PRODUCTION OF BIOMEDICAL POLYMERS

III. Instrumental Analysis to Assure Biocompatibility

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The nature of the interaction of blood with materials foreign to the body is still poorly understood. Indeed, knowledge of the complex sequence of reactions which produce blood clotting or thrombosis under more natural circumstances remains incomplete. Yet synthetic polymers are employed in a wide variety of biomedical devices which contact blood within the body or in some ex vivo situation.

Since the underlying phenomena are unclear, successful production of these polymers is necessarily based on an empirical approach. This presentation will attempt to show that there can be system in this method and that analytical instrumentation is critical to its success.

Among the various prosthetic applications of polymeric materials some of the most demanding are those in which circulatory assistance is supplied to an ailing heart by mechanical means. When this assistance is provided by a flexing elastomeric balloon or diaphragm the polymer must possess excellent physical/mechanical properties as well as a compatibility with the processed fluid, the blood. The ability to withstand a million flex cycles per week must be achieved while maintaining a surface chemical composition and morphology which does not cause excessive blood damage or thrombosis. This combination of restraints eliminates many materials from consideration and was responsible for major difficulties in early work in the field of implantable cardiac assist devices.

Physical/mechanical properties are characteristics considered to be inherent to a particular polymer. It is well-known, however, that to some degree they are functions of the way the material is handled prior to use.

Thermal history during fabrication from the melt can effect tensile properties via degree and distribution of crystallinity. Casting solvents can favor one block in a copolymer and give rise to or eliminate yield points, as well as effecting transport properties in membranes via the microscopic morphology of the bulk. Polymer molecular weight and its distribution are known to effect nearly every measurable physical/mechanical property; strongly in the low range but reaching an asymptote at higher molecular weights.

While these various unit operations or post treatments on the fully reacted polymer are commonly employed by polymer engineers an analogous approach has not been reported for optimizing biocompatibility. To date most researchers have approached the problem of thrombogenicity as if it were an invariant of the material arising primarily from the surface chemical structure. This is doubtless true in the ideal situation in which this property is not overshadowed by gross surface roughness, particulate impurities or the presence of low molecular weight (and therefore surface active) impurities and oligomers.

The diverse and sometimes conflicting hypotheses regarding the requisites of blood compatibility suggest that experimental results are often affected by a randomized factor associated with surface anomalies. It has been shown experimentally that a particulate inclusion a few microns in diameter can easily become the locus of thrombus formation on an otherwise passive surface. It is easy to see why assigning differences in thromboresistance to structural features at the molecular level could lead to confusion under any but the most controlled exper-
mental conditions.

Without question, real understanding of blood/foreign surface interactions will eventually result from additional insight into phenomena on the molecular level. In the interim a better understanding of the way easily controlled bulk properties such as purity and molecular weight ultimately effect surface biocompatibility can produce substantial improvements in device performance, while minimizing trauma to the patient.

This 'unit operations approach to biomedical polymer production' is diagramed in figure one. To be a candidate biomaterial a polymer must possess a basic set of engineering properties suitable for the application. Flexible blood pumps, for instance, require excellent tensile properties and flex life in an elastomer which is easily fabricated and bondable to housing and connectors. This matching of 'physicals' to the end use is common to countless material specification problems and is not at all trivial.

The second requirement shown within the raw material block of figure one is in vivo stability. The candidate material must retain a high percentage of its original properties during prolonged implantation in the rather hostile environment of the body. Hydrolytic stability is a must, since enzymes and the corrosiveness of body fluids can produce extensive degradation of susceptible chemical bonds. Freedom from gross encrustation by plasma components such as lipids is also part of this requirement.

Although this list of basic raw materials requirements might be lengthened, in vivo stability and engineering properties which suit the application are perhaps the most basic. If they are first met it is likely that by applying the appropriate unit operations a satisfactory biomaterial can be produced. Depending upon the application, 'satisfactory' may mean that the material does not elicit inflammatory response when in contact with tissue, or that it does not cause thrombosis when exposed to blood or that it does not elute toxic leachables into the body during use.

In the case of cardiac assist devices previously mentioned, blood compatibility is of primary importance. In devices used in connection with surgery, systemic anti-

coagulants may be withheld since they can interfere with normal healing. In such situations the material itself is severely tested.

The so called segmented polyurethanes are known to possess the two basic properties for candidate biomaterials and are among the few polymers now available for pumps requiring elastomeric blood contact surfaces. Chemically they are the reaction products of polymeric ether glycols and a slight excess of a diisocyanate and a "chain extending" diamine or dihydroxy reagent. As such they are block copolymers possessing soft segments (i.e. low Tg) of polyether and hard segments of urethane and/or urea functionality having a high degree of intermolecular hydrogen bonding. By employing various isocyanates (aliphatic, aromatic) glycols (polyethylene, polypropylene, PTEG) and chain extenders a wide variety of structures is possible. The common feature of typical materials is a two phase micromorphology arising from the thermodynamic incompatibility of the soft and hard segments.

This structure can give rise to thermolabile or 'virtual' crosslinking since the hard urethane domains act as tie points at temperatures below their crystalline melting points but allow the polymer to flow at higher temperatures. The result is a polymer which is a 'snappy' elastomer and effectively 'vulcanized' at use temperatures but which is thermoplastic at elevated temperatures. If stoichiometry during the condensation type polymerization is carefully balanced, the product may be solvent soluble as well.

Nothing in the above description distinguishes segmented polyurethanes intended for implantation into the blood stream from available materials of commerce often used in less demanding applications such as shoe soles and the like. In fact the difference between the two can be entirely a function of the unit operation to which they are subjected. The efficacy of these operations in yielding proper levels of purity and surface properties can be determined by a reasonably straightforward application of instrumental methods of polymer characterization.

Figure one proposes that given the proper raw polymer, a material with improved biocompatibility can be produced by applying traditional chemical engineering
unit operations under the (not necessarily closed loop) control of analytical instruments.

Extraction, for instance, can reduce low molecular weight residues, oligomers and unwanted additives. Filtration eliminates potentially thrombogenic particulate impurities. Distillation and adsorption purify casting solvents so that non-volatiles are not left behind as part of the blood contact surface. Control of the extent of polyfunctional reactions can prevent the occurrence of a gel phase which may produce surface roughness, a known cause of thrombogenicity.

Almost no quantitative information is available relating blood compatibility to the results of these various operations but one generality is possible. That is, any foreign substance which finds its way to the surface of a device intended for use in the blood stream is a potential cause of thrombosis. The exact thrombogenicity of the variety of possible contaminants may be unknown but this general rule seems to be universally applicable. Foreign substance, as used here, means any particulate impurities incorporated in the polymer during manufacture, including any insoluble fractions that may be present. It also means catalyst residues, excess reactants and some processing aids and additives. It may also mean some of the low molecular weight (oligomeric) polymer and, as we will see later, the removal of even medium molecular weight polymer species may improve blood compatibility. This generalized protocol for the preparation of biomedical polymers has emerged during six year of production of Avcothane-51R elastomer which is the material of construction of the Avco Intra Aortic Balloon Pump (IABP).

Avcothane-51R is a hybrid consisting of a segmented polyurethane, a polydimethyl siloxane and a minor amount of urethane/silicone block copolymer. Preparation of Avcothane elastomer involves the extensive purification and characterization of all raw materials. Solvents used in the process are distilled in glass, and dried over molecular sieves to reduce nonvolatile contaminants to one or two parts per million of anhydrous solvent. Polymers are purified and vacuum dried to remove oligomeric impurities and additives. The anhydrous synthesis step is conducted in glass reaction vessels, and the filtered dipping-solution is handled in a closed system until fabricated into devices or packaged for shipment.

The IABP is a temporary cardiac assist device which has been used to treat more than 20,000 patients suffering from cardiogenic shock and other low cardiac output states. The IABP is inserted into the descending aorta and counterpulsed with the patient's own heart beat. Since it is often used in connection with surgery, it must be non-thrombogenic in the absence of systemic anticoagulants. Occasionally, the device is applied for several weeks of continuous operation requiring the balloon to have a good flex life and high resistance to hydrolytic degradation.

Gel Permeation Chromatography (GPC) is employed at three separate stages in the production of Avcothane-51R. Raw polyurethane is characterized according to its molecular weight distribution to determine the level of impurities which must be removed. Following a precipitation purification step the material is again chromatographed as an inprocess check. Finally, the Avcothane prepolymer dipping solution is analyzed to measure the extent of copolymerization between the silicone and urethane components. An optimum degree of copolymerization has been found to exist. Figure 2 shows the effect of extent of reaction on the resultant surface properties of cast films. GPC detects the presence of low solubility long chain branched polymer which can degrade surface smoothness by premature 'skin' formation during solvent evaporation.

GPC has also been very useful in understanding polymer purifications. Precipitation of a polymer solution by the addition of a nonsolvent has been found to give results identical to equilibrium leaching of the undissolved solid in a nonsolvent/solvent mixture of equivalent composition. This is useful for two reasons. First, it means that solvent recovery from the process need not separate solvent from nonsolvent but only liquid from nonvolatile impurities, a simpler operation. Secondly, continuous countercurrent contacting may be employed since the polymer need not be redissolved after each equilibrium stage. This technique can produce purer material more
easily and is analogous to the difference between fractional distillation and a simple flash evaporation.

Figure three shows the GPC's of polyurethane purified by precipitation from THF solution and the fraction removed in the process. Final liquid composition was 65% methanol and 35% THF by volume. Figure four shows again, a pure polyurethane together with fractions removed by equilibrium leachings in three different nonsolvent/solvent mixtures. The GPC's of the fraction removed at 65/35 methanol/THF are the same in both cases.

The polyblend nature of Avcothane-51 elastomer results in an anisotropic distribution of silicone within the urethane rich continuous phase. Degree of copolymerization and ambient conditions can effect the silicone composition of the blood contacting surface of solvent cast films. Since optimum blood compatibility appears to occur at specific silicone surface composition (5) it is necessary to measure this quantity.

Infrared Attenuated Total Reflection spectroscopy (ATR) is used to measure the ratio of the two polymer components present in surface and is routinely employed as a quality control technique. An "IRATR Index" described elsewhere (5) has been defined and found to be inversely proportional to the concentration of silicone in the surface as measured by Electron Microprobe Analysis. Figure five shows the correlation between probe results and the reciprocal 'IRATR index'. Simple transmission IR is also used for identity and purity checks on incoming raw materials used in Avcothane manufacture.

Scanning Electron Microscopy (SEM), in addition to being used to check surface smoothness prior to device implantation, is employed to examine surfaces which have been exposed to blood in animal experiments or clinical situations. The density of adsorbed blood components, such as platelets and white cells, together with their morphology in the adsorbed state can indicate the nature and extent of interaction with the surface.

Related to, but separate from production applications, instrumental polymer characterization can be combined with controlled ex vivo testing to quantify the effects of unit operations on polymer biocompatibility. One such controlled material evaluation method is the Stagnation Point Flow Experiment (SPFE) of Avco Everett Research Lab (6).

This technique exposes polymers to 'non-activated' blood flowing directly from the carotid artery of an anesthetized dog onto the test surface under well controlled conditions of flow geometry. Blood impinges on the flat test sample normal to its surface in a test cell which is mounted in the stage of an optical microscope. Shear forces at the 'stagnation point' are zero and increase with radial distance from this point. White cells which adhere to the test surface at a given distance from the stagnation point are in equilibrium with their adhesive force and the fluid shear forces to which they are subjected. Thus, white cells adhering at points distant from the stagnation point have interacted more intensely with the surface than white cells closer to that point of zero shear.

For materials exhibiting a 'symmetric aggregation' of white cells around the stagnation point, the diameter of the white cell circle which forms is a quantitative measure of the material's blood compatibility.

A series of four molecular weight fractions were obtained from a single aromatic polyurethane and characterized by GPC. After 0.5 micron filtration in scupulously cleaned Millipore filters they were coated onto the glass cover slips which are the sample substrates for the SPFE. Figure six shows the relationship between white cell circle diameter and polymer molecular weight as obtained in a double blind experiment.

Absolute molecular weights are not presently available for the samples but the lowest is believed to have an \( M_W \) of \( \approx 50,000 \), the highest and \( M_W \) of above \( \approx 100,000 \). Little significance should be associated with white cell circle diameters as such. What is interesting is that the ranking, which shows blood compatibility increasing with molecular weight, had a 1/41 (4%) probability of occurring by chance.

Polymeric Pharmaceuticals often do possess optimum activity within a specific range of molecular weight \( M_W \), (7) oral toxicity of homologous series of compounds generally
decreases as molecular weight increases and polydimethylsiloxane oils injected subcutaneously induce tissue inflammation whereas higher MW homologs are 'inert'.(4) It therefore, seems reasonable to expect that some analogous mechanism may be important when blood contacts a 'solid' surface. In fact, polymers containing segments whose Tg's are below physiologic temperature are best considered as viscous liquids(1) so the molecular weight effect seems even more plausible. When one considers low MW species tend to be surface active and possess more entropy per mole than higher MW polymer chains due to less restrictions or segmental motion, then differences in chain length become even more interesting as a possible factor influencing overall blood compatibility.

It is generally agreed that when blood contacts a foreign surface the initial event is the adsorption of plasma proteins onto the surface(2,5) and that the intensity of this interaction determines whether passivation or thrombosis will result. Given this observation the entropy, end group concentration and/or permeability of the surface could be critical. Since these properties are MW related the hypothesis seems worthy of further investigation. Future studies will employ additional samples and more evenly spaced data points, in an attempt to elaborate on this very preliminary experiment.

If one immediately accepts the hypothesis that molecular weight is a significant determinant of blood compatibility and chooses to use high molecular weight materials one risks very little other than some possible added expense and difficulty in handling the higher molecular weight polymer. On the other hand improvement in performance of blood contacting devices could be significant.

The use of existing analytical instruments to characterize polymers and subsequent biocompatibility testing in carefully controlled experiments is more universally applicable then described here. The fact that the same instruments can be used to monitor production of these polymers provides a link between bulk properties controllable by the engineer and the in vivo performance experienced by the surgeon.

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References


Figure 1. Unit operations approach to biomaterials.

Figure 2. Extent of reaction and resultant surfaces.

Figure 3. Purification by precipitation.
FIGURE 4. PURIFICATION BY LEACHING

FIGURE 5. MICROPROBE/IRATR CORRELATION

FIGURE 6.