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Medical Uses for Polyelectrolyte Complexes

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SUMMARY

Polyelectrolyte complexes (PEC) are ionically bonded hydrogels. The resin is synthesized by coreacting linear, water-soluble ionic polymers of opposite electrical charge under carefully controlled conditions. The resulting material is insoluble in water, electrolytes, organic, or common solvents, but soluble in special ternary solvents. Optically clear membranes or shaped articles can be prepared by employing simple solvent casting and drying techniques upon resin dissolution. The equilibrium gel water content of typical, homogeneous complexes can be made to range from 30 to 90% by weight by changing the initial polyanion to polycation ratio. For almost any given charge ratio the water content can be varied from 30 to 90% by initial adjustment of the solvent composition. As the gel water content of a membrane is raised the dialytic, oxygen, and water transport increase. High water content membranes with and without glass reinforcement were shown to be extremely permeable materials. Because these hydrated complexes appeared to be chemically inert and could be tailored to be rich in either polyanion or polycation charged groups, their biocompatability was studied. Extraction, toxicity, tissue compatability, carcinogenicity, and blood contact studies on various polyelectrolyte complexes were carried out.

Intramuscular tissue toxicity tests showed PEC films to be free of irritant effects. Acute systemic toxicity tests indicated that no toxic material was leached from various polyelectrolyte complexes when extracted with normal saline, alcohol and sodium chloride, polyethylene glycol 400 and sesame oil, and these films were shown to be resistant to hydrolysis. Carcinogenic and blood contact studies are in progress. Evaluation of this material for permeable contact lenses, corneal implants, vascular grafts, coatings for other prosthetic devices, and membranes and components for artificial kidneys and blood oxygenators is underway.

INTRODUCTION

Extensive studies of a polyelectrolyte complex (Ioplex 101) prepared from sodium poly(styrene sulfonate), NaSS, and poly(vinylbenzyltrimethyl ammonium) chloride, VBTACl, have been carried out at Amicon (see Fig. 1). Both of these linear, oppositely charged polyelectrolytes are water soluble until coreacted to form an amorphous, infusible complex, or "polysalt." Dissolving this water insoluble complex is possible by employing certain ternary solvent systems; common polymer solvents are completely ineffective.

A typical three-component system would consist of specific combinations of sodium bromide, acetone, and water. Figure 2 depicts the solubility region of a cosolution of NaSS and VBTACl. It is believed that the strongly dissociated component of a ternary system shields the hydrated, oppositely charged groups from each other while the water miscible, organic component solvates the organophilic backbone of the polymer chains.

After casting and sufficiently drying a polyelectrolyte complex, the material is usually placed in a water leaching bath. Once equilibrated in water, these hydrogels must be kept wet to maintain their flexibility. If these materials are first equilibrated in 70% glycerine, however, they can be air dried, and if they are returned to a water environment they will reequilibrate to their original gel water content level.

A newer polyelectrolyte complex (Ioplex 103) has been prepared from NaSS and poly(diallyldimethyl ammonium) chloride, DADACl (see Fig. 3). Hydrogels prepared from these polyions have similar physical characteristics to the 101 materials. However, 103 complexes can be heat sealed, exhibit a high elongation to break, and can be steam autoclaved. These unique

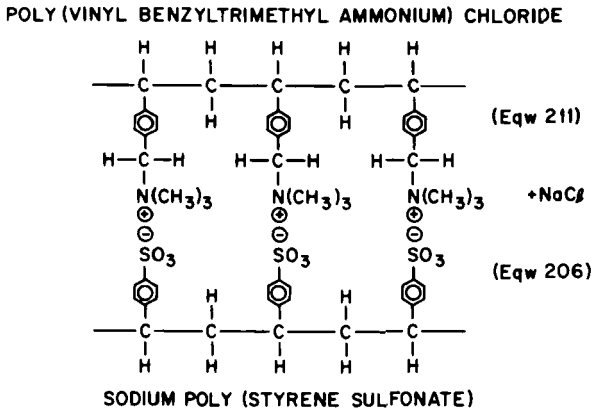

Fig. 1. Ioplex

properties may be attributed to the less rigid and more heat resistant DACACl, which has replaced the rigid styrene, and the more readily degradable trimethylated nitrogen groups of VBTACl.

The mechanical properties (tensile strength, elongation to fail, and modulus) of Ioplex 101 and 103 films are shown in Figs. 4, 5, and 6. ASTM methods were used for these determinations. The samples were kept moist by enclosing them in water-filled thin polyethylene bags. As can be seen from Figs. 4 and 5, there is a tradeoff between tensile strength and elongation, with the former decreasing with increasing gel water content while the latter increases. The optimum gel water content would depend on the application. The tensile strength of the polyelectrolyte complex is considerably lower than many plastic materials but approaches that of dimethyl silicone rubber at low gel water contents [2]. Techniques have been developed to reinforce polysalt materials with porous materials such as fabric, paper, or screens; and methods have been developed to fabricate a variety of shapes from these materials. Sheeting, open and closed-end tubes, and molded articles can be prepared. The polysalt can also be used as a coating. Reinforcement of PEC has been accomplished by the use of impregnating polyester or special glass fabrics so that a range of strengths and stiffness can be made available. Adhesion is only fair on smooth polymeric substrates but is excellent on porous materials such as velour. A stoichiometrically equivalent ratio of two oppositely charged polyelectrolytes yields a neutral hydrogel which has no ion exchange capacity. By suitable adjustment of the ratio of polyanion to polycation used in their preparation, complexes can be formed containing an excess of either polyanion or polycation. (The net ion exchange capacity can be varied

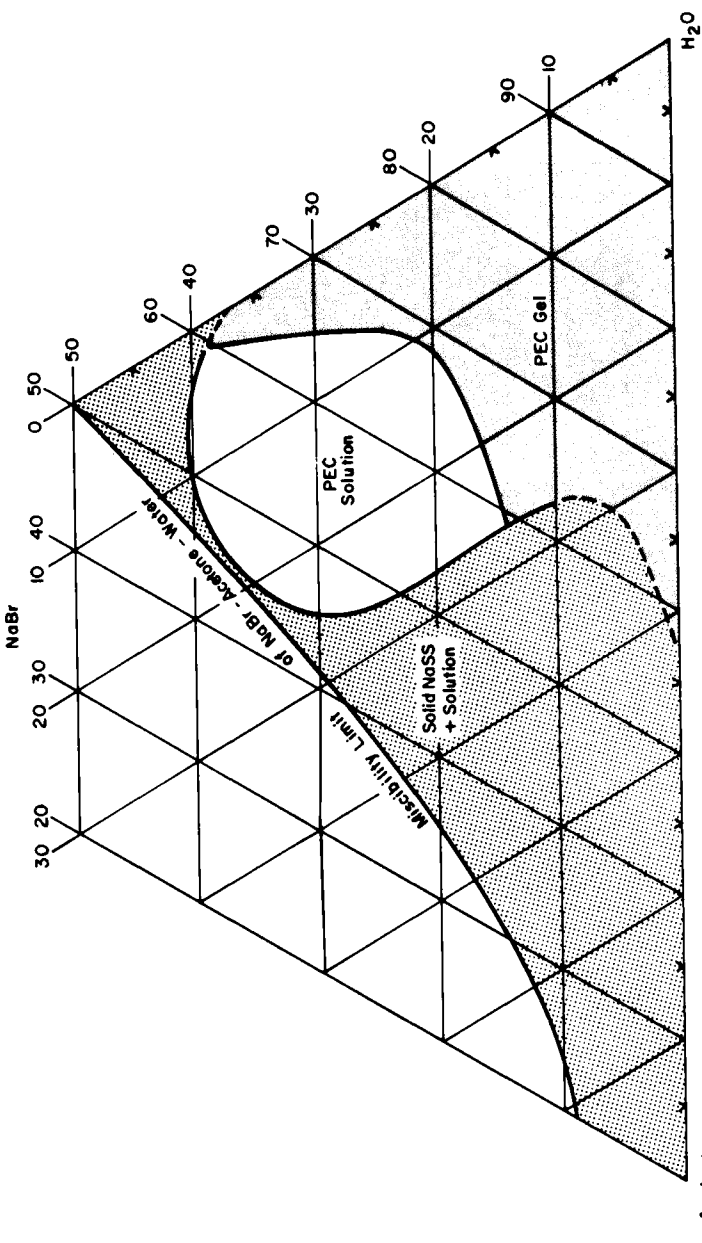

Fig. 2. A typical solvent system for polyelectrolyte complexes. The solution is a cosolution of poly(vinylbenzyltrimethyl ammonium) chloride and sodium poly(styrene sulfonate) at 25-30°C.

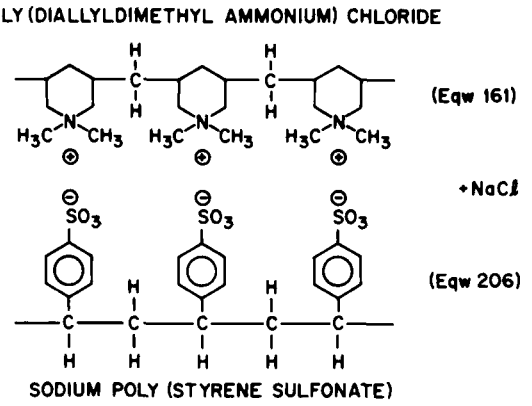

Fig. 3. Ioplex 103.

from 2.0 meq. per dry gram of cation exchange capacity to 2.0 meq. per dry gram of anion exchange capacity.) By adjusting the solvent composition and drying conditions the equilibrium gel water content of a complex for almost any given charge ratio can be varied from 30 to 90% by weight. This is accomplished by incorporating a nonvolatile component, such as sulfuric acid, into the ternary shielding solvent mixture. The nonvolatile substance will not evaporate upon drying, keeping the charged groups apart and hence maximum consolidation is prevented. Larger amounts of a nonvolatile component in a casting solution will result in higher gel water content complexes. The polyion ratio, varied in combination with the water content, can be used to control the net surface charge density of these materials.

BIOMATERIALS EVALUATIONS

Hydrated polyelectrolyte complexes contain both polyanion and polycation groups, and they exhibit a tendency to become hard and extremely brittle when dried. "Excellent" biocompatability of these hydrogels has been based on the finding that polyelectrolyte complex materials are compatible in the biological environment when implanted in various tissue areas and in the bloodstream [3-8]. In the former area, resistance to catabolic attack and immunologic reaction was inferred from numerous animal tests. Complexes have been implanted in such sites as the femoral arteries of cats; the eyes of dogs; and the optic nerves, brains, and subcutaneous areas of rabbits. There has been little or no sign of irritation

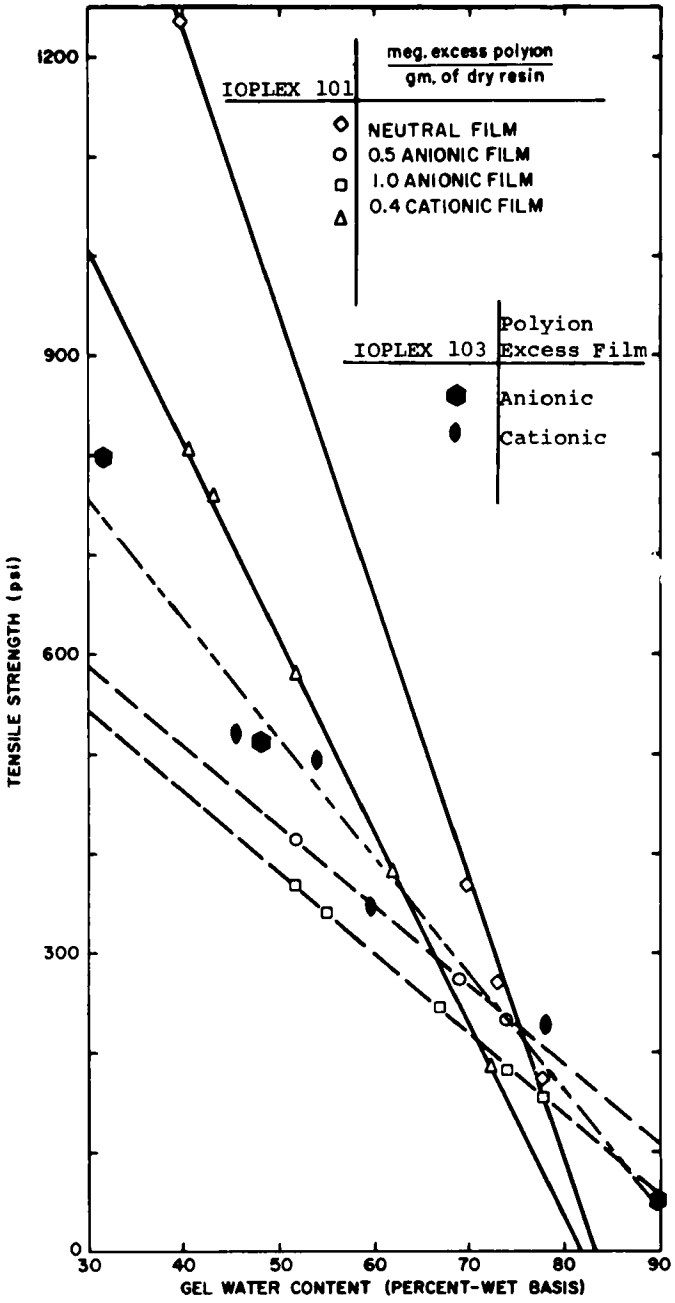

Fig. 4. Tensile strength vs gel water content for homogeneous Ioplex 101 and 103 films.

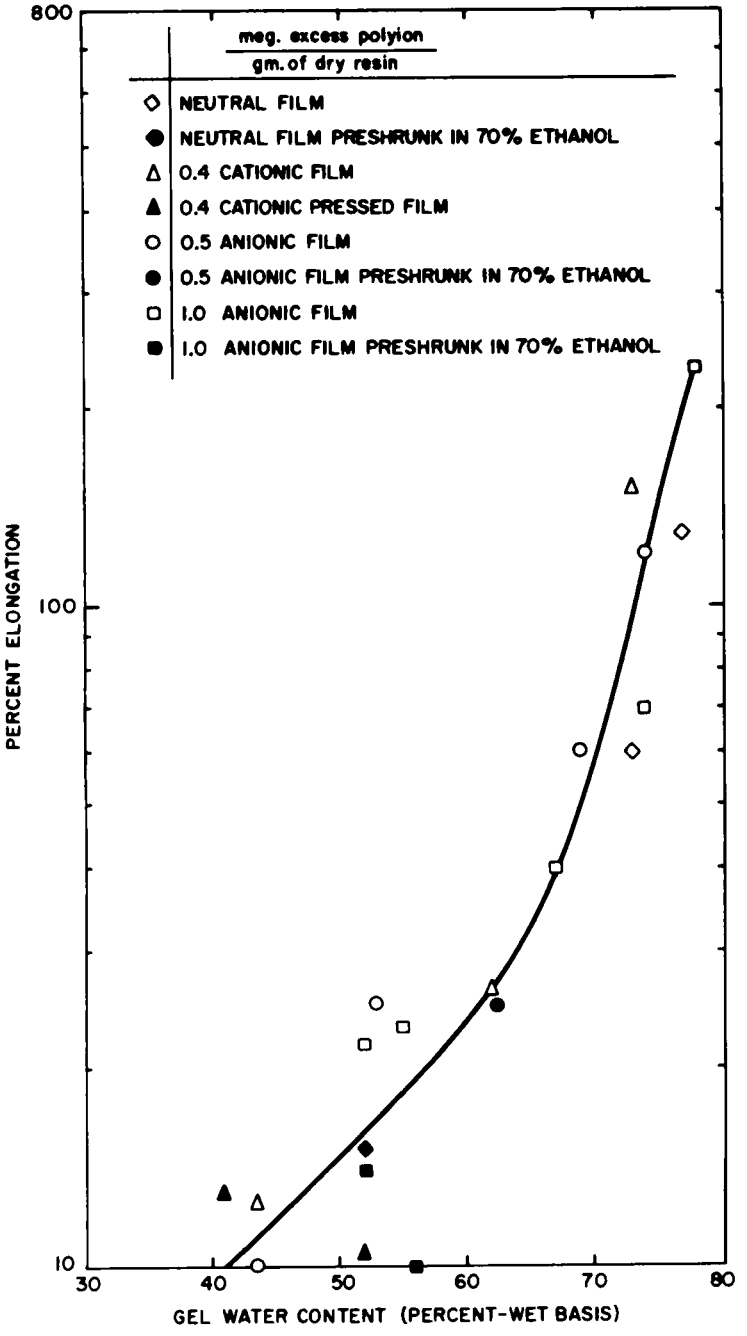

Fig. 5. Per cent elongation vs gel water content for Ioplex films.

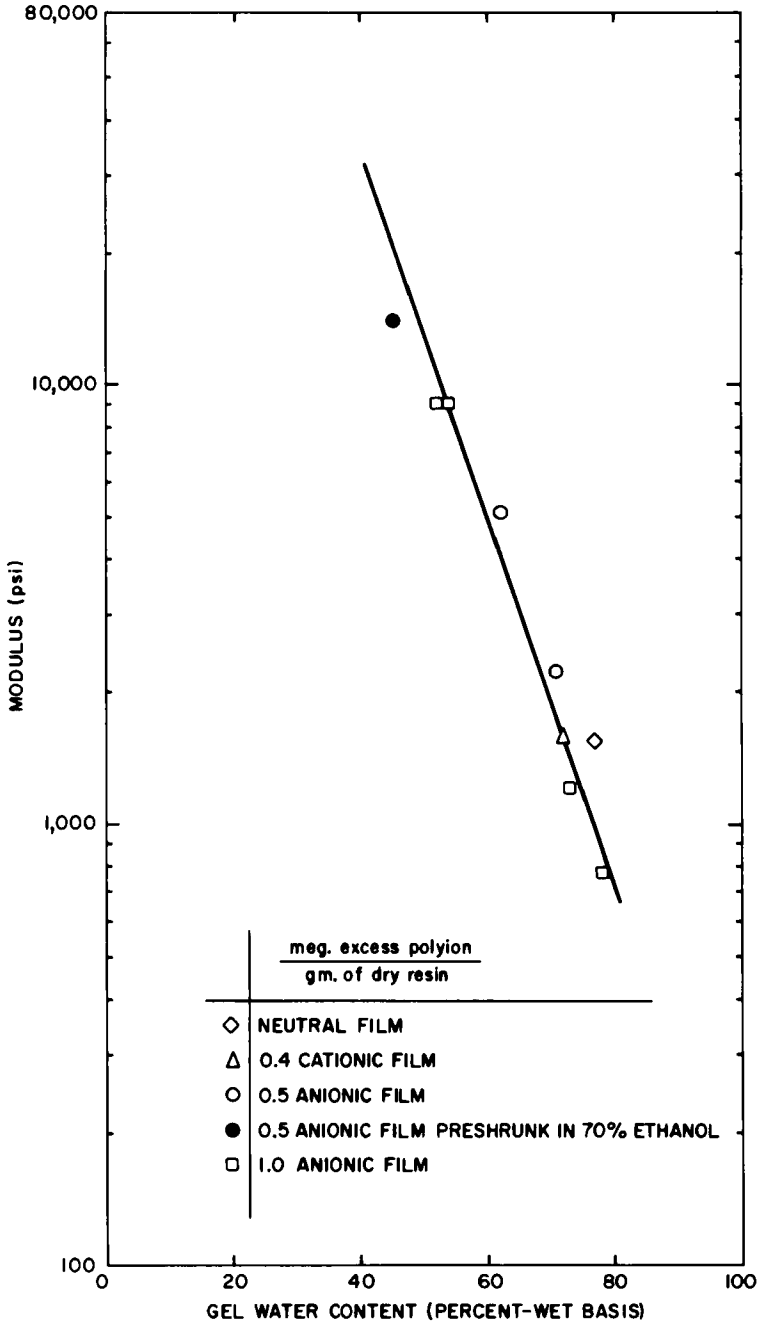

Fig. 6. Modulus vs gel water.

around the implants and no signs of decomposition or dissolution of the implanted material after residences of up to a year. In some cases (particularly when the high gel water content complexes were implanted) there has been actual ingrowth of tissue into the material. Nonthrombogenic behavior has been demonstrated in dogs in in vivo studies.

Extensive extraction, toxicity, and tissue compatability studies were recently performed on sterilized Ioplex at Cutter Laboratories. The usual sterilization procedure employed in these tests consisted of soaking the polyelectrolyte complex film or tube in 3.5 M HCl for 1 hr followed by leaching in sterile saline, sterile 0.1 M NaOH, and sterile saline again. This procedure does not alter the blood clotting characteristics of polyelectrolyte complex materials. That it is effective has been demonstrated by inoculating a sample with spores, pseudomonas, and staphylococcus; sterilizing through the use of the above procedure; and culturing the film sample. No evidence of the contaminating species appeared. (An electron bombardment sterilization technique is currently being evaluated because of its convenience for certain applications.)

Tests conducted at Cutter Laboratories are outlined below.

A. Tissue Compatability Studies

Muscle Implant Test. Acute intramuscular tissue toxicity test NF XII-522 [9]. Male New Zealand Albino rabbits weighing 2.5 to 3.5 kg were used. Each rabbit had five to seven samples implanted on the left side, and two to three control samples implanted on the right side. Each lot was tested in at least three rabbits.

The sterilized control and test samples were inserted into the paravertebral muscles of anesthetized rabbits using aseptic technique.

The rabbits were killed by an overdose of sodium pentobarbital after 7 days and the test sample implantation sites compared to control sites for encapsulation film or hemorrhage. According to the NF XII test, "The tissue immediately surrounding the negative control strips should appear normal and entirely free of hemorrhage, film or encapsulation. The sample meets the requirements of this test if the reaction at three or four sites in each animal is not significantly greater than that at the negative control sites."

Results. All of the Ioplex materials listed were tested and considered to be free of irritant effects.

Samples

No.	Sample	Sterilization procedure	Unusual observations
1	Ioplex 101/0.5X/40[a]	Acid/alkali wash	
2	Ioplex 101/Y/70	Acid/alkali wash	
3	Ioplex 101/0.5Z/50	Acid/alkali wash	
4	Ioplex 103/0.15X/30	Acid/alkali wash	
5	Ioplex 101/0.5X/70	Acid/alkali wash	
6	Ioplex 101/0.5Z/40	Acid/alkali wash	Green coloring after implant
7	Ioplex 101/Y/35	Acid/alkali wash	
8	Ioplex 101/0.8Z/60	Acid/alkali wash	Slight yellowish green after implant
9	Ioplex 103/0.5X/40	Autoclave, under water 121°C, 20 min	
10	Ioplex 103/0.15X/30	Flowing steam for 60 min	Sticky immediately after sterilization
11	Ioplex 103/0.1Z/45	Acid/alkali wash	
12	Ioplex 101/0.8X/55	Acid/alkali wash	
13	Epon 828 diethylene triamine system	Acid/alkali wash	
14	Epon 828 diethylene triamine system plus [Ioplex 101/0.5X/45]	Acid/alkali wash	Tendency of flake after sterilization
	USP negative control plastic, polyethylene	Sterilized in the same manner as test samples	

[a]The coding involves the type of Ioplex in the first term. The second term involves the meq. of cationic or anionic charge per gram of dry resin with "X" equaling anionic and "Z" equaling cation. A "Y" resin is neutral. The third term indicates the per cent gel water.

Extraction Studies

Extraction and Toxicity Studies. (1) Acute Systemic Toxicity Test. Animals and test procedure as listed in NF XII, page 521 with the exception of the PEG 400 dose. This dose was changed from 10 to 5 g/kg because 10 g/kg exceeds the LD_{50} (LD_{50} of PEG 400 = 9.2 g/kg) [10].

(2) Rabbit Intracutaneous Reactivity Test. Animals and test procedure as per NF XII, pages 521-522 [9]. Ten injection sites (0.2 ml) of test sample compared to 5 injection sites (0.2 ml) of the blank. Each test sample compared to blank in two rabbits.

Samples

Sample identification	Results, 4 ext media
Acute Systemic. Mouse, Extracts	
1. Ioplex 101/0.5X/50	Passed
2. Anionic Ioplex 101/1.0X/60	Passed
3. Neutral Ioplex 101/Y/61	Passed
4. Anionic Ioplex 101/0.5X/70	Passed
5. Cationic Ioplex 101/0.5Z/68	Passed
6. Anionic Ioplex 103/0.15X/23	Passed
Rabbit Intracutaneous Test	
1. Ioplex 101/0.5X/50	Passed
2. Anionic Ioplex 101/1.0X/60	Passed
3. Neutral Ioplex 101/Y/61	Passed
4. Anionic Ioplex 101/0.5X/70	Passed
5. Cationic Ioplex 101/0.5Z/68	Passed, except repeat EtOH and NaCl
6. Anionic Ioplex 103/0.15X/23	Passed

Sample Preparation. Extraction of Ioplex 101 materials was carried out at 50°C for 72 hr using 120 cm^2 of film surface area per 20 ml of extracting medium. Two samples and one blank were prepared in each of the following extracting media:

a. Sodium chloride injection.
b. Alcohol (5%) and sodium chloride (0.9%) in water.

c. Polyethylene glycol 400.
d. Sesame oil.
The above media without plastic samples were used as blanks.

Results

Conclusion Test 1. Results of this test indicate that no toxic material leached out of the plastics during the extracting procedure using normal saline, 5% alcohol in normal saline, polyethylene glycol 400, or sesame oil as the extracting medium. Ioplex materials were also resistant to hydrolysis.

Conclusion Test 2. Results of this test indicate that no irritant substance was leached out of the plastic during the extracting process. However, Sample No. 5 (Ioplex 101/0.5Z/68) will be repeated for an alcoholic saline extract because it showed a slight erythema (borderline finding) at all test sample injection sites compared to no erythema of the blank injection sites.

To summarize the results at Cutter Laboratories, intramuscular tissue toxicity tests showed Ioplex films (101, 103, and a 101 epoxy laminate) to be free of irritant effects; acute systemic toxicity tests indicated that no toxic material was leached from 101 and 103 films when extracted with normal saline, 5% ethanol in normal saline, polyethylene glycol 400, and sesame oil; Ioplex films were shown to be resistant to hydrolysis. These results reinforce the position that Amicon's polysalt materials are highly compatible and chemically inert.

In addition to the above studies, physiological compatability was inferred by testing the stability of proteins after they were concentrated by using polyelectrolyte complex ultrafiltration membranes [11]. Results from this study showed that the proteins in human serum and in Cohn fractions III, IV-1, and V were not significantly altered when brought into contact with the Ioplex membranes. Tests performed by Dr. Francis Roe of the Chester Beatty Research Institute, Institute of Cancer Research, London, England, consisted of implanting films into subcutaneous tissue, 16 samples each of anionic, neutral and cationic Ioplex 101 for periods up to 18 months [12]. No evidence of tumors was found in the rats implanted with the anionic or neutral Ioplex although three rats out of 16 implanted with the cationic Ioplex developed sarcomas after a 12-month period. This test will be continued and additional carcinogenic tests on perforated films and powders initiated.

Perhaps the most important testing results to date are those relating to blood compatibility. Dr. Gott's test (where small grafts 9 mm x 8 mm i.d., are inserted into the vena cava of dogs, and after a certain time period, the test vessels are examined for clots) is a very severe one because of the low velocity of blood in the venous segment and because a dog's blood is hypercoagulable compared to human blood. Results of his acute (2-hr) and chronic (2-week) tests show that Ioplex 101 materials of 0.5 meq. of excess polyanion containing 45 to 55% gel water are thromboresistant [8]. In most cases the test vessels are completely clean or show only a small amount of clot formation. The minimal clotting could be due to insertion technique (as a trace quantity of tissue juice will cause clotting) or to turbulence or stagnant areas at the ends of the grafts. In a few cases where thrombus was observed on a section of the test vessel, it was also noted that the thrombus did not propagate as it would on most materials.

In addition to Dr. Gott's tests, blood impingement tests performed by Dr. Madras of Avco-Everett Research Laboratories have shown that this anionic material was one of the least thrombogenic materials he has tested, with no evidence of clotting under slow flow conditions and good performance under rapid flow [13]. Dr. Madras has also shown that neutral resins have some thromboresistance properties, and cationic materials appear thrombogenic. These results correlate extremely well with those of Dr. Gott's (see Table 1 for a brief summary of Dr. Gott's results).

An attempt to predict the thromboresistance of Ioplex polyelectrolyte complexes has proved promising. A series of Ioplexes of minimum water content with various ionic excesses has been made with the results shown in Fig. 7 (i.e., an 0.5 meq. excess polyanion film taken from a distilled water bath will not contain less than 43% water. It may, however, be in an "over-swollen" state and contain more than the minimum amount of water.) The density of sulfonate groups in the (minimum) wet complex versus the milliequivalents of charge on the dry resin is plotted as the solid curve in Fig. 8. Ioplex materials which have actually been tested by Dr. Gott are indicated by the filled squares. These values fall below the solid line curve because the Ioplex samples were in the "over-swollen" state. Three areas under the curve are labelled according to the clotting results of Dr. Gott's in vivo blood compatibility tests. Thus it appears that the clotting tendency of Ioplex 101 is a function of sulfonate density. This theory will be tested further, and if it has validity, it will be used as a guide for selection of candidate polymers for use as components of polyelectrolyte complexes.

Table I

RING TYPE	IONIC STRUCTURE	H_2O CONTENT	2 HOUR IMPLANT RESULTS		
I. MODERATELY ANIONIC	0.5 MEQ. EXCESS POLYANION PER DRY GRAM OF RESIN	55% (WET BASIS)			
II. HIGHLY ANIONIC	1.3 MEQ. ANIONIC EXCESS	80%			
III. NEUTRAL	NEUTRAL	50%			
IV MODERATELY CATIONIC	0.86 MEQ. CATIONIC EXCESS	67%			

RING TYPE	IONIC STRUCTURE	H_2O CONTENT	2 WEEK IMPLANT RESULTS				
I. MODERATELY ANIONIC	0.5 MEQ. ANIONIC EXCESS	55%					

LEGEND:

SIDE VIEW

atrial end

END VIEW

atrial end

(Dark Areas Denote Clotting)

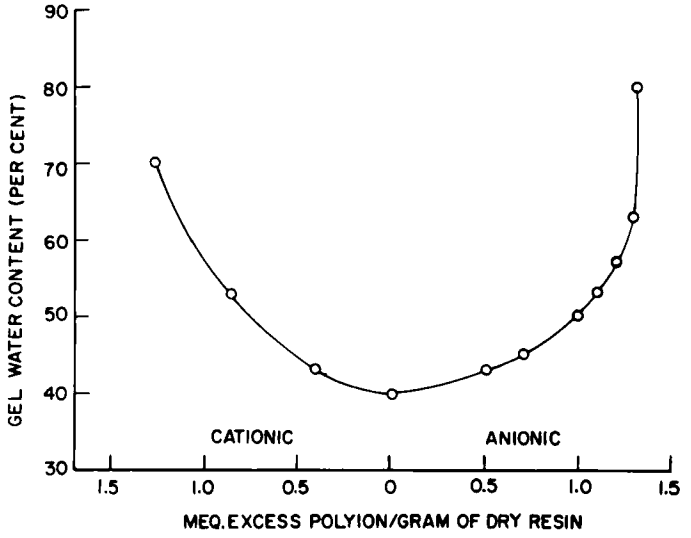

Fig. 7. Equilibrium gel water contents for polyelectrolyte complex resins.

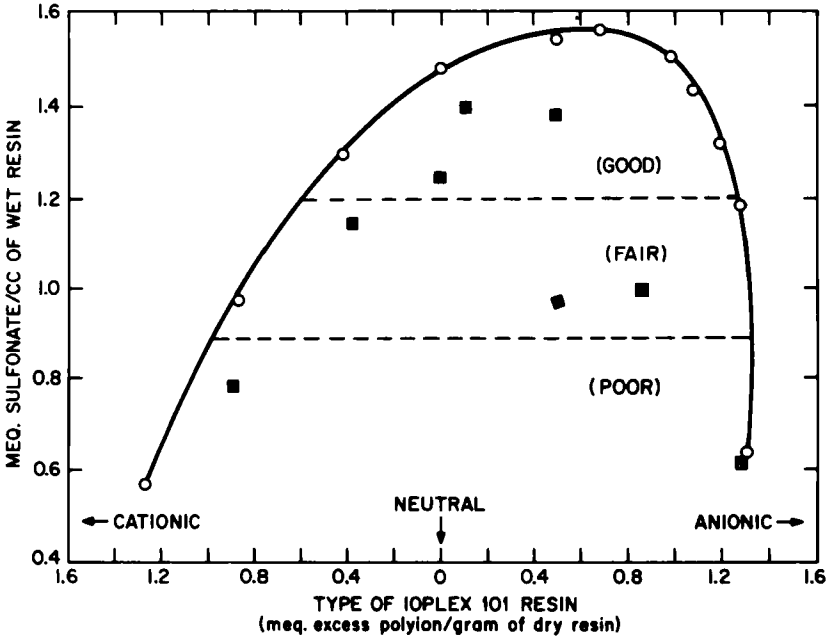

Fig. 8. Milliequivalents of sulfonate per cubic centimeter of wet resin vs type of Ioplex 101 resin.

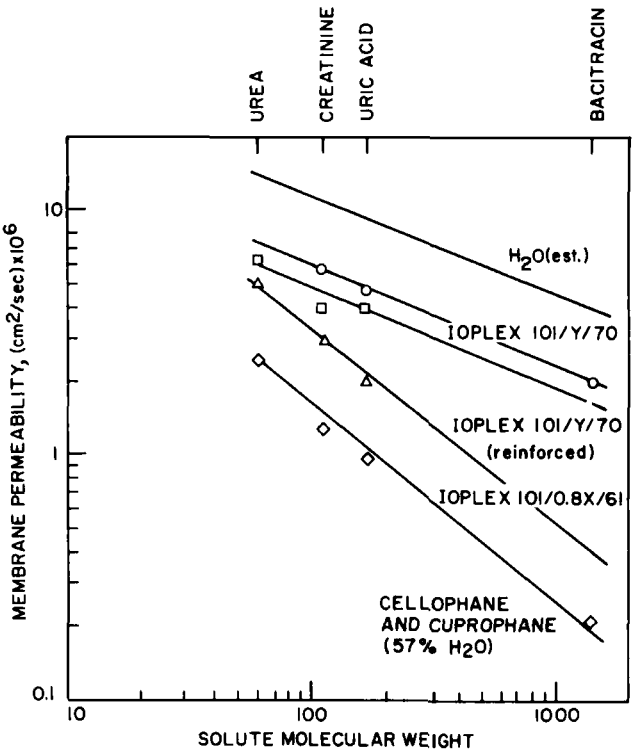

Fig. 9. Dialytic permeability of polyelectrolyte complex membranes.

BIOMEDICAL APPLICATIONS AND CONCLUSIONS

Polyelectrolyte complexes formed with an excess of polyanion over polycation groups appear to simulate the naturally anionic vascular surface (since both surfaces have high water contents, and contain both bound anionic and bound cationic groups, with an excess of the anionic over the cationic). The polysulfonate-rich complex would appear to be thromboresistant, and it has the advantage over surface heparinized polymers of being homogeneous throughout so that the problems of surface abrasion or cracking that might expose a thrombogenic substrate are avoided. In addition, polyelectrolyte complexes are made from totally synthetic, readily available, and relatively inexpensive raw materials.

By varying the water content the permeability to electrolytes and higher molecular weight materials and gases can also be controlled. It has been found that the oxygen permeability of PEC hydrogels is not as high as that of dimethyl silicone but high enough so that the material could be used for a membrane in a blood oxygenator, and the compatibility with blood is far superior to that of dimethyl silicone rubber [14]. The dialysis capabilities of homogeneous PEC membranes were measured, and these values are reported with those of cellophane in Fig. 9. One membrane was supported with an open, lightweight glass mesh to improve its handling characteristics. (Notice that the reinforcing material did not significantly decrease the membrane's permeability.) The high dialysis coefficients of Ioplex membranes would allow more complete blood detoxification. These complexes, coupled with a thromboresistant surface, should be ideal for artificial kidney applications.

The polyelectrolyte complex structure, combined with the gels' extremely high permeabilities to water, gases, and dissolved low molecular weight compounds, would be expected to lead to high compatibility in the body. This compatibility has been well demonstrated in work that has been conducted during the past 4 years on implantation of these materials in sites ranging from muscle tissue to eye tissue, and in tests conducted in vitro which demonstrates that no significant denaturation or alteration of enzymes or other proteins occurs when these are contacted with polysalt materials. Testing results suggest that Ioplex could be used as a material from which to fabricate various types of implants, especially those which require blood contact. Possible uses for PEC hydrogels include corneal replacements, permeable contact lenses, controlled-release encapsulants, artificial kidneys, vascular prostheses, blood fluid duct prostheses, indwelling catheters, coatings for implantable devices, tissue adhesives, and oxygenators.

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