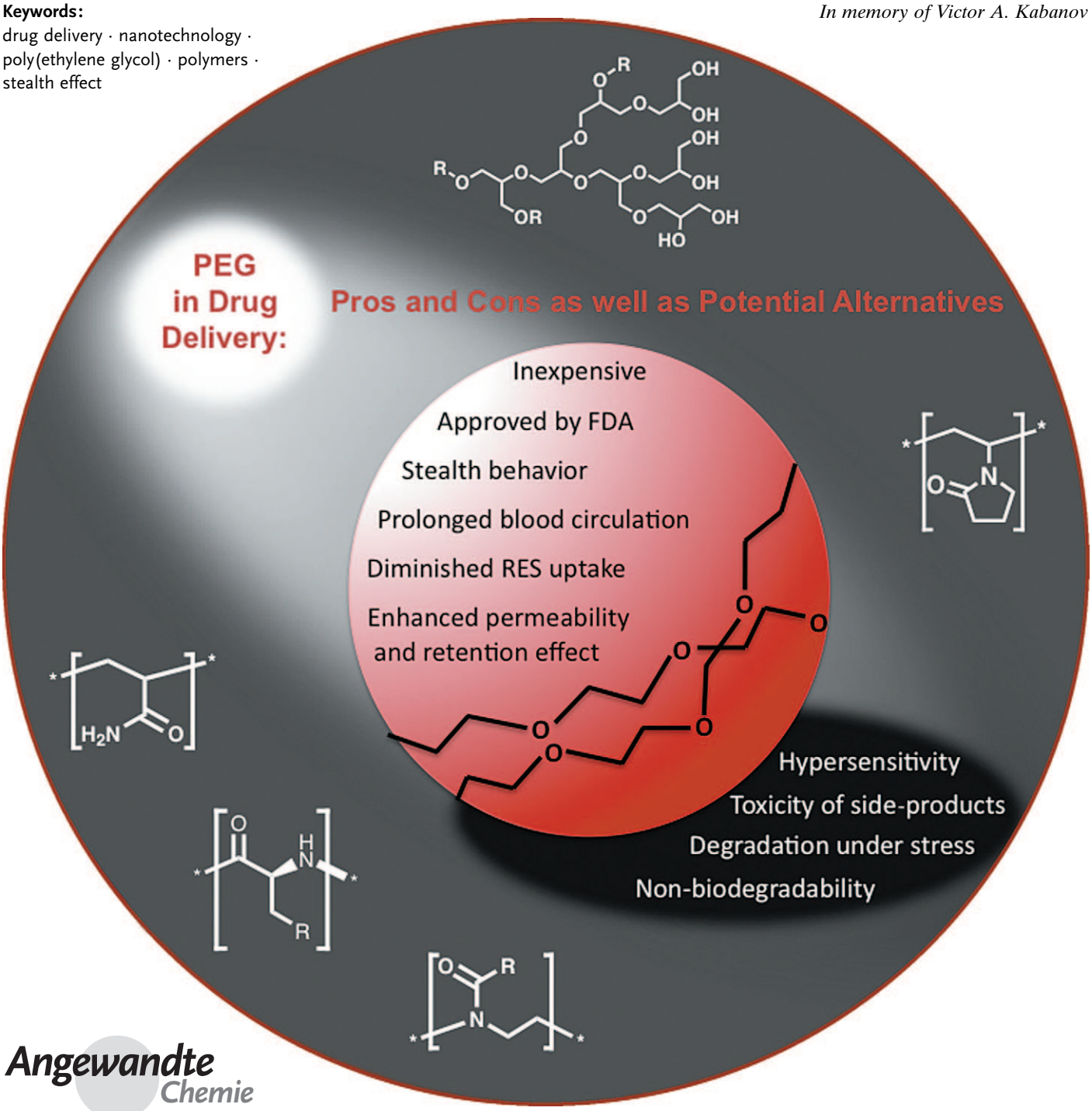
In memory of Victor A. Kabanov



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Poly(ethylene glycol) (PEG) is the most used polymer and also the gold standard for stealth polymers in the emerging field of polymer-based drug delivery. The properties that account for the overwhelming use of PEG in biomedical applications are outlined in this Review. The first approved PEGylated products have already been on the market for 20 years. A vast amount of clinical experience has since been gained with this polymer—not only benefits, but possible side effects and complications have also been found. The areas that might need consideration and more intensive and careful examination can be divided into the following categories: hypersensitivity, unexpected changes in pharmacokinetic behavior, toxic side products, and an antagonism arising from the easy degradation of the polymer under mechanical stress as a result of its ether structure and its non-biodegradability, as well as the resulting possible accumulation in the body. These possible side effects will be discussed in this Review and alternative polymers will be evaluated.

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1. Introduction

Polymeric carriers, which physically entrap molecules of interest, and polymer conjugates, to which such molecules are chemically bound, play an important role in modern pharmaceutical technology. The shared task of carriers and conjugates is the targeted delivery of drugs to specific sites of action in the body. In the case of drug conjugates, in particular, the increase of the molar mass leads to reduced kidney excretion and results in a prolonged blood circulation time of the drug. Shielding of drug carriers and conjugates is required to avoid a fast recognition by the immune system followed by rapid clearance from the body. The suppression of nonspecific interactions with the body, that is, decreased interactions with blood components (opsonization) inducing activation of the complement system, leads to a reduced blood clearance of drug carriers and conjugates, which is known as the stealth effect. Drug-delivery vehicles can be coated with a hydrophilic polymer to allow both inhibition of opsonization and enhancement of water solubility. Poly(ethylene glycol) (PEG) is the most commonly applied non-ionic hydrophilic polymer with stealth behavior. Furthermore, PEG reduces the tendency of particles to aggregate by steric stabilization, thereby producing formulations with increased stability during storage and application.

In the first part of this Review the requirements for hydrophilic polymers in the field of drug delivery will be introduced. In the second part, the overwhelming number of applications of PEG in this field will be briefly discussed, together with the advantages as well as undesired effects observed during the use of this polymer for biomedical purposes. Taking into account these debated deficiencies, potential alternative polymers for forming the hydrophilic shell of carriers for controlled drug release will be introduced and, finally, their actual status will be discussed.

2. Historical Development

The ability of PEG to influence the pharmacokinetic properties of drugs and drug carriers is currently utilized in a wide variety of established and emerging applications in pharmaceutics. The change in the pharmacokinetics of administered drugs by being shielded by or bound to PEG results in prolonged blood circulation times. This consequently increases the probability that the drug reaches its site of action before being recognized as foreign and cleared from the body. Therefore, the majority of conjugated drugs as well as liposomal and micellar formulations on the market or in advanced clinical trials are PEG-containing products.[1] In fact, all polymer-based stealth drug-delivery systems that have been brought to the market up to now contain PEG-functionalized products (PEGylated), and no other synthetic polymer has yet reached this status (Table 1).[1–3]

The concept of PEGylation was first introduced back in the late 1970s; however, it only reached widespread applica-

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tion in different carrier systems in the 1990s (for an overview of drug-delivery systems, see Figure 1).[4,5]

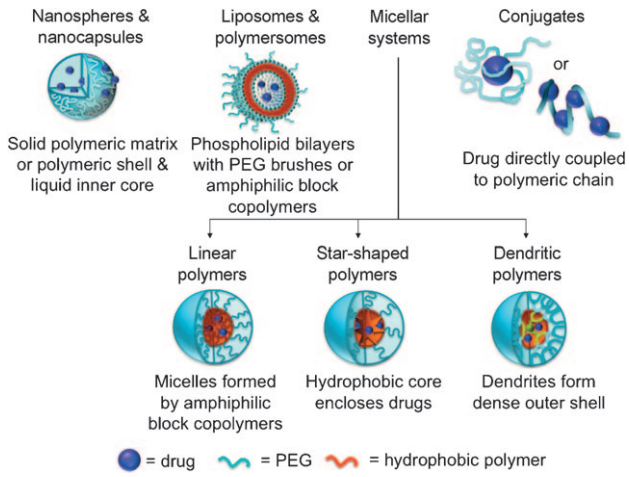

Figure 1. Overview of carrier systems for drug delivery.

The coupling of a protein to PEG was first reported in 1977 by Abuchowski et al. They demonstrated in two studies the non-immunogenicity of PEGylated albumin as well as an extension of the blood circulation time from 12 h to 48 h for PEGylated liver catalase while maintaining the activity of the enzyme.[6,7] A large number of PEG conjugates of proteins, polypeptides, DNA, and RNA as well as of small molecules have since been reported to be more efficient and stable than the native drugs, and several conjugates have reached the market as commercial products. Table 1 shows that PEG conjugates play a very important part in contemporary drug-delivery applications.[1–3] A deeper insight into this topic can be found in two special issues of *Advanced Drug Delivery Reviews*.[8,9]

The effect of PEG surface coverage on the pharmacokinetics of poly(lactic-*co*-glycolic acid) microspheres was reported in 1994 by Gref et al.[10] The authors showed that 66% of the noncoated particles were removed by the liver only 5 minutes after injection, while less than 30% of the 20 kDa PEG-coated nanospheres were captured by the liver 2 h after injection. This study provided the basis for the use of PEG in microsphere technology, whose history already started in the 1950s.[11]

Liposomes have been known since the early 1960s as versatile drug-delivery systems.[12,13] However, a major development was made in 1990 when different research groups reported that the combination of liposome technology and PEGylation by attaching a PEG brush layer to the carriers drastically enhanced blood circulation times of liposomes.[14–16] For example, Klibanov et al. could show that conventional liposomes were completely cleared from blood after 5 h, whereas 49% of the sterically stabilized PEGylated liposomes still circulated in the blood after the same time.[14] This early report provided the basis for the only commercially

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Table 1: Drug-delivery systems stabilized with PEG that have received regulatory approval in the USA and/or the EU.[a]

PEG drug description	Company	Indication	Year of approval
Adagen (11–17×5 kDa mPEG per adenosine deaminase)	Enzon Inc. (USA & Europe)	severe combined immunodeficiency	1990 (USA)
Oncospar (5 kDa mPEG-L-asparaginase)	Enzon Inc. (USA)/ Rhône–Poulenc Rorer (Europe)	acute lymphoblastic leukemia	1994 (USA)
Doxil/Caelyx (SSL formulation of doxorubicin)	Alza Corp. (USA)/ Schering-Plough Corp. (Europe)	Kaposi's sarcoma, ovarian cancer, breast cancer, multiple myeloma	1995 (USA) 1999 (USA) all 1996 (EU)
PEG-Intron (2×20 kDa mPEG-interferon-α-2a)	Schering- Plough Corp. (USA & EU)	chronic hepatitis C	2000 (EU) 2001 (USA)
Pegasys (12 kDa mPEG-interferon-α-2b)	Hoffmann-La Roche (USA & EU)	chronic hepatitis C	2002 (USA & EU)
Neulasta (20 kDa mPEG-G-CSF)	Amgen Inc. (USA & EU)	febrile neutropenia	2002 (USA & EU)
Somavert (4–6×5 kDa mPEG per structurally modified HG receptor antagonist)	Pfizer (USA & EU)	acromegaly	2002 (EU) 2003 (USA)
Macugen (2×20 kDa mPEG- anti-VEGF- aptamer)	Pfizer (EU)/OSI Pharm. Inc. and Pfizer (USA)	age-related macular degeneration	2004 (USA) 2006 (EU)
Cimzia (2×40 kDa mPEG- anti-TNFα)	UCB S. A. (USA & EU)	Crohn's disease, rheumatoid arthritis	2008 (USA) 2009 (USA) 2009 (EU)

[a] mPEG: methoxypoly(ethylene glycol), SSL: sterically stabilized liposome, G-CSF: granulocyte-colony stimulating factor, HG: human growth, VEGF: vascular endothelial growth factor, TNF: tumor necrosis factor.

available particulate drug-delivery system—Doxil/Caelyx—the stealth liposome encapsulated doxorubicin (Table 1).[17–19]

Even though the use of micelle-forming amphiphilic polymers as drug-delivery vehicles was already proposed by Ringsdorf et al. in the 1970s, Kabanov et al. were the first to propose the use of PEG as a hydrophilic part of linear block copolymers for micellization in 1989.[20] Kwon and Kataoka finally pushed forward the development of PEG-containing block copolymer micelles to drug-delivery carriers.[21] This progress led to the development of dendritic and star-shaped amphiphilic structures, which exhibit enhanced control over the architecture, size, shape, and surface functionality of the micelles at the cost of higher complexity compared to linear block copolymers.[22]

The enormous progress achieved during the last two decades in gene therapy stimulated the development of efficient vectors for gene transfection, but required the polymer to have special properties because of the charged nature of DNA. However, the cationic charge of the nonviral vectors which is necessary for electrostatic interaction with the negatively charged DNA is responsible for toxicity and a low half-life of the carriers in the body. The PEGylation of gene carriers resulted in a decrease in the disposition in the lung as well as lower initial toxicities compared to unmodified complexes.[23–25] This positive influence is most likely related to a decreased interaction with blood constituents, a lower tendency of the complexes to aggregate, and, therefore, a lower rate of filtration by pulmonary capillaries. Furthermore, PEGylated carriers are also characterized by a slower uptake by the organs (liver and spleen) of the reticuloendothelial system (RES).[23,26] A comparison between 25 kDa poly(ethylene imine) (PEI) and a PEGylated derivative grafted with 50 molecules of 550 Da PEG demonstrated that 15 minutes after intravenous (i.v.) injection, the PEGylated copolymer reached only 50% of the values of the unmodified polycation in the liver and spleen. This was correlated with a prolonged circulation of the PEGylated PEI in the blood through an increased (+63%) area under the curve (AUC) and an elevated terminal elimination phase compared to unmodified PEI. This effect of PEGylation could also be proved with other cationic polymers. By using PEGylated poly(L-lysine) (PLL) the amount of polyplex circulating in the blood shifted to 69% from 15% for the non-PEGylated polymer.[23]

PEGylated drugs, liposomes, and nanocarriers are characterized by reduced renal filtration, decreased uptake by the RES, and diminished enzymatic degradation. For this reason, PEGylated drugs show a prolonged half-life in the body and, thus, an enhanced bioavailability. Hence, the frequency of

drug administration and the amount of drug can be diminished, which improves the life quality of the patient and reduces clinical costs.[1,27]

The excretion of PEG conjugates and PEGylated carriers by the kidneys is reduced by using drugs with a higher molar mass, and the enhanced permeability and retention (EPR) effect can be exploited. This EPR effect, discovered by Maeda et al., is mostly observed in cancerous or inflamed tissues.[28] These tissues are marked by hypervascularization and a leaky vasculature. These unorganized and loosely connected endothelial cells allow nanoscopic particles to enter the neoplastic tissue and remain inside as a result of missing or decreased lymphatic drainage (Figure 2). Additionally, an increased

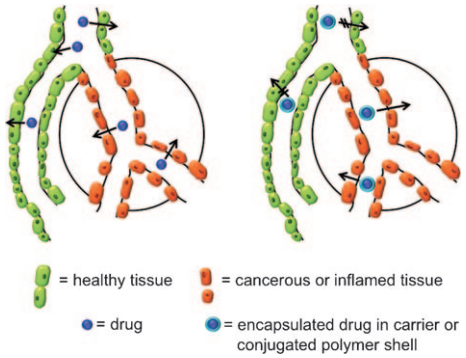


Figure 2. Schematic representation of the EPR effect.

production of vascular permeability enhancing factors is observed in tumor tissue, further augmenting the extravasation of macromolecules within the tumor. The EPR effect is also called passive targeting, and forms the basic principle that causes the functioning of targeted polymeric drug delivery in different diseases, such as cancer, infection, and inflammation, that show more permeable endothelia.[28–30]

Some polymers show a nonlinear behavior in response to an external stimulus, such as a change in temperature or pH value. This response, which could, for example, be a decrease of solubility, can be taken advantage of in drug-delivery applications. The extracellular matrix of cancerous tissue has a decreased pH value of 6.5 to 7.2 compared to blood with a pH value of 7.35 to 7.45. This drop in the pH value can induce precipitation of the polymer and the associated trapping of the polymer and a potentially bound drug or carrier within the cancer tissue. This approach of stimuli-responsive polymers includes manifold stimuli and various responses by the polymers which are ouside the scope of this Review. The interested reader is referred to reviews discussing this topic.[31–34]

These selected examples of applications clearly demonstrate the rising importance of polymers, and in particular of PEG, in biomedical domains such as drug delivery.

3. Advantages of PEG

Not every non-ionic hydrophilic polymer can provide stealth behavior. A number of structural parameters influence the biological and stabilizing effects and have to be carefully taken into consideration.[35]

The molar mass as well as the polydispersity of the polymer has been shown in many applications to be important for biocompatibility and stealth behavior. The molar mass of PEG used in different pharmaceutical and medical applications ranges from 400 Da to about 50 kDa. PEG with a molar mass of 20 kDa to 50 kDa is mostly used for the conjugation of low-molar-mass drugs such as small molecules, oligonucleotides, and siRNA. This results in fast renal clearance being avoided by increasing the size of the conjugates above the renal clearance threshold. PEGs with lower molar masses of 1 kDa to 5 kDa are often used for the conjugation of larger drugs, such as antibodies or nanoparticulate systems. In this way, opsonization and subsequent elimination by the RES is avoided, enzymatic degradation is reduced, and cationic charges are hidden. PEG of about 3 kDa to 4 kDa is given orally as a laxative (as GoLYTELY and MoviPrep).

From a theoretical point of view, a biodegradable polymer would be more beneficial in applications, since difficulties in achieving complete excretion would be avoided, although other issues, such as the toxicity of degradation products and the limited shelf live, would need to be considered. However, it should be kept in mind that the excretion of the polymer is not directly dependent on the molar mass of the polymer, but rather on the hydrodynamic volume, which is affected by the architecture of the polymer. For example, star-shaped polymers and dendrimers show lower hydrodynamic volumes than linear polymers with similar molar masses.[36,37]

In general, a low polydispersity index (PDI) is a basic prerequisite for the polymer to have pharmaceutical applications. A PDI value below 1.1 provides a polymer with an acceptable homogeneity to ensure reproducibility in terms of body-residence time and immunogenicity of the carrier system.[1,38] This demand is readily fulfilled by PEG, since very well defined polymers with PDIs around 1.01 are readily accessible by the anionic polymerization of ethylene oxide.

Furthermore, PEG shows a high solubility in organic solvents and, therefore, end-group modifications are relatively easy. At the same time, PEG is soluble in water and has a low intrinsic toxicity that renders the polymer ideally suited for biological applications. When attached to hydrophobic drugs or carriers, the hydrophilicity of PEG increases their solubility in aqueous media. It provides drugs with a greater physical and thermal stability as well as preventing or reducing aggregation of the drugs in vivo, as well as during storage, as a result of the steric hindrance and/or masking of charges provided through formation of a "conformational cloud".

This "conformational cloud" is generated by the highly flexible polymer chains, which have a large total number of possible conformations. The higher the rate of transition from one conformation to another, the more the polymer exists statistically as a "conformational cloud" which prevents interactions with blood components as well as protein

interactions such as enzymatic degradation or opsonization followed by uptake by the RES.[39] The formation of an efficient sterically hindering cloud on the surface of particles is not only dependent on the polymer, but is also influenced by other factors such as the molar mass of the PEG, the surface density, and the way the PEG is attached to the surface (for example, brush-like or mushroom-like).[40,41]

The diminished interactions with the body result in PEGylated products showing less immunogenicity and antigenicity; hemolysis and aggregation of erythrocytes can also decrease, as can the risk of embolism. The steric hindrance has the additional advantage that the charge in charged carrier systems is shielded and the resulting zeta-potential and charge-induced interactions within the body are decreased. As a consequence, recognition by the immune system through opsonization is suppressed. These favorable properties of PEG in pharmacokinetics are known under the name of the stealth effect, in reference to stealth planes.

Acute and short-term studies as well as pharmacokinetic studies of PEG have been carried out on a wide range of animal species such as rats, mice, guinea pigs, monkeys, and dogs. The gastrointestinal absorption of PEG is decreased as the molar mass increases. Whereas PEGs with a molar mass of 4 kDa to 6 kDa are not absorbed over 5 h in rat intestine, low-molar-mass PEGs of about 1 kDa show a slight absorptive effect of about 2%. The excretion of PEGs is mainly accomplished by the kidneys. In humans, 85% and 96% were excreted in urine in 12 h after intravenous injection of 1 g of 1 kDa and 6 kDa PEG, respectively. LD_{50} values after oral intake were higher than 50 $g\,kg^{-1}$ body weight for 6 kDa PEG (50% solution in water) in mice, rats, rabbits, and guinea pigs. After intraperitoneal (i.p.) administration, the LD_{50} value was 5.9 and 6.8 $g\,kg^{-1}$ in mice and rats, respectively.

In short-term studies in monkeys (*Macaca fascilaris*), daily doses of 2–4 $mL\,kg^{-1}$ of 200 Da PEG were administered over a 13 week period. Intratubular deposition of small numbers of oxalate crystals in the renal cortex were observed, but not related to other clinical or pathological findings. Long-term studies with albino rats with doses of 0.06 $g\,kg^{-1}$ 1 kDa PEG and 0.02 $g\,kg^{-1}$ 4 kDa PEG per day did not cause any significant adverse effects over a two-year period.[42] Toxic effects to the kidney resulting from high PEG doses of 200–600 Da have been observed in laboratory animals and in burn patients whose injured skin was treated topically with PEG.

Evaluating the relative safety of PEG solutions used for bowel cleansing prior to colonoscopy concludes that, in the absence of preexisting renal disease, PEGs are associated with similarly low risks of renal impairment. No significant adverse effects from low-molar-mass PEGs have been observed in inhalation toxicology studies, carcinogen testing, or mutagen assays. Biondi et al. reported that low-molar-mass PEGs of about 200 Da have a genotoxic effect after metabolic activation. However, this was evaluated by induction of chromosome aberrations in CHEL and CHO cells only in the presence of S9 mix. The findings suggested a potential mutagenic risk for PEGs of similar size.[43]

In conclusion, PEGs of different molar masses have essentially similar toxicities, with the toxicity being inverse to the molar mass since the absorption from the gastrointestinal tract decreases with increasing molar mass. The level that caused no toxicological effect in rats was 20000 ppm in a diet equivalent to 1 $g\,kg^{-1}$ body weight. The estimate of the acceptable daily intake for man is 0–10 $mg\,kg^{-1}$ body weight.

The success of PEG in drug-delivery applications also led to its use in other medical fields. Thus, PEG is used in blood and organ storage, where it reduces the aggregation of red blood cells and improves the blood compatibility of poly(vinyl chloride) bags.[44–47] PEG copolymers that are implanted as cardiovascular devices, such as stents, decrease thrombosis.[48]

Furthermore, PEG is not only used in pharmaceutical preparations as an excipient for parenteral, topical, nasal, and ocular applications, it is also used as the active principle in laxatives. The suppression of interactions with biomolecules also led to a variety of antifouling and antiadhesion applications, such as in Merrifield syntheses,[49] ultrafiltration,[50] and the protection of contact lenses from pathogenic bacteria and fungi.[51,52] PEG chains attached to hydrophobic molecules, such as oleic acid, can act as a surfactant, and are found as surface-active, viscosity-increasing, and skin-conditioning agents in all kinds of cosmetics—from toothpaste to cleansing agents, such as shampoos, body and bath soaps, to fragrance, aftershave lotion, face powder, and eye shadow.[53,54] These examples show that PEG, with its special properties, is not only very popular in pharmaceutical applications, it is also a daily consumer product, and is omnipresent in our everyday life.

4. Drawbacks of PEG Polymers

The increasing use of PEG and PEGylated products in pharmaceutical research as well as clinical applications not only provides new insight into the underlying mechanism of the beneficial properties of PEG, it also increases the likelihood of encountering potential side reactions.

The potentially unfavorable effects that might be caused by PEG can be divided into several groups: Adverse side effects in the body can be provoked by the polymer itself or by side products formed during synthesis that lead to hypersensitivity. In addition, unexpected changes in the pharmacokinetic behavior can occur with PEG-based carriers. Furthermore, an antagonism arises from the non-biodegradability of PEG in combination with its relatively easy degradation upon exposure to oxygen. All these potential drawbacks and their importance will be discussed in the following.

4.1. Immunological Response

4.1.1. Intravenous Administration

It was already shown in early studies in 1950 that PEG has the propensity to induce blood clotting and clumping of cells, which leads to embolism. This finding indicates nonspecific interactions of PEG with blood.[42] Since then, it has been shown that PEG, which is not supposed to show any opsonization, can nevertheless induce specific as well as

nonspecific recognition by the immune system, thereby leading to a response of the body to intravenously administered PEG formulations such as liposomal and micellar carrier systems or conjugates.

It was shown that adverse reactions of PEG often occur through complement (C) activation, which leads to hypersensitivity reactions (HSR) that can provoke an anaphylactic shock.[55,56] The complement system, which is part of the immune system, is a biochemical cascade that is started by the hydrolysis of C3, a protein present in blood, whose fragmentation can be triggered by a change in the conformation upon adsorption on a surface. This hydrolysis reaction leads to a biochemical cascade that results in the generation of different C3 and C5 fragments that bind to the surface, thereby labeling the identified foreign body. Leucocytes, mast cells, and macrophages that carry receptors for these complement factors will be activated to remove the foreign body and release inflammatory mediators, such as histamine and proinflammatory cytokines.[57] The release of histamine does not imperatively lead to the hypersensitivity reaction; an additional special susceptibility to one of the other steps is also necessary.[56,58] In earlier studies it was proposed that surface-exposed PEG hydroxy groups provide molecular sites where C3b can covalently bind to the surface and, thus, initiate the pathway of complement activation.[59] However, it should be noted that the vast majority of all currently used PEGylated products are based on methoxy-PEG (mPEG), thereby disproving the validity of this hypothesis.

Although the exact trigger for this phenomenon has not yet been clarified, an immediate HSR in 5–10% of treated patients was shown for different PEG-containing liposomal carriers.[60] Complement activation with subsequent HSR was demonstrated with ^{99m}Tc-labeled 2 kDa mPEG-liposomes for the treatment of Crohn's disease.[61] The Doxil/Caelyx (commercial distearoylphosphatidylethanolamine (DSPE) 2 kDa mPEG) liposome formulation of doxorubicin (Table 1) used in anticancer therapy also causes HSR in up to 25% of the patients, despite pretreatment with corticosteroids and antihistamines and without prior sensitization.[55,58] However, the conclusion that the adverse reaction is only caused by PEG can not be drawn conclusively. In fact, depending on the composition and size of the liposomal formulation, PEG-liposomes cause complement activation even without doxorubicin encapsulation, but Doxil is a more-efficient complement activator than empty PEGylated liposomes (Table 2).[60]

Investigations of the hypersensitivity from side reactions caused by sterically PEG-stabilized liposomes revealed rather opposing results, with complex causal relationships found between PEGylation, size, loading, preparation of the formulation, and different other parameters.[60] For example, small PEGylated liposomes with diameters of less than 70 nm showed no complement activation, in contrast to larger ones.[60] In addition, the beneficial pharmacokinetic effects of covering liposomes with PEG are sometimes absent. Parr et al. found only slight differences in the rates of plasma clearance for PEGylated and non-PEGylated liposome formulations of doxorubicin;[62] Metselaar et al. observed that liposomes without PEG showed the same or even longer circulation half-lives as PEGylated liposomes (36 h and 22 h, respectively).[60]

In summary, these studies indicated complement activation by PEG attached to liposomes, but further investigations are necessary to draw definite conclusions on the mechanism involved and the influence of the various factors that seem to affect the HSR.

The adverse reaction of intravenously administered PEG can also be observed in the application of different contrast agents for echocardiography. Anaphylaxis as a result of hypersensitivity to PEG is observed with SonoVue (commercial contrast agent containing PEG), but not with Optison and Definity (commercial contrast agents without PEG).[63] De Groot et al. reported three cases of anaphylactic shock as a reaction to SonoVue.[64] Dijkmans et al. admit that SonoVue might contain a triggering factor responsible for three fatal cases (0.002% of the treated patients with advanced coronary artery disease as a predisposition) and 18 of 19 adverse anaphylactic or vasovagal reactions (fainting) (0.012%); no such adverse reactions were observed with Optison.[65]

All together, a conclusive statement can not be given as to whether PEG alone or a combination of several factors causes hypersensitivity; further investigations are required. Even though these results argue for a nonspecific recognition of PEG by the body, the binding of antibodies—the specific immune response to PEG—was also observed. In 2005, a case study appeared that showed a severe IgE antibody mediated hypersensitivity reaction to intravenously administered 4 kDa PEG.[66] However, in 1983 Richter et al. already reported the formation of antibodies to PEG conjugates in rabbits. The response to PEG itself was very low, but antibodies were observed for the conjugate of ovalbumin with 6 mPEG chains with a molar mass of 11 kDa anti-PEG as well as anti-ovalbumin. Although the formation of antibodies was highly dependent on the degree of substitution of the protein by PEG and the proportion of animals showing antibody response varied (17% to 50%), this study showed initially that PEG could act as a haptene.[67] Later, the same authors reported that the subcutaneous injection of a mPEG-modified rag-

Table 2: Severity of adverse reactions of different carriers containing PEG and anticancer drugs in a porcine model.[a] Adapted from Ref. [60].

Liposomes	Lipid dose [μmol kg^{-1}]	Frequency of adverse reaction	Severity of adverse reactions		
			mild	severe	lethal
DPPC, PEG-DSPE, Chol	0.17–1.39	4/6	1	1	2
DPPC, PEG-DSPE, Chol	0.16–1.97	2/4	0	1	1
DPPC, Chol (90 nm)	0.16–1.85	5/11	3	2	0
DPPC, Chol (60 nm)	0.16–1.54	0/8	0	0	0
Doxil/Caelyx	0.02–0.27	12/14	3	8	1
DaunoXome	0.18–0.73	7/8	2	1	4

[a] DPPC:dipalmitoyl phosphatidylcholine, PEG-DSPE: 2 kDa mPEG-conjugated distearoyl phosphatidylethanolamine, Chol: cholesterol.

weed allergen in humans triggered the formation of IgM isotype antibodies to PEG, but the only moderate humoral response was classified as not significant for clinics.[68]

However, preexisting IgG and IgM anti-PEG antibodies were identified in over 25% of the healthy donors, and anti-PEG antibodies were induced in 5 of 13 patients in the clinical trial of PEG-asparaginase.[69] The presence of anti-PEG antibodies was strongly related to the rapid blood clearance of PEG conjugates; this effect was also observed for PEG-uricase in 5 of 8 patients.[70]

In summary, PEGylation will continue to be of significant value in medicine to decrease immunogenicity, antigenicity, and toxicity as well as reducing renal clearance. However, it is important to recognize that PEG may possess antigenic and immunogenic properties as haptenes, and the close interaction between complement activation and antibody response should be kept in mind. Further comprehensive studies are required to fully elucidate the effect of anti-PEG antibodies on PEG conjugates.

4.1.2. Oral Administration

Hypersensitivity reactions not only occur when PEG is intravenously injected, but also during the preparation of patients for colonoscopy by oral administration of PEG as a laxative. In general, the gastrointestinal adsorption of PEG decreases as the molar mass increases. Whereas 4 kDa to 6 kDa PEGs are not absorbed over 5 h in rat intestines, low-molar-mass PEGs of about 1 kDa show a slight adsorptive effect of about 2%.[42]

MoviPrep, one of the commercial 3.35 kDa PEG solutions for colonoscopy preparation, is reported to cause hypersensitivity and rash uticaria upon administration. The low absorption rate of 0.2% of high-molar-mass PEG by intestinal mucosa was suggested to be sufficient to cause angioedema as a result of systemic HSR to PEG in susceptible patients.[71] Similarly, GoLYTELY, another 3.35 kDa PEG preparation for colonoscopy, was reported to cause anaphylactic reaction without prior disposition in three separate case studies.[72–74]

4.1.3. Dermal Application

Different examples indicate that cutaneous application of PEG can also cause allergic reactions, such as contact dermatitis. This contact allergy was observed for PEG with molar masses between 4 kDa and 20 kDa used in, for example, dentifrice.[75] Another study found that 8 kDa and 20 kDa PEG present in multivitamin tablets caused hypersensitivity that culminated in unconsciousness in a 36 year old man without predisposition.[76]

Contact dermatitis as a result of hypersensitivity was also reported by Fisher in four patients when drugs containing PEG ranging from 200 to 400 Da were used as an excipient.[77] Quartier et al. reported contact dermatitis to the moisturizing 1 kDa PEG-dodecylglycol block copolymers in 19 of 21 patients.[78] However, both Le Coz et al. and Quartier et al. note a connection between contact dermatitis and 1,4-dioxane, an industrial side product of the PEG synthesis.[54,78]

4.2. Changes in Pharmacokinetic Behavior

Another potential immune reaction to the presence of PEG is the accelerated blood clearance (ABC) phenomenon. Dams et al. first reported that the 2 kDa mPEG liposome concentration in rats was drastically decreased after 4 h compared to a previously injected liposome dose [from (52.6 ± 3.7)% to (0.6 ± 0.1)% after the second injection].[79] Kiwada and co-workers later observed that the ABC phenomenon also occurred when the second injection was administered within five days. This finding indicated that a preceding injection of PEGylated liposomes can alter the circulation time of repeatedly injected PEG liposomes.[80] In addition, it was also reported that previously administered PEG-containing micelles with a size of at least 30 nm can also induce the ABC reaction,[81] thus indicating that the size of the PEGylated particles is also an important parameter for the reaction. On the other hand, it has been demonstrated that very high doses (5 μmol phospholipid per kg rat) of unprotected liposomes also cause this enhanced blood clearance.[82] This finding shows that the induction and magnitude of the phenomenon is not only determined by PEG, but also by the size and surface of the carrier.[83]

Additionally, the amount of PEGylated lipid can affect the ABC phenomenon. Liposomes containing 0, 5, 10, or 15 mol% PEGylated lipid were tested in rabbits. The ABC phenomenon was found with 5 mol% PEG-covered liposomes to reach a maximum, and with the effect decreasing at higher coverage rates. This observation is in good agreement with the production of anti-PEG as well as anti-ovalbumin antibodies in the presence of an ovalbumin conjugate with 6 molecules of 11 kDa PEG. No antibodies were produced by conjugation with 20 molecules of 11 kDa PEG molecules per ovalbumin molecule.[67]

This ABC phenomenon not only affects the bioavailability of the drug, but passive targeting is also decreased: the second dose was shown to preferentially end up in Kupffer cells of the liver.[79,80,84] This observation proves an involvement of the immune system. This can cause severe liver damage in the case of highly toxic anticancer therapeutics.

The mechanism of ABC is still not fully understood, but it has been suggested that the formation of anti-PEG IgM antibodies by the spleen occurs upon the first injection; the IgM binds to the PEG of the second dose and activates the complement system, thereby leading to opsonization with C3 fragments of PEG and an enhanced uptake by Kupffer cells.[83,85] Since non-PEGylated liposomes can also induce this phenomenon, it seems clear that the mechanism of the occurrence of ABC is much more complex. In any case, these unexpected changes in the pharmacokinetic behavior are undesirable and complicate the therapeutic use of PEGylated liposomes and micelles.

An additional pharmacokinetic irregularity that is shown by PEGylated liposomes is the loss of long-circulating behavior at very low doses (approximately 0.5 mmol kg^{-1}).[82] This observation was made with doses much lower than those used during normal therapeutic application (4 to 400 mmol of lipid kg^{-1}); nevertheless, it is important in nuclear medicine, where only trace amounts are

administered.[86] The mechanism accounting for this unexpected behavior is unknown and the question as to whether the loss of long-circulation time is connected to the ABC phenomenon remains unanswered.

4.3. Non-Biodegradability of PEG

A disadvantage of PEG is its non-biodegradability. Therefore, the use of low-molar-mass PEGs would be preferable. However, oligomers with a molar mass below 400 Da were found to be toxic in humans as a result of sequential oxidation into diacid and hydroxy acid metabolites by alcohol and aldehyde dehydrogenase. The oxidative degradation significantly decreases with increasing molar mass and, therefore, a molar mass well above 400 Da should be used.[87,88]

On the other hand, the molar mass should not exceed the renal clearance threshold to allow complete excretion of the polymer. A molar mass limit of 20–60 kDa is reported for nondegradable polymers (corresponding to the albumin excretion limit and a hydrodynamic radius of approximately 3.5 nm).[1,27,38,89–91] Pasut and Veronese assumed that a molar mass below 40–60 kDa is required to prevent accumulation in the liver,[1] but the renal clearance threshold of PEG is not easy to determine.[38] It seems that PEG with a molar mass below 20 kDa is easily secreted into urine, while higher molar mass PEG is eliminated rather slowly, and clearance through the liver becomes predominant.[1] To overcome these uncertainties multiarm and branched biodegradable PEGs were investigated that form low-molar-mass PEGs which can be excreted more easily after cleavage in the body.

Studies concerning toxicity and excretion of PEG mostly date back to the 1950s to 1970s and, therefore, need to be updated with contemporary knowledge and methods.[92–94] In particular, the fate of PEG and PEGylated delivery systems at the cellular level is not known and needs further investigation. It is common practice to assume a fate similar to PEG for PEGylated delivery systems, which are, in general, chemically modified PEGs. Thus, the majority of studies seem to ignore the biological fate of the polymers after disintegration of the liposomes or micelles from which they originate.[91,95]

In fact, there are no systematic long-term studies that show 1) whether PEG is excreted completely or partly remains in the body, 2) where it is accumulated, and 3) its effects at the sites of accumulation.[96]

4.4. Degradation under Stress

The stability of a polymer used for drug delivery is an important factor in achieving and maintaining the stability and therapeutic properties of drugs during storage as well as during treatment.[97] Instabilities observed in polymers can result from chemical changes induced by oxygen, water, and energy such as heat, radiation, or mechanical forces.[97] The effect of these exogenous factors on PEG stability will be discussed in the following.

Mechanical stress on polymers and subsequent degradation can arise during several processes, such as the simple flow of solutions, stirring, or ultrasound treatment. In addition to shear stress during production processes or by injection with a syringe, shear stress can also occur in biological systems. Significant flow of aqueous fluids occurs in the human body, with shear stresses of up to 5 Pa, but the shear behavior of polymers for biomedical applications under these conditions has hardly been considered.[98] Therefore, an examination of the processes that lead to degradation, occurrences that happen during degradation, and the products formed during scission are an important part of the evaluation of polymers for biomedical applications.

Up to now, stress studies have only been carried out on industrial PEG samples with molar masses ranging from 50 kDa to 4000 kDa that are not used in drug-delivery applications. Similarly, shear stresses up to 9 kPa were applied, which significantly exceed the forces occurring in vivo. These forces in vivo are generally around 1 Pa, with maximal shear stresses of around 5 Pa in capillaries and arterioles.[99–104] Even though the investigations on shear stress induced degradation of PEG were not performed with biologically relevant polymers and conditions, the partial degradation of PEG-based therapeutics during prolonged circulation can not be excluded. General findings such as the involvement of oxygen in the rupture of the ether bond and the faster degradation of PEG compared to polymers with a carbon backbone, such as poly(acrylic acid) (PAA) and poly(vinylpyrrolidone) (PVP), should be kept in mind.[103]

PEG is also observed to undergo remarkable degradation under heating in the solid state and solution.[105] Scheirs et al. noted a decrease in the molar mass of solid-state PEG from 100 kDa to 10 kDa after aging for 30 days at 60 °C under air. The authors found by measuring IR spectra that the degradation resulted in the formation of appreciable quantities of aldehyde, carboxylic acid, and alcohol functional groups.[106]

The heating of PEG probes of various molar masses (1–4000 kDa) under a non-oxidative atmosphere at 50 °C also showed slight chain scissions. This finding led the authors to the conclusion that these degradations are induced at so-called weak scissions which have their origin at previously formed peroxides.[107,108] This observation is consistent with others that show that neither the addition of antioxidants nor free radical inhibitors can totally prevent thermal degradation of PEG under inert conditions.[108] Although the discussed conditions might have only limited relevance for biological media, they should be kept in mind during the preparation of the carrier systems.

Even though PEG does not absorb light above 300 nm, it is very sensitive to photooxidation, because of the oxidizability of the α-carbon atom by chromophoric impurities.[107] UV degradation of PEG in the range of 55 to 390 kDa through the formation of ester and formate end groups occurs much faster than in other hydrophilic polymers such as PAA and PVP in the same molar mass range.[103]

Although none of the studies concerning the mechanical stability of PEG involved pharmaceutical grade polymers and the conditions were harsher than those occurring in vivo, it

can be concluded that PEG is more sensitive to degradation than vinylic polymers because of its ether structure and the possible formation of hydroperoxides. These factors have to be taken into consideration, in particular during storage of the polymer as well as the drug formulation.

4.5. Toxicity of Side-Products

The most prominent side product formed during the synthesis of PEG is the cyclic dimer of ethylene oxide, 1,4-dioxane. Currently, 1,4-dioxane is stripped off from the product under reduced pressure. Dioxane is classified by the International Agency for Research on Cancer (IARC) in group 2b (that is, as being possibly carcinogenic in humans with sufficient evidence from animal experiments). Therefore, the European Pharmacopoeia (Ph. Eur.) limits the dioxane content to 10 ppm for pharmaceutical applications. Nonetheless, an evaluation of dioxane by the US Department for Health and Human Services revealed that rats exposed over two years to 111 ppm of 1,4-dioxane in air did not show any evidence of dioxane-caused cancer or any other health effects.

Furthermore, PEG can also contain residual ethylene oxide from polymerization that is classified by the IARC in group 1 (carcinogenic in humans), as well as formaldehyde, which is in the same group. As a consequence, the Ph. Eur. limits the content of ethylene oxide to 1 ppm and the amount of formaldehyde to 30 ppm in PEG for pharmaceutical applications.

The toxicity of these potential side products clearly demonstrates the necessity of using pharmaceutical grade PEG for biomedical applications.

5. Summary of PEG

PEG is a very popular polymer with an overwhelming number of positive properties, as is easily confirmed by searching the literature. These advantageous qualities have led to a very broad usage of PEG in everyday products, industrial applications, as well as in many biomedical drug-delivery systems. Its success in the latter field is well reflected by numerous pharmaceutical products that have reached approval by the Food and Drug Administration (FDA) and European Medicines Agency (EMEA) during the last 20 years (Table 1).

In publications on the use of PEG in drug delivery, an overwhelming enthusiasm is often evident and possible disadvantages are hardly mentioned, with potential difficulties that might be faced with this polymer concealed. Although, the possible disadvantages of PEG are highlighted here, this Review does not wish to create the impression that PEG should be avoided. On the contrary, we believe that PEG is of utmost importance for the development of new drug-release systems that will improve the quality of life. In addition, most of the discussed side effects and instabilities of PEG were only observed in a limited percentage of patients and are not as well investigated and documented as the numerous positive properties. Therefore, we want to increase the awareness that PEG might also exhibit some limitations to complement the multitude of reviews that focus on all the beneficial properties of PEG.

The limitations of PEG include the non-biodegradability and the resulting, and in most studies ignored, fate of PEG after in vivo administration. Many biological and toxicological data evaluating those points date back to the 1950s and 1970s and need to be updated and evaluated with contemporary knowledge, especially in terms of the fate at the molecular and cellular level, such as tissue vacuolization and fusion of membranes. At the same time, the polyether structure provides easy targets for peroxide degradation, and although investigations have not been performed under biologically relevant conditions, PEG can be relatively easy degraded compared to polymers with a carbon backbone.

From a medicinal viewpoint, the unpredictable complement activation, which can lead to hypersensitivity reactions and unclear pharmacokinetics after a second dose (the so called ABC phenomenon) complicate the use of PEG therapeutics. Although PEG alone seems to be immunologically harmless, the immunogenicity of PEG is highly dependent on the degree of PEGylation and to which molecule the PEG is coupled.

Nevertheless, the positive properties of PEG cannot be dismissed and strongly outweigh the sometimes observed negative effects discussed. As a consequence, PEG remains the most used polymer and the gold standard in biomedical applications; potential alternatives with even better properties are difficult to find at the moment. However, the search for alternative polymers is also driven by the strained patent and marketing situation of PEG, since numerous patents protect its applications.

6. Potential Alternatives to PEG

The discussed disadvantages of PEG intensified the search for alternative polymers for use in therapeutics. This section will present the most promising hydrophilic polymers that have been investigated as synthetic alternatives to PEG for different biomedical applications and their properties and potentials are compared to PEG.

A variety of natural polymers such as heparin,[109] dextran,[110,111] and chitosan[112] have also been used in a wide range of drug-delivery systems. However, they fall beyond the scope of this Review, which focuses on synthetic polymers, and will not be discussed here.

6.1. Biodegradable Polymers

6.1.1. Poly(amino acid)s

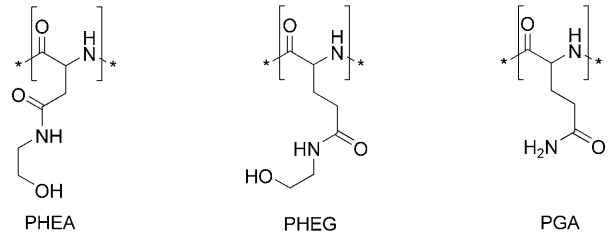
Different synthetic poly(amino acid)s are currently being investigated as alternatives to PEG and are in different stages of development. Poly(glutamic acid) (PGA), which was first investigated by Li and Wallace, has already entered a phase III clinical trial in the form of a 40 kDa PGA-paclitaxel conjugate (37 wt%; Table 3).[113] Poly(hydroxyethyl-L-aspar-

Table 3: Drug-delivery systems containing alternative polymers to PEG and their current status in clinical trials.[a],[3,196,218,219]

Polymeric drug description	Manufacturer	Indication	Status
36 kDa PG-paclitaxel (21 wt%) (CT 2103, Opaxio)	Cell Therapeutics Inc.	NSCLC, ovarian, colorectal, breast and esophageal cancers	phase III
33 kDa PG-camptothecin (37 wt%) (CT 2106)	Cell Therapeutics Inc.	colorectal, lung and ovarian cancers	phase I/II
28 kDa PHPMA-doxorubicin (8.5 wt%) (PK1, FCE 28068)	Pfizer, Cancer Research Campaign, UK	NSCLC and breast cancers	phase III
25 kDa PHPMA-platinate (8.5 wt%) (AP 5280)	Access Pharmaceuticals	ovarian cancer	phase II
25 kDa PHPMA-doxorubicin (7.5 wt%)-galactosamine (PK2, FCE 28069)	Pfizer, Cancer Research Campaign, UK	hepatocellular carcinoma	phase I/II
25 kDa PHPMA-DACH-platinate (8.5 wt%) (AP 5346)	Access Pharmaceuticals	ovarian, melanoma and colorectal cancers	phase I/II
18 kDa PHPMA-camptothecin (10 wt%) (PNU 166148)	Pfizer, Cancer Research Campaign, UK	refractory solid tumors	phase I, discontinued
17 kDa PHPMA-PGA (37 wt%)-paclitaxel (5 wt%) (PNU 166945)	Pfizer, Cancer Research Campaign, UK	refractory solid tumors	phase I, discontinued

[a] NSCLC: non-small cell lung cancer; DACH: diaminocyclohexyl chelating ligand.

agine) (PHEA) and poly(hydroxyethyl-L-glutamine) (PHEG) were tested for drug delivery in different studies, in particular by Romberg et al. (Scheme 1).[96]



Scheme 1. Structures of poly(hydroxyethyl-L-asparagine) (PHEA), poly-(hydroxyethyl-L-glutamine) (PHEG), and poly(glutamic acid) (PGA).

PGA, PHEA, and PHEG are degraded in vivo to their corresponding amino acids, which can be metabolized by physiological pathways. Their degradation kinetics have been studied in vitro by using different enzymes, which lead either to complete decomposition into single amino acids or degradation to oligomers with 4 to 9 repeating units.[96,114] Biodegradability is the main strength of these polymers together with a prolonged blood circulation time of particles with poly(amino acid)-modified surfaces. This extension was similar to liposomes modified with 5 kDa PEG, 4 kDa PHEG, and 3 kDa PHEA. Similar elimination rates were measured for the poly(amino acid) liposomes as for the PEG liposomes. Additionally, a decrease in the ABC phenomenon has been shown when low doses were injected into rats (Figure 3).[96,115–117]

However, PHEG and PHEA showed increased SC5b-9 (SC5b-9 = complement factor formed by hydrolysis of C3) levels in ELISA tests, thus indicating the explicit activation of the complement system.[96] In addition, the antigenicity of polymers with more than three amino acids in the chain complicates their use in vivo.[118] Nevertheless, both polymers have been used in different drug carriers, such as 100 kDa PHEG-mitomycine conjugate (5.4 wt% mitomycine),[119] histidine-conjugated PHEA as a micelle-forming amphiphilic agent for doxorubicin,[120] or in combination with hyaluronic acid as a hydrogel for the administration of thrombin.[121]

In contrast to PHEA and PHEG, poly(glutamic acid) is already approved by authorities and widely used as a thickener in food and cosmetics, as a wetting agent in cosmetics, and as a fertilizer which slowly releases nitrogen.[122] Despite the known antigenicity of poly(amino acid)s, the paclitaxel-PGA conjugate was the first non-PEG polymer-drug conjugate to reach a phase III clinical trial (under the name Opaxio, formerly Xyotax (CT-2103); Figure 4).[113] It showed less side effects and improved drug efficiency than nonconjugated paclitaxel in some tumors. Although clinical trials have been carried out since 2005, all of them have failed to meet the primary end-point of extended survival compared to gemcitabine or vinorelbine for non-small cell lung cancer (NSCLC in patients with a poor performance status (PS2) is incurable with the therapy available). However, beneficial

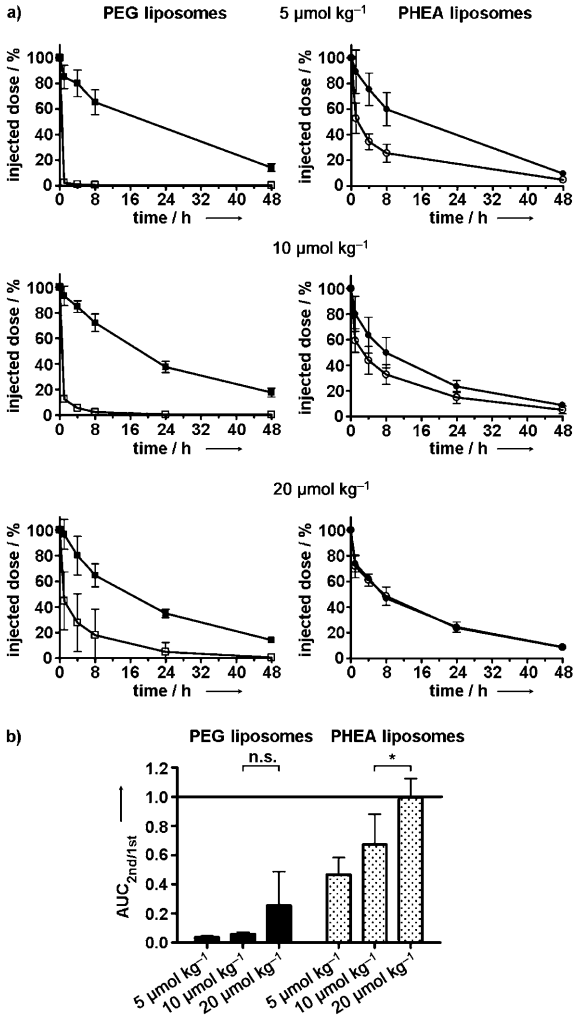


Figure 3. Effect of the polymer-lipid concentration on the pharmacokinetic behavior of PHEA and PEG liposomes after the first and second injection. a) Circulation kinetics of PHEA- (DPPC/cholesterol/PHEA-DODASuc) and PEG liposomes (DPPC/cholesterol/PEG-DSPE) (% of injected dose versus time). The closed symbols represent the results after the first injection; the open symbols those after the second injection of liposomes. b) Ratio of $AUC_{0-48\,h}$ of the second injection to the $AUC_{0-48\,h}$ of the first injection ($AUC_{2nd/1st}$) at the different lipid doses [$AUC_{0-48\,h}$ values were calculated from (a)]. Filled bars represent the $AUC_{2nd/1st}$ of PEG liposomes, dotted bars represent the $AUC_{2nd/1st}$ of PHEA liposomes. All results are expressed as the mean ± standard deviation ($n = 3$–4). *$p < 0.05$; n.s. = not significant, DODASuc = succinyldioctadecylamine.[115] Reproduced from Ref. [115] with permission from Elsevier B.V.

tolerability, convenience, and safety, such as lower requirement for red blood cell transfusions, fewer hematologic and gastrointestinal adverse events as well as lower incidence of alopecia, fatigue, and weight loss were found.[123] Superior survival was observed among women less than 55 years old, and presumably premenopausal, upon treatment with Opaxio. This effect is attributed to the increased release of paclitaxel.[124,125] Hypersensitivity reactions were only rarely observed, and those that did occur were only mild to moderate.[113] Therefore, Cell Therapeutics, Inc., the pharmaceutical company holding the rights to Xyotax, received fast-

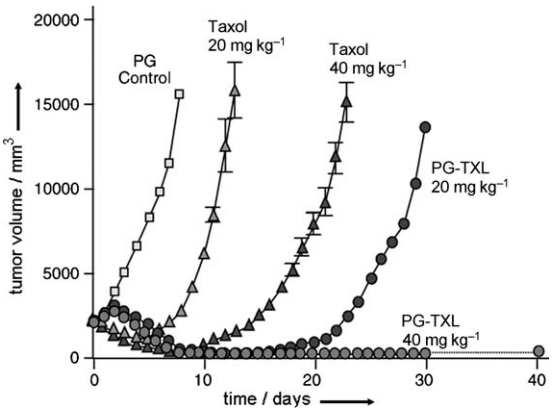


Figure 4. Antitumor activity of PGA-taxol in rats bearing rat breast tumor 13762F (PG = poly(glycerol), TXL = taxol). Each drug was injected intravenously in a single dose at the indicated equivalent paclitaxel concentration. Data are presented as the mean and standard deviations of the tumor volume.[113] Reproduced from Ref. [113] with permission from Elsevier B.V.

track designation from the FDA for paclitaxel-PGA in the indication of advanced non-small cell lung cancer in patients with a poor performance status. Currently, Cell Therapeutics has withdrawn its European marketing application of Opaxio for NSCLC after an EU panel raised concerns over the trial design.[126]

In summary, poly(amino acid)s combine a number of advantageous properties for drug-delivery applications such as prolonged blood circulation, decreased ABC clearance, and—particularly importantly—biodegradability. Their major drawback is complement activation; however, this effect may be tolerable in clinical trials, since it apparently leads to only moderate hypersensitivity reactions.

6.2. Non-Biodegradable Polymers

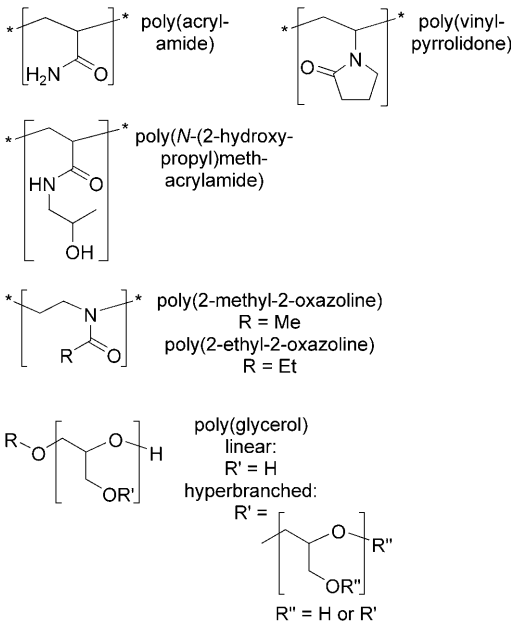
As the promising biodegradable polymers show an activation of the immune system, non-biodegradable polymers have also been taken into further consideration as alternatives to PEG for drug-delivery applications (Scheme 2).

6.2.1. Polymers with Heteroatoms in the Main Chain

6.2.1.1. Poly(glycerol)

The close structural similarity of poly(glycerol) (PG) to PEG renders the polymer predetermined for biological applications. Indeed, linear as well as hyperbranched PG (HPG) with molar masses ranging from 150 Da to 540 kDa have already been used as hydrophilic shells for conjugates and liposomes, reverse micelles, and hydrogels.[127–131]

The stealth effect and biocompatibility of these polymers have been evaluated in several studies. A prolonged blood circulation time of HPG liposomes compared to unmodified liposomes has been found for poly(glycerol)s with molar masses in the range of 150 Da to 750 Da.[128] Surfaces covered



Scheme 2. Structures of the discussed non-biodegradable polymers.

with 1.5–5 kDa HPG showed similar or better protein repulsion than PEG of the same molar mass, probably because of its dense brush-like structure.[132,133]

Only very low in vitro cytotoxicity has been observed at concentrations of 10 mg mL^{-1} after 48 h incubation for HPGs with molar masses between 106 kDa and 870 kDa. In vivo studies on mice did not show any signs of toxicity.[134,135]

The hemocompatibility of HPG has been proven by examination of platelet activation and by coagulation studies.[127,134–136] By examining the generation of C3a, the complement activation of the immune system by poly(glycerol)s has been found to be in the same range as that in saline and PEG (Figure 5). However, since only C3a levels were examined, a direct comparison with the results obtained by Szebeni et al. on PEG is not possible, as their studies were based on SC5b-9 levels. A comparative study between linear PG and HPG of 6.4 kDa showed no significant difference in red blood cell aggregation, complement activation, and cell viability for the two polymer architectures in vitro as well as in vivo. No decrease in the biocompatibility and no increase in complement activation, because of the lower molar mass, was observed relative to the results found with 106 kDa and 870 kDa HPG.[134]

The same non-biodegradability in vivo can be speculated for PG and PEG because of their comparable polyether structures. Michael and Coots found no signs of catabolism of PG, and the predominant excretion in urine after oral administration is similar to PEG.[137] Additionally, accumulation in the liver and spleen (but not in the kidney, lung, or heart) was found for the high-molar-mass HPG, and only very low excretion in urine was reported over 30 days in mice (Figure 5).[131] However, HPGs of lower molar mass were not investigated in terms of their accumulation, thus making an estimation of the excretion limit for HPG impossible. In

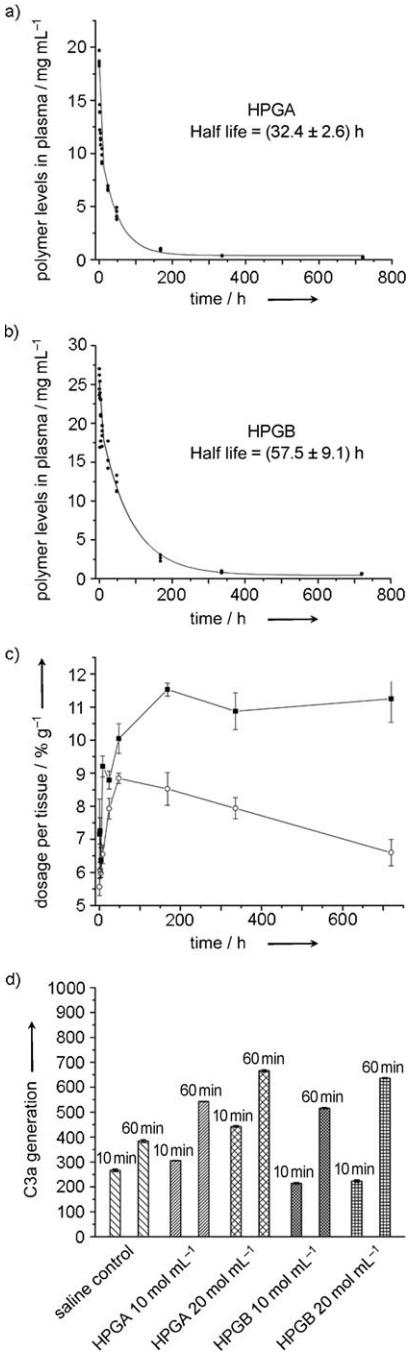
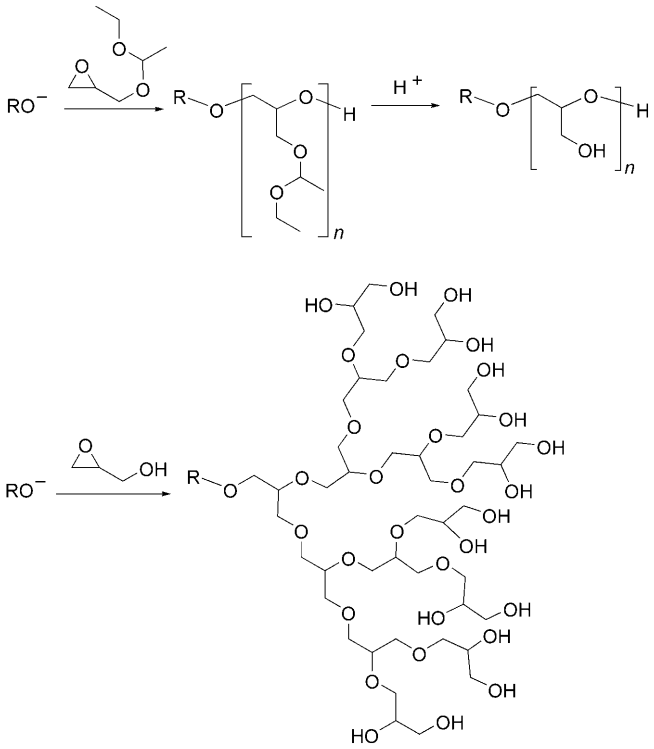


Figure 5. a) b) Plot of the polymer concentration in plasma versus time after intravenous injection into female Balb/C mice. c) Levels of polymer accumulated over time in liver injected intravenously into female Balb/C mice (blank squares: HPGA, filled squares: HPGB).[131] Reproduced from Ref. [131] with permission from Elsevier B.V. d) Generation of the C3a fragment upon incubation of polymers with PPP.[135] Reproduced from Ref. [135] with permission from Elsevier B.V. Abbreviations: HPGA = hyperbranched poly(glycerol) of 106 kDa, HPGB = hyperbranched poly(glycerol) of 540 kDa, PPP = platelet-poor plasma.

contrast to PEG, no degradation studies under mechanical stress have been reported for PG, but as PG possesses a similar ether structure as PEG, an analogous susceptibility to oxygen-induced degradation might be assumed.

The use of glycidol or epichlorhydrine as latent AB_m monomers permits better control over the polymerization to give more defined hyperbranched poly(glycerol)s with the PDI value reduced from 5 to 1.8.[138] While hyperbranched poly(glycerol)s are accessible from glycidol or epichlorhydrine monomers, linear polymers are available by protecting the free hydroxy group of glycidol to prevent branching. The polymerization step is followed by deprotection of the hydroxy groups.[134] The functionalization of PG is feasible via the initiator, and since poly(glycerol)s are hydroxy-rich polymers, all the general substitution reactions of hydroxy groups are possible and result in high degrees of functionalization (Scheme 3).[129,136,139] Diglycerol, PG-3, and PG-4 are commercially available oligomers. Esters of up to PG-10 are approved by the FDA as food and pharma additives.[140]



Scheme 3. Synthesis of linear and hyperbranched poly(glycerol).

In conclusion, since PG possesses a similar structure to PEG it shows comparable advantages and disadvantages. An additional interesting possibility is PG branching, since the hyperbranched arrangement allows very high degrees of functionalization, although some end groups will be sterically hidden. The high degree of branching is also advantageous for the circulation time, since branched structures are not as quickly excreted as their linear analogues. Furthermore, highly branched polymers have low intrinsic viscosity and are, therefore, expected to increase the blood viscosity only slightly, which has been shown to cause a variety of complex physiological effects.[141]

6.2.1.2. *Poly(2-oxazoline)s*

The hydrophilic poly(2-methyl-2-oxazoline) (PMeOx) and poly(2-ethyl-2-oxazoline) (PEtOx) were discovered in the 1960s.[142–145] Since then a wide range of chemistry has built up around this class of polymers including the living polymerization method, which yields very low PDI values, and versatile end-group chemistry.[146,147]

Nonetheless, the application of poly(2-oxazoline)s in biomedical fields arose only recently, and although both types of polymers have been quite widely tested for different drug-carrier applications, only a few basic biological and stability studies have been reported.[148,149] Drug-transport systems with poly(2-oxazoline) were developed, for example, based on micelles of PLA-PEtOx-PLA [PLA = poly(lactic acid)] as carriers of doxorubicin.[150] PEtOx-poly(ε-caprolactone) micelles with paclitaxel have been shown to possess the same efficiency as Cremophor EL formulated paclitaxel.[151] A cytosine arabinose conjugate of PEtOx showed IC_{50} values in HeLa cell viability tests in a similar range as the corresponding PEG conjugate.[152] PMeOx-coated surfaces have been shown to possess the same protein repellency as PEG.[153,154]

One of the few fundamental biological studies was performed by Veronese et al. They showed erythrocyte compatibility for PEtOx with molar masses of 5, 10, and 20 kDa at polymer concentrations of 5 mg mL^{-1}. They also showed that 20 kDa PEtOx was safe and nontoxic for intravenous administration every second day at doses of up to 50 mg kg^{-1} over a period of 2 weeks (control: saline).[155] The hydrophilic shells of PMeOx and PEtOx prolonged the blood circulation times of liposomes in the same range as PEG.[156]

In addition, Zalpinsky et al. documented similar prolonged blood circulation of PEG-, PMeOx- and PEtOx-modified liposomes with 5 mol% of phospholipid and about 40 repeating units of each polymer.[157] Additionally, they found that three different types of liposomes showed a similar tissue distribution profile after 24 h, which means there is preferential distribution in the liver, spleen, and kidney (Figure 6).[157] Analogous results have been found for ^{111}In-labeled 4 kDa PMeOx and 4 kDa PEtOx, which showed an augmented blood circulation time but also an increased occurrence of the polymer in the kidney and bladder.[158] Furthermore, PEtOx possesses a lower critical solution temperature (LCST), which can be used for enhanced targeting of specific tissue.[149,159]

The biodegradation of PEtOx was investigated by using proteinase K, a nonhuman enzyme. A partial degradation to PEI was found on incubation, but whether this degradation also takes place in humans was not investigated.[160] Cleavage of this side group generates the cationic derivative PEI, which was shown to be cytotoxic as well as to induce erythrocyte aggregation and hemolysis depending on the molar mass, branching, and number of cationic groups. The lower the molar mass and the degree of branching of the PEI, the higher the bio- and hemocompatibility.[161,162]

In summary, PMeOx and PEtOx show a behavior comparable to PEG in terms of blood circulation time, opsoniza-

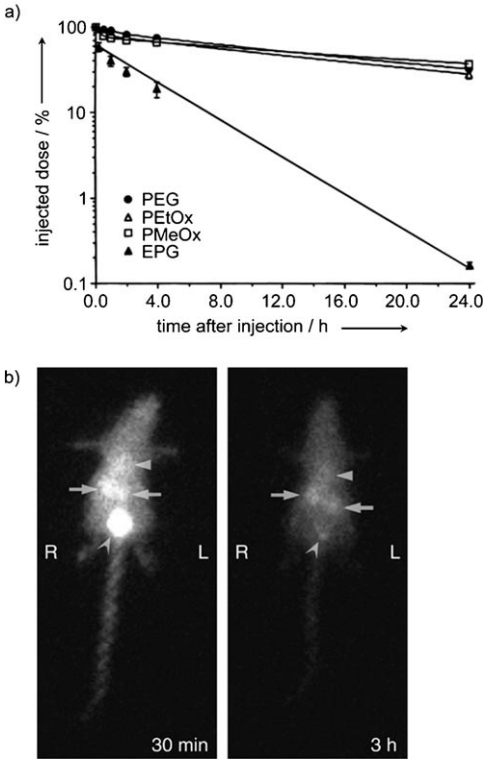


Figure 6. a) Blood lifetimes of ^{67}Ga-labeled liposomes [(90 ± 5) nm] prepared from the EPC, cholesterol, and DSPE conjugate of either PEG, PMeOx, or PEtOx, as well as a control EPG; molar ratio 1.85:1:0.15. Four Sprague–Dawley rats were injected with each liposomal preparation in the tail vein. Samples obtained by retroorbital bleeding at various times were used to determine the radioactivity in the blood (EPC: egg phosphatidylcholine, EPG: egg phosphotidylglycerol).[156] Reproduced from Ref. [156] with permission from the American Chemical Society. b) γ-Camera imaging of the in vivo distribution of $PMeOx_{48}$PipDOTA[^{111}In] in a CD1 mouse 30 min and 3 h after intravenous injection (PipDOTA: piperazine-thiouryl-*p*-benzyl-1,4,7,10-tetraazacyclododecane-*N*′,*N*,*N*,*N*-tetraacetic acid). The highest concentrations were in the bladder (thin arrowhead), the kidneys (arrows), and the blood pool in the heart (thick arrowhead).[158] Reproduced from Ref. [158] with permission from Elsevier B.V.

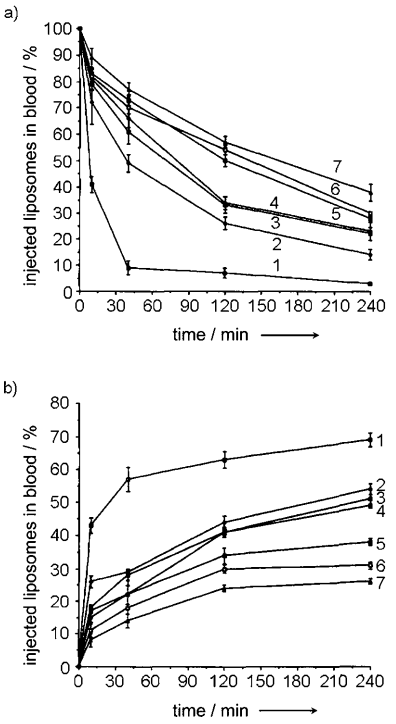


Figure 7. a) Liposome clearance from the blood of mice; b) liposome accumulation in the liver. 1) "plain" liposomes; 2) PVP-L-P-liposomes (2.5 mol% PVP); 3) PAAm-L-P-liposomes (2.5 mol% PAAm); 4) PEG-liposomes (2.5 mol% PEG); 5) PVP-L-P liposomes (6.5 mol% PVP); 6) PAAm-L-P liposomes (6.5 mol% PAAm); 7) PEG liposomes (6.5 mol% PEG) (L: molar mass of polymer 6–8 kDa, P: with terminal palmityl group). Reproduced from Ref. [163] with permission from Elsevier B.V.

tion, and organ distribution. Nevertheless, important details of immune activation and mechanical stability need further investigation to further evaluate the potential of poly(2-oxazoline)s as alternatives to PEG.

6.2.2. *Vinyl Polymers*

6.2.2.1. *Poly(acrylamide)*

Torchilin et al. reported that liposomes covered with 7 kDa poly(acrylamide) (PAAm) showed prolonged blood circulation compared to unmodified liposomes (Figure 7).[163] Microspheres of PAAm containing 5-fluorouracil,[164] hydrogels for ibuprofen release, and ultrafine hydrogel nanoparticles with *meta*-tetra(hydroxyphenyl)chlorin for photodynamic therapy (PDT) have also been tested.[165] Hemoglobin-containing PAAm microspheres have also been investigated as oxygen carriers.[166]

PAAm is often affirmed in such studies to be non-immunogenic, highly protein resistant,[167,168] and not to show cytotoxic effects.[169] However, other reports state an inflammatory response upon implantation of PAAm hydrogels.[170–172]

Other reported drawbacks of PAAm are the following:

1) PAAm can degrade to acrylamide by thermal and photolytic effects;[171]
2) it has a carbon backbone and, as a result, is not biodegradable;[171]
3) a preferential distribution of 7 kDa PAAm liposomes in the liver;[163]
4) PAAm is synthesized by polymerization of acrylamide, a monomer which is known to induce a variety of severe neurotoxic effects[167] so that residual monomer may account for adverse reactions.[171,173]

In view of the controversies and the described disadvantages of PAAm, the wide application of PAAm seems to be surprising. Although PAAm seems to improve blood clearance rates of liposomes, it activates the immune system and even worse, the monomer shows very distinct toxic side effects—it is classified by the International Agency for Research on Cancer in group 2b (meaning the agent is possibly carcinogenic to humans)—and is produced during

thermal and photolytic degradation of the polymer. These drawbacks limit the widespread use of PAAm for biomedical applications.

6.2.2.2. *Poly(vinylpyrrolidone)*

Poly(vinylpyrrolidone) (PVP) is commercially available, for example, under the brand name Kollidon from BASF. In the cosmetic and pharmaceutical industry it is used as, for example, tablet coating and binder as well as an excipient for the formulation of poorly water soluble drugs. PVP is also used in adhesives, coatings and inks, photoresists, paper, photography, textiles, and fiber applications. PVP was used as a plasma expander in the first half of the 20th century, and the iodine complex (Povidone-iodine) possesses disinfectant properties. As a food additive, PVP is used as a stabilizer and has the E number E1201.[40]

This wide range of oral applications indicate that there is already potential compatibility to biomedical fields and, indeed, investigations concerning its suitability for drug-delivery applications look very promising. Its highly hydrated structure makes it suitable to increase the water content of other polymeric materials.[174,175] It is possibly through this high hydration that an interaction with the immune system is suppressed, and distribution studies on poly(hydroxyethyl methacrylate) (PHEMA) and PHEMA-PVP copolymer hydrogels suggest that PVP shows no C3a activation.[176] This leads to the prolonged blood circulation of 6 kDA and 7 kDa PVP liposomes and 6 kDa PVP-superoxiddismutase (SOD) conjugates (Figure 7).[163,177,178]

Nevertheless, contradictory results have also been reported that show an enhanced protein adsorption on 6 kDa PVP-uricase conjugates compared to native uricase as well as the formation of PVP antibodies.[179] Similar observations have been made with PVP-conjugated D-*N*-acetylhexosamidase A, which can interact strongly with antibodies of the native protein.[179] Despite this finding, PVP has been tested in several drug-delivery systems, including a SOD conjugate[178] and liposomes with a stabilizing, hydrophilic PVP shell.[163,177,180] In addition, PLA-PVP micelles[181,182] and microspheres[183] as well as PVP-gelatin hydrogels[184] and PVP have been studied for their formulation assistance.[185,186] PVP has also been used as a gene-delivery system as it can bind presumably through hydrogen bonds with DNA to form a complex. PVP increased the stability and half-life of DNA in vivo by shielding the negative charge and protecting it against enzymatic degradation.[187]

Another promising aspect of PVP is its slower degradation compared to PEG under UV or ultrasound irradiation,[103,188] even though the formation of peroxides during drying can not be prevented.[189] The Ph. Eur. limits the peroxide content of this polymer to 400 ppm. As for PEG, the slow in vitro peroxide-mediated degradation of the polymer is in contrast to its in vivo non-biodegradability. PVP possesses a carbon backbone that is not degraded on exposure to enzymes. This led to the removal of PVP as a plasma expander from the market: patients who received PVP with a molar mass above 25 kDa, which cannot be excreted from the body developed a "PVP storage disease".[190]

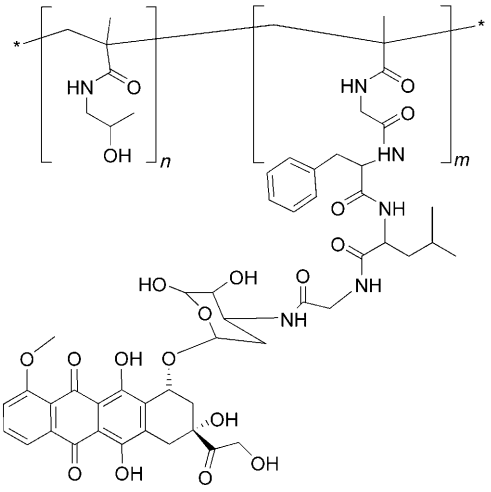
PVP can be synthesized by free-radical polymerization of vinylpyrrolidone as well as by controlled radical polymerization methods.[174] The latter method leads to improved PDI values below 1.2,[182,191–193] variable end groups,[182,194] and—most importantly—to the prevention of high-molar-mass PVP. The vinylpyrrolidone monomer is presumed to be a carcinogen and should be removed carefully from the polymer.

In conclusion, the biocompatibility of PVP looks quite promising for polymers with molar masses below the kidney threshold. Nevertheless, PVP has similar problems as PEG: an unclear immunological behavior and non-biodegradability, which leads to accumulation of the polymer above the excretion limit.

6.2.2.3. *Poly(N-(2-hydroxypropyl)methacrylamide)*

The bio-application of poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) was established by Kopecek et al. in the 1970s.[195] The application of PHPMA was further developed by Rihova et al. and Duncan, which led to the use of PHPMA conjugates in clinical trials.[195]

The most successful conjugate, a 28 kDa PHPMA-doxorubicin copolymer (8.5 wt%; doxorubicin PK1; clinical trial FCE 28068 phase III; Scheme 4) was tested against various cancers (Table 3) and showed activity against NSCLC, colorectal cancer, and breast cancer.[196] Additionally, neither cardiotoxicity nor multidrug resistance was observed in these studies, no liver and spleen accumulation was noted, and no immunogenicity or polymer-related toxicity was detected.[195]



Scheme 4. A doxorubicine-HPMA conjugate.

In addition, 25 kDa PHPMA conjugates with doxorubicin galactosamine (7.5 wt%), carboplatinate (8.5 wt% Pt), and DACH platinate (8.5% w/w) successfully entered phase I/II trials for various cancers.[195] However, the fact that the clinical trials for the PHPMA-paclitaxel and PHPMA-camptothecin conjugates were discontinued because of a lack of antitumor activity shows that biological events that result in efficient drug-delivery systems in vivo are not easily understood and not yet fully explored (Table 3).

Other HPMA-conjugated systems for cancer therapy were also investigated, but have not yet entered clinical trials. These include a glutathione derivative to inhibit human glyoxalase and a cyclohexanone derivative coupled to HPMA with a molar mass of 3–30 kDa and with various drug contents. In vitro studies with murine B16 melanoma cells showed the conjugate was less efficient than the free drug, but this result is not surprising, since the success of conjugates is based on the retarded, slower release of the drug in vivo.[197] A study of 16 to 50 kDa geldanamycin-HPMA conjugates also revealed reduced toxicities in A2780 ovarian cancer cells as well as prostate cancer cell lines (PC-3 and DU145) and endothelial cells (HUVECs). The improved in vivo tolerance of mice against the conjugate was investigated, and showed a tolerance for 80 mg kg^{-1} with no signs of toxicity (compared to 30 mg kg^{-1} for the free drug).[198,199]

Conventional conjugation by PHPMA occurs by binding to the multiple side chains of the polymer. For conjugates with proteins, conjugation of one end of the PHPMA chain (instead of the multiple side groups to yield starlike architectures) turned out to be more advantageous. The PDI value above 3.5 for a conventional protected SOD conjugate was reduced to 2 by conjugation with semitelechelic PHPMA. This probably causes the improved biocompatibility, as significantly larger numbers of antibodies were formed against the classic form of PHPMA-SOD conjugate than the star-shaped PHPMA-SOD conjugate.[200,201]

Drug-delivery systems with different architectures, such as starlike doxorubicin conjugates, conjugation with multiple side groups, or the use of the polymer as an excipient all showed a decreased efficiency in vitro against A2780 ovarian carcinoma cells compared to the free doxorubicin.[202] HPMA conjugates were prepared for active targeting by a Fab antibody, and mesochlorin was introduced as the active principle for photodynamic therapy. This study showed an inhibition of the growth of ovarian carcinoma cells under irradiation.[203] Complexes of poly(L-lysine) and DNA with semitelechelic 5.5 kDa and 8.5 kDa PHPMA were found to display an increased in vitro stability in salt solutions against albumin-induced aggregation, decreased albumin binding, and reduced phagocytic uptake, but have not shown any prolonged circulation times in vivo. The reason for that remains to be elucidated.[204] The blood circulation time for PLL-PEI complexes was found to increase from 5 to 90 minutes by modification with multivalent PHPMA.[23]

Liposomes with PHPMA hydrophilic shells that would transport calcein were prepared.[205] Whiteman et al. showed that liposomes modified with 4.3 kDa PHPMA have longer blood circulation times than unmodified ones (Figure 8).[206] Different types of hydrogels of PHPMA were also tested as drug carriers,[207] for example, for doxorubicin[208] or for PHPMA-adriamycine conjugates to overcome multidrug resistence.[208] PHPMA can be prepared by either free or controlled radical polymerization mechanisms (such as atom-transfer radical polymerization (ATRP) or reversible addition-fragmentation chain transfer (RAFT)),[209–211] and different end groups for chemical modifications were obtained.[212]

The degradation of the polymer under thermal heating was studied.[213] Again, similar to all vinyl polymers, they are degraded under stress, but in general they are not biodegradable under physiological conditions.[214] Nonetheless, it has been shown that a molar mass of 30 kDa ensures elimination of the carrier from the body.[195] The excretion limit is 45 kDa, and long-circulating carriers end up in the liver and spleen.[214–216] However, a DOX-PHPMA conjugate with a molar mass above 30 kDa tested in mice showed a diminished doxorubicin concentration in the heart, but an augmented presence of the conjugate was found in the liver and spleen.[217]

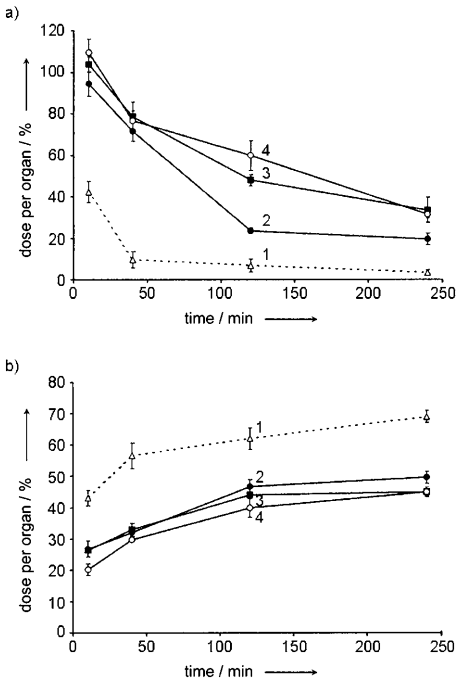


Figure 8. a) Blood clearance and b) liver accumulation for 1) plain liposomes and 2) liposomes with 0.3 mol% PHPMA-oleic acid, M_w 4300 Da; 3) liposomes with 3 mol% PHPMA-oleic acid, M_w 4300 Da; 4) liposomes with 3 mol% PHPMA-oleic acid, M_w 2900 Da.[206] Reproduced from Ref. [206] with permission from Informa Healthcare, Taylor & Francis Group.

In summary, PHPMA conjugates have already entered clinical trials and the results look very promising. However, immunological and stability questions as well as the excretion limit have not yet been investigated and the results of the clinical trials have to be awaited.

7. Conclusions

PEG is currently the most used polymer in the biomedical field of drug delivery and the only polymeric therapeutic that has market approval for different drugs. The success of PEG is based on its hydrophilicity, decreased interaction with blood components, and high biocompatibility. However, scientific results obtained in recent years show that it may also have possible drawbacks, such as interaction with the immune system, possible degradation under stress, and accumulation in the body above an uncertain excretion limit. Furthermore, many of the studies on the biocompatibility of PEG date back to the 1950s to 1970s and, therefore,

additional investigations are required that exploit contemporary techniques and analytical possibilities, in particular at the cellular level.

If an alternative polymer to PEG has to be chosen, a wide range of chemically very different synthetic polymers are available, although only a limited number are water soluble. These water-soluble polymers have to compete with the very high requirements of the gold standard—PEG. It becomes very clear when considering the potential alternatives that, in comparison to PEG, none of the alternative polymers are supported by sufficient studies concerning their biocompatibility, degradation under stress, and excretion limit. Even though the difficult patent situation of PEG pushed the search for alternative polymers, none of them have yet achieved approval for application. Most of the hydrophilic polymers cannot be considered as alternatives because they undergo severe interactions with the immune system and, therefore, are not able to prolong drug-carrier circulation times in the body. The most promising polymers that do show enhanced circulation time are poly(glycerol)s, poly(amino acid)s, poly(vinylpyrrolidone), poly(2-oxazoline)s, and poly(*N*-(2-hydroxypropyl)methacrylamide).

Clearance by the kidneys can be favored by using biodegradable polymers. However, the only polymers that provide both a biodegradable structure and a stealth effect are synthetic poly(amino acid)s. All other considered polymers show the same disadvantage as PEG, namely non-biodegradability and the associated unknown fate after disaggregation of the drug carrier, in particular after frequently repeated administrations. Poly(amino acid)s are the only polymers not to excite the accelerated blood clearance phenomenon, which is an advantage over PEG. An evaluation is not possible for all the other presented polymers, as investigations on this topic have not yet been reported. It should also be considered that the presented polymers, with their rather different chemical structures, might follow different degradation pathways, which may lead to new chemical species of yet unknown biocompatibility.

Degradation under stress has barely been investigated for all of the alternative polymers; thus, conclusions can not be drawn except in the case of PAAm, which degrades to its toxic monomer and is, therefore, unsuitable for biomedical applications. Interestingly, most of the monomers are toxic compounds, whereas the resulting polymers are biocompatible.

Considered as a whole, it appears that when all the polymers are judged with the same severe criteria, PEG remains the gold standard in the field of polymeric drug delivery, as it is the best investigated polymer. However, further studies with more systematic investigations may lead to a different view. In fact, possible substitutes are showing promising results and just need further investigations to allow proper evaluation and comparison with PEG.

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