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REVIEW

BIOMEDICAL APPLICATIONS OF MALEIC ANHYDRIDE COPOLYMERS

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This review rehearses and groups the biomedical applications of maleic anhydride/maleic acid copolymers. Maleic anhydride copolymers proved suitable for this application, being functional polymers with an alternating, well defined and reproducible chemical structure and a variable hydrophilic/hydrophobic balance. The anhydride cycle offers the possibility to obtain different drug-polymer conjugates by chemical reactions in mild conditions. These copolymers can be used in many different ways: as drugs (especially with antitumor activity), in drug controlled release systems (conjugates, films, solid dispersions, micro/nano particles), as components in biomaterials, in dental applications or in tissue engineering as support for bioactive molecules.

INTRODUCTION

The current uses of polymers consist mainly in plastics, elastomers, fibres/yarns, coatings, paintings and foams, sealants or gaskets, functional polymers, thermal or electric insulators. Besides these traditional fields, new advanced applications of polymers are being developed. Functional polymers have gained much attention over the past four decades as more and more polymers find applications beyond their traditional use in commodity. The range of functional polymers available today is large: from very simple structures obtained in a single step to polymers with complex architectures prepared through multi-step syntheses.[1] Many of these polymers have been applied specifically, as for example solubilizing agents, nanoparticulate formation, surface modification, macromolecular drug carriers, diagnostic imaging agents and implants. The majority of the functional polymers show a multitude of biological activities.

Polymers have numerous applications for biomedical purposes, as for instance: in prostheses, medical devices, dental materials, contact lenses, and pharmaceutical excipients. Their applications as drugs, drug-conjugates, enzyme-conjugates or gene delivery systems entered less in clinical practice. To obtain the authority approval for the clinical testing of a therapeutic system, at least two conditions have to be fulfilled: i) to be active as drug and with reasonable price, ii) to be safe and efficacious to justify administration to patient. Therefore, only a small number of therapeutic systems based on polymers get to be produced and commercialized.[2] A literature search evidenced that the number of papers dedicated to this topic considerably increased particularly in the last two decades (Figure 1).

The copolymers obtained by radical copolymerization of maleic anhydride (MA) with various acrylic or vinyl comonomers were investigated for this purpose from the '50s. In Scheme 1 is presented the general chemical structure of a binary maleic anhydride copolymer. It is easy to observe that these polymers are functional, even multifunctional. Firstly they possess the anhydride rings, from which the acid or the salt form can be prepared. Then maleic copolymers include one or more comonomers, also

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with different functional groups, such as N-vinylpyrrolidone, vinyl acetate, alkyl vinyl ethers, and so on. The medical and pharmaceutical applications of maleic copolymers are also favored by several properties such as: biocompatibility, water solubility, generally well-defined structure, the possibility to vary the hydrophilic/hydrophobic balance by the proper choice of the comonomer or by further chemical reactions on the copolymer. In addition, their chemistry is convenient: the copolymer synthesis is relatively easy and the monomers are cheap; the maleic anhydride cycle is versatile, allowing mild reactions with various partners (drugs, proteins, enzymes). Not in the least, the copolymers receive carboxylic functions after hydrolysis or maintain a part of these functions after chemical reaction, being anionic polyelectrolytes with enhanced solubility in water or in body fluids and with higher ability to form complexes with cationic partners. In the following, the MA copolymers, maleic acid copolymers or their derivatives (salts) will be denoted as maleic copolymers.

There are many data in the literature regarding the applications of MA copolymers in medical or pharmaceutical field. Most of them can be classified as: i) maleic copolymers with *per se* activity, ii) drug-polymer systems (conjugates), iii) drug formulations. The last two groups belong to the topic of controlled delivery systems. Another more recent application of maleic copolymers is in the fabrication of biomaterials or in tissue engineering.

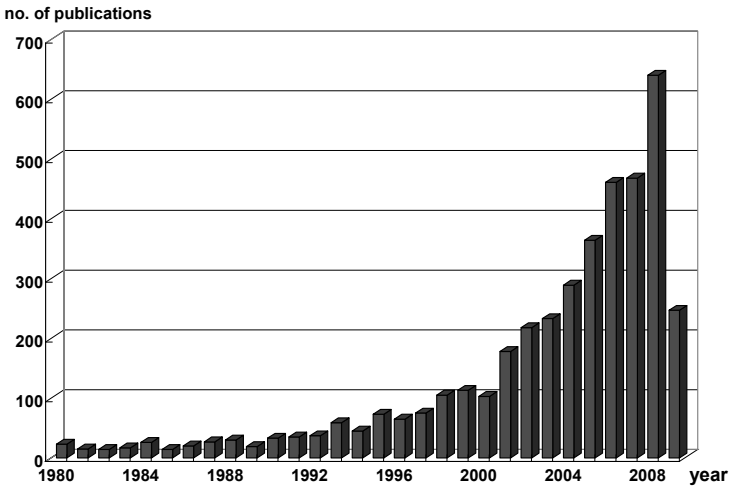
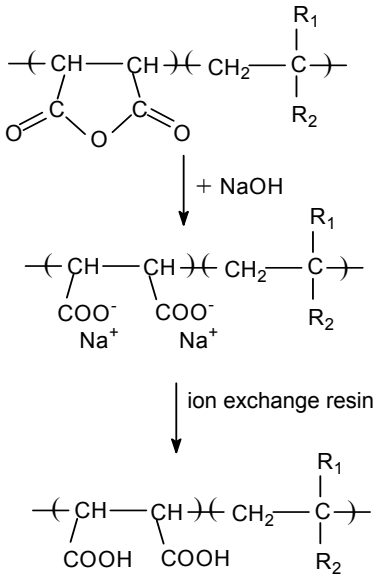


Fig. 1 – Evolution of literature citations concerning the biomedical applications of polymers.



Scheme 1 – Chemical structure of maleic copolymers. From top to bottom: MA, sodium maleate and maleic acid copolymers, respectively. R_1 can be H atom or CH_3 radical. R_2 is an organic radical/group such as methyl, phenyl, carboxymethyl etc.

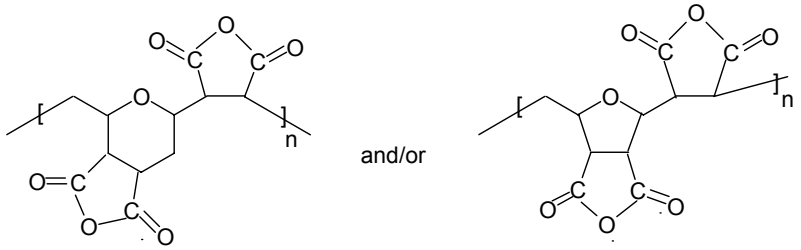
General reviews on the synthesis and characterization of maleic anhydride copolymers are already available[3-6] and will not be discussed in this paper even if this knowledge is very useful. We will mention only that, like other synthetic polymers, the toxicity of maleic copolymers increases with molar mass and is strongly influenced by the impurities (unreacted monomers, oligomers, solvent traces) which are toxic and have to be removed by advanced purification. Generally, lower molar mass and narrow molar mass distribution are required in order to provide at the same time suitable biological activity and reduced toxicity.[7,8]

MALEIC COPOLYMERS WITH *PER SE* BIOLOGIC ACTIVITY

Some of the maleic copolymers can have biologic activity by themselves (*per se*), especially antitumor activity. The drugs used in cancer chemotherapy should: i) have a good distribution in the body (bioavailability), which can be obtained by

means of a proper equilibrium between hydro- and liposolubility and ii) be targeted only to the tumor tissue as much as possible. Unlike low molecular drugs, polymers may combine with large molecules (plasma albumin) in the biological medium and may be slowly released into the biological fluids, giving an effective concentration over a longer period of time. The molar mass of the polymers should not be so high because they cannot be eliminated by the kidney and therefore accumulate in the body, achieving the efficient level but resulting as well in toxic reactions. The balance between efficiency and toxicity (selective toxicity) is a delicate task.[9-11]

Maleic anhydride – divinyl ether copolymer, in form of sodium salt, known as DIVEMA or Pyran, has a broad spectrum of biological activities among which: antitumor activity, interferon inducing, activation of macrophages, antiviral and antibacterial activity.[12] DIVEMA copolymer was synthesized in 1951 by G.B. Butler by radical copolymerization of MA with divinyl ether. It was obtained a cycle containing copolymer with alternating structure in which the mole ratio of the comonomers is 1:2 (divinyl ether: MA). ^{13}C NMR studies indicated that the copolymer contains tetrahydropyran and tetrahydrofuran cycles, as shown in Scheme 2, depending on the solvent(s) used by copolymerization.[13-16]



Scheme 2 – Chemical structure of DIVEMA copolymer.

DIVEMA is one of the most studied polymers with *per se* activity. It has been used since the '70s in numerous *in vitro* and *in vivo* experiments in the form of Na salt. The preliminary results have shown DIVEMA as active against adenocarcinoma 755, Lewis lung carcinoma, Friend leukemia virus, and Dunning ascites leukemia.[12] It was admitted that antitumor activity can be explained by DIVEMA copolymer capacity to activate the macrophage cells becoming toxic against the tumor cells.[17] DIVEMA copolymer can also be used as adjuvant in chemotherapy.[18]

The antiviral activity of DIVEMA copolymer was evaluated against over 20 viruses, including a number of cancer-inducing viruses: Friend leukemia, Rauscher leukemia, Moloney sarcoma, and so forth. It has also been observed that the prophylactic treatment with DIVEMA protects the body from the viral infection. That is why, for a considerable time, it was thought that the interferon induction activity of DIVEMA was responsible for the prophylactic action, but it has been demonstrated that the protection was kept long after any circulating interferon can be found in the blood stream. Then, the effect of DIVEMA against Rauscher leukemia was studied on both normal and immunosuppressed mice and in both cases it was found out that DIVEMA caused a dramatic reduction in the virus titer, suggesting that the copolymer activity was not only the result of stimulating host immune response.

The antibacterial activity of DIVEMA was demonstrated against both gram-positive[19] and gram-negative[20] bacteria. The antifungal activity was demonstrated against *Cryptococcus neoformans,* lethal yeast which attacks the lungs and brain of mice.

The tested copolymers have had a wide distribution of the molar mass and showed a number of toxic side effects as liver damage, depression of the reticuloendothelial system and others. Breslow showed that copolymer toxicity increases with the increase of the molar mass.[12] It was necessary to identify the molar mass range (as narrow as possible) in which satisfactory biological activity and reduced toxicity are provided at the same time. In the same connection it can be mentioned that the calcium salt of DIVEMA was proved to be less toxic than the sodium salt.[21]

Other maleic anhydride copolymers with *per se* activity are presented in Table 1.

Table 1

MA copolymers with *per se* activity

Copolymer	Activity	Ref.
MA–2-cyclohexyl-1,3-dioxap-5-ene	antitumor activity, immunostimulator, activation of macrophages	22-26
MA–2-isopropenyl-1,3-dioxap-5-ene	antitumor activity	27
MA–ethylene	antitumor and antiviral activity	28-31
Maleic acid–acrylic acid	antitumor activity	32,33
MA–dihydropyran, MA–dihydrofuran	antitumor activity	34,35
MA–vinyl adenine	activation of macrophages	36,37
MA–styrene, (MA–styrene)–block-styrene, (MA–styrene)–block-styrene derivatives with mannose or glucose	inhibition of HIV-1 infection	38
MA–styrene	inhibitor of spermatozoa motility, damaging the spermatozoa membrane	39-41

DIVEMA and other MA copolymers could be used as well after encapsulation, for example in liposomes, provided that the polyanions do not cause any changes in the liposomal membranes. It was evidenced that DIVEMA and MA–2-cyclohexyl-1,3-dioxap-5-ene copolymers do not perturb the liposomal membrane.[23,25] Ottenbrite *et al* used liposomes covered with polysaccharides for the encapsulation of MA–cyclohexyl-dioxapene copolymer then the macrophage activation was evaluated by measuring of superoxide anion (O^{2-}) released from the peritoneal macrophage cells.[24,26]

Maleic anhydride–styrene copolymer is used in male contraception as dimethyl sulfoxide solution.[40] In contact with water from the spermatic fluid, the maleic anhydride copolymer is hydrolyzed, forming the maleic acid copolymer. The product is called RISUG® (Reversible Inhibition of Sperm Under Guidance) and is in phase III clinical trials in India.[41]

CONTROLLED RELEASE POLYMERIC SYSTEMS

The formulation of a drug can have a significant effect on its efficiency. The drugs have an optimal concentration range, in which the benefit is maximal. In higher concentration they can be toxic and in lower concentration they would not produce the expected therapeutic effect. The formulation additives can be very different: water soluble polymers, microparticles based on natural or synthetic polymers, microcapsules, cells, lipoproteins, liposomes or micelles. The carriers can be slowly degradable, stimuli (pH, temperature) sensitive and even targeted.

The controlled drug release systems based on polymers can be prepared by a proper combination of a natural or synthetic polymer with a drug which is then released in a pre-designed manner. The release may be constant over a long period of time, cyclic, or triggered by the external conditions. The goal of the controlled release is to achieve a more efficient therapy concomitantly with the elimination of both under- and overdosing. Other advantages of using controlled-delivery systems are: the maintenance of drug level within desired range, the reduction of the number of administrations, and not in the least the increase of patient compliance.[42] The controlled delivery systems are efficient when the drug is in high enough local concentration at the target.

The drugs can be included into polymer matrices or shells by physical interactions or can be chemically attached to the polymer, when the so called polymer-drug conjugates are obtained. In the polymer-drug conjugates the drug pharmacokinetics is altered by the increase of effective molar mass brought by the polymer. The drug is released by chemical or enzymatic hydrolysis. In the systems based on physical interactions between polymer and drug (solid dispersions or encapsulations) the drug can be released by diffusion, degradation or swelling followed by diffusion. The release can be also sensitive to the environmental conditions such as temperature, pH, ionic strength, certain chemical substances (glucose, urea, uric acid, etc.), light, electric or magnetic field.

Conjugates of maleic copolymers with therapeutic agents

Polymer-drug conjugates (prodrugs) are obtained by covalent binding of the drug to the macromolecular support. Polymer-drug conjugates are used at least for two main purposes: (i) to improve the properties of the concerned drug (absorption, bioavailability, duration of action, safety, solubility, stability or taste) or/and (ii) to provide targeting drug delivery by enhancing selective concentration in the target tissue or by selective cleavage of the drug-support bond, that can be produced for example by an enzyme that is present or concentrated only in the target tissue.[43] Macromolecular prodrugs are built from three main parts: the polymeric carrier, the active moiety and the spacer between them, the last one being not compulsory. The polymeric supports can be: (i) natural, such as dextran, pullulan, mannan, dextrin, chitosan, hyaluronic acid, (ii) synthetic, such as poly(ethylene glycol), N-(2-hydroxypropyl)methacrylamide copolymers, poly(ethylenimine), poly(vinylpyrrolidone), etc. or (iii) pseudo-synthetic, such as poly(glutamic acid), poly(l-lysine), poly(aspartamides).[44] The use of natural polymers can raise some problems (immunogenic response, sensibility to chemical modification, uncontrolled biodegradation, the variety of the metabolites formed by biodegradation). Therefore, sometimes it is preferable to use synthetic polymers. To develop a system from which the drug can be released, suitable bonds between support and spacer and between spacer and drug should be chosen. The macromolecular prodrug should be stable during the transport in the blood circulation or other body fluids, on one hand, and should have an optimal rate of drug release at the site of action, on the other hand.

The alternant copolymer of maleic anhydride with N-vinyl-pyrrolidone (NVP) was one of the first synthetic polymeric supports studied. This polymer fulfills the requirements of a drug carrier: biocompatibility, reproducible chemical structure, suitable molar mass, narrow molar mass distribution, availability of attachment sites, water solubility.[45-47] In Table 2 are presented the prodrugs in which the drug is directly coupled to MA copolymers. In Table 3 are presented the systems in which the drug is coupled by them *via* a spacer.

Table 2

Conjugates from therapeutic agent and maleic copolymers

Copolymer	Drug	Pharmacological activity of the drug	Coupling mode (P-polymer, D-drug)	Ref.
MA–NVP	3-(2-methoxy phenoxy)-1,2-propanediol; quinidine	muscle relaxant/ antispasmoid; antiarrhythmic	P-CO-O-D	48
MA–NVP	melphalan; p-phenylene-diamine-mustard; dibromodulcitol	cytostatic; cytostatic; cytostatic	P-CO-NH-D P-CO-NH-D P-CO-O-D	48,49
MA–10-chloro undecene, MA–acryloyl chloride	chloramphenicol	antibiotic	P-CO-O-D	50
DIVEMA	adryamycin	antitumor activity	P-CO-NH-D	51
MA–methacryloyl -5-fluorouracil, MA–p-vinyl benzoyl -5-fluorouracil	fluorouracil	antitumor activity	P-CO-NH-D	52
MA–styrene, MA–styrene crosslinked with divinyl benzene	salicylic acid; eugenol; paracetamol	antiseptic; antiseptic, anesthetic; analgesic, antipyretic.	P-CO-O-D	53
MA–vinyl acetate, MA–styrene, MA–NVP	thymol eugenol	antiseptic; antiseptic	P-CO-O-D	54
MA–NVP, MA–styrene	phenothiazine derivatives	antipsychotic	P-CO-O-D, P-CO-NH-D	55
MA–styrene	4-Amino-6-hydroxypyrazolo[3,4-d]pyrimidine	antihypertensive	P-CO-NH-D	56
MA-methyl vinyl ether	S-nitrosothiols	donnor of nitric oxide, used in wound healing	P-CO-NH-D	57

The attachment of the drug to maleic anhydride copolymers can be easily carried out by reactions of the anhydride cycle or by the reactions of the comonomer reactive groups.[50] The conjugate can be obtained by the reaction of the comonomer with the drug, followed by copolymerization,[51] which is a less convenient way of synthesis. The release of the drug can be very slow, so the activity of the conjugate is lower than the free drug. In the case of DIVEMA–adryamycin, MA–methacryloyl-5-fluorouracil or MA–p-vinyl benzoyl-5-fluorouracil conjugates the prodrug activity *in vivo* is higher than the free drug.[51,52]

4-Amino-6-hydroxypyrazolo[3,4-d]pyrimidine (AHPP) is one of the most potent inhibitor of xanthine oxidase, an enzyme that catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid, generating superoxide anions (O_2^-). AHPP inhibit the oxidation of xanthine and thus the production of O_2^- in the vascular system. This drug is insoluble in water and can not be used for *in vivo* applications, but a water-soluble polymeric conjugate was obtained by reaction of AHPP with MA–styrene copolymer.[56] The conjugate presented an inhibitory activity against xanthine oxidase comparable with native AHPP. *In vivo* experiments showed that the conjugate can be used as antihypertensive agent.

A new series of derivatives of MA- methyl vinyl ether copolymer with S-nitrosothiols were used in the form of hydrogen-bonded interpolymer complex with poly(vinyl pyrrolidone) in the controlled release of nitric oxide. The interpolymer complex enhanced the storage stability of S-nitrosothiols. *In vitro* test on diabetic rats showed that this system accelerates the wound healing by the release of nitric oxide.[57]

Table 3

Covalent coupling to maleic copolymers *via* spacers

Copolymer	Drug and pharmacologic activity	Spacer/ spacer & aminoacid	Ways in which the drug release can be achieved	Ref.
AM–NVP	quinidine (antiarrhytmic)	6-aminohexanoyl-	hydrolysis	58
AM–NVP	quinidine (antiarrhytmic)	6-aminohexanoyl-L-phenylalanyl-	chemical hydrolysis or enzymatic hydrolysis with chymotrypsin	49
AM–NVP, semiamide and diamide of AM–NVP with different alkyl chains	p-nitroanilide (model drug)	6-aminohexanoyl-L-phenylalanyl-	enzymatic hydrolysis with chymotrypsin	59-61
AM–NVP	p-nitroanilide (model drug)	Gly-Gly-Val-Phe Gly-Val-Phe Gly-Gly-Phe	enzymatic hydrolysis with chymotrypsin	62
AM–NVP	6-purinethiol (antileukemic) transformed in 2-(6-purinyldithio) ethanol	6-aminohexanoyl-	hydrolysis	63
AM–NVP, AM–methyl vinyl ether	5-aminosalicylic acid (inflammatory bowel diseases)	4-(6-aminohexanoyl-) phenylazo-	N=N bond was broken by the anaerobic bacteria from the bowel	64

Gly – glycine, Val – valine, Phe – L-phenylalanine

The introduction of the spacer between polymer and drug can increase the solubility and the rate of releasing.[58] When L-phenylalanyl radical is introduced between the spacer and the drug, the link between aminoacid and drug can be easily broken by enzymatic hydrolysis. So, the release rate was found ten times higher.[49] Enzymatic hydrolysis depends on the pH,[59] on the charge distribution, on the polymer hydrophobicity and on the steric hindrance and macromolecule conformation.[60,61] Because the MA–NVP copolymer accumulates in the bone tissues, it is a suitable support for the antileukemic drugs that need to be released in the bones.[63]

One of the recent tendencies is the development of polymeric supports that can cross the cell membrane and release the active agent into cytoplasm or nucleus. For example, the derivatives of MA–styrene copolymer with alkyl amines were proposed as vectors for intracellular drug or even RNA release[65,66] because the reaction with amines having hydrophobic substituents increases the maleic copolymer affinity to the cell membrane.[67,68] The ability of those polymers to

destabilize the cell membrane depends on the alkyl chain length, on the conversion and on the molar mass. By adjusting these parameters, polymers with suitable hydrophilic/hydrophobic balance and dissociation constant can be obtained, able to disrupt the membrane at endosomal pH range. The anhydride groups not reacted with alkyl amines can be used for subsequent reactions with drugs or other biomolecules. If the therapeutic cargo is extremely hydrophilic, the polymeric support should have a high hydrophobicity to cross the cell membrane.

Maleic copolymers can also be conjugated with other active agents such as proteins or enzymes. In that case it seems that the obtained conjugate does not release the therapeutic agent, acting as a whole.

The only conjugate of maleic copolymers that is nowadays used in the clinical practice is the so-called SMANCS (marketed as Zinostatin stimalamer), a conjugate of neocarzinostatin with poly(maleic anhydride–co–styrene) partially esterified with n-butyl alcohol. Neocarzinostatin (NCS) is a protein with anticancer activity, presenting some limitations such as *in vivo* and *in vitro* instability and high toxicity. This maleic copolymer was chosen because anhydride groups react with the amidic groups of the protein while the styrene units bring certain hydrophobicity.[69,70] The alkyl chains also influence the conjugate properties. It was shown that n-butyl groups increase the hydrophobicity thus improving the lipid solubility and the affinity to albumin.[71]

SMANCS conjugate produces the inhibition of DNA synthesis, like free NCS, but it is also capable of activating macrophages and inducing interferon-γ. The activation of macrophages is maximal when the MA-St copolymer is in n-butyl ester form. If the copolymer would be esterified with longer alkyl chains, the water solubility would be decreased, that is why the n-butyl ester was chosen as the most proper one.[72,73] SMANCS conjugate keep the anticancer activity of the NCS protein and, by conjugation with the polymer, the pharmacological properties are improved: the *in vitro* and *in vivo* stability are increased, the capacity to concentrate in the tumor tissue is increased and the lipid solubility also increased.[72,74] SMANCS conjugate attaches to the cells faster than NCS, so the action to inhibit the malign cell development is enhanced. The hydrophobic character of SMANCS leads to an increased interaction with the cell membrane by penetrating the lipid bilayer.[75]

Generally, the conjugation with polymer leads to the increase of immunogenic properties and toxicity of a drug/protein. The free NCS and the SMANCS conjugate possess low immunogenicity.[76] The targeting of the anticancer agent only to the tumor tissues not only enhances the efficiency of the drug but also reduces the side effects.[77] The SMANCS conjugate is targeted to the tumor due to several factors: (i) tumor angiogenesis resulted in hypervasculature, (ii) enhanced permeability of SMANCS from blood capillary to tumor tissues, (iii) the lymphatic system operated poorly, with thus little drainage recovery *via* the lymphatic systems. Normally, when the body is healthy, proteins from sanguine plasma and macromolecules with molar mass higher than 50000 Da are retained in the blood capillary. That doesn't happen in the tumor tissues, and proteins and large macromolecules accumulate in the interstitial space. A small protein as free NCS does not present such behavior, but SMANCS conjugate behaves as a protein with molar mass in the range of 80000 Da. In the normal tissues, the proteins and lipids from the interstitial space are recovered by the lymphatic system and returned to the circulatory system. In the tumor tissues, the lymphatic capillaries are missing. SMANCS or albumins from the interstitial space are not recovered and they accumulate in those tissues. These phenomena are called "enhanced permeability and retention" (EPR) effect.[76,78-80]

SMANCS can be dissolved in water, organic solvents and, very importantly, it is lipophilic. Most often it is used dissolved in Lipiodol, an iodinated fatty acid, but also in other vegetable oils or triglycerides.[81] Lipiodol can be detected by X ray scanning and was demonstrated to accumulate selectively in the lymphatic system. SMANCS conjugate dissolved in Lipiodol was often injected into the arteries (hepatic artery for the treatment of hepatic or pancreatic cancer, bronchic artery in the case of pulmonary or bronchic cancer).[82-84] The administration of SMANCS/Lipiodol formulation can offer two distinct benefits: i) the selective delivery of the anticancer drug to the target tumor; ii) a potential value in the accurate determination of the size and location of the tumor by various X-ray systems and in the further monitoring of the tumor. SMANCS/Lipiodol system was also clinically tested by patients having colorectal or gastric cancer with lymphatic node metastases and was proved to have a remarkable anticancer effect

when the arterial administration was used.[85] The intravenous administration of SMANCS conjugate in aqueous solution was also efficient.[86,87]

Neocarzinostatin (NCS) was also conjugated with Pyran (divinyl ether–maleic anhydride) copolymer. The citotoxic activity of the conjugate was found not higher than the free drug, but the toxicity was decreased by conjugation. So, the dosage of the conjugate can be increased, enhancing the treatment efficiency.[88] The EPR effect observed on SMANCS conjugate was not observed in the case of Pyran–NCS conjugate, probably because the molar mass was too low.

Tumor necrosis factor (TNF-α) is a bioactive protein that causes a hemorrhagic necrotic effect against solid tumors. The conjugation of TNF-α with DIVEMA copolymer was done after protecting a large part of the amino groups of TNF with 2,3-dimethylmaleic anhydride, by reacting the protein with the copolymer and then by deprotecting the protein. [89] The DIVEMA–TNF-α conjugate tested in low doses on mice revealed an antitumor effect approximately 100 times higher than the native TNF-α.

Superoxide dismutase (SOD) is an enzyme with antioxidant effect that decomposes the superoxide radical anion according to the scheme below:

$$2O_2^{-\bullet} + 2H^+ \xrightarrow{SOD} O_2 + H_2O$$

$O_2^{\cdot-}$ is very reactive and causes damaging of the cells and tissues, being recognized as the main factor of the inflammatory diseases. Thus SOD is expected to be a candidate for the treatment of inflammatory diseases, ischemia, ulcerative colitis, etc. The anti-inflammatory activity of SOD is not significant because of his short half-life *in vivo* (4-5 min). By conjugation of this enzyme with polymers, the molar mass increases and its elimination is retarded. Hirano and co-workers covalently bound SOD to DIVEMA copolymer.[90] If all the 20 amino groups of the protein were reacted with anhydride groups of the copolymer, a massive crosslinked gel would be formed. To restrict the number of the amino groups reacting with the copolymer, a pH-reversible protective agent (2,3-dimethylmaleic anhydride) was used. Thus the DIVEMA–SOD conjugate retained almost the same enzymatic activity as the native SOD. Polymer conjugation was found not to affect the protein structure (revealed by circular dichroism measurements), but enhanced the thermal stability of the enzyme and decreased the antigenicity of the conjugate.[91] DIVEMA–SOD conjugate can remove the $O_2^{\cdot-}$ radicals produced as an overreaction of the immune response of the host in the infection with influenza virus.[92] It was also used in the treatment of the rats with re-expansion pulmonary edema.[93] Another feature of DIVEMA–SOD derivative is to have a higher stability against H_2O_2 than free SOD.[94]

The conjugation of another enzyme, l-asparaginase, with the comb shape copolymer of MA with poly(oxyethylene) allyl methyl diether led to the stabilization of the enzyme toward heat, urea and acidity.[95]

Other controlled release systems

Beside the controlled release systems where the drug is covalently attached to the polymer, there have been developed many systems in which the drug interacts not chemically with the polymer: solid dispersions, micro or nanospheres/capsules/discs, polymeric films. Consequently the drug can be released mainly by three mechanisms: diffusion, degradation, and swelling followed by diffusion. As in the case of polymer-drug conjugates, the polymers used in the formulations with controlled release should be biocompatible, biodegradable, nontoxic, and should not induce cancer, allergic or pyrogenic reactions. Their biodegradation products should fulfill the same requirements.

Solid polymer-drug dispersions can be obtained by co-precipitation, co-dissolution and solvent evaporation, co-melting or by mixing. They can be used to improve the solubility and bioavailability of the drugs with low water solubility if the drug is dispersed in water soluble polymers. Such polymers can be copolymers of maleic anhydride with alkyl vinyl ethers, especially in half-ester form, which are soluble only over a certain pH. The pH of the dissolution medium and the dissolution rate of these copolymers can be influenced by the length of ether alkyl chain of the parent copolymer and/or by the esterification of MA units.[96] Generally, the increase of the alkyl chain length or the increase of the esterification degree led to the increase of the hydrophobicity and thus the copolymer can be tailored to be dissolved at desired pH value among the gastrointestinal tract.[97,98] In Table 4 are summarized the controlled release systems in which maleic copolymers are used in solid dispersions with drugs. MA–MVE is the maleic anhydride–methyl vinyl ether copolymer.

Table 4

Solid dispersions based on maleic copolymers

Polymer	Drug/active agent	Therapeutic effect	Ref.
Half esters of MA–MVE	hydrocortisone	anti-inflammatory effect	98,99
Half esters of MA–MVE	pilocarpine	ophthalmologic use	100,101
Isopropyl monoester of MA–MVE	timolol maleate	treatment of high blood intraocular pressure;	102
MA–MVE	hydrocortisone hydrochlorothiazide; indomethacin; nalidixic acid; reserpine	compensate a deficit in cortisone diuretic; anti-inflammatory; anti-bacterial; anti-hypertensive	103
MA–MVE and its half esters	griseofulvin	antifungal	104,105
MA–MVE and its ethyl half-ester	phenylbutazone	anti-inflammatory	106
Ethyl monoester of MA–MVE	theophylline	therapy of asthma	107
MA–MVE	clofazimine	antileprotic	108
n-propyl and n-butyl half ester of MA–MVE linear or plasticized with Tween 20	tripelennamine; isoniazide; hydrochlorothiazide	antihistaminic; antituberculous; diuretic	109
blend from MA-MVE and poly(ethylene oxide)	metronidazole	antibiotic, active against anaerobic bacteria and protozoa	110

The drug solubility is increased or the insoluble drugs become soluble by dispersion with maleic copolymers.[103-105,108] MA–MVE copolymer and the half esters of these copolymers are soluble mainly in ionized form (over a certain pH value), that is why the drug is released by erosion of polymer matrix and less by diffusion. The polymer erosion takes place at the interface. If the total area is not significantly changed in time, the drug release is constant and respects zero order kinetics.[98,100,101,108] When the maleic copolymer is crosslinked and totally insoluble, the drug release is a diffusion process and respects first order kinetics.[109] An interesting approach is the use of blends from poly(ethylene oxide) and maleic anhydride copolymer that form *in situ* intermolecular complexes. The hydrolysis of the maleic anhydride groups led to the formation of hydrogen-bonded complexes between the two polymer chains. The duration of polymer erosion is extended and the drug release is retarded.[110]

Films for tablet coating can be obtained from maleic copolymers solutions in organic solvents followed by solvent evaporation. The films can be directly deposed on the tablets by their immersion in the copolymer solution[111] or by spraying the solution on the tablets.[112] Films can also be obtained by casting the copolymer solution, with or without plasticizers, on a glass or inert polymer surface followed by film detaching for characterization purpose.[113,114] The release of the drug from MA–MVE copolymer films was studied *in vitro* and it was found that their permeability can be controlled by varying the polymer molar mass, the hydration of the film or the crosslinking agent content. The increase of the pH over the value of dissociation constant of the first carboxylic group from the maleic units led to the increase of the film permeability, which is an advantage when the retardation of the drug release in the intestinal tract is desired. Generally, the maleic copolymer is in the anhydride form (soluble in organic solvents), but it can be partially transformed in acid form with a higher water-solubility.[111] *In vivo* studies showed that the covering of the tablets with MA–MVE copolymer films impeded the disintegration in the stomach for only 15-30 min.[111] In Table 5, maleic copolymer applications in tablet coating are summarized.

Table 5

Controlled release systems across maleic copolymers films

Polymer	Drug/active agent	Therapeutic effect / application	Ref.
MA–MVE	Dicalcium phosphate, barium sulfate	radiocontrast agents for X-ray imaging	111
Half esters of MA–MVE	aspirin	antipyretic , anti-inflammatory effect	112
Half esters of MA–styrene with ethanol	erythromycin	antibiotic	114

Controlled release of drugs can be also achieved with polyelectrolyte complex films in which maleic copolymers are the anionic partner. The polyelectrolyte complex between chitosan and the copolymer of maleic acid with poly(oxyalkylene)vinyl methyl diether was used to obtain drug loaded films.[115-117] For preparation purpose the polyelectrolyte complex solution together with the model molecule (ibuprofen, salicylic acid, phenol or glucose) was cast on a plane surface and the solvent was evaporated. The drug release depends on the film composition (the ratio between the polyanion and polycation) and is also sensitive to the temperature or pH.

Beads with the polymer matrix made from poly(maleic acid–co–methyl vinyl ether) crosslinked with divalent ions as Ca^{2+} or Zn^{2+} and incorporated drug were obtained by extrusion method.[118,119] If the beads are treated by microwave irradiation, the drug-polymer and polymer-polymer interactions are enhanced, and the drug release is retarded.

Microcapsules are spherical particles with size between 0.05 and 2000 μm, the shell being a polymer, and the core being a bioactive substance.[120] A more precise definition of micro/nanoparticles describes micro/nanospheres as being formed from a solid core with a dense polymeric network and micro/nanocapsules as being formed from a thin polymeric shell that surround a hole that can be a "reservoir".[121] In practice, the "micro/nanoparticle" term is used instead of "micro/nanospheres". In Table 6 are presented microcapsules based on maleic copolymers.

Table 6

Microcapsules obtained from maleic copolymers

Polymer	Drug/active agent	Therapeutic effect	Ref.
n-butyl half-ester of MA–MVE copolymer	phenacetin	analgesic	122
n-butyl half-ester of MA–MVE copolymer	hydrochlorothiazide	diuretic	123
MA–MVE copolymer	triclosan	antibacterial, antifungal	124
MA–MVE copolymer	indomethacin	anti-inflammatory	125
Monoamide of MA–vinyl acetate copolymer with dodecylamine	enzyme	-	126
Polyaddition product between maleic anhydride–styrene copolymer and polyamines	dodecyl acetate, dodecanol	model compounds for sex pheromones of insects	127
polyelectrolyte complex between MA–styrene copolymer and gelatin	-	-	128

Microcapsules based on maleic copolymers can be obtained by different methods: (i) coacervation, when by adding salt the polymer is taken out of solution as viscous drops that tend to the deposition on the surface of drug particles suspended in the system;[122] (ii) interface precipitation: drug powder is suspended in liquid paraffin, then the polymer solution is added and the polymer is deposited on the surface of drug particles, forming microcapsules;[123] (iii) evaporation of the solvent from oil in water in oil double emulsions;[124] (iv) crosslinking of maleic polymers with divalents ions;[125,126] (v) polyadition at the interface between maleic copolymer dissolved in organic phase dispersed in a continuous aqueous phase containing polyamine;[127] (vi) interface complexation: drops of organic phase are dispersed in aqueous solution of MA–styrene copolymer. The maleic copolymer with hydrophobic groups is adsorbed to the interface with phenyl groups oriented to the organic phase drops and carboxylic groups outside the drops. When aqueous solution of gelatin is added, the complexation occurs. To crosslink the gelatin, glutaraldehyde can be added.[128]

Hollow microcapsules/shells with semi-permeable membrane can be used to encapsulate drugs. They can be obtained using layer-by-layer (LbL) deposition technique. LbL allows the fabrication of multilayers by physical adsorption of partners with opposite charge. This technique was used initially in the self-assembly of opposite charge polyelectrolytes, then it was extended to other materials bearing electric charges.[129] The basic principle of this technique is to expose a substrate with electric charge to oppositely charged polyelectrolytes, alternatively. Every adsorption inverses the electric charge of the surface resulting in a multilayered polyelectrolyte complex, stabilized by strong electrostatic forces. Colloidal particles can also be covered by LbL technique and after the removal of the core, hollow microcapsules with polyelectrolyte multilayer shells are obtained.[130]

If these small particles have the size in range 0.001 – 0.1 μm (1 – 100 nm) they are called nanoparticles (nanospheres or nanocapsules). In this case the drug loading capacity is lower, but the ability to cross cell membrane is enhanced, the risk of undesired clearance from the body through the liver or spleen is reduced and the uptake by the reticuloendothelial system is minimized.[131]. Nanoparticles can be obtained by various techniques: emulsion polymerization, "salting-out" effect, heat denaturation of proteins and crosslinking in emulsion, desolvation and crosslinking in aqueous medium, deposition to the interface, layer-by-layer deposition, auto-assembling, and so forth.

Nanoparticles based on MA–methyl vinyl ether copolymer were obtained by solvent displacement method:[132-135] into a solution of the maleic copolymer in acetone, an ethanol:water mixture was added, then the solvents were eliminated under reduced pressure, and the resulting nanoparticles were centrifuged and lyophilized. Nanoparticles can also be obtained by removal of acetone from acetone-water solution of the copolymer. Antigens or allergens can be entrapped in such nanoparticles and the maleic copolymer from the nanoparticles can be crosslinked with difunctional amines.[135-137] Nanoparticles based on MA–butylvinylether copolymers (hemiesterified, grafted with PEG segments and/or antifibrin monoclonal antibody) obtained by coprecipitation technique are used to entrap enzymes with fibrinolytic activity.[138] In this technique the low water solubility of the copolymer and the interaction of the copolymer with protein molecules led to microphase separation and nanoparticle formation.

The compressed fluid technology is a new method for the production of nanoparticles. An organic solution of the MA–methyl vinyl ether copolymer is sprayed into a current of CO_2 that attained into a precipitation chamber filled with CO_2 and the organic solvent. The antisolvent effect of the compressed CO_2 over the solution caused the precipitation of the micro- or nanoparticles.[139]

A frequently used technique for obtaining nanoparticles is the generation of nano-drops of an anionic polyelectrolyte solution mixed with the active substance followed by the contact with a cationic partner solution. When the solutions have contact, polyelectrolyte complexes are formed as a shell of nanoparticles in suspension.[140] For example, non-stoichiometric polyelectrolyte complexes between poly(maleic acid–alt–α - methylstyrene) and poly(diallyldimethylammonium chloride) were used to obtain nanoparticles by consecutive centrifugation and redispersion.[141,142]

Polyelectrolyte complexes based on poly(N-vinylpyrrolidone)–block–poly(styrene-alt-maleic anhydride) and poly(N-vinylpyrrolidone)–block–poly(N,N-dimethylaminoethyl methacrylate) were used to obtain nanoparticles/micelles with the core formed by polyelectrolyte complex and incorporated drug and shell from hydrophilic poly(N-vinylpyrrolidone). The drug release was strongly controlled by the surrounding pH.[143] Micelles loaded with a water soluble drug are obtained by complexation of double-hydrophilic block copolymer poly(N-vinylpyrrolidone)–block–poly(styrene–alter–maleic anhydride) with chitosan.[144] Micelles obtained by self-assembling of amphipilic triblock copolymer poly(N-isopropylacrylamide)–block–poly(styrene–alt–maleic anhydride) can also be loaded with drugs.[145] In this case, the drug release is pH and thermo-responsive.

Nano-sized water soluble micelles can be formed based on hydrophobic interactions between styrene units from MA–styrene copolymer and hydrophobic or amphiphilic drugs: doxorubicin,[146] pirarubicin,[147] zinc-protoporphyrin.[148,149] Those drugs are used in cancer therapy and the formed micelles present "enhanced permeability and retention" effect *in vivo*, that led to the selectively target to tumor tissue.

OTHER BIOMEDICAL APPLICATIONS

Immobilization of viruses or antibodies

Maleic anhydride copolymers can easily react with amines or proteins and can be used to immobilize viruses or antibodies.[150-153] Therefore a new method to determine total tyrosine was developed using polystyrene tubes covered with MA–styrene copolymers.[154]

Dental applications

In vitro and *in vivo* studies[155,156] showed that a combination between MA–vinyl methyl ether copolymer, pyrophosphate anion, and NaF reduced with 57% calculus formation and did not damage developing enamel surfaces. Pyrophosphate anions inhibit hydroxyapatite formation, but in the presence of phosphatases from the oral cavity, those anions are hydrolyzed and lose efficiency as

anti-calculus agents. Maleic acid–methyl vinyl ether copolymers inhibit the enzymatic activity of alkaline phosphatase.[157] It can also be used in the composition of denture adhesive[158] or dental cements.[159]

Components in biomaterials

Using MA–vinyl acetate copolymer in the hydrothermal synthesis of hydroxyapatite, new biocompatible composites hydroxyapatite–copolymer were obtained.[160,161] By adding higher amount (10-20%) of maleic copolymer, organic-inorganic composites with nano-sized dimension grains were obtained, whose biocompatibility was better than that of the commercial hydroxyapatite. In the same context, MA–methyl methacrylate copolymer was investigated in bone cements composition, but the bending strength of those materials was not satisfactory.[162]

Hemodialysis membranes from high-density polyethylene were functionalized by grafting with maleic anhydride–vinyl acetate copolymer performed by means of gamma rays. Maleic anhydride cycles can be then hydrolyzed and transformed in sodium salt (enhancing the membrane hydrophilicity) or can easily react with amines or amine derivatives to introduce pyridine or sulfamic acid groups, enhancing the membrane functionality. The grafted membranes have good mechanic properties, better hydratation capacity, enhanced permeability and reduced protein adsorption.[163]

Tissue engineering

Maleic copolymers can also be used in the field of tissue engineering, which has witnessed great progress over the past few decades. Films from maleic copolymers can be obtained by spin coating onto aminosilanized glass or SiO_2 surfaces. The amidic linkage formed between maleic copolymer film and the solid surface was transformed into five membered imidic cycles by heating at 120°C. The unreacted anhydride groups can be reacted with difunctional amines and then reacted at 120°C with compounds with $–NH_2$ and –OH groups when hydroxyl-containing polymer surfaces are obtained[164] or after the reaction with diamines, a spacer (polyethyleneoxide) can be introduced.[165] Bioactive molecules immobilization onto the polymeric film can be controlled by choosing the comonomer of maleic copolymer (styrene, propene, ethylene, octadecene) and by varying the conversion of maleic groups.[165] Increasing the environmental pH from 3 to 7.4 led to the swelling of maleic copolymers films and consequently to the increase of their reactivity.[166]

Proteins with different functions can be immobilized on the surfaces covered with maleic copolymer. Thus, fibronectin is immobilized onto maleic copolymers film by covalent linkage and by hydrophobic interactions. Fibronectin interacts more strongly with MA–hydrophobic comonomer, while the weaker interaction with MA hydrophilic copolymers led to the reorganization of fibronectin in fibrils.[165-168] The physical-chemical characteristics of the surface covered with maleic copolymers will modulate the anchorage of immobilized fibronectin that will promote the growing of endothelial cells as monolayers or as capillary networks.[169] Collagen[170], thrombin, human serum albumin or lysozyme[171] can also be immobilized onto maleic copolymers film.

Polystyrene multi-well plates used in biochemical and cell culture assays can also be functionalized with maleic anhydride copolymers for biomolecule attachment. In this case, free amino groups are generated on the polystyrene surface by low pressure ammonia plasma treatment and thin films of maleic anhydride copolymers were covalently attached to the surfaces by the same reactions as in the case of aminosilanized SiO_2 surfaces. Micrometer-sized lateral patterns of the functional coatings can be obtained by plasma etching. Fibronectin was attached only onto the micro-patterns that led to a controlled cell culture.[172]

CONCLUSIONS

Maleic anhydride or maleic acid copolymers can be used in different ways: as drugs, in drug controlled release systems (conjugates, micro/nano particles), as component in biomaterials, in hemodialysis membrane functionalization, in stomatology, or in tissue engineering as support for bioactive molecules.

The biomedical application of maleic copolymers is based on their structure, properties and activity. Generally, maleic anhydride copolymers have an alternating, well defined and reproducible structure. The hydrophilic/hydrophobic character and the charge density can be varied by the proper choice of the comonomer(s). The anhydride cycle offers the possibility to obtain different drug-polymer conjugates by chemical reactions in mild condition (low temperatures, without catalysts), avoiding contamination with

impurities. Maleic anhydride copolymer conjugates have often a pH-sensitive solubility that can be used to release the drug in certain segments of the gastrointestinal tract. Practically, maleic copolymers can become less or non toxic after an advanced purification that removes the monomers or other auxiliary products.

The first maleic copolymer with *per se* activity that became clinically tested as anticancer agent was the maleic anhydride–divinyl ether copolymer in sodium salt form, known as DIVEMA or Pyran. That copolymer has a broad spectrum of biologic activities among which antitumor activity, interferon inducing, activation of macrophages, antiviral and antibacterial activity. Anticancer activity of other maleic anhydride copolymers with ethylene, acrylic acid, cyclohexyl-1,3-dioxepene, dihydropyran, dihydrofuran, vinyladenine or styrene was studied, but those did not reach the performance of DIVEMA.

Maleic copolymers were also studied as support for drug-polymer conjugates used for controlled delivery of drugs. Maleic anhydride–N-vinyl pyrrolidone copolymer was intensively studied for prodrug obtaining. The copolymers with divinyl ether or with methyl vinyl ether were also used but these drug conjugates never surpassed the phase of *in vitro* testing. The only product based on maleic copolymer that passed the clinical tests and is used in the medical current practice in Japan is SMANCS, a conjugate of neocarzinostatin with the butyl ester of the poly(maleic anhydride–co–styrene). Other synthesized and tested conjugates with enzymes were DIVEMA conjugates with neocarzinostatin, tumor necrosis factor-α or superoxide dismutase.

Other pharmaceutical applications of maleic anhydride copolymers, especially with alkyl vinyl ethers in half-ester form, are in controlled drug release formulations where the polymer does not interact chemically with the drug. These formulations can be: solid dispersions, films for tablet coting, micro/nano-particles. The development of controlled radical polymerization methods led to the obtaining of triblock copolymers that auto-aggregate into micelles that can be used in drug release. Other tested applications of maleic copolymers were as components in analysis kits, biomaterials, and tissue engineering.

From the authors' point of view, many biomedical applications of maleic anhydride copolymers have only reached the synthesis stage. The most significant chemical structures were synthesized and tested (not exhausted), as well as the conjugates with various drugs or proteins. The formulation of drugs by different ways with maleic anhydride copolymers was also investigated in many contributions. Nevertheless, the research effort to examine and ascribe the toxicity of these polymers was definitely lower. The purification of the copolymers before testing in a bioapplication was also insufficiently studied, except a series of valuable pioneering papers. The mechanism of maleic copolymers action was much less investigated taking into account their behavior as polyelectrolyte.

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