Synthesis and Characterization of Biodegradable and Charged Salen-Based Polymers

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ABSTRACT: The goal of this study was to synthesize novel biodegradable charged polymers for medical applications. The polymers synthesized were metal-coordinated salicylidene-based copolymers. The linear copolymers were prepared by polycondensation of the metal-coordinated salicylidene monomer with acyl or aryl dichloride. Structure analysis was carried out by ^{1}H and ^{13}C NMR, FTIR, and elemental analysis. Physicochemical evaluation was carried out using DSC and thermogravimetry. The surface properties were analyzed by contact angle measurements and the crystallinity was determined by polarizing microscopy and AFM. Finally, polymer electrical conductivity and biocompatibility were examined. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 2568–2577, 2006

Key words: conducting polymer; biocompatibility; synthesis; biodegradable; metal co-ordinated salicylidene-based copolymers

INTRODUCTION

About 40 years ago, it seemed a paradox that such well-known insulators as organic compounds could become electrically conductive. In the 1960s, it was pointed out that complex formation between electron acceptors and electron donors increases the conductivity by several orders of magnitude. During this period, conductive polymers with conjugated double bonds were also being developed, including polymers such as polypyrroles and polythiophenes.[1] More recently, conductive metallopolymers have been developed.[2] Within this group, the salicylidene-based copolymers are of special interest, because of their various applications; namely, as catalysts[3–6] or free radical scavengers,[7–9] and as material to make electrodes.[10,11] The study of their nonlinear optical properties has also aroused interest.[12,13]

The need for biodegradable and biocompatible charged and conductive copolymers, in the biomedical field, has been pointed out by several studies. For example, conductive polymers can be used as materials in tissue engineering, including bone and nerve regeneration as a means to deliver electric stimuli while providing cell scaffolds.[14] Cationic polymers (conductive or not) are also of great interest for the delivery of genetic material.[15] In the context of pharmaceutical and biomedical applications, biocompatibility and biodegradability are important parameters. Polypyrroles and polythiophenes, the most studied conductive polymers, are not degradable. It is also the case with salen-based conductive polymers produced by electrochemical polymerization.[16,17] On the other hand, noncharged and nonconductive aliphatic polyesters are widely used in agricultural, biomedical, pharmaceutical, and environmental applications; they exhibit excellent biodegradability and good biocompatibility.[18] Their degradation yields nontoxic by-products which are readily metabolized in the human body. Polypyrrole oligomers, linked by ester linkage, has been reported to be both semiconductive (10^{-4}–10^{-5} less conductive than polypyrrole) and degradable.[19] The aim of this study was to synthesize biodegradable copolymers of salicylidene. The synthesis was carried out through the addition of a biodegradable functional ester monomer to salicydenes moieties, to obtain a copolymer of aliphatic and aryl chain and metal complexed Schiff bases. This should represent a new family of conductive and charged biodegradable heteropolymers to be used for pharmaceutical or biomedical applications. This report will focus on the synthesis and characterization of this new polymer family.

EXPERIMENTAL

General

All reagents and solvents were obtained from commercial sources and used without further purification.

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Structure and composition of the synthesized compounds were determined using elemental analysis by the Laboratoire d'analyze élémentaire of the Université de Montréal with a Fisons Instrument, model EA 1108 CHN; by ^{1}H NMR and ^{13}C NMR using a Brüker ARX 400 MHz spectrometer and by IR-FTIR using a PerkinElmer spectrometer. Gel permeation chromatography analysis were performed on a Waters system equipped with a refractometer detector, Waters Styragel GPC columns, and using THF as the mobile phase at a rate of 1 mL/min. Characterization of crystallinity of the copolymers was carried out by polarization microscopy using a polarizing microscope model Olympus B201 mounted with a CCD camera for image acquisition. Degradation onset was determined, by thermogravimetry (TGA) using a Universal 2.5H TA instruments. DSC scans were acquired using a Seiko RDC-220 instrument. Samples were analyzed at a heating rate of 10°C/min using crimped aluminum pans. The instrument was calibrated for heat flow and temperature using gallium (Goodfellow, 99.99% pure), indium (Goodfellow, 99.999% pure), and tin (NIST, SRM2220). The melting temperature was obtained using an Electrothermal apparatus. A Contact Angle Meter Tantec model Cam-Micro was used to determine the contact angles of the copolymers. Surface properties were imaged by AFM using a Nanoscope IIIa Dimension 3100 atomic force microscope (Digital Instruments, Santa Barbara, CA) in tapping in air mode, using nanoprobe RTESPE7, with spring constant of 20–100 N/m and resonance frequency of 200–400 kHz. Cantilever length was 125 μm. AFM measurements were conducted on thin polymer pellets prepared by using tablet press. To measure conductivity, a home-built system was used consisting of an auto-ranging picoamperometer (Keithley Instruments, Cleveland, OH), a constant current generator, and a chip of compacted polymers (thickness 0.6–1.2 mm) to which copper wire sticks were attached on each face with silver epoxy glue and connected in series. Conductivity (G) is expressed in Siemens per meter and is calculated with the formula: $G = I/(V \times m)$, where I is the current intensity measured in Ampere, V is the tension in Volt, and m is the polymer chip thickness in meter.[1]

Synthesis of (*R,R*)-*N,N′*-bis(2,5-dihydroxysalicylidene)-1,2-cyclohexanediamine (1a)

To a solution of potassium carbonate (12.64 g, 91 mmol) and *trans*-1,2-diaminocyclohexane (5.58 mL, 45.5 mmol) in water (20 mL) ethanol was added (65 mL) at room temperature. The resulting mixture was stirred until the reflux temperature had been reached (75°C). A solution of 2,5-dihydroxybenzaldehyde (12.57 g, 91 mmol) in ethanol (30 mL) was then added dropwise to the above reaction mixture. Stirring was refluxed for 14 h. Then, the mixture was cooled to room temperature. The solvent was removed by rotary evaporation. The mixture was extracted four times with ethyl acetate and water. The organic fractions were combined and the solvent was removed by rotary evaporation to give 9.68 g (60%) of golden crystals; mp 202–204°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 1.4 (m, 4H, —CH_2—, cyclohexane), 1.6 (m, 4H, —CH_2—CHR—, cyclohexane), 1.8 (m, 2H, —CH_2—C*H*R—, cyclohexane), 6.8–7.1 (m, 6H, ArH), 8.5–8.6 (m, 2H, —C*H*=N—), 8.6–8.8 (m, 2H, OH ext) and 9.0–9.2 (m, 2H, OH int); ^{13}C NMR (DMSO-d_6) δ (ppm) 25 (—CH_2—CH_2—CH_2—, cyclohexane), 34 (—*C*H_2—CHR—, cyclohexane), 73 (—CH_2—*C*HR—, cyclohexane), 117.1 (CH—*C*H—C(OH)int—), 117.4 (CR—*C*H—C(OH) —), 119 (CH—*C*H—C(OH)ext—), 121 (—*C*R=CH—C(OH) —), 150 (—N=*C*H—), 154 (=*C*(OH)ext—), and 166 (=*C*(OH)int—); HRMS (FAB) *m/z* 355.1646 (M^+); $C_{20}H_{23}N_2O_4$ ($[M+H]^+$) 355.1652; Elemental analysis calculated: C, 67.8%; H, 6.3%; N, 7.9%. Found: C, 66.1%; H, 6.5%; N, 7.5%.

***N,N′*-bis(2,5-Dihydroxysalicylidene)-ethylenediamine (1b)** was synthesized as compound **1a** with ethylenediamine instead of *trans*-1,2-diaminocyclohexane to give 10.9 g (80%) of mauve crystals; mp 210–213 °C; ^{1}H NMR (DMSO-d_6) δ (ppm) 3.8 (m, 4H, —*N*—CH_2—CH_2—N), 6.6–6.8 (m, 6H, ArH), 8.5 (s, 2H, —CH=N—), 8.7 (m, 2H, OH ext) and 8.9 (m, 2H, OH int); ^{13}C NMR (DMSO-d_6) δ (ppm) 60 (=N—CH_2—CH_2—N=), 117.2 (—(OH)int—*C*H—CH—), 117.6 (CR—*C*H—C(OH)ext), 119 (C(OH)ext—*C*H—CH—), 121 (—C(OH)int=*C*R—CH—), 150 (—N=*C*H—), 154 (C(OH)ext), and 167 (C(OH)int); Elemental analysis calculated: C, 64%; H, 5.4%; N, 9.3%. Found: C, 63.5%; H, 5.6%; N, 9.3%.

(*R,S*)-*N,N′*-bis(2,5-Dihydroxysalicylidene)-*meso*-1,2-diphenylethylenediamine (1c) was synthesized as compound **1a** with *meso*-1,2-diphenylethylenediamine instead of *trans*-1,2-diaminocyclohexane to give 12.3 g (60%) of dark yellow crystals; mp 133°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 5.0 (m, 2H, ph—C*H*R—N=), 6.6–6.8 (m, 6H, Ar(H)), 7.2–7.5 (m, 10H, —ph(H)) 8.1–8.3(m, 4H, Ar(OH)ext and CH=N) and 8.9 (m, 2H, Ar(OH)int); ^{13}C NMR (DMSO-d_6) δ (ppm) 79 (ph—*C*HR—N=), 117.2 (—C(OH)int—*C*H—CH—), 117.6 (C(OH)ext—*C*H—CR—), 119 (C(OH)ext—*C*H—CH—), 121 (CH—*C*R—C(OH)int), 127–129 (—CH=), 141 (=CH—*C*=CH—) 150 (—CH=N—), 154 (—C(OH)ext), and 166 (—C(OH) int); HRMS (FAB) *m/z* 453.1825 $C_{28}H_{25}N_2O_4$ ($[M+H]^+$) 453.1809; Elemental analysis calculated: C, 74.3%; H, 5.3%; N, 6.2%. Found: C, 72.8%; H, 5.7%; N, 6.2%.

***meso*-1,2-bis(4-Hydroxyphenyl)ethyl-1,2-diamine (d)** was prepared using the method of Kurzand and Johnson.[20] To a solution of aluminum chloride (0.39 g, 2.9 mmol) and *meso*-1,2-diphenylethylenediamine (4.56 g, 0.02 mmol) in water (100 mL) hydrogen peroxide was added (50%) (0.05 mL, 1.45 mmol) at 0°C. The resulting mixture was stirred for 3 h at 0°C, and

then 15 mL of HCl was added dropwise to the above reaction mixture. Stirring was continued for 1 h. The mixture was filtered and the precipitate was collected to give 4.8 g (98%) of a white powder; mp 264°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 4.9 (m, 2H, NH2—C*H*R—), 7.4 (m, 4H, —C(OH)=C*H*—), 7.7 (m, 4H, —C(OH)=CH—C*H*=) and 9.0 (m, 4H, NH_2); ^{13}C NMR (DMSO-d_6) δ (ppm) 57 (NH2—CRH—), 129 (C(OH)—*C*H), 130 (C(OH)—CH—*C*H), 131 (—CH—*CR*—CH—), and 134 (C(OH)); Elemental analysis calculated: C, 68.8%; H, 6.6%; N, 11.5%. Found: C, 57.3%; H, 6.7%; N, 9.4%.

(***R,S*)-*N,N′*-(2-Hydroxysalicylidene)-1,2-bis(4-hydroxyphenyl)ethyl-1,2-diamine (1d)** was synthesized as compound **1a** with *meso*-1,2-bis(4-hydroxyphenyl)ethyl1,2-diamine instead of *trans*-1,2-diaminocyclohexane. After the reaction, the mixture was filtered and the precipitate was collected to give 11.1 g (54%) of a yellow powder; mp 209°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 5.0 (s, 2H, ph(OH)—C*HR*—N=), 6.8 (m, 4H,C(OH)—C*H*—CH—), 7.2–7.5 (m, 16H, —CH=) and 8.4 (s, 2H, —CH=N—);^{13}C NMR (DMSO-d_6) δ (ppm) 80 (ph(OH)—CRH—N=), 117 (—C(OH)—*C*H—CH—) 119 (—CH=*C*H—CH=CH—), 120 (C(N)—*C*(CH)=C(OH)), 129 (CH—*C*H—C(CH)—CH—) 130 (C(CN)—*C*H—CH—) 133 (—CH—CH—CH—C(OH)), 140 (C(OH)ext) 160 (C(OH)int) and 167 (—*C*H=N—). Elemental analysis calculated: C, 74.3%; H, 5.3%; N, 6.2%. Found: C, 79.6%; H, 5.9%; N, 6.6%.

Synthesis of (*R,R*)-*N,N′*-bis(2,5-dihydroxysalicylidene)-1,2-diaminocyclohexane manganese (2a)

A reaction flask containing manganese acetate tetrahydrate (5.44 g, 22 mmol) in ethanol (50 mL) was warmed gently to reflux temperature (75°C). To this warm solution, a solution of **1a** (2.59 g, 7.3 mmol) was added dropwise in ethanol (25 mL). The reaction mixture was stirred and refluxed for 14 h. The mixture was then cooled to room temperature, and the solvent was removed by rotary evaporation. The mixture was washed two times with acetone followed by water. The precipitate was collected to give 2.85 g (95%) of a green powder; mp > 300°C. Elemental analysis calculated: C, 59.0%; H, 4.9%; N, 6.9%. Found: C, 55.6%; H, 5.2%; N, 5.7%.

(***R,R*)-*N,N′*-bis(2,5-Dihydroxysalicylidene)-1,2-diaminocyclohexane zinc (2b)** was prepared as compound **2a** with zinc acetate dihydrate instead of manganese acetate tetrahydrate to give 2.9 g (96%) of a green powder; mp > 300°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 1.4–1.5 (m, 4H, —CH_2—, cyclohexane), 1.6–1.7 (m, 4H, —CH_2—CHR—, cyclohexane), 1.8 (m, 2H, —CH_2—C*H*R—, cyclohexane), 6.8–7.1 (m, 6H, ArH), 8.6 (m, 2H, —N=CH—), and 8.8 (m, 2H, AR(O*H*)ext); ^{13}C NMR (DMSO-d_6) δ (ppm) 24 (—CH_2—, cyclohexane), 26 (—CH_2—CHR, cyclohexane), 65 (—CH_2—*C*HR, cyclohexane), 119 (=C(O)—*C*H=CH—), 123 (C(OH)ext—*C*H—CH), 145 (*CR*—CH—C(OH)—), 162 (—*C*H=N—), 165 (—*C*(OH)ext—), and 176 (=*C*(O—Zn)int—); Elemental analysis calculated: C, 57.5%; H, 4.8%; N, 6.7%. Found: C, 42.2%; H, 4.0%; N, 3.9%.

(***R,R*)-*N,N′*-bis(2,5-Dihydroxysalicylidene)-1,2-diaminocyclohexane copper (2c)** was prepared as compound **2a** with copper acetate monohydrate to give 2.7 g (88%) of a green powder; mp > 300°C; Elemental analysis calculated: C, 57.8%; H, 4.8%; N, 6.7%. Found: C, 49.1%; H, 4.4%; N, 5.4%.

***N,N′*-bis(2,5-Dihydroxysalicylidene)-ethylenediamine manganese (2 days)** was prepared as **2a** with **1b** instead of **1a** to give 1.4 g (56%) of a pink powder; mp > 300°C; Elemental analysis calculated: C, 54.4%; H, 4.0%; N, 7.9%. Found: C, 49.2%; H, 4.2%; N, 6.6%.

***N,N′*-bis(2,5-Dihydroxysalicylidene)-ethylenediamine zinc (2e)** was prepared as compound **2b** with **1b** instead of **1a** to give 2.4 g (90%) of a pink powder; mp > 300°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 3.7 (m, 4H, —*N*—CH_2—), 6.4 (m, 4H, ArH), 6.6 (m, 2H, —C(OH) —C*H*—CR, Ar), 8.2 (s, 2H, —CH=N—) and 8.4 (m, 2H, Ar(OH)ext); Elemental analysis calculated: C, 52.8%; H, 3.9%; N, 7.7%. Found: C, 51.9%; H, 3.1%; N, 7.4%.

***N,N′*-bis(2,5-Dihydroxysalicylidene)-ethylenediamine copper (2f)** was prepared as **2c** with **1b** instead of **1a** to give 0.7 g (25%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) 3400, 1558, 1432, 1406, 1340, 1238, 1047, 931, and 823; Elemental analysis calculated: C, 53.1%; H, 3.9%; N, 7.7%. Found: C, 50.2%; H, 3.3%; N, 6.9%.

(*R,S*)-*N,N′*-bis(2,5-Dihydroxysalicylidene)-*meso*-1,2-diphenylethylenediamine manganese (2g) was prepared as **2a** with **1c** instead of **1a** to give 3.7 g (80%) of a green powder; mp > 300°C; Elemental analysis calculated: C, 66.5%; H, 4.8%; N, 5.5%. Found: C, 60.5%; H, 4.8%; N, 6.0%.

(*R,S*)-*N,N′*-bis(2,5-Dihydroxysalicylidene)-*meso*-1,2-diphenylethylenediamine zinc (2h) was synthesized as **2b** with **1c** instead of **1a** to give 0.4 g (10%) of a yellow powder; mp 295°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 4.9 (d, 2H, ph—C*HR*—N=), 6.3–6.5 (m, 2H, —C(O)int—C*H*—CH—), 6.7 (d, 2H, C(O)int—CH—C*H*—), 6.9 (d, 2H, C(OH)ext—C*H*—CR—), 7.1 (m, 5H, ArH), 7.3 (m, 5H, ph(H), and 8.3 (m, 2H, —C*H*=N—); Elemental analysis calculated: C, 65.2%; H, 4.3%; N, 5.4%. Found: C, 56.2%; H, 3.7%; N, 4.3%.

(***R,S*)-*N,N′*-bis(2,5-Dihydroxysalicylidene)-*meso*-1,2-diphenylethylenediamine copper (2i)** was synthesized as **2c** with **1c** instead of **1a** to give 3.7 g (87%) of a brown powder; mp > 300°C; Elemental analysis calculated: C, 65.4%; H, 4.3%; N, 5.4%. Found: C, 59.1%; H, 4.8%; N, 5.7%.

(*R,S*)-*N,N′*-(2-Hydroxysalicylidene)-1,2-bis(4-hydroxyphenyl)ethyl-1,2-diamine manganese (2j) was synthesized as for compound **2a** with **1 day** instead of 1a to give 2.9 g (80%) of a brown powder; mp 223°C; Elemental analysis calculated: C, 66.5%; H, 4.4%; N, 5.5%. Found: C, 66.2%; H, 5.0%; N, 4.9%.

(*R,S*)-*N,N′*-(2-Hydroxysalicylidene)-1,2-bis(4-hydroxyphenyl)ethyl-1,2-diamine zinc (2k) was synthesized as **2b** with **1d** instead of **1a** to give 3.7 g (99%) of a yellow powder; mp 274°C; ^{1}H NMR (DMSO-d_6) δ (ppm) 5.2 (m, 2H, A), 6.7 (d (*J*; 5.8 Hz), 2H, B), 6.8 (dd (*J*; 5.8 and 3.1 Hz), 4H, C), 6.8–7.0 (d (*J*; 3.1 Hz), 2H, D), 7.1–7.3 (m, 8H, E) and 8.2 (s, 2H, F); Elemental analysis calculated: C, 65.2%; H, 4.3%; N, 5.4%. Found: C, 55.8%; H, 4.8%; N, 3.9%.

(*R,S*)-*N,N′*-(2-Hydroxysalicylidene)-1,2-bis(4-hydroxyphenyl)ethyl-1,2-diamine copper (2l) was synthesized as **2c** with **1d** instead of **1a** to give 2.3 g (60%) of a pink powder; mp > 300°C; Elemental analysis calculated: C, 65.4%; H, 4.3%; N, 5.4%. Found: C, 64.9%; H, 4.6%; N, 5.4%.

Synthesis of copolymer (*R,R*)-*N,N′*-bis (2,5-dihydroxysalicylidene)-1,2-diaminocyclohexane manganese-*co*-adipoyl (3a)

To a suspension of **2a** (0.64 g, 1.56 mmol) in acetone (25 mL) adipoyl dichloride (0.45 mL, 3.1 mmol) and triethylamine (0.43 mL, 3.1 mmol) was added. The resulting mixture was stirred at reflux temperature (50°C) for 2 h. Then, the mixture was cooled to room temperature and the solvent was removed by rotary evaporation. The mixture was washed three times with water, then with acetone, and was collected to give 0.3 g (30%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1747; Elemental analysis calculated: C, 60.4%; H, 5.1%; N, 5.9%. Found: C, 61.9%; H, 5.9%; N, 6.4%.

The copolymer **(*R,R*)-*N,N′*-bis(2,5-dihydroxysalicylidene)-1,2-diaminocyclohexane-zinc-*co*-adipoyl (3b)** was synthesized as **3a** with **2b** instead of **2a** to give 0.3 g (40%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1746; ^{1}H NMR (DMSO-d_6) δ (ppm) 0.5 (m, 4H, —CH_2—, cyclohexane), 1.3 (m, 4H, —CH_2—CHR—, cyclohexane), 1.7 (m, 4H, —CH_2—CH_2—COO—, adipoyl), 2.0 (m, 2H, —CH_2—CHR—, cyclohexane), 2.3 (m, 4H, —CH_2—CH_2—COO—), 5.8 (m, 2H, —C(O)int—CH—CH—), 6.5 (m, 2H, C(O)int—CH—CH—), 7.2 (m, 2H, C(OH)ext—CH—CR—) and 8.2 (m, 2H, —N=CH—); ^{13}C NMR (DMSO-d_6) δ (ppm) 24 (—CH_2—, cyclohexane), 25 (—CH_2—CHR, cyclohexane), 26 (—CH_2—CHR, cyclohexane), 31 (CH_2—CH_2—COO—, adipoyl) 120 (—C(OH int) —CH =CH—), 124 (C(Oext) —CH=CR—), 128 (—CO=CH-CH=), 145 (—CO=CR—), 164 (=C(O—Zn)—), 176 (—CH=N—), 192 (—CH_2—COO—), and 207 (—CH2—COO—); Elemental analysis calculated: C, 59.2%; H, 5.0%; N, 5.3%. Found: C, 52.7%; H, 5.2%; N, 3.8%.

The copolymer **(*R,R*)-*N,N′*-bis(2,5-dihydroxysalicylidene)-1,2-diaminocyclohexane-copper-*co*-adipoyl (3c)** was synthesized as **3a** with **2c** instead of **2a** to give 0.4 g (47%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1746; Elemental analysis calculated: C, 59.4%; H, 5.0%; N, 5.3%. Found: C, 57.2%; H, 5.7%; N, 4.9%.

The copolymer ***N,N′*-bis(2,5-dihydroxysalicylidene)-ethylenediamine-manganese-*co*-adipoyl (3d)** was synthesized as **3a** with **2 days** instead of **2a** manganese to give 0.58 g (90%) of brown powder; mp > 300°C; Elemental analysis calculated: C, 56.8%; H, 4.8%; N, 6.0%. Found: C, 54.4%; H, 5.0%; N, 8.4%.

The copolymer ***N,N′*-bis(2,5-dihydroxysalicylidene)-ethylenediamine-zinc-*co*-adipoyl (3e)** was synthesized as **3a** with **2e** instead of **2a** to give 0.67 g (91%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1695; ^{1}H NMR (DMSO-d_6) δ (ppm) 1.7 (m, 4H, —CH_2—CH_2—COO—), 2.1 (m, 4H, —CH_2—CH_2—COO—), 3.7 (m, 4H, —N—CH_2—), 6.4 (m, 4H, ArH), 6.6 (m, 2H, —C(OH) —CH—CR, Ar), 8.2 (s, 2H, —CH=N—) and 8.4 (m, 2H, Ar(OH)ext); Elemental analysis calculated: C, 55.5%; H, 4.7%; N, 5.8%. Found: C, 54.8%; H, 5.4%; N, 6.2%.

The copolymer ***N,N′*-bis(2,5-dihydroxysalicylidene)-ethylenediamine-copper-*co*-adipoyl (3f)** was synthesize as for compound **3a** with **2f** instead of **2a** to give 0.60 g (80%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1708; Elemental analysis calculated: C, 55.8%; H, 4.7%; N, 5.9%. Found: C, 53.2%; H, 4.0%; N, 6.2%.

Synthesis of copolymer (*R,S*)-*N,N′*-bis (2,5-dihydroxysalicylidene)-*meso*-1,2-diphenyl-ethylenediamine-manganese-*co*-terephtaloyl (4a)

The copolymer (*R,S*)-*N,N′*-bis(2,5-dihydroxysalicylidene)-ethylenediamine maganese-*co*-terephtaloyl was synthesized as **3a** with terephtaloyl chloride instead of adipoyl chloride, and with **2g** instead of **2a** to give 0.98 g (99%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1789; Elemental analysis calculated: C, 68%; H, 3.8%; N, 4.4%. Found: C, 58.4%; H, 5.7%; N, 4.6%.

The copolymer **(*R,S*)-*N,N′*-bis(2,5-dihydroxysalicylidene)-*meso*-1,2-diphenylethylenediamine zinc-*co*-terephtaloyl (4b)** was synthesized as **4a** with **2h** instead of **2a** to give 0.33 g (33%) of a yellow powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1791; Elemental analysis calculated: C, 66.9%; H, 3.7%; N, 4.3%. Found: C, 66.8%; H, 5.4%; N, 5.1%.

The copolymer **(*R,S*)-*N,N′*-bis(2,5-dihydroxysalicylidene)-*meso*-1,2-diphenylethylenediamine copper-*co*-terephtaloyl (4c)** was prepared similarly to **4a** with **2i** instead of **2a** to give 0.94 g (94%) of a brown

powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1789; Elemental analysis calculated: C, 67%; H, 3.7%; N, 4.3%. Found: C, 62%; H, 4.2%; N, 3.8%.

The copolymer **(*R,S*)-*N,N′*-(2-hydroxysalicylidene)-1,2-bis(4-hydroxyphenyl)ethyl-1,2-diamine- manganese-*co*-terephtaloyl (4d)** was synthesized d **4a** with **2j** instead to **2a** to give 0.98 g (99%) of a brown powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1789; Elemental analysis calculated: C, 68%; H, 3.8%; N, 4.4%. Found: C, 60%; H, 7.1%; N, 5.6%.

The copolymer **(*R,S*)-*N,N′*-(2-hydroxysalicylidene)-1,2-bis(4-hydroxyphenyl)ethyl-1,2-diamine-zinc-*co*-terephtaloyl (4e)** was synthesized as **4a** with **2k** instead of **2a** to give 0.98 g (25%) of a beige powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1789; Elemental analysis calculated: C, 66.9%; H, 3.7%; N, 4.3%. Found: C, 63.7%; H, 3.4%; N, 1.1%.

The copolymer **(*R,S*)-*N,N′*-(2-hydroxysalicylidene)-1,2-bis(4-hydroxyphenyl)ethyl-1,2-diamine-copper-*co*-terephtaloyl (4f)** was synthesized as described for **4a** with **2l** instead of **2a** to give 0.99 g (99%) of a gray powder; mp > 300°C; IR (KBr, cm^{-1}) C=O ester 1789; Elemental analysis calculated: C, 67%; H, 3.7%; N, 4.3%. Found: C, 64.6%; H, 5.0%; N, 4.1%.

Cytocompatibility studies

Cell lines

RAW 264.7 murine macrophage cell lines (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and penicillin/streptomycin (Gibco, USA). The cells were grown in tissue culture flasks and incubated at 37°C in a 5% carbon dioxide atmosphere.

Proliferation assays

Polymers dissolved in 10 μL dimethyl sulfoxide (DMSO) were added in the wells on a 96 well-flat bottomed microplate (Corning, NY), in triplicate. The amounts tested were: 250; 100; 10; 1; 0.1; and 0.01 μg. DMSO was subsequently removed under vacuum. RAW 264.7 cells were diluted in complete medium at a final concentration of 5×10^5 cells/mL and plated (100 μL/well). The plates were incubated for 24 h after which cell proliferation was assessed with MTT assay.[21] Briefly, 10 μL of thiazolyl blue tetrazolium bromide (Sigma, St. Louis, MO) dissolved in PBS (10 mM, pH 7.4) at a concentration of 5 mg/mL and filtered on 0.22 μm sterile filter (Millipore, Bedford, MA), was added to each well. After 3 h incubation time at 37°C in 5% carbon dioxide atmosphere, 50 μL of solution (Isopropanol, 10% Triton X100, 0.1*N* HCl) was added to each well to dissolve the dark blue formazan crystals. Absorbance was read at a 570-nm wavelength on a microplate reader (SAFIRE, Tecan, Austria). To evaluate the presence of potential toxic leachable components, polymer extracts were prepared. Briefly, 12–20 mg of polymers or salens were incubated in 1 mL DMEM supplemented with serum at 37°C for 2–72 h. Extracts were sterile filtered and serially diluted. Extracts were added to RAW 264.7 cells plated on a 96-wells microplate. After 24 h incubation, cell proliferation and cell lysis were assessed using MTT and LDH assays. Cell growing pattern was followed in routine examination with optical inverted microscope (Axiovert S100, Zeiss, Germany).

Lysis assays

The presence of lactate dehydrogenase (LDH) in the supernatant obtained from proliferation assays was used as an indicator of cell lysis and death and determined using a commercial dosing kit (Sigma, St. Louis, MO) used as directed by the manufacturer. Briefly, 5 μL of supernatant were transferred to a new microplate and incubated with the reaction mixture for 30 min. The reaction was stopped with 0.1*N* HCl. Microplates were read using a microplate reader (SAFIRE, Tecan, Austria), at the wavelength of 450 nm (reference wavelength at 690 nm). Results are plotted in reference to positive control wells, 100% Triton X100 lysed cells.

RESULTS AND DISCUSSION

General synthesis approach

Four different Schiff bases were synthesized based on the salicylidene tetradentate ligand derived from salicylaldehyde and diamines. Moderate to good yields of salicylidene monomer were obtained using a modified Larrow and Jacobsen method.[22] Different reagents were used, such as 2,5-dihydroxybenzaldehyde, to obtain salen structure with additional alcohol groups. Some modifications to the Jacobsen purification process were done to adapt it to the polarity of these new compounds. The structures of the monomers were confirmed by ^{1}H NMR; for example, loss of the aldehydic proton on the 2,5-dihydroxybenzaldehyde at 9.9 ppm and replacement by the proton signal at 8.5 ppm, due to the proton (—N=CH—). In addition, the four protons (3.5 ppm) of the reactive amines have also disappeared, as expected (spectra not shown). The typical 2 N—H bond bands of the reactive amine at vibration wavelength 3300 cm^{-1} disappeared in the FTIR spectrum of final salicylidene compounds. Elemental analysis confirmed the proposed structure. For example, the compound **1a** has 67.8% of carbon, 6.3%; of hydrogen, and 7.9% of nitrogen and we found respectively, 66.1% of C, 6.5% of H, and 7.5% of N which

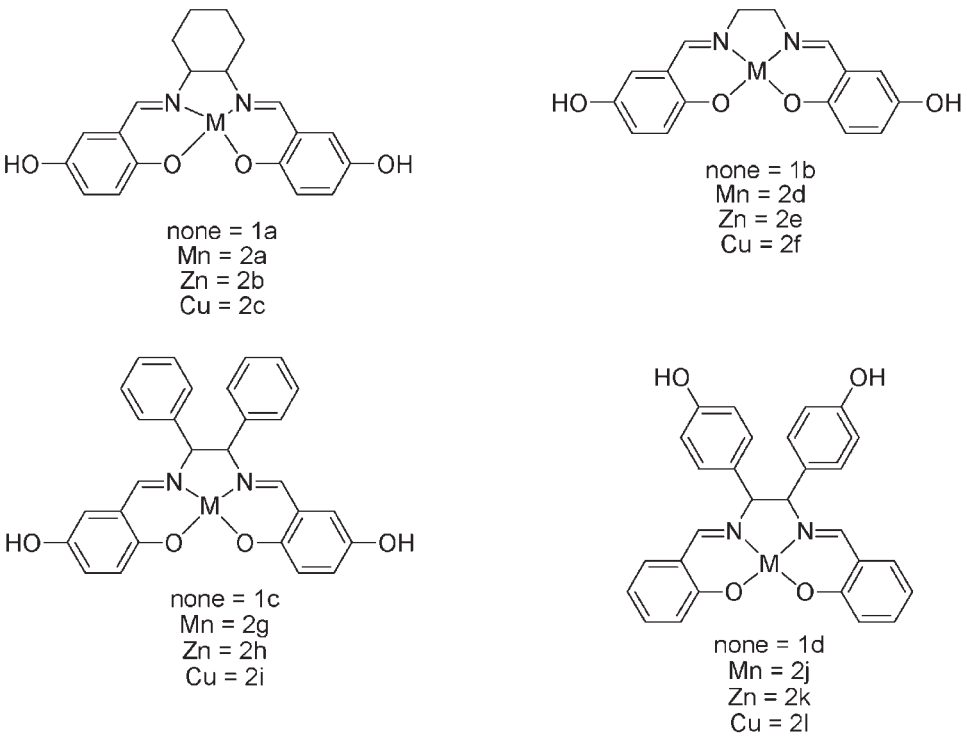

Figure 1 The salicylidenes and metal complexes.

is within the 5% error margin. However, the elemental analysis of compound **d** showed a large difference with the predicted values, probably due to the presence of HCl impurity.

Salicylidenes were thus complexed with selected metals (Fig. 1). As the complexes with Mn and Cu are paramagnetic and they are unsuitable for NMR characterization, complexes with Zn (diamagnetic) were synthesized to serve as a model compound to enable characterization by ^{1}H and ^{13}C NMR. Prior to metal complexation, the hydroxyl proton NMR signals of salicylidene consisted of a multiplet peak at 9.0–9.2 for the two 2,2′ protons (internal) that are hydrogen-bonded and another multiplet peak at 8.6–8.8 for the 5 and 5′ protons (external). Proof of complexation can be seen by ^{1}H NMR with the disappearance of the two hydroxyl protons at positions 2 and 2′ for compound **2b**. Only the proton signal corresponding to the external protons is visible after complexation (spectra not shown). Confirmation of the composition of the coordinated salicylidene metal complexes was obtained by elemental analysis. However, for some complexes, water molecules are coordinated to the metal: two molecules of water for complexes **2a**, **2b, 2d, 2g, 2i** and four for **2c, 2h**, **2k**.

The linear coordinated metal copolymers were formed by polycondensation through esterification of the alcohol on Schiff base with different diacids under basic conditions (Fig. 2). These reactions are mechanistically well understood. Each reaction involves nucleophilic addition to the carbonyl group, facilitated by the polar nature of the carbon–oxygen double bond, the ability of the carbonyl oxygen atom to assume a formal negative charge, and the planar configuration of the trigonal carbon that minimizes steric hindrance. Confirmation of the polymerization can be seen by ^{1}H NMR, with the disappearance of the hydroxyl proton of the metal complex at 8.8 ppm (spectra not shown). The molecular weights have been determined by gel permeation chromatography, giving M_w between 30 and 60 kDa (Table I). Forma-

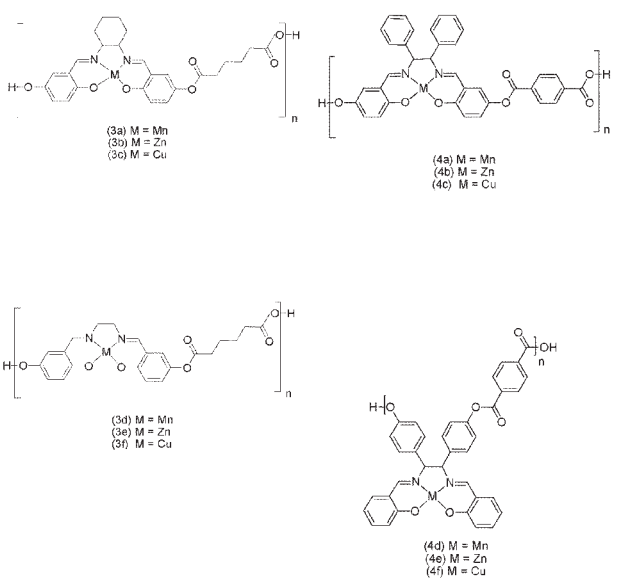

Figure 2 The alternating linear copolymers.

TABLE I
Gel Permeation Chromatography Results

Sample	M_n	M_w	PD
3a	40,400	58,700	1,45
4c	44,200	57,500	1,30
4d	31,200	36,200	1,16
4f	23,800	31,100	1,30

M_n, molecular number; M_w, molecular weight; PD, polydispersity.

tion of the new ester bond was detected by FTIR, with peaks in the range of 1638–1791 cm^{-1} characteristic of the C=O stretch vibration. The copolymers based on terephtaloyl could not be characterized by NMR because they are poorly soluble in organic solvents, but GPC (Table I) and FTIR results confirmed their polymeric nature. Elemental analyses of the linear copolymers are consistent with the proposed structures. However, as observed for metal complex monomer, water molecules were coordinated to the metal complex present in the copolymer (two molecules of water for polymers **4a**, **4c**, **4d** and four for polymer **3b** per metal complex). Hydrates are thus conserved after polymerization.

Thermal analysis

Experimentally, the melting points of the Schiff bases were about 200°C. When Schiff bases were metal-complexed, the melting point increased to 223°C. The thermal stability is dependent on the aromaticity of salicylidene. Complexation increases its aromaticity and, therefore, its stability follows the same trend.

Thermal analysis using DSC shows high T_g values for all polymers (150°C ± 10°C) with no significant differences between the coadipoyl and coterephtaloyl polymers. The high T_g values can be explained by the rigidity of the aromatic rings and intermolecular interactions. Thermal stability depends on the bond energies of each molecule. The vibrational energy increases proportionally with temperature to reach the breaking point. Breaking the C—C bonds of linear polymer requires less energy than to break the bonds of polymer with cycloaromatic groups, since electronic delocalization is high in these groups and the bonds are thus stronger. For a polymer to be considered thermally stable or heat resistant, it should not decompose below 400°C and it should retain its useful properties at temperatures near the decomposition temperature.[23] Thermogravimetric analysis suggests that all the copolymers have good thermal stability since their temperatures of decomposition are above 200°C. However, these copolymers cannot be considered heat resistant. The decomposition of the adipoyl and the terephtaloyl segments starts respectively, at 250 and 300°C, except for compound **4c** for which TGA analysis showed a decomposition temperature starting at 800°C. A second signal at 1000°C corresponding to the decomposition temperature of metal complexes can be observed. The TGA results suggested that terephtaloyl copolymers have improved stability relative to the coadipoyl-based copolymers.

Surface analysis

All the copolymers were found to be semicrystalline as evidenced by the presence of some crystals and some amorphous zone under polarizing microscopy (data not shown). AFM was used to characterize polymer surface microstructure in topography [Figs. 3(a) and 4(a)] and phase mode [Figs. 3(b) and 4(b)]. While topography gives height cartography of the polymer surface, the phase signal is most sensitive to mechanical properties.[27] The surface analysis using AFM tapping in air mode confirmed the semicrystalline character of the polymer **4d** [linear structures on Fig. 3(a)]. Similar structures were observed for polymer **4f** [Fig. 4(a)], however they were less clear. In phase detection

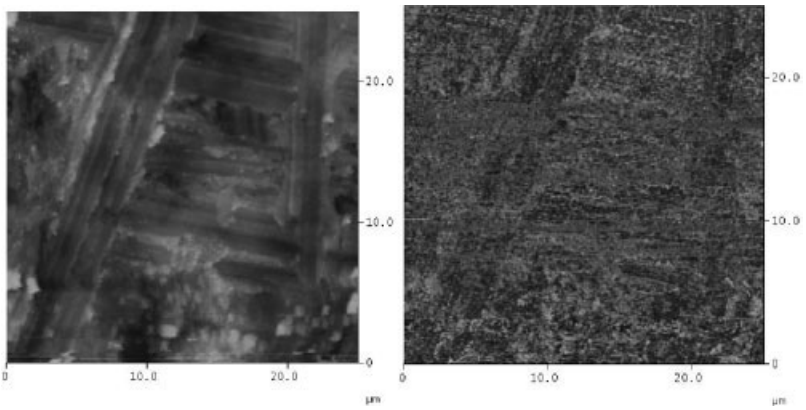

Figure 3 AFM topographic (a) and phase (b) images of polymer **4d** slab, illustrating the semicrystalline character of polymer.

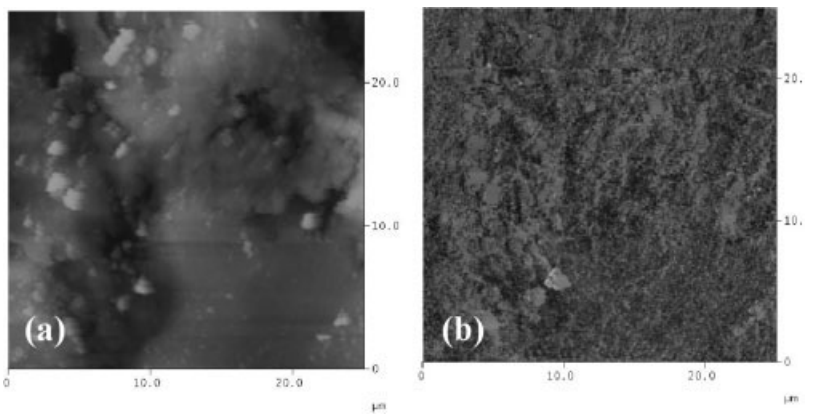

Figure 4 AFM topographic (a) and phase (b) images of polymer **4f** slab, illustrating the presence of metal in the light region in the AFM phase image. The particles present in the AFM topographic image are light in phase images indicating the presence of salicylidene metallic complexes.

mode, the surface was found nonhomogeneous, showing phase separation. In a previous study on salen-grafted polymers, we have shown that lighter zones in phase detection are characteristic of the presence of metal co-ordinated salen.[24] We, indeed observed these light zones indicating the presence of metal complex section.[24] Meanwhile, dark zones in phase images [Figs. 3(b) and 4(b)] may indicate the presence of hydrophobic terephthalic rings.

Static contact angle was performed to determine the polymers wettability. This property greatly influences the biomaterial interactions with cells and proteins. Contact angles from 40° to 80° have been reported to favor general cell adhesion.[19,25] A drop of water was deposited on a polymer chip, allowed to equilibrate, and the measurements were then carried out ($n = 10$). All polymers displayed contact angles between 40° and 75°, thus potentially favoring cell adhesion (Fig. 5). The hydrophobic character is due to the presence of the adipoyl and aromatic groups and can be predicted from the structure. The terephtaloyl polymers are more hydrophobic than the aldipoyl polymers. However, we were not able to correlate the type of the metal with the wettability of the polymer.

Conductive nature of polymers

The conductivity of polymers is not a well-understood phenomenon. However, some molecular arrangements are recognized to favor conductivity. In general, conductive polymers contain conjugated double or triple bonds on aromatic moieties to allow delocalization of the charge[1,23] and hence continuous charge transfer along the backbone or the polymer chain.[23] The presence of electro-attractive or electro-donating agents increases the conductivity. The configuration, conformation, and molecular arrangement also influence the conductivity.[1,23] The polysalicylidene copolymers were expected to be intrinsically conductive polymers since they allow electron mobility. A constant current was applied between opposite face of compacted polymer disc, and the current was measured to calculate conductivity values (Table II). Polymers **4a**, **4d,** and **4f**,

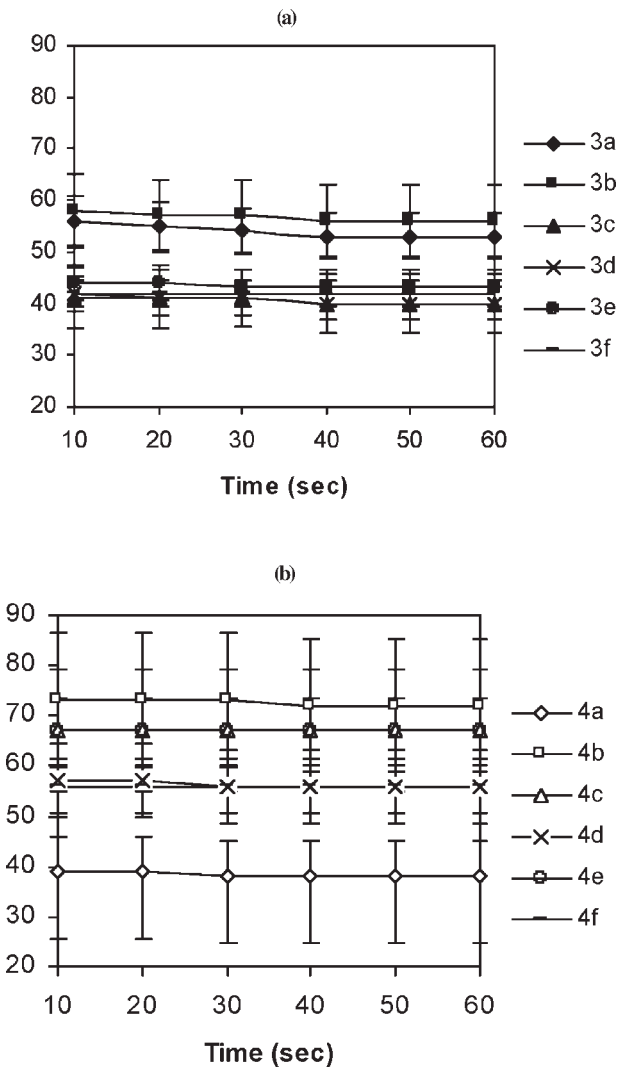

Figure 5 Contact angle average and standard deviation for (a) polymers **3a**–**3f** and (b) polymers **4a**–**4f**.

TABLE II
Conductivity Results

Compound	Current intensity (A)	Conductivity, *G* (S/m)
Reference[a]	0.7643	–
3a	1.82×10^{-09}	1.61×10^{-09}
3b	4.3×10^{-10}	3.38×10^{-10}
3c	1.12×10^{-08}	8.80×10^{-09}
3d	1.24×10^{-08}	7.31×10^{-09}
3e	8.99×10^{-08}	5.30×10^{-08}
3f	3.2×10^{-08}	3.77×10^{-08}
4a	7.6×10^{-06}	5.97×10^{-06}
4b	1.5×10^{-09}	8.84×10^{-10}
4c	3.7×10^{-09}	3.27×10^{-09}
4d	1.46×10^{-04}	1.86×10^{-4}
4e	2.8×10^{-10}	1.37×10^{-10}
4f	3.34×10^{-05}	3.94×10^{-05}

[a] Reference: closed circuit (see Fig. 5).

showed conductivity readings between 10^{-4} and 10^{-6} S/m and can be considered as clearly semiconductive (comparable to thiophene polymers[28]), while, **3c**, **3d**, **3e,** and **3f** with conductivity around 10^{-8} S/m can be found weakly semiconductive. Other polymers with conductivity readings between 10^{-9} and 10^{-10} S/m can be considered close to insulators (comparable to glass).[1] The linear polymers with terephtaloyl as comonomer have the greater conductivity. This could be explained by their higher aromaticity and conjugation compared to the adipoyl-based polymers.[26] The conductivities of polymers with manganese or copper as coordinated metals were about the same (Table II). Taking together results of conductivity and AFM surface analysis, seem to indicate that conductivity is not related to the crystallinity. It was also found that conductivity is not related to M_w (Table I).

Cytocompatibility assays

Cytocompatibility of the conductive polymer **4a**, **4d,** and **4f** was determined as described in the experimental section. Ester bonds can be cleaved in biological media, so we included here testing of the different monomers or potential degradation products (salens and terephthalic acid (TA)) which constitute the polymers. MTT colorimetric assay was used for the assessment of cell proliferation since it measures the tetrazolium ring cleavage by active mitochondria of living cells,[21] whereas, LDH measurement in cell supernatant indicates the level of cell lysis. Blank experiments showed no significant polymer absorbance at detection wavelength and in the concentration range studied. The graph of the cell proliferation and cell lysis assays showed that the polymer and its degradation products are nontoxic in concentration lower than 100 μg/mL [Figs. 6(b) and 7(b)]. At higher concentrations, all showed low toxicity except the **2g** salen which seemed to be more toxic than the others [Fig. 6(a)]. In the cell proliferation study, the salens are less toxic than the corresponding polymers. This suggests that the polymerization with TA increased the toxicity or the resulting polymer had some residual reactant or solvent. In cell lysis assays, the toxicity of salen and corresponding polymer are the same [Fig. 6(a and b)]. The mix of TA and salen **2g** could represent the degradation products of **4a**. We found similar results for each polymer and their corresponding degradation mixtures. Microscopic observations of the macrophage monolayer during the study, showed no changes in cell morphology or growing patterns.

To test the hypothesis that the observed toxicity at high concentrations was due to leachable materials, i.e., solvent or unreacted products, polymer extracts were prepared and incubated with macrophages (data not shown). Indeed, the results showed that the toxicity observed at high polymer concentration seem to be due to leachable materials, probably unreacted monomers or solvents. A potential source of toxic materials is the salen co-ordinated metal, but is unlikely as these bonds are known to be very stable. The other possibility is the toxicity of the degradation products, but it is also unlikely as we found the same profile of toxicity regardless of the incubation length (2 or 72 h). Although a complete degra-

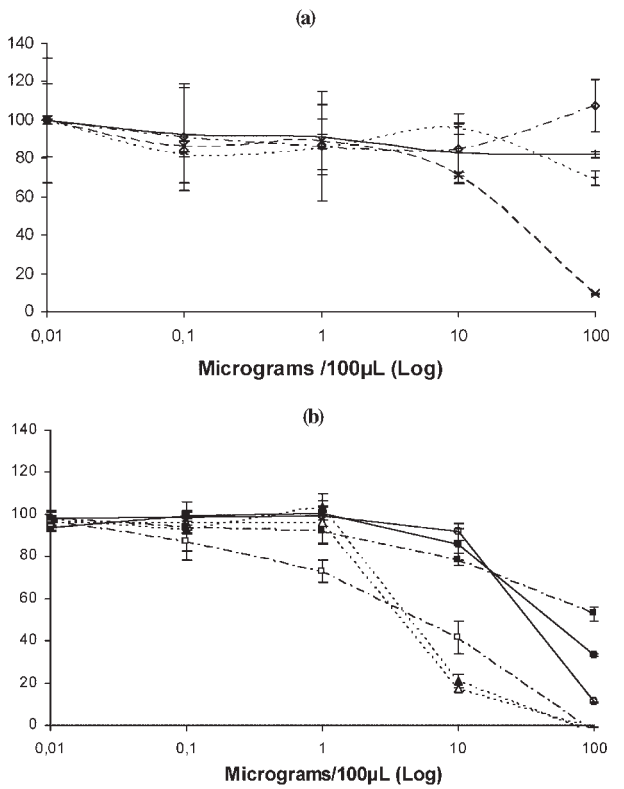

Figure 6 Cell proliferation assays. (a) MTT assay of TA (+), of salens (**2g** (×), **2j** (——), **2l** (◇). (b) MTT assay of polymer (**4a** (○), **4d** (△), **4f** (□)) and the mixture of degradation product of **4a** (

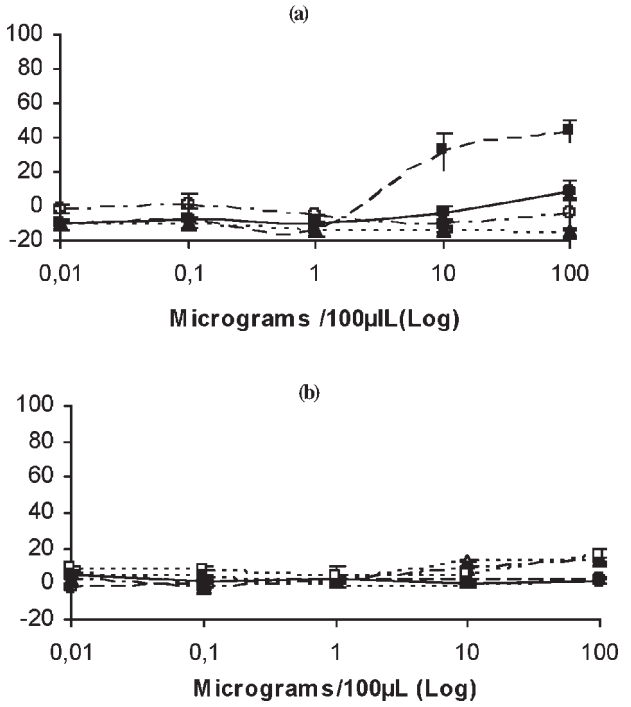

Figure 7 Cell lysis assays. (a) LDH assay of TA (+), of salen (**2g** (×), **2j** (——), **2l** (◇). (b) LDH assay of polymer (**4a** (○), **4d** (△), **4f** (□)) and the mixture of degradation product of **4a** (

dation study of the polymers is yet to be completed, further investigations are also underway to identify these products. These results are encouraging but the tests are preliminary, and cytocompatibility will have to be assessed more thoroughly *in vitro* and *in vivo* considering the hydrophilicity of the polymers and the potential toxicity of the chelating metal. However, it is now possible to use these polymers in further studies to test biological applications.

CONCLUSIONS

Synthesis and characterization of new family of charged semicrystalline polymers based on salicylidenes have been reported, and the structural characterizations have validated the proposed polymer structures. The hydrophilic/hydrophobic character, conductivity properties, and thermal stability were determined. The surface properties are compatible with cell adhesion and growth and the *in vitro* biocompatibility studies showed promising results. Three copolymers based on TA showed significant conductive properties. This article constitutes the first report on a new family of charged biodegradable polymers. These polymers could have several applications in bone and nerve regeneration and DNA complexation and delivery. We recently described DNA complexed with salens-grafted-PLA, similarly, linear salicylidene polymers can be tested for their abilities to form DNA complexes.[24]

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