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Biologically active polymeric sequestrants: Design, synthesis, and therapeutic applications*

Pradeep K. Dhal‡, Chad C. Huval, and S. Randall Holmes-Farley

Drug and Biomaterials R&D, Genzyme Corporation, 153 Second Avenue, Waltham, MA 02451, USA

Abstract: In recent years, functional polymers exhibiting inherently biological activities have been receiving increasing attention as polymer-based human therapeutic agents. These polymeric drugs exhibit unique pharmaceutical properties that are fundamentally different from their traditional small-molecule counterparts. However, unlike polymeric drug delivery systems, examples of polymers possessing intrinsically therapeutic properties are relatively scarce. By virtue of their high-molecular-weight characteristics, these polymeric drugs can be confined to the gastrointestinal (GI) tract, where they can selectively recognize, bind, and remove target disease-causing substances from the body. Being confined to the GI tract and non-biodegradable, these polymeric drugs are free from toxic effects that are associated with traditional systemic drugs. This report highlights recent developments in the rational design and synthesis of appropriate functional polymers that have resulted in a number of promising polymer-based therapeutic agents, including some marketed products.

Keywords: polymers; drugs; sequestrants; non-absorbed; GI tract.

INTRODUCTION

The design and development of functional polymers as key agents in biomedicine have attracted significant attention in recent years [1]. The use of polymers as biomaterials has found applications in such areas as artificial organs, tissue engineering, components of medical devices, and dentistry [2]. Another growing aspect of the field of biomedical polymers is their therapeutic applications, where polymers have been utilized as carriers for delivery of active pharmaceutical agents [3,4]. There have been significant developments in the area of polymeric delivery systems and polymeric pro-drugs, where functional polymers have been utilized to prepare conjugates of small molecule and protein to improve therapeutic indices of original drugs. While such polymeric drug delivery systems have been around for many years, *intrinsically* bioactive polymers (polymers as active pharmaceutical ingredients) are relatively scanty [5]. Although polymers, in general, do not fit "drug-like" definitions in a traditional sense, a reevaluation of the attributes of polymers reveals a number of promising advantages associated with polymeric pharmaceuticals that cannot be achieved with traditional small-molecule drugs. For example, when taken orally, high-molecular-weight characteristics of polymers render them to be non-absorbed through the gastrointestinal (GI) tract. However, confinement of these functional polymers to the GI tract offers unique opportunity, where it is desirable to restrict a therapeutic agent from systemic exposure. By combining this characteristic with the ability of polymers to selectively recognize and bind molecular and macromolecular components in intestinal fluids, it could be possible to discover and develop

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‡Corresponding author

a new class of therapeutic agents that can selectively bind and remove detrimental species from the GI tract. Furthermore, the ability to incorporate a variety of binding sites as well as a high density of such groups along a polymer chain can lead to "polyvalent" interaction with target ligands, which would result in highly potent drugs [6].

Coverage of the field of polymer-drug conjugates and/or polymeric drug delivery systems is outside the scope of this article. Several recent books and review articles have adequately covered most aspects of this area of research [7,8]. Instead, this article will focus primarily on the research and development challenges and successes in the field of inherently biologically active polymers as human therapeutic agents. Furthermore, the report will mainly highlight authors' own experience in the discovery and development of polymer drugs that have transitioned from laboratory to clinic to marketplace for treating different human diseases.

POLYMERIC SEQUESTRANTS FOR MOLECULAR AND MACROMOLECULAR AGENTS

A number of potentially detrimental substances, which are either small molecules or macromolecules, are present in the GI tract. These molecules and pathogens are implicated for a number of human diseases. Effective removal of these species in a selective manner offers a promising opportunity for the treatment of a number of ailments. Biocompatible polymeric sequestrants represent an ideal class of drugs that can be used either prophylactically or therapeutically. Non-absorption of polymers through the intestinal wall should offer minimal toxicity, while the incorporation of appropriate functional groups and manipulation of polymer physicochemical characteristics provides opportunities to tailor-make polymers with high selectivity and potency toward target molecules. A number of functional polymers have been discovered, which have been found to be effective in removing undesired agents from the body at acceptable therapeutic doses.

Sequestration of dietary phosphate: A treatment for renal disease

The safe and effective management of elevated levels of serum phosphorous (hyperphosphatemia) is a critical goal of physicians for treating patients with chronic and end-stage renal diseases [9]. The consequences of inadequately controlled serum phosphate levels can lead to various pathologies of clinical importance and are major risk factors for mortality among patients undergoing dialysis.

Since the primary route for the elimination of excess phosphate from the body is through the kidneys, patients with impaired kidneys accumulate phosphate systemically, which leads to elevated plasma phosphate. The consequences of poor control of serum phosphate level can lead to a number of clinically significant pathological conditions. These conditions include soft tissue calcification (leading to cardiac calcification and other cardiac-related complications), renal bone disease (leading to reduced bone density), and secondary hyperparathyroidism. Phosphate binder therapy has been the primary treatment for patients suffering from hyperphosphatemia. Traditional phosphate binders include metal salts, such as those based on calcium, aluminum, lanthanum, and iron [10]. These metal salts remove phosphate from the human body through the formation of insoluble phosphate salts within the GI tract. However, since metal ions have the propensity to be systemically absorbed, their long-term use can lead to undesirable toxic side effects.

The use of cationically charged polymers to sequester dietary phosphate ions in the GI tract offers an attractive and safe approach to treat hyperphosphatemia in renal failure patients. The binding of phosphate ions by poly-cationic compounds has been extensively studied by researchers in the area of molecular recognition and supramolecular chemistry [11]. Many research efforts have been directed toward the design and synthesis of novel compounds such as oligomeric and macrocyclic amines, ammonium salts, and guanidinium compounds as synthetic receptors for different kinds of phosphate-containing anionic guests [12,13]. By utilizing the underlying principles of the physical organic chemistry of this anion recognition process, polymeric drugs were designed and synthesized as non-absorbed,

cationic, polymeric hydrogels (such as polymeric amines and guanidinium compounds) that show affinity toward phosphate ions derived from dietary sources. Being non-absorbed, these polymeric sequestrants are confined to the GI tract. Therefore, they act as effective therapeutic agents (free from the side effects associated with calcium and related metal salt-based phosphate binders) to treat hyperphosphatemia.

Like low-molecular-weight phosphate receptors, the complexation process involves electrostatic interaction between the polymer-bound ammonium groups and phosphate anions. As a result, the optimum level of protonation of amine groups along the polymer chain is a key to achieving maximum binding capacity. Furthermore, the divalent (and possibly trivalent) nature of phosphate anions requires favorable display of ammonium groups to provide strong binding strength toward phosphate through chelation. Due to higher local concentrations of amine groups in polymeric systems, the protonation of neighboring amine groups depends on the architecture of the polymer chain. By considering the factors laid out as above, a series of amine-containing cross-linked polymers were designed and synthesized. These amine-functionalized hydrogels were prepared either by cross-linking of amine polymers, or by the cross-linking polymerization of amine-containing vinyl monomers [14,15]. Cross-linked polymers provide inherent safety advantages since they are confined to the GI tract. The general method for syntheses of polymeric amine gels by cross-linking of soluble polymers is shown in Fig. 1. These amino functional polymers are believed to bind phosphate anions through electrostatic and possibly through hydrogen-bonding interactions (see Fig. 2). A systematic structure–activity study enabled us to identify epichlorohydrin cross-linked polyallylamine as the candidate for subsequent preclinical and clinical development. The compound was approved in the United States by the Food and Drug Administration (FDA) in 1998 under the generic name of sevelamer hydrochloride and has been marketed since under the brand name Renagel®. Since its approval, Renagel has demonstrated effective and long-term control of serum phosphate levels in renal failure patients and has led to longer life expectancy for these patients [16,17].

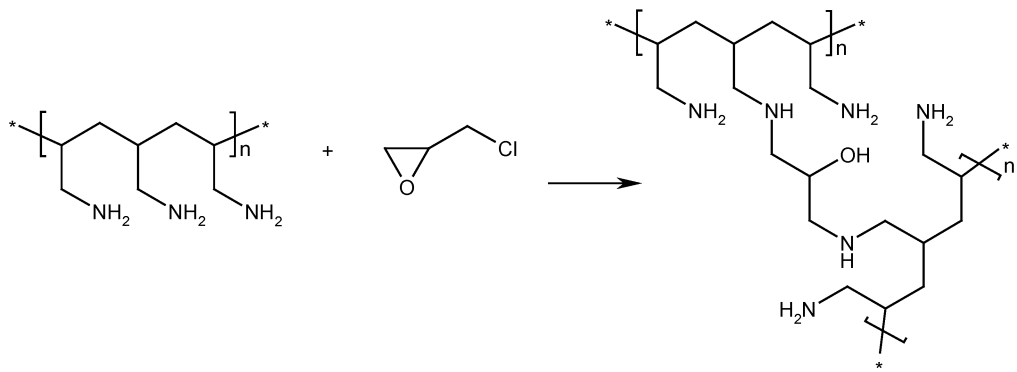


Fig. 1 Synthesis of amine-containing hydrogels by modification of soluble polymers.

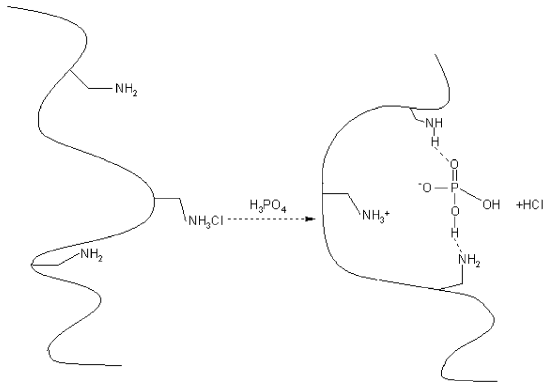


Fig. 2 Schematic representation of binding of phosphate ions to amine polymers.

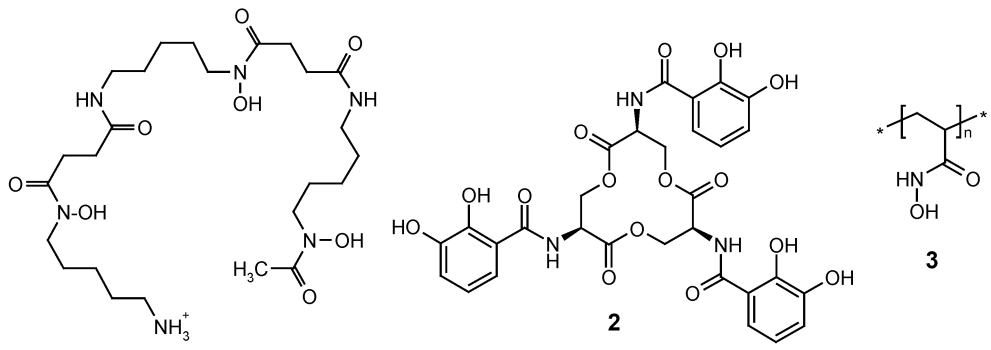
Iron-sequestering polymers: Polymeric approaches for iron overload disorders

Although iron is an important metal ion that is needed for many critical human biological processes, the presence of excess iron in the body leads to toxic effects. Excess iron catalyzes the transformation of molecular oxygen to oxygen-derived free radicals such as hydroxyl radicals, which in turn cause damage to many vital biological molecules in the human body. Such iron-mediated biological events are responsible for a number of pathological conditions [18,19]. Under normal physiological conditions, iron metabolism is tightly conserved with the majority of the iron being recycled within the body. Normal physiology does not provide a mechanism for iron loss through the process of excretion in urine, feces, or bile. Certain genetic disorders can, however, lead to an increased systemic uptake of dietary iron (as in the case of hemochromatosis) or transfusion-induced iron overload (as in the case of β-thalassaemia and sickle cell anemia) [20,21].

Thus, the development of orally available and systemically non-absorbed iron chelators that selectively sequester and remove excess dietary iron from the GI tract may help relieve such iron overloads. The current standard of care for iron chelation therapy is desferrioxamine. Desferrioxamine has reportedly been associated with several drawbacks, including a narrow therapeutic window and lack of oral bioavailability. As a result, it requires administration for 8–12 h per day by parenteral infusions [22]. Thus, there is a clear need for the discovery and development of a new generation of orally active iron chelating agents for the treatment of iron overload conditions.

Non-absorbed polymeric ligands that selectively sequester and remove dietary iron from the GI tract appear to be attractive therapeutic agents for the treatment of certain iron overload conditions. Because of the importance of other metal ions for normal human physiology, clinically acceptable polymeric ligands must possess high affinity, capacity, and selectivity toward iron in both oxidation states. Furthermore, such a polymer needs to be biocompatible and non-absorbed. Additionally, for clinical applications, the key desirable properties of chelators include metal ion selectivity and high stability constant for the ligand–metal complex. Therefore, the design of pharmaceutically relevant polymeric iron chelators has been based on the knowledge of low-molecular-weight chelators.

For Fe(II), soft donor atoms (e.g., nitrogen-containing ligands such as bipyridine and phenanthroline) can be employed. Although these ligands are selective for Fe(II), they also possess affinity toward other biologically important divalent metal ions such as Zn(II) and Cu(II). On the other hand, oxyanions like hydroxamates and catecholates are selective toward Fe(III) and these ligands in general show higher selectivity toward trivalent metal ions over divalent metal ions. Nature offers a precedent: natural iron chelators like siderophores such as desferrioxamine (**1**) and enterobactin (**2**) contain hydroxamate and catechol groups, respectively, which exhibit selectivity toward Fe(III) [23].



1 **2** **3**

Using the above knowledge of iron coordination chemistry, cross-linked polymeric hydrogels containing hydroxamic acid and catechol moieties as well as cross-linked polymeric amines were prepared and evaluated as iron chelators [24]. Under in vitro conditions, all of these polymers sequester iron at high pH. At lower pH, the polymers containing hydroxamic acids maintained their iron-binding properties, while other polymers showed poor iron-binding properties. In vivo studies using rodents have shown that the use of a hydroxamic acid-based hydrogel (**3**) has arrested intestinal absorption of dietary iron. The polymers were well tolerated by the test animals, indicating their overall biocompatibility [25].

Recently, Hider and coworkers reported the synthesis and biological evaluation of a series of dendritic polymers containing hydroxypyridinone and catechol moieties on their surfaces. Building on the possibility of presenting excess iron chelating sites on the dendrimer surface, they designed and synthesized a number of polymeric hexadentate ligands for iron. These iron chelating dendrimers were found to possess a high affinity and selectivity toward Fe(III). A functional dendrimer terminated with hydroxyl-pyridinone groups was highly efficient at reducing systemic absorption of iron from the rat intestine [26].

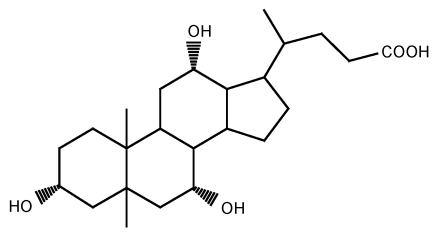
Bile acid sequestrants: Polymeric drugs as cholesterol-lowering agents

Increases in the total cholesterol and low-density lipoprotein cholesterol (LDLc) in the blood are the primary underlying causes for the development of cardiovascular diseases. Therapeutic approaches to reduce elevated LDLc in the serum are the most common treatments for this disease [27]. The most widely used cholesterol-lowering drugs belong to the class of compounds called HMG-CoA reductase inhibitors (more commonly known as statins). However, in spite of their successes, long-term potential safety issues associated with statins have mandated the need for the discovery and development of newer therapies to reduce blood LDLc [28].

Cholesterol is biotransformed into bile acid in the liver, and the bile acid is subsequently secreted to the gall bladder. From the gall bladder, bile acids enter the GI tract, where they act as biological surfactants for the digestion of food. Since bile acid is present in the cholesterol metabolism pathway, effective removal of the bile acids from the bile pool is an attractive approach to treat cardiovascular diseases. Removal of bile acids from the GI tract up-regulate bile acid biosynthesis from the circulating cholesterol, thereby leading to an overall drop in plasma cholesterol levels. Bile acid sequestrants (BASs) are cross-linked polymeric cationic gels that bind anionic bile acids in the GI tract, restrain their reuptake, and result in their elimination from the body [29]. Being non-absorbed, these polymeric drugs do not exhibit the systemic side effects that are associated with statins. Despite the appeal of their safety profiles, a very high dose (~20 g/day) of these cationic polymers are needed to produce any meaningful therapeutic effect. As a result, the two first-generation BASs (cholestyramine and colestipol) have

shown poor patient compliance. This low clinical efficacy of BASs has been ascribed to competition for bile acids with the active bile acid transporter system of the GI tract [30]. From a physical chemistry standpoint, it appears that, for a cationic polymer to be a potent BAS, it must exhibit high binding capacity, strong binding strength, and selectivity toward bile acids in the presence of competing desorbing forces of the GI tract. In other words, a potent BAS needs to exhibit slow off-rates of bound bile acids from the cationic polymer gel to effectively counteract against the active transport of bile acids from the GI tract.

Careful consideration of the structures (**4**) and physicochemical features of bile acids provided valuable insights for the design of the next generation of polymers as potent BASs. Typically, a bile acid possesses an anionic group and a hydrophobic core, which impart their detergent properties. Thus, while electrostatic interaction is the primary driving force behind complexation of bile acids with cationic polymers, hydrophobic interaction between the polymeric sequestrant and the bile acid is an important secondary attractive force contributing to enhanced binding. Furthermore, to enhance the accessibility of binding sites in the polymer gel, favorable swelling characteristics of these cationic hydrogels in physiological environments are required. Thus, the key features of a potent polymeric BAS constitute a balanced combination of hydrophilicity (high capacity) and hydrophobicity (to slow down the rate of desorption), along with an optimum density of cationic groups.



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Synthetic exploitation of the above-mentioned design rules led to the discovery of a number of BASs over the last decade [31–33]. Key structural characteristics of some of the representative new BASs are shown in Fig. 3. The common features of these hydrogels are the presence of amine/ammonium groups in adequate densities along polymer chains. Additional structural features of these polymers include the presence of hydrophobic chains. Different kinds of polymer backbones including vinyl and allyl amine polymers, (meth)acrylates, (meth)acrylamide, styrene, carbohydrates, polyethers, and other condensation polymers have been used to synthesize these hydrogels, which were prepared either by cross-linking copolymerization of appropriate monomers or by post-polymerization chemical modification [34,35]. Although numerous polymers have been synthesized and tested in preliminary in vitro and in vivo studies, very few of these polymers have entered preclinical development and subsequent human clinical trials. From a systematic structure–activity study, a novel hydrophobically modified polymeric amine structure (**5**) was identified as the candidate for clinical development. This polymer showed significantly higher potency than cholestyramine in clinical trials and has been approved under the generic name of colesevelam hydrochloride. It is being sold under the trade name WelChol™ [36].

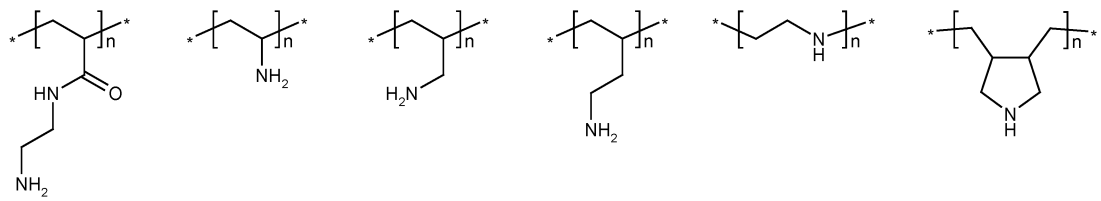
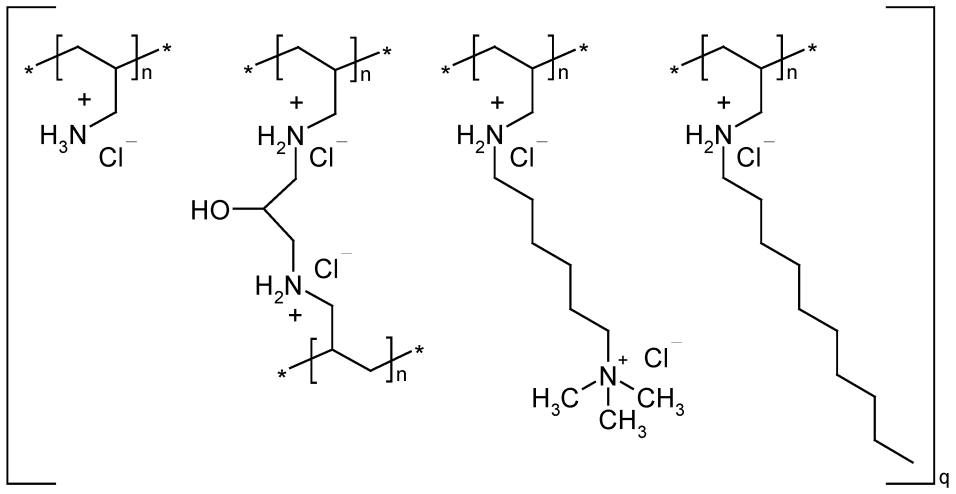


Fig. 3 Representative amine polymers used as building blocks for BAS synthesis.



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Sequestration of toxins: Polymeric anti-infective agents

Toxins are produced by pathogenic microorganisms within a host's body. There are two types of toxins: exotoxins and endotoxins [37]. Exotoxins are generally proteinaceous materials released by these microorganisms. Endotoxins are lipopolysaccharides and consist of polysaccharide segments and glycolipid segments that constitute the outer cell membranes of all Gram-negative bacteria.

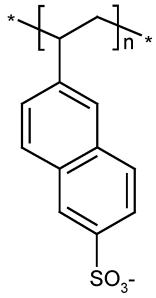
Upon secretion from microorganisms, these toxins can travel within a host organism and can cause damage in organs remote from the initial site of infection. Common pathogenic effects of bacterial toxins include diarrhea, hemolysis, destruction of leucocytes, paralysis, and septic shock. The outcomes of these events can sometimes be life-threatening [38].

Clostridium difficile (*C. difficile*) is a microorganism that releases life-threatening toxins in the GI tract, which are responsible for large numbers of episodes of diarrhea that arise from antibacterial treatment. *C. difficile* releases two high-molecular-weight proteins (toxins A and B), which are primary causes of *C. difficile*-associated diarrhea [39]. The traditional approach to treat *C. difficile*-associated diarrhea has been the use of one of two antibiotics: metronidazole or vancomycin. Although the use of antibiotics is effective in eliminating *C. difficile* infection initially, its repeat use sterilizes the gut, thereby increasing the chance of further infection. Furthermore, there is an increasing chance of antibiotic resistance that further complicates the situation.

Since the disease symptoms are attributed to the toxins released by *C. difficile*, selective removal of these toxins by a polymeric sequestering agent would be potentially an attractive, antibiotics-free therapy to treat *C. difficile* infection. These protein-derived toxins possess multiple binding sites (part of various amino acid side chains) that can effectively interact with multifunctional polymers (carrying complementary binding sites) through simultaneous polyvalent interactions [40]. Polyvalent inter-

actions are characterized by the simultaneous binding interaction between multiple ligands on one molecular entity with multiple receptors on another (cells, viruses, proteins, etc.). These phenomena are frequently encountered in biological systems. Due to a multitude of chelating effects, polyvalent interactions can be significantly stronger than the corresponding monovalent interactions. Polymeric systems, wherein a collection of similar or different ligands can be covalently linked together on a single polymer chain, provide a unique scaffold, which leads to binding of macromolecular targets by the polymeric ligands with high binding strength. This translates into longer-lasting and more potent therapeutics.

Systematic studies in our laboratories have led to the discovery of some novel polymeric multivalent ligands as non-antimicrobial sequestrants that effectively bind and neutralize these toxins. Amongst various polymers, a series of high-molecular-weight, water-soluble anionic polymers (e.g., **6** and **7**) have proven to be particularly effective in sequestering and neutralizing *C. difficile* toxins. The general characteristics of these polymers are the presence of sulfonic acid groups and their high molecular weight. Careful in vitro experiments revealed that the binding constants of complexes between this polymer and toxins A and B were 133 nM and 8.7 μM, respectively. More importantly, these polymers, under in vivo conditions, prevented the mortality of 80 % of hamsters with severe *C. difficile* colitis [41].



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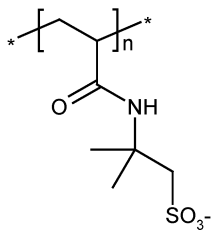

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From an extensive structure–activity relationship (SAR) study, a potent polymer was selected as the lead candidate for clinical development. This compound, under the generic name "tolevamer", has been successful in a phase II human clinical trial. In this clinical study, tolevamer was found to rapidly resolve *C. difficile*-associated diarrhea with a potency similar to vancomycin. The compound is currently undergoing a pivotal phase III human clinical trial.

POLYMERIC DRUGS: OTHER DEVELOPMENTS

Functional polymers have been increasingly evaluated as therapeutic agents for the treatment of a variety of disease conditions. Due to space limitation, it is not possible to elaborate on these examples. One of the most significant polymeric compounds is the amino acid-derived random copolymer, Copaxone, which has been widely used for the treatment of multiple sclerosis. Other examples include a dual-acting lipase inhibitor-fat binder for the treatment of obesity, polyvalent antiviral and antimicrobial agents, and polymers for the treatment of sickle cell anemia [42].

CONCLUDING REMARKS

The design and syntheses of functional polymers showing inherent therapeutic efficacy is an exciting field of biomedical polymer research. The discovery and development of newer biologically active

polymers will lead to novel human therapeutics. In the present article, we have attempted to illustrate, with specific examples, the extraordinary potential of some of the polymers. By careful consideration of both disease targets and their mechanisms of action, polymers exhibiting pharmacological properties can be developed into marketed products. Capitalizing on the unique physicochemical properties of polymer materials, new classes of therapeutically active drugs can be developed, which cannot be achieved with traditional small-molecule drugs. Finally, the ability to control the structural and functional diversities of polymers in combination with an improved understanding of the mechanism of various complex diseases such as cancer should lead to the next generation of polymeric drugs for systemic applications.

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