This article was downloaded by:
On: *24 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

AMINE FUNCTIONALIZED POLYETHERS AS BILE ACID SEQUESTRANTS: SYNTHESIS AND BIOLOGICAL EVALUATION

Chad C. Huval[a]; Matthew J. Bailey[a]; S. Randall Holmes-Farley[a]; W. Harry Mandeville[a]; Karen Miller-Gilmore[a]; Robert J. Sacchiero[a]; Pradeep K. Dhal[a]
[a] GelTex Pharmaceuticals, Inc., Waltham, MA, U.S.A.

Online publication date: 30 November 2001

To cite this Article Huval, Chad C. , Bailey, Matthew J. , Holmes-Farley, S. Randall , Mandeville, W. Harry , Miller-Gilmore, Karen , Sacchiero, Robert J. and Dhal, Pradeep K.(2001) 'AMINE FUNCTIONALIZED POLYETHERS AS BILE ACID SEQUESTRANTS: SYNTHESIS AND BIOLOGICAL EVALUATION', Journal of Macromolecular Science, Part A, 38: 12, 1559 — 1574
To link to this Article: DOI: 10.1081/MA-100108405
URL: http://dx.doi.org/10.1081/MA-100108405

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

J. MACROMOL. SCI.—PURE APPL. CHEM., A38(12), 1559–1574 (2001)

AMINE FUNCTIONALIZED POLYETHERS AS BILE ACID SEQUESTRANTS: SYNTHESIS AND BIOLOGICAL EVALUATION

Chad C. Huval,* Matthew J. Bailey, S. Randall Holmes-Farley, W. Harry Mandeville, Karen Miller-Gilmore, Robert J. Sacchiero, and Pradeep K. Dhal*

GelTex Pharmaceuticals, Inc., 153 Second Avenue, Waltham, MA 02451

Downloaded At: 12:06 24 January 2011

Dedicated to the memory of Professor Sukant K. Tripathy.

ABSTRACT

A series of amine functionalized polymers based on polyether backbones was prepared by the chemical modification of poly(epichlorohydrin) and poly(2-chloroethylvinyl ether). Nucleophilic substitution of pendant chloroalkyl groups offers a versatile route to prepare hydrophilic, cationic polymers. Through the choice of appropriate experimental conditions, including solvent, temperature, and amine reagent, a high degree of substitution at the chloromethyl groups can be achieved. Depending on the nature of the amine used, both water-soluble and amphiphilic cationic polymers were obtained. Crosslinked hydrogels were prepared by either subsequent crosslinking of the amine functional polyethers or by reaction of chloroalkyl polyethers with multifunctional amines. These amine functional polyethers exhibited promising bile acid sequestration properties during *in vivo* experiments using hamsters as animal models, providing a novel approach for treating hypercholesterolemia. Some of these polymers show efficacy superior to commercially available bile acid sequestrants. The results suggest that these novel polyammonium gels may be useful as cholesterol lowering agents.

*Corresponding authors.

Copyright © 2001 by Marcel Dekker, Inc. www.dekker.com

Key Words: Polyether; Cationic hydrogel; Polymeric amine; Cholesterol lowering; Polymeric drugs

INTRODUCTION

Bile acid sequestrants are a class of cationically-charged, biomedical polymers that serve as important therapeutic agents for the treatment of hypercholesterolemia [1, 2]. The primary mechanism of action of these polymers involves electrostatic interactions between anionically-charged bile acids and cationically charged polymer gels in the gastrointestinal (GI) tract and the excretion of the resulting polymer-bile acid complexes with the feces. In the liver this phenomenon triggers an increase in the rate of bile acid biosynthesis from cholesterol. Since most of the cholesterol is derived from the plasma, the net effect is manifested in a reduction of serum cholesterol [3]. These polymeric drugs are nonabsorbed, and as a result they do not present systemic side effects that are associated with other well-known cholesterol lowering agents such as hydroxymethyl glutamate coenzyme A (HMG-CoA) reductase inhibitors (e.g. statins).

The success of a cationic polymer as an efficient bile acid sequestrant depends upon its high binding capacity and strong binding strength towards bile acids in the competing desorbing forces of the GI tract. Thus, a potent bile acid sequestrant needs to exhibit slow off-rates of bound bile acids from the polymer resin to effectively overcome the active transport of bile acids from the lumen through the ileum wall. Research efforts aimed at enhancing the effectiveness of bile acid sequestrants through the rational design of polymer structures that incorporate high swelling in physiological environments (to increase capacity) and additional binding interactions like hydrophobic interactions (to slow down the rate of desorption) has led to the discovery of a number of highly potent bile acid sequestrants [4, 5].

We have pursued a concerted effort to discover potent bile acid sequestrants by incorporating desired features in various polymer backbones [6, 7]. In this paper, we describe the syntheses and characterization of a series of amine functionalized polymer gels based on polyether backbones and their evaluation as bile acid sequestrants.

MATERIALS AND METHODS

General

Poly(epichlorohydrin) (PECH) (MW = 700,000) was purchased from Aldrich Chemical Co. Unless stated otherwise, all reagents and solvents were obtained from Aldrich. Solvents were of analytical grade and were used as received. Elemental analyses were carried out at QTI Laboratories.

Downloaded At: 12:06 24 January 2011

Functionalization of Poly(Epichlorohydrin) with Various Amines

The modification of PECH with different amines was carried out under similar conditions. Some typical examples of synthesis are given below.

Functionalization of Pol(Epichlorohydrin) with Dimethylamine

To a 1-L, three-necked, round-bottomed flask equipped with a jacketed condenser with circulating water/ethylene glycol mixture chilled to –10°C, a mechanical stirrer, a heating mantle and a thermometer was added 20.0 g of finely cut PECH, 200 mL of 40% aqueous solution of dimethylamine, and 200 mL of water. The reaction mixture was stirred and heated to 70°C for 24 hours. After 24 hours, a white jelly-like solid had formed. The reaction mixture was transferred to a blender along with 100 mL of 40% dimethylamine solution. The mixture was blended at high speed for one minute, breaking up the solid and forming a white, smooth, and thick mixture. This mixture was transferred to the reaction flask. The blender was rinsed with 100 mL of the 40% dimethylamine solution and was combined with the reaction mixture. The mixture was subsequently heated to 70°C.The mixture became extremely thick. The heating was continued for another 96 hours. A 200 mL portion of 40% dimethylamine was then added and the mixture was stirred with a mechanical stirrer. The temperature was then increased to 100°C for 2.5 hours. After cooling the reaction mixture to room temperature, the solvent was removed using a rotary evaporator. Subsequently, the residue was dried overnight in a forced air oven at 60°C, yielding 31.4 g of the polymer as an off-white solid.

Downloaded At: 12:06 24 January 2011

Modification of Dimethylamine Functionalized Poly(Epichlorohydrin) with 1-Bromodecane

To a 1-L, three-necked, round-bottomed flask equipped with an air condenser, a mechanical stirrer, a heating mantle and a thermometer was added 10.0 g of dimethylamine functionalized polyepichlorohydrin, 44.2 g of 1-bromodecane, 150 mL of isopropanol and 50 mL of water. The resulting mixture was heated to 70°C. The pH was adjusted to 12 by adding 6.3 g of 50% aqueous sodium hydroxide. Concentrated HCl (1.2 g) was then added to adjust the pH to 10.8.After 1 h, the pH decreased to 8.25. The pH was adjusted to 10.6 by adding 1.1 g 50% aqueous NaOH, and heating was continued at 70°C. After 3 more hours, the pH was 8.20 and was adjusted to 11.5 by adding 0.7 g 50% aqueous NaOH.Concentrated HCl (0.5 g) was then added to lower the pH to 10.07. After an additional 90 minutes, the pH was 9.095, and 0.2 g 50% aqueous NaOH was added to increase the pH to 11.6. Conc HCl (2 drops) was then added to adjust the pH to 10.9. The reaction mixture was heated for an additional 24 hours. After cooling down to room

temperature, the reaction mixture was filtered. The residue was washed sequentially three times with 750 mL of methanol and two times with 750 mL of 2 M NaCl, and once with 4.0 L of deionized water. It was subsequently stirred in 1.0 L of deionzied water.The stirring slurry had a conductivity of 0.37 mS/cm and a pH of 5.13.Concentrated HCl (3.0 mL) was then added and the stirring continued for 20 minutes. After filtration, the residue was dried at 60°C in an oven for 16 hours yielding 8.4 g of an off-white solid.

Functionalization of Poly(Epichlorohydrin) with Ammonia, Followed by Crosslinking with Epichlorohydrin

To a 1 L, three-necked, round-bottomed flask equipped with a jacketed condenser with a circulating water/ethylene glycol mixture chilled to –10°C, a mechanical stirrer, a heating mantle and a thermometer was placed 10g of PECH and 400 mL of DMF. After the polymer was completely dissolved, the flask was cooled using an ice bath. To this cold polymer solution 10 g of ammonia gas was transferred. The reaction mixture was stirred overnight at room temperature. The solution was subsequently heated to 90°C. After 24 hours, a cloudy pink solution was obtained. The reaction mixture was cooled to room temperature. At this point, the pH of the reaction mixture was 10.4.After removing the solvent under reduced pressure, the residue was suspended in a mixture of 40 mL of deionized water and 50 mL of methanol and heated to 70°C. Aqueous sodium hydroxide (50%) was added to bring the pH to 6.3. The solvent was decanted, and 40 mL of DMF was added to the remaining sludge. The mixture was heated to dissolve the solids and then cooled to room temperature, yielding a deep rose-colored solution of pH 10.2.Epichlorohydrin (0.497 g, 0.00537 mol) was then added and the solution was covered with plastic wrap and stirred at room temperature for three days. An additional 0.497 g (0.00537 mol) of epichlorohydrin was then added and the solution was heated gently in a water bath. After several hours, the mixture gelled. The gel was then blended with 500 mL of deionzed water, filtered and washed several times with water. The residue was dried at 60°C in an oven yielding 4.8 g of light pink solid.

Modification of Ammonia Functionalized Poly(Epichlorohydrin) with (4-Chlorobutyl)dimethyldodecyl-ammonium Bromide

To a 500 mL, three-necked, round-bottomed flask equipped with an air condenser, a mechanical stirrer, a heating mantle and a thermometer was added 4.2 g of ammonia functionalized crosslinked PECH gel,34.9 g of 70% methanolic solution of (4-chlorobutyl)dodecyldimethylammonium bromide and 62.7 mL of water. The pH of the mixture was adjusted to about 10 by adding 50% aqueous NaOH. The resulting mixture was heated to 70°C. After 30 minutes, the pH had dropped

Downloaded At: 12:06 24 January 2011

to 7.4.The pH was adjusted to about 10 with 50% aqueous NaOH.Heating at 70°C was continued for a total of 22 hours, after which the pH was about 2.0. The reaction mixture was transferred to a blender and blended gently for one minute. The blended reaction mixture was transferred back to the flask and 34.9 g of (4-chlorobutyl)-dodecyldimethylammonium bromide (70% solution in methanol) was added. The pH was adjusted to 9.9 with 50% aqueous NaOH. After 2 hours, the pH was about 1.5 and was adjusted to about 11.1 with 50% aqueous NaOH. After stirring at 70°C for 16 hours, the reaction mixture was cooled to room temperature and filtered, yielding a brown, gummy solid. The residue was washed sequentially with methanol (3 X 500 mL), 2 M NaCl (3 × 350 mL), and deionized water (3 × 500 mL). After filtration, the residue was stirred in 1 L of deionized water for 30 minutes. The conductivity of the suspension was 0.45 mS/cm and the pH was 5.0. Concentrated HCl (2.0 mL) was then added to adjust the pH to 2.5. The mixture was filtered and dried at 60°C in a forced-air oven for 16 hours, yielding 3.2 g of the polymer.

Downloaded At: 12:06 24 January 2011

Crosslinking of Poly(Epichlorohydrin) with Diaminododecane Followed by Alkylation with Trimethylamine

To a 2 L, three-necked, round-bottomed flask equipped with a jacketed condenser with circulating water/ethylene glycol mixture chilled to –10°C, a mechanical stirrer, a heating mantle, and a thermowatch was added 20.0 g of PECH and 240 mL DMF. The resulting mixture was stirred to dissolve the polymer. The solution was heated to 70°C and 21.7 g 1,12-diaminododecane was added. The solution gelled after about two hours.The heating was continued for 4 hours. After cooling down to room temperature, the reaction mixture was stirred for 64 hours at room temperature. After adding 470 mL DMF, the mixture was stirred for 3 hours forming a swollen gel. Isopropanol (50 mL) was added and the mixture was cooled in an ice bath. After bubbling in 160.2 g of trimethylamine the reaction mixture was stirred for 14 hours at room temperature. The temperature was gradually raised to 70°C over the course of 8 hours, and the reaction mixture was stirred at this temperature for 14 hours. The temperature was increased to 80°C for 4 hours, to 90°C for 14 hours, and finally to 95°C for 18 hours. After cooling down the reaction to room temperature, the contents were transferred to an 8 L bucket. Water (4 L) was added and the mixture was stirred for 2 hours. The aqueous suspension was acidified with 35.6 g concentrated HCl (to pH 1.74).The suspension was allowed to settle for several hours, and the supernatant was poured and reserved in a clean beaker. The solid residue was dried at 60°C in an oven for 14 hours, yielding 10.4 g of the polymer. The supernatant was allowed to settle for 72 hours, the solvent was decanted, and the residue was dried at 60°C in an oven for 14 hours, yielding 9.3 g of the polymer, Thus, the total isolated yield of the modified polymer was 19.7 g.

Simultaneous Crosslinking and Functionalization of Poly(Epichlorohydrin) with Diaminododecane

A mixture of PECH (10.0 g) and DMF (120 mL) was heated at 90°C for 4 hours until all of the PECH has dissolved. Then, 1,12-diaminododecane was added, and the mixture was heated at 70°C for 32 hours. A gel formed after the first 3 hours of heating. After cooling to room temperature, the polymer gel was blended with methanol (1.5 L), and the mixture was allowed to settle. The top layer was decanted from the bottom layer, and the bottom layer was filtered. The filtered polymer was suspended in deionized water (1/2 L) and conc HCl was added to the suspension until pH 1.4. After stirring for 10 minutes, the suspension was filtered, and the wet polymer was dried in a forced-air oven at 60°C to provide 12.4 g of a solid.

Synthesis of Poly(2-Chloroethyl Vinyl Ether)

Downloaded At: 12:06 24 January 2011

To an oven-dried, 250-mL, three-necked, round-bottomed flask was added 100 mL of anhydrous dichloromethane and 25 g of freshly distilled 2-chloroethylvinyl ether. The reaction mixture was stirred and bubbled with a slow stream of nitrogen for 30 min at room temperature. While stirring under nitrogen, it was cooled to –70°C. At –70°C, 0.5 mL of borontrifluoride diethyl etherate was added to the reaction mixture. After stirring at –70°C for 1 hour, the reaction mixture was allowed to warm to room temperature. The reaction mixture was poured into 500 mL of MeOH and stirred for 30 minutes. The solvent was removed and the residue was redissolved in 150 mL of THF. The resulting solution was poured into 500 mL of MeOH. The solvent was removed and the precipitate was dried at 50°C under vacuum for 24 hours, yielding 21 g of the polymer as an off-white rubbery solid.

Modification of Poly(2-Chloroethyl Vinyl Ether) with Diethylenetriamine

To a 250-mL, three-necked, round–bottomed flask was added 10 g of poly(2-chloroethyl vinyl ether). The polymer was dissolved in 125 mL of THF. To the polymer solution was added 10 g of diethylenetriamine. The reaction mixture was stirred at room temperature for 30 minutes, and then the temperature was raised to 65°C. The solution gelled after 2 hours. The reaction temperature was maintained at 65°C for 24 hours, and was subsequently allowed to cool to room temperature. The solid mass was filtered. The residue was dispersed in 200 mL of isopropanol and stirred for 30 minutes. The polymer was filtered, dispersed in 250 mL of methanol and stirred for 30 minutes. After filtration, the polymer was dispersed in 300 mL of deionized water and the resulting suspension was stirred for 30 minutes and filtered. The filtered polymer was dispersed in 250 mL of deionized water and

to it was added 100 mL of 2M HCl. The suspension was stirred for 30 minutes and filtered. The filtered polymer was dried in a forced-air oven at 60°C, yielding 18 g of the product as an off white solid.

Modification of Poly(2-Chloroethyl Vinyl Ether) with 1,4-bis(3-Aminopropyl) Piperazine

To a 250 mL, three-necked, round–bottomed flask was added 5.5 g of poly(2-chloroethyl vinyl ether). The polymer was dissolved in 60 mL THF. To the polymer solution was added 2.8 g of 1,4-bis(3-aminopropyl)piperazine. The reaction mixture was stirred at room temperature for 30 minutes, and the temperature was subsequently raised to 65°C. The reaction temperature was maintained at 65°C for 24 hours, and was subsequently allowed to cool. The polymer gel was broken into smaller pieces and was dispersed in 400 mL of methanol. After stirring the dispersion for 30 minutes, the polymer was filtered. This methanol treatment was repeated one more time and the filtered polymer was dispersed in 300 mL of deionized water. The polymer suspension was stirred for 30 min and filtered. The filtered polymer was dispersed in 250 mL of deionized water and to it was added 100 mL of 2 M HCl. The suspension was stirred for 30 minutes and filtered. The filtered polymer was dried in a forced-air oven at 60°C yielding 10 g of the product as an off white solid.

Downloaded At: 12:06 24 January 2011

Evaluation of *In Vivo* Bile Acid Sequestration Properties

After a week of acclimation to the facility, hamsters were transferred to special cages that separate urine and feces. In order to synchronize their urge for food as a group, the animals were given only water for 24 hours. Following the 24-hour fast, they were presented a casein-based purified feed with 10% fat added plus a predetermined amount of the drug. The control group was given no drug. The food was presented for a 72-hour period. Fecal material was collected for 63 hours; from the 9^{th} to the 72^{nd} hour. The fecal material was freeze-dried to remove water. The dry feces were pulverized with an amalgamator to a uniform powder, and 1 g was placed in the extraction cell. A solution of 80% methanol in 100 mM NaOH was used as the extraction solvent since it is a solvent in which most bile acids are sufficiently soluble and is basic enough to hydrolyze bile acid esters. The esters commonly occur in the feces and become difficult to extract if not hydrolyzed. The extraction was accelerated by holding the sample and solvent at 100ºC under a pressure of 1500 psi. A portion (0.25 mL) of the extract was evaporated and reconstituted in bovine calf serum. The sample was then analyzed like a standard serum sample, enzymatically, for bile acid concentration [8]. The concentration was multiplied by four times the volume of extract and expressed as the concentration per gram of feces.

RESULTS AND DISCUSSION

We have presumed that the following structural features of a cationic polymer gel are necessary for potent bile acid sequestration: (a) adequate swelling characteristics in the physiological environment; (b) adequate cationic charge density and; (c) incorporation of additional binding features to enhance self aggregation of bile acids on polymer resins [9]. Cholestyramine (**1**) and colesevelam hydrochloride (**2**) are two examples of FDA approved commercial bile acid sequestrants that are used as pharmaceutical agents for cholesterol reduction. While both of these polymers contain quaternary amine groups, colesevelam hydrochloride is a far superior bile acid sequestrant. A comparison of their structural features reveals that both sequestrants contain hydrophobic regions: cholestyramine contains an aromatic ring as the hydrophobe and colesevelam hydrochloride contains aliphatic hydrophobic carbon chains. However, colesevelam hydrochloride possesses a higher charge density per repeat monomer unit than cholestyramine. The high charge density of colesevelam hydrochloride provides more cationic sites for the anionic groups of bile acids and the aliphatic hydrophobic chains of colesevelam hydrochloride are considered to lend additional hydrophobic interactions to the steroid skeletons of bile acids. These combined features are manifested in an increased affinity of colesevelam hydrochloride toward bile acids, slowing down the desorption of bound bile acids by effectively competing with active transport in the GI tract.

We felt that cationic polymer gels with more hydrophilic backbones and with hydrophobic side chains would constitute an interesting class of bile acid sequestrants. While hydrophilic backbones would impart improved aqueous swelling properties, the hydrophobic side chains would provide enhanced binding to the steroid backbone of bile acids. These features would result in an overall increase in potency of these polymers as bile acid sequestrants.

Downloaded At: 12:06 24 January 2011

Synthesis and Characterization of Amine Functionalized Polyethers

We chose to investigate poly(alkyleneglycol) based polymer backbones with pendant amines and hydrophobic groups as target polymer structures for bile acid sequestration. Chemical modification of reactive polymers to prepare novel multifunctional polymers is well known in the literature [10]. Our synthetic strategy involved using polymers of 1,2-epoxy-ω-haloalkanes as precursor materials. The pendant alkyl halide groups of these polymers can be reacted with amines to produce amine-functionalized polyethers (see Figure 1). For example, commercially available poly(epichlorohydrin) (PECH) is a reactive elastomer. Nucleophilic substitution reaction at the carbon-chlorine bond of PECH (**3**) with nucleophiles such as phenolates has been carried out to produce functional polymers [11]. We felt that the potential reactivity of the chloromethyl groups of PECH towards amines would provide a novel synthetic strategy to prepare our proposed bile acid seques-

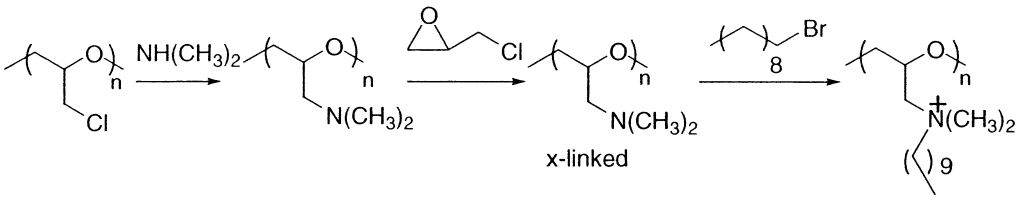

Figure 1. Chemical modification of poly(epichlorohydrin) to prepare amine functionalized hydrogel.

trants. Furthermore, we have utilized poly(2-chloroethyl vinyl ether) (**4**) as another polyether based chloroalkyl functional polymer as the precursor to prepare amphiphilic amine containing functional polymers.

Commercial PECH of number average molecular weight 700, 000 is an elastomeric material. This polymer is soluble in various organic solvents such as toluene, N,N-dimethylformamide (DMF), low molecular weight ketones (e.g. acetone), chloroform, tetrahydrofuran (THF), etc. On the other hand, the polymer is insoluble in water, methanol, ethanol, acetonitrile, ethers, and hydrocarbon solvents such as hexane. Our initial attempts to prepare amine functional polyethers by reacting PECH with low molecular weight amines such as dimethylamine and ammonia in hot DMF, THF, or dioxane at 70°C were largely unsuccessful, in agreement with earlier observations of the poor reactivity of the chloromethyl group of this polymer towards nucleophilic substitution reactions. The poor reactivity of the chloromethyl group of this polymer was attributed to the modest leaving group characteristic of aliphatic chlorides coupled with the role of the β-branch point in depressing the reactivity [12]. By carefully optimizing experimental conditions such as temperature and solvents, however, it became possible to substitute the chloromethyl group of PECH with both dimethylamine and ammonia. Thus, an adequate degree of functionalization was achieved (without sophisticated high-pressure reactors) by reacting an aqueous suspension of finely cut particles of PECH with aqueous solutions of the amine by adding the latter in several portions. After a desired amount of reaction time, the polymer was isolated from the reaction medium. If additional substitution was desired, the isolated polymer was subjected to further reaction by dispersing it further in fresh aqueous solutions of the amines. These amine-functionalized polyethers were washed and dried. Depending on the degree of substitution, the amine functionalized polyether derivatives were either swellable or soluble in water. These polymers can be subjected to further modifications. For example, reaction of these polymers with epichlorohydrin led to insoluble hydrogels, which were subsequently reacted with long chain alkyl halides (e.g. 1-bromodecane) producing amphiphilic cationic hydrogels. The cascade of chemical modification reactions to produce these cationic polyether based hydrogels is illustrated in Figure 1.

Amphiphilic amine functionalized polyethers were prepared in a single step by reacting PECH with long chain alkyl amines. These reactions were generally

Downloaded At: 12:06 24 January 2011

carried out by reacting PECH and the appropriate amine in DMF at elevated temperatures. We were able to substantially substitute PECH with various primary and secondary alkyl amines (e.g. dodecylamine, butylamine, and N-methyloctadecylamine) by carrying out reactions in DMF in the temperature regions of 70-90°C. The extent of substitution in all cases was established from elemental analyses of the modified polymers (C/N molar ratio). Unfortunately, our attempts to react PECH with tertiary amines such as dimethyldodecyl-amine or dimethylbutylamine were unsuccessful. These reactions usually led to degradation of the polymer (based on low polymer recoveries and very high C/N molar ratios). The results of these modification reactions such as compositions of amine functionalized PECH, reaction conditions, polymer solubility, etc., are summarized in Table 1.

PECH could be crosslinked by reaction with difunctional primary and secondary amines, such as 1,12-diaminododecane, 1,4-diaminobutane, or N,N'-dimethyl-1,6-hexanediamine. In line with our earlier observation, its reaction with bifunctional tertiary amines (e.g. *N,N,N'N'*-tetramethyl-1,6-hexanediamine) was unsuccessful. These crosslinked gels were insoluble but swelled in solvents that dissolve the linear polymer, such as DMF. The cross-linked gels could be further substituted by reacting the swollen gels with different primary or secondary amines.

Since the effectiveness of an amine functional polymer as a bile acid sequestrant depends on the concentration of amine groups in the polymer chain, it is

Downloaded At: 12:06 24 January 2011

Table 1. Syntheses of Amine Modified Polyether Based Cationic Hydrogels by Chemical Modification of Poly(Epichlorohydrin)

Polymer	Amine Reagents	Temp (ºC)	Reaction Time (h)	Solvent	Composition (C/N)
P1	Dimethylamine	90	120	H_2O	4.98
P2	Polymer **P1** + 1-bromodecane	70	30	Isopropanol + H_2O	13.01
P3	1,12-diaminododecane + trimethylamine	95	101	DMF	6.91
P4	1,12-diaminododecane	70	32	DMF	9.92
P5	1,12-diaminododecane + 1-aminobutane	70	96	DMF	5.17
P6	1,12-diaminododecane + 1-(dimethyl)aminobutane	70	96	DMF	17.15
P7	Polymer **P4** + CH_3I	25	72	CH_3OH	11.92
P8	1-aminododecane	70	96	DMF	28.17
P9	N,N'-dibutyl-1,6-hexanediamine	70	12	DMA	32.00
P10	4,4'-methylenebis-(cyclohexylamine)	70	12	DMA	11.41
P11	1,12-diaminododecane + 1-aminododecane	70	31	DMF	n.d.

desirable to incorporate a higher concentration of amine groups per repeat units of the polymer chain. One appealing way to incorporate a higher concentration of amine groups onto a polyether chain is by reacting PECH with unsymmetrical bifunctional di- and tri- amines (containing primary/secondary and tertiary amine groups). Since PECH is inert towards tertiary amines but react with primary or secondary amines, these reactions produce amine functional polyethers with pendant tertiary amine groups. For example, reaction of PECH with 3-dimethylaminopropyl-diamine produced a water-soluble polymer. The resulting polymer was crosslinked with epichlorohydrin and was subsequently alkylated with bromodecane to produce a hydrophobically modified cationic hydrogel. Crosslinking of PECH with 1,12-diaminododecane followed by reaction with 3-dimethylaminopropylamine also offered hydrophobically modified cationic gel suitable for bile acid sequestration purpose. The reaction steps for the syntheses of this class of amine functionalized polyethers are illustrated in Figure 2. This polymer exhibited significant swelling in water.Results of the syntheses of these amine-functionalized hydrogels are summarized in Table 2.

Interestingly, the reaction of PECH with equimolar amounts of symmetrical diamines has been found to be a convenient way to introduce the necessary ammonium groups and the hydrophobic groups to polyether backbones simultaneously. During the reaction of PECH with equimolar amounts of symmetrical diamines, a portion of the diamines constitute crosslinking units while the remainder result in pendant ammonium groups tethered to the polyether backbone. This reaction scheme is illustrated in Figure 3. Following this procedure, several novel bile acid sequestrants were synthesized.

We have also utilized poly(2-chloroethyl vinylether) as another reactive polyether resin to prepare amine-functionalized polymers. This polymer **(4)** contains alkyl halide groups that can be subjected to nucleophilic substitution reactions with various amines. The polymer **4** has a carbon-carbon backbone bearing a pendant ether group. The polymer was prepared by cationic polymerization of 2-chloroethyl vinyl ether using borontrifluoride-diethyl etherate as the initiator. Purification by repeated dissolution and reprecipitation offered the polymer **4** as an elastomeric solid.

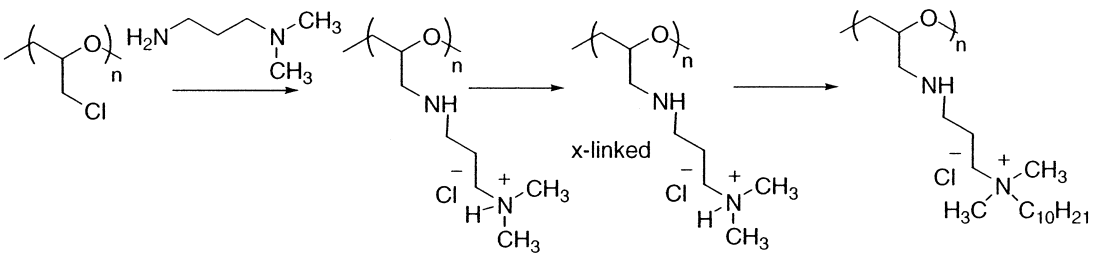

Figure 2. Chemical modification of poly(epichlorohydrin) to prepare polyamine gels with high cationic charge density.

Downloaded At: 12:06 24 January 2011

Table 2. Syntheses of Amine Modified Polyepichlorohydrin Based Cationic Hydrogels Bearing Mutifunctional Pendant Amine Substituents

Polymer	Amine Reagents	Temp (°C)	Reaction Time (hr)	Solvent	Composition (C/N)
P12	1,12-diaminododecane 3-dimethylaminopropyl-amine	+70	42	DMF	3.79
P13	3-dimethylaminopropyl-amine	70	23	DMF	8.20
P14	Polymer **P12** + epichlorohydrin	25	18	H_2O	8.34
P15	Polymer **P13**+ 1-bromodecane	75	18	Isopropanol + H_2O	8.35
P16	tris(2-aminoethyl)-methylamine	70	12	DMA	3.49
P17	N,N'-bis(3-aminopropyl)-ethylenediamine	70	12	DMA	4.07

Downloaded At: 12:06 24 January 2011

Transformation of **4** to amine functionalized polymers was achieved by adapting different well-known polymer modification reactions. For example, primary amine groups were introduced to the polymer chain by the Gabriel pthalimide synthesis protocol (see Figure 4) [13]. The resulting poly(2-aminoethyl vinyl ether), **5**, is a water-soluble polymer. Reaction of **5** with 1,4-butanediol diglycidyl ether gave a crosslinked gel, which was subsequently reacted with 1-bromodecane to produce the target hydrophobically modified cationic hydrogel.

Besides the above procedure, reaction of **4** with multifunctional amines offers amine-containing polyethers similar to PECH. Thus, **4** was reacted with diethylene triamine and 1,4-bis(3-aminopropyl)piperizine in a manner analogous to our PECH modification. The reactions produced hydrophilic polymer gels.

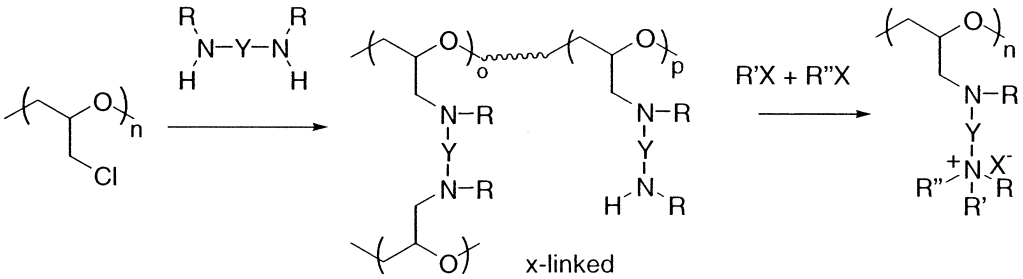

Figure 3. Simulatneous crosslinking and functionalization of poly(epichlorohydrin) to amine functionalized hydrogel.

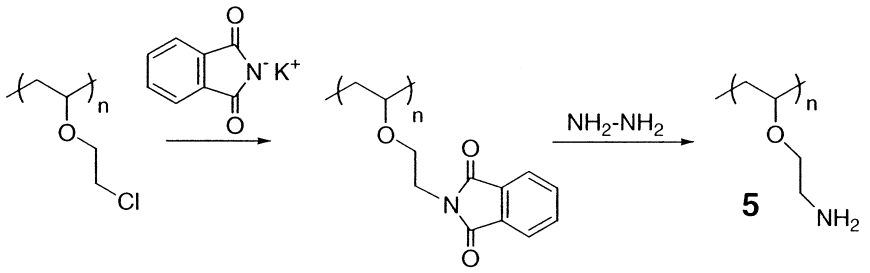

Figure 4. Transformation of poly(2-chloroethyl vinyl ether) to poly(2-aminoethyl vinyl ether).

Evaluation of *In Vivo* Bile Acid Sequestration Properties

The bile acid sequestration properties of these polyammonium polyether resins were evaluated *in vivo* using hamsters as the animal models. Most of the published literature on the development of novel bile acid sequestrants has relied on *in vitro* experiments. Some of these publications claim that the binding measurements were carried out under conditions that are likely to be encountered *in vivo* [14]. However, in addition to the presence of a gradient of pH and ionic strengths in the GI tract, active uptake of bile acids is known to be mediated by transporters in the GI tract. None of these *in vitro* experiments have taken this transporter mediated uptake factor into consideration, which would significantly affect the *in vivo* bile acid binding efficacies of these sequestrants. Therefore, we prefer to evaluate the bile acid binding abilities of newly synthesized sequestrants under true biological conditions. We feel such a study provides a better picture of the potency of sequestrants.

The bile acid binding properties of these polymers were determined by feeding hamsters with polymers and measuring the bile acid contents of their feces. An increase in bile acid contents of the feces of treated animals (in comparison to control animals) provides a direct measurement of the sequestering abilities of these polymers under physiological conditions. The fecal bile acid output of polymer treated animals are summarized in Table 3. Both bile acid mass excreted (μmol/g) as well as percentage above the untreated control groups are given for comparison. Furthermore, the relative sequestering efficacies of different polymers can be visualized from Figure 5.

Perusal of the data provides some insight on the structure-activity relationships with respect to these amine functionalized polyether resins.PECH functionalized with shorter chain alkyl amines showed modest bile acid sequestration properties (Table 3, polymers **1**, **3** and **16**). However, the addition of hydrophobic chains to these polymers brought about a significant increase in efficacy.The hydrophobic groups were incorporated either by quaternization of the pendant tertiary amine groups on the PECH or by the reaction of PECH with longer chain alkyl amines. For example, dimethylamine modified PECH (polymer **1**) was found to sequester 162% bile acid over the control. This polymer upon subsequent

Downloaded At: 12:06 24 January 2011

Table 3. *In Vivo* Bile Acid Sequestration Results for Amine Functionalized Polyether Based Cationic Hydrogels

Polymer	Dose in Feed (w/w in %)	Bile Acid Mass Excreted in Feces (μmol/g)	% Above Control
P1	0.2	2.44	162
P2	0.2	4.60	395
P3	0.2	1.55	66
P4	0.2	3.79	524
P7	0.2	3.23	246
P10	0.1	2.77	211
P11	0.2	4.41	241
P15	0.2	5.70	511
P16	0.1	1.68	89
P17	0.1	1.63	83
Cholestyramine	0.3	3.00	125
Colestipol	0.3	2.31	110

Downloaded At: 12:06 24 January 2011

modification with 1-bromodecane resulted in 395% bile acid sequestration over the control (polymer **2**). The increased efficacy of these amine functionalized polyethers towards bile acid sequestration (more than two-fold) by incorporating hydrophobic substituents is significant. The synergistic effect of hydrophobic alkyl substituents on *in vivo* bile acid sequestration appears to be a general trend for these polymers. For example, the simultaneous crosslinking/functionalization of PECH with 1,12-diaminododecane also brought about more than a two-fold increase in sequestration in comparison to corresponding amine polymers substi-

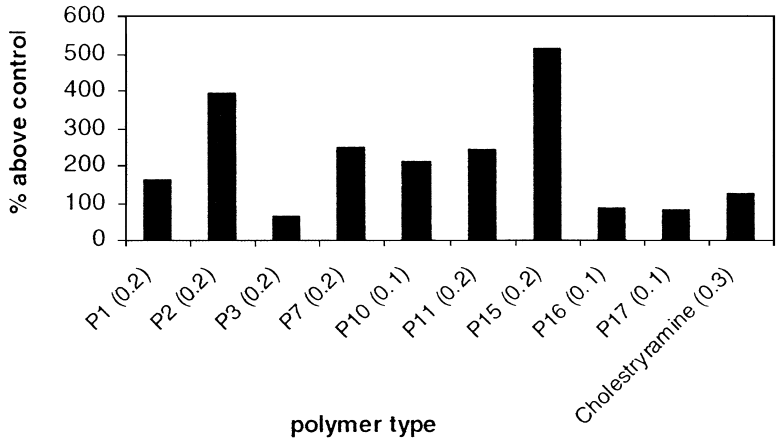

Figure 5. *In vivo* bile acid sequestration properties of various amine functionalized polyether hydrogels.

tuted with short chain hydrophilic amines (Table 3, polymers **15** *vs.* **16**). Some of these polymers exhibit far superior bile acid sequestration properties than commercially available sequestrants such as cholestyramine and colestipol.

Enhanced *in vivo* sequestration properties of hydrophobically modified cationic polyether based hydrogels over corresponding polymers modified with hydrophilic shorter chain alkyl amines can be explained on the basis of the physiology of bile acids in the GI tract. Cationic polymer resins bind anionically charged bile acids in the lumen of the intestine. Under *in vitro* conditions, where competing factors like transport mediated active uptake are absent, the electrostatic interaction may be adequate to show improved binding strength. That may be the reason for the discrepancy observed for *in vitro* and *in vivo* activities of cholestyramine. In order to enhance the binding strength between the sequestrant and the bile acid, incorporation of features to the polymer that enhances secondary binding interactions is useful. One such feature is the hydrophobic interaction between the steroid skeleton of the bile acid and the hydrophobic groups in the polymer chain. This design strategy appears to have paid off, since the amine functionalized polyethers modified with hydrophobic alkyl chains have shown enhanced *in vivo* activity. Since both polymers have the same number of cationic charge per repeat unit, the hydrophobic interaction in combination with primary ionic interaction seems to have enabled these sequestrants to better overcome the desorbing forces of the GI tract.

Downloaded At: 12:06 24 January 2011

CONCLUSION

A new class of amine functionalized polymers has been prepared by chemical modification of polyethers bearing pendant alkyl halide substituents. By optimizing experimental conditions, it has become possible to modify the chloromethyl groups of PECH, which are usually difficult to react. Multistep modification reactions enabled the preparation of amphiphilic cationic hydrogels with complex polymer architectures. Both soluble and crosslinked polymers were produced. From preliminary *in vivo* studies it appears that these amine functionalized polyether hydrogels are excellent bile acid sequestrants and promising cholesterol lowering agents. By judiciously designing polymer structures, it is possible to synthesize polymer compositions that show better bile acid sequestration properties than some of the commercially available agents.

REFERENCES

1. Mandeville, W. H.; Goldberg, D. I. The Sequestration of Bile Acids, a Non-Absorbed Method for Cholesterol Reduction. Current Pharmaceuticals Design **1997**, *3*, 15-28.
2. Zarras, P.; Vogl, O. Polycationic Salts as Bile Acid Sequestering Agents. Prog. Polym. Sci. **1999**, *24*, 485-516.

3. Stedronsky, E.R. Interaction of Bile Acids and Cholesterol with Non-Systemic Agents Having Hypocholesterolemic Properties. Biochimica et Biophysica Acta **1994**, *210*, 255- 287.
4. Tsuchida, E.; Yamamoto, K.; Miyatake, K.; Endo, K. Molecular Assembly of Cholesterol Bearing Poly(allylamine) for Binding Bile Salts in Water. Macromolecules **1997**, *30*, 4235-4237.
5. Zhang, L-H.; Janout, V.; Renner, J.L.; Uragami, M.; Regen, S.L. Enhancing the Stickiness of Bile Acids to Crosslinked Polymers: A Bioconjugate Approach to the Design of Bile Acid Sequestrants. Bioconjugate Chem. **2000**, *11*, 397-400.
6. Mandeville, W.H.; Holmes-Farley, S.R. Method for Removing Bile Salts from a Patient with Alkylated Amine Polymers. US Patent 5,679,717, 1997.
7. Huval, C.C.; Bailey, M.J.; Braunlin, W.H.; Holmes-Farley, S.R.; Mandeville, W.H.; Petersen, J.S.; Polomoscanik, S.C.; Sacchiero, R.J.; Chen, X.; Dhal, P.K. Novel Cholesterol Lowering Polymeric Drugs Obtained by Molecular Imprinting. Macromolecules **2001**, *34*, 1548-1550.
8. Setchell, K.D.; Lawson, A.M.;Tanida, N.; Sjovall, J.General Methods for the Analysis of Metabolic Profiles of Bile Acids and Related Compounds in Feces. J. Lipid Res. **1983**, *24*, 1085.
9. Mandeville, W.H.; Braunlin, W.; Dhal, P.; Guo, A.; Huval, C.; Miller, K.; Petersen, J.; Polomoscanik, S.; Rosenbaum, D.; Sacchiero, R.; Ward, J.; Holmes-Farley, S.R. Three Generations of Bile Acid Sequestrants. Mat. Res. Soc. Symp. Proc. **1999**, *550*, 3-14.
10. Iizawa, T.; Nishikubo, T.; Ichikawa, M.; Sugawara, Y. J. Polym. Sci. Polym. Chem. Edn. **1985**, *23*, 1893.
11. Perez, M.; Ronda, J.C.; Reina, J.A.; Serra, A. Synthesis of Functional Polymers by Chemical Modification of PECH and PECH-PEO with Substituted Phenolates. Polymer **2001**, *42*, 1-8.
12. Zussman, M.P.; Shih, J.S.; Wicks, D.A.; Tirrell, D.A. Synthesis and Reactions of Halogenated Polyethers and Polysulfides. Am. Chem. Soc. Symp. Ser. **1988**, *364*, 60-71.
13. Sparrow, J.T. An Improved Polystyrene Support for Solid Phase Peptide Synthesis. J. Org. Chem. **1976**, *41*, 1350-1353.
14. Benson, G.M.; Alston, D.R.; Hickey, D.M.B.; Jaxa-Chamiec, A.A.; Whittaker, C.M.; Haynes, C.; Glen, A.; Blanchard, S.; Cresswell, S.R.; Suckling, K. E.SK&F 97426-A: A Novel Bile Acid Sequestrant with Higher Affinities and Slower Dissociation Rates for Bile Acids *In Vitro* Than Cholestyramine. J. Pharm. Sci. **1997**, *88*, 76-81.

Downloaded At: 12:06 24 January 2011